Applied Food Research 5 (2025) 100963

Contents lists available at ScienceDirect

Applied Food Research

journal homepage: www.elsevier.com/locate/afres

Combination of pulsed electric fields and ultrasounds technologies for mycotoxins mitigation in grape juice

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ARTICLE INFO

Keywords:
Pulsed electric fields (PEF)
Ultrasounds (USN)
Aflatoxins
Ochratoxin A
Enniatins
LC-MS/MS

ABSTRACT

Grape juice has increased market share due to its rich phenolic compounds, linked to preventive effects against cardiovascular diseases and cancer. However, concerns arise as studies report the presence of mycotoxins in juices, posing significant health risks. Pulsed electric fields (PEF) and ultrasounds (USN) have been explored as an eco-friendly tool for contaminants mitigation. The present study explores the effect of the sequential combination (PEF + USN) for mycotoxins mitigation in grape juice. For this, grape juice was elaborated and spiked individually with Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Ochratoxin A (OTA), Enniatin B (ENNB), and Enniatin B1 (ENNB1) at a concentration of 100 μg/L. Then, mycotoxins were extracted from treated samples and non-treated controls employing Dispersive Liquid-Liquid Microextraction (DLLME) and determined by Liquid Chromatography couple to mass spectrometry (LC-MS/MS). The reductions obtained were up to 48 % for ENNB1, 36 % for ENNB, 21 % for OTA, and 15 % for both of AFB1 and AFB2. Thus, the combination of PEF + USN showed interesting potential to be applied for mycotoxins mitigation.

Industrial relevance: The combination of pulsed electric field (PEF) and ultrasounds (USN) has shown great potential in reducing mycotoxins in grape juices. Concretely, a reduction up to 48 % for enniatins (ENNs), 21 % for ochratoxin A (OTA) and 15 % for aflatoxins were achieved. Overall, the need for innovative non-thermal technologies that do not affect the organoleptic and nutritional quality of the product and the growing interest in the consumption of products with a lower carbon footprint make this work a valuable starting point to be further applied into food industry.

1. Introduction

In recent years, there has been a shift in food consumption trends and consumer choices are now revolving around nutritional content, health advantages, food safety, and the environmental impact of processing, rather than just price and taste (FAO, 2022; Herrero et al., 2021).

Grapefruit also contains bioactive compounds (anthocyanins, phenolic acids, stilbenes, flavanols, and proanthocyanidins) with several health benefits, such as gut microbiota-modulating, hepatoprotective, and anti-inflammatory effects. Hence, grape juice is an important source of phenolic compounds that provides excellent nutritional properties. Phenolic compounds from grapefruit are related with preventive effect against cardiovascular diseases and cancer. These effects could be attributed to the antioxidant activity and antiproliferative properties of polyphenols (da Silva et al., 2016; Zhou et al., 2022).

However, the European Food Safety Agency (EFSA) and also the U.S. Food and Drug Administration (FDA) have reported the presence of mycotoxins in several foodstuff including fruit juice (EFSA, 2017; U.S. FDA, 2022). Mycotoxins are substances naturally produced as secondary metabolites by some filamentous fungi, under certain temperature and humidity conditions contaminating food chain, mainly during cultivation, but also during transport and storage. The main mycotoxin-producing genera are *Aspergillus, Fusarium,* and *Penicillium* that are frequently reported mainly in plant origin food and therefore pose a serious public risk health (FAO, 2023; Neme and Mohammed, 2017).

The occurrence of mycotoxins in fruit juice have been widely evaluated (Carballo et al., 2018; Mandappa et al., 2018; Pallarés et al., 2019;

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https://doi.org/10.1016/j.afres.2025.100963
Received 23 July 2024; Received in revised form 5 May 2025; Accepted 11 May 2025
Available online 16 May 2025

Torović et al., 2017). The occurrence of 30 mycotoxins in 80 commercial fruit juice samples from Valencia (Spain) has been already investigated and the mycotoxin exposure in children and adults through fruit juice consumption have been evaluated by comparing the Estimate Daily Intakes (EDIs) with Tolerable Daily Intakes (TDIs) (Pallarés et al., 2019). The results appointed to an increasing risk for children, and data in this sense were also reported by Torović et al. (2017) for children population.

Aflatoxin B1 (AFB1) and Aflatoxin B2 (AFB2) are considered the most harmful mycotoxins to human health due to their high occurrence and toxicity. Their main adverse effects are listed in Table 1. In 2020, EFSA released a scientific opinion on the risk assessment for human health associated with the presence of aflatoxins in food and notified that the AFB1 mean concentrations ranged from 0.64 to 0.97 μg/kg on the "fruit and fruit products" category (Schrenk et al., 2020a). Information on the levels of AFB1 and AFB2 in grape juices is quite scarce. Nonetheless, a study by El Khoury et al. (2008) reported a maximum AFB1 concentration of 0.46 μg/L in handmade must (EL Khoury et al., 2008).

