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Efficiency of cold atmospheric plasma under ozone (O_3) and nitrogen oxide (NO_x) regimes on the degradation of aflatoxins and ochratoxin A in solid state and in spiked pistachio kernels

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ABSTRACT

Mycotoxins are the primary contaminants in food that have a significant impact on global food safety and security. Cold atmospheric plasma (CAP) has demonstrated significant potential in reducing mycotoxins among innovative technologies. This study aimed to assess the effectiveness of a surface dielectric barrier discharge (SDBD) CAP system in reducing aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), and ochratoxin A (OTA). The distinctive feature of the device here used was the ability to operate at two different powers, resulting in two different plasma reactive species ambients: ozone (O_3) and nitrogen oxides (NO_x) regimes. CAP treatments exhibited a significant reduction of all investigated pure mycotoxins, which was regime, molecule chemical structure, distance from the source and time-dependent. In particular, O_3 regime was more effective (AFB1 and AFG1 99%, AFB2 and AFG2 60%, OTA 70% reduction, respectively, at 60 min, 4 cm distance from the plasma source). As low-moisture foods pistachio kernels (*Pistacia vera* L. seeds) were selected to evaluate the matrix effect. As expected, the mycotoxins were reduced to a much lesser extent than pure molecules. Worthy of note was the 23% reduction of OTA. To our best knowledge, this is the first investigation about OTA degradation in pistachio food matrix by cold plasma. It should also be noted that the pistachios have been artificially contaminated with spots of a mixture of mycotoxins, to best represent a probable real contamination.

1. Introduction

The production of foods with plant and animal origins has expanded with the world population's rapid growth. In this context, maintaining food security is still essential to meeting the market's increasing need for healthy food. It has been estimated that approximately 14% of food produced, before it even reaches the retail level or the consumers, is lost due to food contamination (FAO, 2019). In particular, fungi are responsible for losing up to 30% of crop products through disease and spoilage processes (Avery, Singleton, Magan, & Goldman, 2019), and climate change can exacerbate matters by creating conditions that make foodborne hazards more likely to emerge and spread, with a shift in mycotoxin-producing fungi and a change in global patterns of mycotoxin incidence (Delgado-Ospina, Molina-Hernandez, Chaves-López, Romanazzi, & Paparella, 2021; Eskola, Kos, Elliott, Hajšlová, & Krska, 2020). In fact, there is evidence of contamination by toxigenic fungi raising concerns about the safety of products (FAO, 2022). The European Rapid Alert System for Food and Feed (RASFF) reported that mycotoxin presence in food was the third most notified hazard category in 2021, accounting for 450 cases, and most of the mycotoxin notifications were related to aflatoxins detected in nuts. Alshannaq and Yu (2021), analysing data from papers published over 10 years since 2010, highlighted that almost 95% of U.S. mycotoxin RASFF notifications were reported for foods and only 5% for feeds. It is well known that different climate conditions favour different mycotoxin producing fungi species, leading to a highly variable mycotoxins prevalence worldwide.

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Although prevention of fungal contamination in farming products is the key to mitigation of the impact of mycotoxins on human and animal health, current agricultural and manufacturing practices cannot guarantee the absence of these compounds in food commodities (Wielogorska et al., 2019).

Many strategies have been suggested for reducing mycotoxin formation like physical methods (i.e., dehulling, sorting, sieving, floatation, washing, steeping, milling, ultraviolet, gamma treatments, and thermal treatments), chemical (acids and bases such as ammonia, hydrogen peroxide, food preservatives or anti-fungal agents or herbal extracts), and biological control methods (a non-toxigenic microbial strains) (Basaran, Basaran-Akgul, & Oksuz, 2008; Misra, Yadav, Roopesh, & Jo, 2019; Nunes, Moosavi, Mousavi Khaneghah, & Oliveira, 2021; Shanakhat et al., 2018). However, despite the progress achieved by using these methods, they have several disadvantages, including low efficacy and the requirement of expensive chemicals and sophisticated equipment. As a result, some of these methods are usually considered impractical, costly, not completely wholly efficient, and time-consuming, especially for the large-scale treatment of food or feed products (Feizollahi, Misra, & Roopesh, 2020).

Novel detoxification strategies, including high-pressure processing (HPP), pulsed electric fields (PEF), ultrasound (USN), supercritical carbon dioxide (SC–CO_2), and cold plasma (CP) processing, have been found to have good potential for mycotoxins reduction (Gavahian & Khaneghah, 2020). Among these innovative technologies, the atmospheric cold plasma (CAP) treatment has shown promising results for detoxifying the most problematic mycotoxins (Ziuzina & Misra, 2016). Plasma, often defined as the fourth state of matter, is an ionised state of the gas produced by increasing the internal energy of the gas and which exhibits unique properties (Lieberman & Lichtenberg, 2005). Plasma has been induced using several gases, from ambient air, oxygen, and nitrogen to noble gases such as helium, argon, and their combinations. Plasma can be produced using several equipment configurations, including corona discharge, plasma jet, gliding arc discharge, and dielectric barrier discharge reactors (Charoux et al., 2021). Among the different configurations, the surface dielectric barrier discharge (SDBD) results one of the most promising. The key aspects of the SDBD that make it more attractive are: (1) the application of relatively low voltage, (2) highly efficient production of reactive species, (3) high density of micro-discharges, (4) the operation in ambient air with no need for a noble gas admixture (Abdelaziz, Ishijima, Osawa, & Seto, 2019; Pavlovich, Clark, & Graves, 2014).