Ochratoxin A (OTA) has also been widely studied for its toxic effects (Table 1) and for its presence in grapefruit, grape juice, and wine (Bellí et al., 2002; Pallarés et al., 2019). Yusefi et al. (2018) analyzed the OTA levels in grape juice samples from Iran and observed 55.7 % of positive samples with mean level of 0.52 μg/L (Yusefi et al., 2018). Terra et al. (2013) detected OTA in 38.24 % of the grape juice samples from Brazil and the mean OTA content determined was 2.65 ± 0.05 μg/L. The Commission Regulation of 25 April 2023 establishes maximum levels for OTA in grape juice at 2 μg/kg being the OTA, the unique mycotoxin legislated by the European Commission in grape juice (European Commission, 2023).

Emerging mycotoxins are the latest incorporated mycotoxins to risk characterization studies. In this sense, data on toxicity of Enniatin B (ENNB) and Enniatin B1 (ENNB1) are limited and seem to indicate that they are quite harmless at dietetic exposure doses (EFSA, 2014). At present, no data are available regarding the presence of Enniatins in grape juices. However, several studies have reported the occurrence of *Fusarium* species—the fungi responsible for Enniatin production—in grapes, emphasizing the need to re-evaluate the potential presence of these metabolites in grape-derived products (Bolton et al., 2016; Gonçalves et al., 2020).

Pulsed electric fields (PEF) is non-thermal technology associated with food contaminants mitigation with minimum impact on nutritional quality parameters under operational processing conditions (Pinheiro et al., 2021).

PEF treatment uses electrical pulses of short duration, ranging from nanoseconds to milliseconds, with electric fields strength ranging between 20 - 80 kV/cm. It is important to note that this type of sustainable treatment is limited to fluids that meet suitable conductivity conditions while for solid food, water immersion turns necessary (Urszula Tylewicz, 2020).

Table 1
Toxicological information of Aflatoxin B1 and B2, Ochratoxin A, and Enniatin B and B1.

Mycotoxin	Toxigenic fungi	Main adverse effect	IARC group	Reference
AFB1 AFB2	*Aspergillus*	Nephrotoxic, hepatotoxic, carcinogenic	Carcinogenic to humans (Group 1)	(Schrenk et al., 2020a)
OTA	*Aspergillus* and *Penicillium*	Nephrotoxic and genotoxic	Possibly carcinogenic to humans (Group 2B)	(Schrenk et al., 2020b)
ENNB ENNB1	*Fusarium*	Cytotoxic, immunotoxicity (in vitro)	No data available	(EFSA, 2014)

AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, OTA: Ochratoxin A, ENNB: Enniatin B, ENNB1: Enniatin B1, IARC: International Agency for Research on Cancer.

This technology is based on the principle of electroporation and has several applications in the food industry, such as improving freezing and dehydration processes, reducing the oil absorption capacity of potatoes, and extracting nutrients and bioactive compounds from food matrices and by-products (Barba et al., 2015; Martí-Quijal et al., 2021). PEF has also been investigated for the reduction of mycotoxins in various foods (barley, rice, and potato), achieving high reduction rates (up to 90 % for AFB1 in potato) (Adebo et al., 2021; Gavahian et al., 2020).

The main advantages of this treatment include relatively low energy costs and the ability to preserve heat-sensitive compounds. Therefore, the PEF technology is presented as an alternative to conventional heat treatments (Arshad et al., 2021; Soltanzadeh et al., 2022).

Several studies have already assessed the influence of PEF treatment in grape juice from different points of view. In this sense, Marsellés-Fontanet et al. (2013) showed that PEF treatment had no statistically significant impact on the concentrations of vitamin C, total polyphenol, cinnamic acid, free catechin and non-flavonoids in grape juice.

Ultrasounds (USN) based on oscillating sound pressure waves, is also explored as a sustainable technology for food processing (Taha et al., 2023). USN by its frequency enhances the transfer process between interfaces and require a physical medium to propagate, unlike electromagnetic waves that can propagate in a vacuum, and is classified according to its intensity and frequency (Gallo et al., 2018).

From a technological perspective, high-intensity, low-frequency ultrasound (USN) is particularly valuable due to two key phenomena: rarefaction and cavitation. The generation, expansion, and subsequent implosion of gas bubbles during ultrasound treatment cause localized collapses on the surface of food matrices, releasing intense pressure and heat. This process results in the formation of shock waves and microfractures (Gavahian et al., 2018). Rarefaction refers to the alternating compression and decompression of the medium caused by sound waves, leading to enhanced energy release, turbulence, and mass transfer. Cavitation, on the other hand, involves the formation, growth, and violent collapse of bubbles, which also contributes to the release of significant energy (Bhargava et al., 2021).The most studied applications are microbial inactivation of spores, fungi, and bacteria in vegetables and fruits, ultrasound-assisted freezing to reduce damage to cell structure, extraction of compounds by cell disruption and improvement of foam properties by increasing stability (Astráin-Redín et al., 2021; Chavan et al., 2022; Salgado-Ramos et al., 2022).

Several studies have explored the impact of the USN as an innovative and sustainable technology for the extraction of bioactive compounds from grapes (Carrera et al., 2012; Morata et al., 2021; Salgado-Ramos et al., 2022). In this sense, the study of Lieu & Le (2010), revealed that USN improved the grape juice quality, as the sugar content, total acidity and phenolic content, as well as the color density of the grape juice.

However, there is limited information on the effectiveness of ultrasound (USN) in reducing mycotoxin levels in juice (Sebastià et al., 2023). The mechanisms behind the two proposed treatments—Pulsed Electric Fields (PEF) and USN—differ significantly. PEF appears to contribute to mycotoxin reduction through the breakdown of structural molecules, such as amino acid fragments (e.g., HyLv, Val, Ile, or Phe) in the case of emerging mycotoxins, as well as through chemical modifications like the addition of OH^- and H^+ groups to double bonds or the loss of a methylene group ($-CH_2$) in AFB2 (Pallarés et al., 2021a, 2020). In contrast, USN can promote the release and degradation of contaminants in aqueous media through cavitation effects (Yuanfang Liu et al., 2019). When applied sequentially, these treatments may produce a synergistic effect, enhancing overall mycotoxin reduction. The aim of this study is to assess the synergistic effect of PEF and USN in reducing mycotoxins. To this end, grape juice was spiked with AFB1, AFB2, OTA, ENB and ENB1 and subsequently treated by PEF and USN.

2. Materials and methods

2.1. Chemicals and reagents

Merck (Germany) provided the solvents chloroform ($CHCl_3$), acetonitrile (ACN), and methanol (MeOH) of 99 % grade. Alfa Aesar (Germany) supplied ethyl acetate (EtOAc) of HPLC grade. The deionized water, with resistivity greater than 18 MΩ cm-1, required for preparing the mobile phase, was obtained from the Milli-Q SP® Reagent Water System (Millipore Corporation, Bedford, MA, USA). Before use, all mobile phase solvents were filtered using a 0.45-μm cellulose filter acquired from Scharlau (Barcelona, Spain). VWR Chemicals (Leuven, Belgium) provided sodium chloride (NaCl), Panreac Quimica S.A.U. (Barcelona, Spain) supplied ammonium formate (99 %), and Sigma-Aldrich (St. Louis, MO, USA) provided formic acid (reagent grade 95 %). All samples were filtered through a 13 mm/0.22 μm nylon filter from Membrane Solutions (TE, USA) before injection. Sigma (St. Louis, MO, USA) provided the AFB1, AFB2, OTA and, ENNs (ENNB and ENNB1) standards. Standard working solutions were prepared from the methanol stock solutions at a concentration of 1000 mg/L. All solutions were stored at 20°C.

2.2. Grape juice preparation

Grape juice samples were prepared in the laboratory following the method described by (Pallarés et al., 2021b), replicating a commercial formulation. The manufacturing procedure is outlined in Fig. 1. The formulation per 100 mL of juice consisted of 60 mL of grape juice, 40 mL of water, 7.5 g of sugar, 0.3 g of pectin, and 0.1 g of citric acid.

The total grape juice volume prepared was 1.5 L, from which 1.2 L was spiked individually by AFB1, AFB2, OTA, ENNB, and ENNB1 at a concentration of 100 μg/L. Three aliquots from spiked volumes were employed as untreated control and aliquots of 200 mL were treated. All experiments were performed in triplicate and all juice samples were treated after their elaboration.

2.3. Sample treatments

The samples were subjected to PEF treatment followed by USN, as illustrated in Fig. 2. The treatment parameters for both PEF and USN were established based on previous studies conducted by our research group, as well as findings from other laboratories (Mortazavi et al., 2015; Pallarés et al., 2020; Sebastià et al., 2023).

PEF treatment was performed employing the PEF system PEF-Cellcrack III (German Institute of Food Technologies (DIL)) equipment (ELEA, Quakenbruck, Osnabruck, Germany with a treatment chamber with a 10 cm of space between electrodes. The voltage set was 30 kV, the specific energy was 500 kJ/Kg and the field strength was 3 kV/cm. The pulse shape remained consistent as a unipolar square wave pulse, with a pulse duration of 100 ms and a frequency of 2 Hz. Approximately, 240 pulses were applied for about 5 min. Temperatures were monitored with the ProfiLine Cond 3310 conductometer (WTW, Xylem Analytics, Weilheimin Oberbayern, Germany) and did not exceed 70°C. The conductivity was also checked with ProfiLine Cond 3310 conductometer and the values ranged from 700 to 800 μS/cm. The sample volume treated were 200 mL of grape juice.