Mycotoxin degradation using atmospheric cold plasma relies on the production of a diverse mix of components, including ultraviolet radiation, reactive oxygen species (ROS) such as ozone (O_3), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), peroxyl (ROO•) and hydroxyl radicals (•OH), reactive nitrogen species (RNS) including nitric oxide (NO•), peroxynitrite (ONOO–) or peroxynitrous acid (OONOH) and strong electric field (Misra, Yepez, Xu, & Keener, 2019; Pai et al., 2018; Szili, Hong, Oh, Gaur, & Short, 2018). Each of these species can affect independently but it has been highlighted that the effectiveness of plasma treatment is due to the synergic effect of the plasma components, which makes the plasma unique. Even though the numerous offers of the SDBD for ozone production, namely ozone "regime", the performance of nitrogen oxides (NO_x) "regime" has not yet been thoroughly investigated (Pavlovich et al., 2014). Therefore, the purpose of this study was to investigate the effect of SDBD under two different regimes of plasma (O_3 and NO_x) at different exposure treatment times and different distances from the source on the degradation of aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), and ochratoxin A (OTA). The investigation started with the pure mycotoxin molecules, exposed in solid state, varying the application parameters of the device, in order to optimize the operative conditions. Subsequently, samples of artificially contaminated pistachio kernels (*Pistacia vera* L. seeds) were subjected to optimised CAP treatments to evaluate the matrix effect.

2. Material and methods

2.1. Chemicals and reagents

The standards of mycotoxins aflatoxin B2, aflatoxin G1, aflatoxin G2 and ochratoxin A were purchased from Sigma-Aldrich (Missouri, USA). Aflatoxin B1 was purchased from Acros Organics (Italy). The working standard mixtures were prepared by appropriate dilution in methanol (MeOH) and stored at −20 °C. Ultrapure water (H_2O), formic acid, MeOH and acetonitrile (ACN) were UPLC-MS grade and were purchased from VWR (Pennsylvania, USA). Ammonium formate was purchased from Sigma-Aldrich (Missouri, USA).

2.2. Device configuration

The Surface Dielectric Barrier Discharge (SDBD) device used in this study was previously described (Molina-Hernandez et al., 2022). The plasma formed produced an indirect treatment. The high voltage generator produced a sinusoidal waveform with a peak voltage of 6 kV and a fixed frequency of 23 kHz; two different operative conditions, so two different regimes, were obtained using a duty-cycle (i.e. pulsing the high voltage signal with repeated on and off periods). The NO_x regime was obtained using a duty cycle equal to 100% (425.35 ± 25.79 W), while the O_3 regime was obtained with a duty cycle equal to 10% (42.54 ± 2.58 W).

2.3. Optical absorption spectroscopy (OAS) and temperature analysis

Capelli et al. (2021) previously described a similar setup for OAS. Two LEDs were used as a light source, one operating at 255 nm and the other at 400 nm. The light beam was focused on the mesh to investigate the concentration of RONS in the closed chamber; the beam was then collected into a 500 mm spectrometer (Acton SP2500i, Princeton Instruments) and spectrally resolved. OAS acquisitions were performed using a grating with 150 mm^{-1} resolution and 10 μm width for the inlet slit of the spectrometer. A photomultiplier tube (PMT-Princeton Instruments PD439), with a fixed amplification factor connected to a fast oscilloscope (Tektronix MSO46), was used as a detector to allow for fast acquisitions (time resolution of 40 ms).

The quantitative evaluation of the species concentrations from absorption measurements was performed according to the Lambert-Beer law:

$$\frac{I}{I_0} = e^{(-L\sigma n)} \quad (1)$$

where the concentration of the absorbers (n) is correlated with the light absorbed after an optical path of length (L) and expressed as the ratio between the intensity of the incident light (I_0) and the residual light intensity after the absorption (I). The absorption cross-section σ is a function of the light wavelength ($\sigma = \sigma(\lambda)$). In the experiments, the optical path was 25 cm long. The contributions of background radiation and spontaneous plasma emission were duly taken into account in the data processing, subtracting them from the acquired values of I and I_0. The wavelengths selected to perform the study and the corresponding absorption cross-sections for O_3 and NO_2 as absorbers are reported in Table 1. Following Moiseev et al. (2014), these wavelengths were defined to maximise the absorption of the molecules relevant to our study, minimising the contribution of other absorbing molecules, i.e. the

Table 1
Absorption cross-sections in cm^2 of the species of interest at each selected wavelength.