USN treatment was performed in an ultrasonic bath (A Branson 5200), the conditions assessed were 20 kHz of frequency and 100 W of power at 50°C temperature. The duration of the treatment was 30 min. The volume of juice sample treated was also 200 mL.

2.4. Mycotoxin extraction by DLLME

Mycotoxin extraction was performed using Dispersive Liquid–Liquid Microextraction (DLLME), as detailed in Fig. 3. The extraction process was conducted in two sequential steps. In the first step, acetonitrile (ACN) served as the dispersing solvent and ethyl acetate (EtOAc) as the extracting solvent. In the second step, methanol (MeOH) and chloroform ($CHCl_3$) were used as the dispersing and extracting solvents, respectively. This methodology was previously validated by our research group (Pallarés et al., 2019). The limits of detection (LOD) and quantification (LOQ) were 0.3 μg/L and 1 μg/L, respectively, for AFB1, AFB2, OTA, and ENNB1, and 0.15 μg/L and 0.5 μg/L, respectively, for ENNB. In this previous paper, recovery experiments were performed in a juice matrix at concentrations of 50, 100 and 200 μg/L with satisfactory recoveries ranging from 64 to 115 % for AFB1 and from 69 to 83 % for AFB2. For OTA, the recoveries obtained ranged from 71 to 90 %, for ENNB between 66 and 104 %, and for ENNB1 ranged from 67 and 110 %.

Regarding matrix effect experiments, the signals suppression enhancement (SSE) obtained for AFB1, AFB2, OTA, ENNB, and ENNB1 were 48, 80, 64, 66 and 54 %, respectively.

2.5. Mycotoxins analysis by UHPLC-MS/MS

For mycotoxins analysis by UHPLC-MS/MS a Sciex TRIPLE QUAD 6500+ mass equipped with electrospray ionization (ESI) coupled to an Agilent 1260 HPLC UHPLC system (degasser, quaternary pump, and column oven) with an Eksigent ULC 100 HTC-xt autosampler was employed. The column used was a BEH® C18 Column (1.7 μm 100 Å, LC Column 50 × 2.1 mm, Waters). The mobile phases were (A) H_2O 5 mM ammonium formate and 0.1 % formic acid, and (B) methanol (0.1 % formic ac.). The linear gradient was: 0 min (95 % A), 2 min (95 % A), 13 min (0 % A), 15 min (0 % A), 15.1 min (5 % A), and 18 min (5 % A) with an injection volume of 5 μL and a constant temperature of 30°C in the column. The mass spectrometer was used in positive ionization mode and in multiple Selected Reaction Monitoring (SRM) mode, with a turbo Spray IonDrive ionization source, and the following conditions: curtain gas (CUR) 30 psi, ion sputtering voltage (IS) at 4.5 kV, temperature of 300°C, and ion source gas (GS) 1 and 2 at 55 psi. The UHPLC-MS/MS

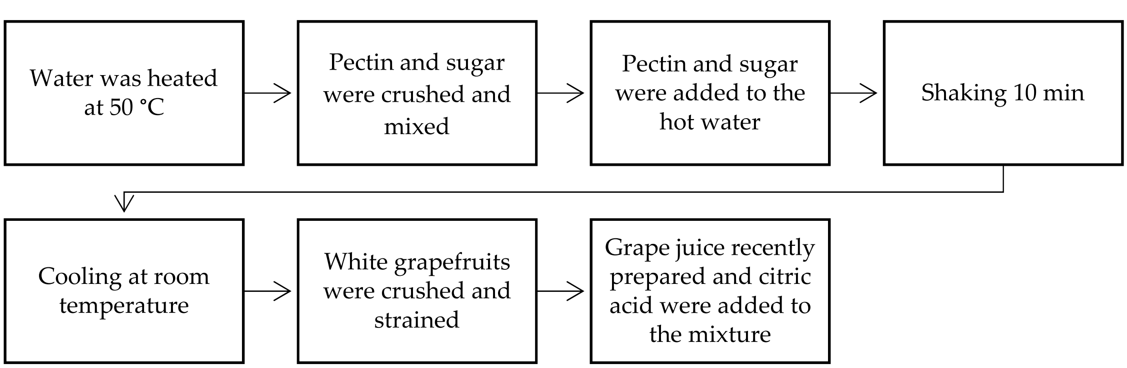

Fig. 1. Description of the grape juice manufacture in the laboratory.

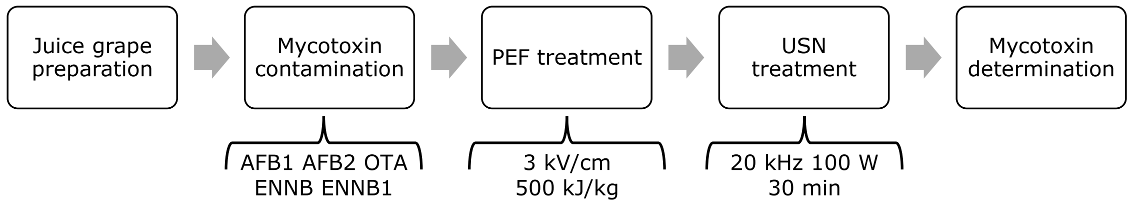

Fig. 2. Schematic representation of the treatment methods applied to grape juice samples spiked with mycotoxins. Treatments include Pulsed Electric Fields (PEF) followed by Ultrasound (USN). Mycotoxins: AFB1 – Aflatoxin B1; AFB2 – Aflatoxin B2; OTA – Ochratoxin A; ENNB – Enniatin B; ENNB1 – Enniatin B1.

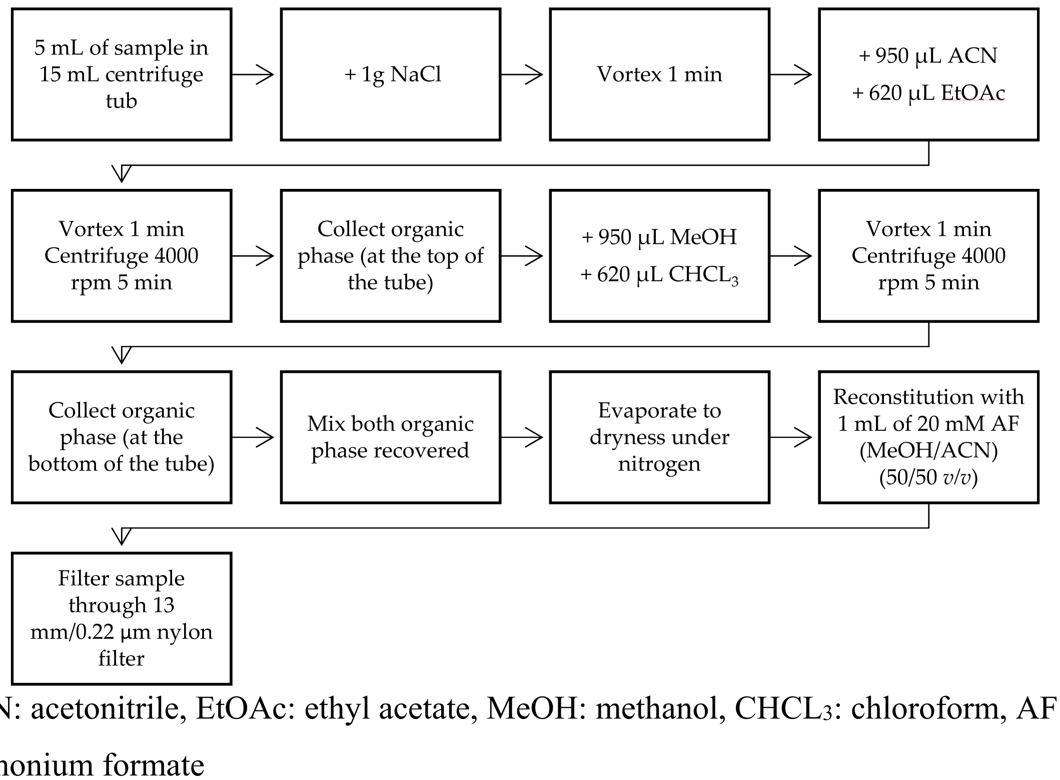

Fig. 3. Scheme of Dispersive Liquid Liquid Micro Extraction procedure for Aflatoxin B1 and B2, Ochratoxin A, Enniatin B and B1 analysis in grape juice. ACN: acetonitrile, EtOAc: ethyl acetate, MeOH: methanol, $CHCL_3$: chloroform, AF: ammonium formate.

parameters for each compound are listed in Table 2.

2.6. Statistical analysis

Data were analyzed using analysis of variance (one-way ANOVA) to determine the significance of differences between treatments and control samples for each mycotoxin individually. A $p < 0.05$ was considered significant. All analyses were performed with the software GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA). Data were expressed as mean ± standard deviation in all cases. All analysis performed were applied in triplicate.

3. Results

The combination of PEF and USN treatments resulted in AFB1, AFB2, OTA, ENB, and ENB1 mitigation in grape juice. The highest reduction percentage has been achieved by ENNB1 with a 47.88 % while the reduction of ENNB reached 35.91 %, which is statistically significant ($p \leq 0.01$). The influence of the treatments was less in the case of aflatoxins, where the reduction percentages achieved were 14.73 % and

Table 2
Optimized mass spectrometry parameters for the determination of Aflatoxin B1, Aflatoxin B2, Ochratoxin A, Enniatin B and Enniatin B1.

					Quantification ion (Q)			Confirmation ion (q)		
Mycotoxin	t_R (min)	DP	EP	Precursor ion (*m/z*)	CE	Product ion (*m/z*)	CXP	CE	Precursor ion (*m/z*)	CXP
AFB1	8.1	106	10	313.1	33	285.2	16	91	128.1	10
AFB2	7.7	96	10	315.1	37	287.2	18	43	259.2	18
OTA	10.1	91	10	404	37	239	16	105	102	14
ENNB	12	81	10	657.5	45	196.3	18	47	214.1	18
ENNB1	12.2	111	10	671.4	43	196	12	41	210	12

t_R: retention time, DP: declustering potential, EP: entrance potential, CE: collision energy, CXP: collision cell exit potential, AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, OTA: Ochratoxin A, ENNB: Enniatin B, ENNB1: Enniatin B1

14.97 % for AFB1 and AFB2, respectively. Finally, OTA obtained intermediate reduction percentage with a reduction of 20.63 %. Table 3 details the mycotoxin final concentration after the treatments and Fig. 4 shows the percentage reductions obtained after the treatments.

Fig. 5 displays the chromatograms of grape juice spiked by AFB2 and ENNB obtained by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS-IT). A PEF+USN treated sample and untreated control of AFB1 and ENNB are shown in the Fig. 5.

4. Discussion

Several authors listed in Table 4 have previously explored the effect of these technologies on the mitigation of mycotoxins in some solid and liquid matrix used as food. PEF technology was also effective in reducing aflatoxins in potato dextrose agar, juices, and milkshakes with reduction percentages up to 80-90 % (Pallarés et al., 2021a; Vijayalakshmi et al., 2018). On the other hand, USN extraction was also shown to reduce the presence of AFs, Deoxynivalenol (DON), Zearalenone (ZEA) and OTA in aqueous solutions between 41-97 % when a frequency of 20 kHz was maintained, but with differences in application time (10-80 min) and intensity (6.6-11 W/cm3) (Liu et al., 2019a; Mortazavi et al., 2015; Liu et al., 2019b). The highest reductions were obtained in aqueous solution and potato dextrose agar after USN and PEF treatment, this could be since the matrix is simpler and allows a better performance of the treatment. Thus, it was observed how both technologies were effective in attenuating mycotoxins when applied alone or in combination due to electroporation and cavitation phenomena. These processes proved to be more effective than the application of High Hydrostatic Pressure (HPP), a technology widely used in the preservation of liquid foods under pressure. For several mycotoxins (AFB1, AOH, ENNs, AFs, PAT) in vegetable and fruit juices, a reduction of about 7-75 % was achieved by applying a pressure of 400-600 MPa for 5 minutes (Avsaroglu et al., 2015; Hao et al., 2016; Pallarés et al., 2022).

For this reason, the study of PEF and USN technologies to mitigate mycotoxins and increase food safety in liquid matrices is of great interest as it shows high reduction percentages, as well as the combination of PEF + USN, whose results are consistent with those observed in several studies. In this sense, the effects of the combination of USN+PEF and PEF+USN were presented in a previous study published by Sebastià et al. (2023), which showed that the reduction of mycotoxins (OTA, ENNB, ENNB1) ranged from 19 to 37 % (USN+PEF) and from 34 to 50 % (PEF+USN), respectively, so that the combination PEF + USN was the one with the highest reduction percentages. In addition, Pallarés et al. (2021a) pointed out that the percentage of AFB2 and AFG1 attenuation after PEF in grape juice was 72 and 84 %, respectively, while only 24 % was obtained for AFG2. In other study, Pallarés et al. (2020) employing similar PEF treatment conditions obtained reduction percentages ranged from 43 to 53 % for ENNs in grape juice too. These results indicate that the attenuation caused by the methodology depends on the structure of the mycotoxin studied, with different degradation products identified after PEF treatment, possibly due to the addition of OH - and H+ groups to the bonds of the AFB2 structure and the loss of the methylene group ($-CH_2$).

However, new research focuses on the influence of the sequence and order of application of innovative technologies, as well as on the reduction mechanism, which is partially unknown. Stranska et al. (2023) suggested that PEF technology reduces mycotoxin content in most of the studied mycotoxins due to possible structural changes in the molecule, although the number of studies on degradation products is very limited. This fact, in conjunction with the cavitation phenomenon causing both microfractures and chemical changes, would be interesting for application to mycotoxins.

Table 3
Concentration (μg/L) of Aflatoxin B1 and B2, Ochratoxin A, Enniatin B and B1 in grape juice after the treatments, the initial concentration was 100 μg/L. * indicates a significant reduction regarding the untreated control ($p \leq 0.01$).

	AFB1	AFB2	OTA	ENNB	ENNB1
PEF + USN	85.28 ± 1.45	85.03 ± 14.37	79.38 ± 10.67	64.1 ± 6.27*	52.12 ± 18.5*

AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, OTA: Ochratoxin A, ENNB: Enniatin B, ENNB1: Enniatin B1

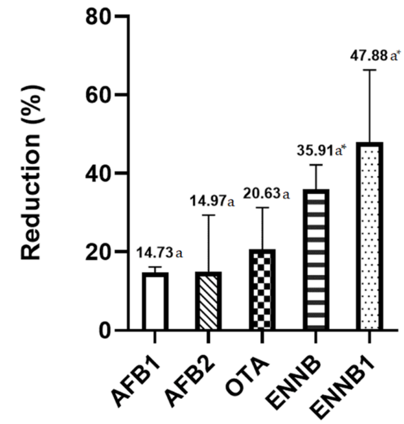

Fig. 4. Reduction percentage of Aflatoxin B1 and B2, Ochratoxin A, Enniatin B and B1 in grape juice after pulsed electric field and ultrasounds treatments. Different letters indicate different percent reduction rates between mycotoxins. * indicates a significant reduction regarding the untreated control ($p \leq 0.01$). AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, OTA: Ochratoxin A, ENNB: Enniatin B, ENNB1: Enniatin B1; PEF: pulsed electric field, USN: ultrasound.

Another innovative mycotoxins detoxification strategy is through fermentation. Several studies detail that *Lactobacillus, Streptococcus, Bacillus and Saccharomyces cerevisiae* are capable of degrading mycotoxins such as OTA, PAT, DON, ZEA and AFs. In addition, other studies show PEF increases the fermentation time of these microorganisms and increases enzymatic activity. In this way, PEF technology would act directly by degrading mycotoxins and indirectly by improving fermentation (Gavahian et al., 2021; Mota et al., 2018; Shanakhat et al., 2018; Shetty and Jespersen, 2006).

AFs are very stable to high temperatures showing thermal resistance to treatments that reach 250°C (Milani and Maleki, 2014). The resilience of ENNs following exposure to heat treatment has been studied in pasta, fish, and medicinal plants. It is important to note that ENNs are not heat-resistant, therefore thermal processes involving temperatures above 100°C and durations of 5 to 10 minutes can reduce ENNs up to 100 % (de Nijs et al., 2016; Serrano et al., 2013; Tolosa et al., 2017). Nevertheless, this also leads to the compromise of the nutritional and sensory qualities of the juices and smoothies (Galiassi and Ramirez, 2022; Gunes and Turan, 2017).

Regarding OTA, Dahal et al. (2016) documented the remarkable thermal stability of OTA when subjected to water heated to 100°C. Furthermore, other study about OTA reduction in roasted oat-based cereals revealed that after exposure to 120°C for 30 minutes, OTA reduction was less than 2 % (Lee, 2020).

4. Conclusions

This study reaches the conclusion that the combination of PEF and USN may be useful for AFs, OTA and ENNs mitigation in grape juice. The parameters set in both treatments have proven to be efficient in the reduction of mycotoxins in grape juice. The results obtained pointed out a 21 % reduction for OTA and a 15 % reduction for AFB1 and AFB2 in grape juice spiked by 100 μg/L. The highest reduction percentages were observed in ENNB and ENNB1 (36 and 48 %, respectively). In addition, significant differences were observed between control and treated samples for these emerging mycotoxins. PEF and USN are sustainable

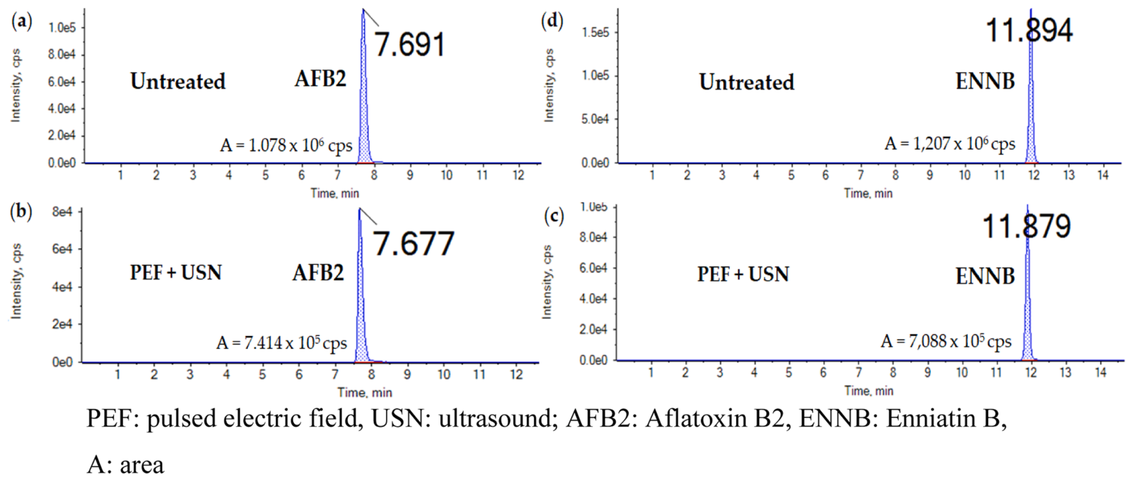

Fig. 5. LC-MS/MS-IT chromatograms of grape juice spiked by Aflatoxin B2 untreated (a); spiked by Aflatoxin B2 treated by PEF + USN (b); spiked by Enniatin B untreated (d); spiked by Enniatin B treated by PEF + USN (c). The area and the retention time (min) also are indicated.
PEF: pulsed electric field, USN: ultrasound; AFB2: Aflatoxin B2, ENNB: Enniatin B, A: area.

Table 4
Overview of the reduction percentages observed for mycotoxins in food products.

Food matrix	Mycotoxin	Treatment	Parameters	Reductions (%)	Ref.
Grape juice	AFs	PEF	30 kV voltage| 3 kV/cm EFS| 500 kJ/kg SE	24 to 84	(Pallarés, Berrada, et al., 2021a)
		HPP	500 MPa pressure| 5 min	14 to 29	
	AFB1		600 MPa pressure| 5 min	13	(Pallarés, Sebastià, et al., 2021b)
	AOH			37	
	ENNs	PEF	30 kV voltage| 3 kV/cm EFS| 500 kJ/kg SE	43 to 53	(Pallarés et al., 2020)
	BEA			46	
Others juices and milkshakes	AFB1	HPP	600 MPa pressure| 5 min	7 to 24	(Pallarés, Sebastià, et al., 2021b)
	AOH			28 to 37	
	ENNs			11 to 75	(Pallarés et al., 2022)
	PAT			17 to 26	(Hao et al., 2016)
	PAT		400 MPa pressure| 5 min	29	(Avsaroglu et al., 2015)
	ENNs	PEF	30 kV voltage| 3 kV/cm EFS| 500 kJ/kg SE	56 to 60	(Pallarés et al., 2020)
	BEA			70	
	ENNs	USN + PEF	30 kV voltage| 3 kV/cm EFS| 500 kJ/kg SE	19 to 47	(Sebastià et al., 2023)
	OTA		20 kHz frequency | 100 W power | 30 min	30 to 50	
Malting barley	*Fusarium* and *Alternaria* mycotoxins	PEF	500 pulses| 9 kV/cm voltage| 10 μs pulse width |10 Hz frequency	up to 46	(Stranska et al., 2023)
Aqueous solution	AFB1, DON, ZEA, OTA	USN	20 kHz frequency | 6.6 W/cm^3 intensity | 50 min	61 to 97	(Liu et al., 2019a)
	AFB1		20 kHz frequency | 6.6 W/cm^3 intensity | 80 min	85.1	(Liu et al., 2019b)
	AFs		20 kHz frequency | 1000 W power | 10 min	41	(Mortazavi et al., 2015)
Potato dextrose agar	AFs	PEF	20-60 % output voltage | 4-32 μs pulse width | 110°C	79 to 96	(Subramanian et al., 2017)
			20-60 % output voltage | 4-32 μs pulse width | 4-10 pH	77 to 97	(Vijayalakshmi et al., 2018)

PEF: Pulsed Electric Fields, USN: Ultrasounds, HPP: High-Pressure Processing, AF: Aflatoxin, AFB1: Aflatoxin B1, ENN, Enniatin, BEA: Beauvericin, DON: Deoxynivalenol, ZEA: Zearalenone, OTA: Ochratoxin A, AOH: Alternariol, PAT: Patulin

technologies and have a minimal impact on the nutritional and organoleptic quality of the product. In this sense, the data reported in this work helps to understand the synergistic effect of electroporation and cavitation mechanisms, though further studies are needed. The integration of PEF and USN technologies into food processing practices may become a standard approach, enhancing food safety and quality while reducing reliance on industrial processes. However, more studies are necessary to understand the combination of these technologies and the combination with other unconventional strategies. Furthermore, more information is needed regarding the nutritional and organoleptic characteristics after the treatments.

CRediT authorship contribution statement

Albert Sebastià: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Mara Calleja-Gómez:** Writing – original draft, Investigation, Formal analysis, Data curation. **Noelia Pallarés:** Writing – original draft, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Juan Manuel Castagnini:** Writing – review & editing, Supervision, Software, Methodology, Conceptualization. **Emilia Ferrer:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Houda Berrada:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research was supported by the Spanish Ministry of Science and Innovation project (PID2020-115871RB) and the project given by the Generalitat Valenciana (Spain) AICO/2021/037. The authors also thank the Generalitat Valenciana for the financial support (IDIFEDER/2018/046). Albert Sebastià would like to acknowledge the pre-PhD scholarship program of the University of Valencia "Atracció de Talent". Juan Manuel Castagnini is beneficiary of the grant (ZA21-028) for the requalification of the Spanish university system from the Ministry of Universities of the Government of Spain, modalities "Maria Zambrano", financed by the European Union, NextGeneration EU.

Ethical statement

This work does not involve animal or human studies.

Data availability

Data will be made available on request.

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