Selected wavelength	O_3 cross-section	NO_2 cross-section
253 ± 1.2 nm	(1.12 ± 0.02) E−17	(1.1 ± 0.3) E−20
400 ± 1.2 nm	(1.12 ± 0.08) E−23	(6.4 ± 0.2) E−19

disturbance. The concentration of O_3 and NO_2 was evaluated at different distances from the source (4 cm and 20 cm). In addition, the temperature inside the treatment chamber was measured during the discharge time employing a fiber optic temperature sensor (opSense, OTG series).

2.4. Evaluation of mycotoxins standards degradation operated by CAP treatment

An aliquot (5 μL) of a mycotoxin solution stock (10 μg/mL) was centrally added into a polystyrene six-well plate. After solvent evaporation at room temperature, the plates were placed in the treatment chamber at 20 and 4 cm from the ground electrode and subjected to plasma for 15, 30, and 60 min at two different regimes: O_3 and NO_x. Following the exposures, the residual mycotoxins were dissolved in 1 mL of methanol and transferred into vials for HPLC-MS/MS analysis. The mycotoxins percentage decrease was evaluated by $((C_0\text{-}C_t)/C_0)*100)$, in which C_t referred to the mycotoxin concentration after exposure to plasma for time t, and C_0 was the initial concentration of untreated mycotoxins. All the analyses were performed in triplicate.

2.5. Evaluation of matrix effect on mycotoxins degradation by CAP treatment

In order to assess the degradation of mycotoxins in food matrix, the most efficient plasma regime (O_3) and the optimal distance from the source were selected for treating pistachio kernels purchased from the local market. To explore whether the size and shape of the sample could influence the treatment outcomes, whole and chopped pistachios were deliberately contaminated.

A certified control material was purchased from Test Veritas S.r.l. (Padua, Italy). It was a sample of pistachio slurry contaminated by AFB1 23.00 μg/kg, AFB2 2.14 μg/kg, AFG1 5.09 μg/kg, AFG2 0.40 μg/kg, OTA 7.35 μg/kg. Samples of 7.50 ± 0.01 g were spread into Petri dishes of 60 mm and treated as follows.

2.5.1. Sample preparation

Non contaminated batches of whole and chopped pistachios were artificially contaminated into Petri dishes of 60 mm. Samples of 2.00 ± 0.01 g were spiked with 100 μg/kg of AFB1, AFB2, AFG1, AFG2, and OTA, from a stock solution containing their mixture. The samples obtained were allowed for 30 min under a biosafety cabinet at room temperature to let the solvent evaporate. Thus, the Petri dishes were placed in the treatment chamber at a distance of 4 cm from the ground electrode and subjected to plasma in O_3 regime for 30 and 60 min. After treatment, the mycotoxins were extracted as described in the following section.

2.5.2. Mycotoxins extraction and clean-up

The extraction of mycotoxins from the contaminated pistachios was performed according to Akinfala et al. (2020) with some modifications. A tested sample (2.00 ± 0.01 g) was extracted with 8 mL of methanol-water-acetic acid (79/20/1 v/v/v) for 90 min on a rotary shaker (Centomat BS-T, B. Braun, Milan, Italy) at 200 rpm and centrifuged for 10 min at 5200 rpm. The supernatant was collected for subsequent clean-up procedures. An aliquot of the upper layer (4 mL), equivalent to 1 g sample, was diluted by adding 28 mL phosphate-buffered saline (PBS, 50 mM) at pH 7.4 and homogenised by vortex-mixing for 5 s. The 32 mL of the diluted extract were passed through the column COMBI-IAC AFLA & OTA 3 mL (CHROMAtific) at a flow rate of about one drop per second. The column was washed with 5 mL of 50 mM PBS, and bound toxins were eluted two times with 1 mL of methanol-acetic acid (98/2 v/v) as eluent. The eluate was filtered through a 0.22 μm PTFE filter and analysed by High-Performance Liquid Chromatography coupled with tandem Mass Spectrometry (HPLC–MS/MS). All analyses were carried out in triplicate.

2.6. HPLC–MS/MS analysis

Mycotoxins analysis was performed using a Nexera XR LC system (Shimadzu, Tokyo, Japan) coupled to a Qtrap 4500 mass spectrometer (Sciex, Toronto, ON, Canada) equipped with a heated ESI source (V-source). A Kinetex 2.6 μm XB-C18 column (100 × 2.1 mm) equipped with a security guard cartridge was used. As mobile phases, H_2O 5 mM ammonium formate (A) and 50:50 ACN: MeOH 5 mM formic acid (B) were used. The gradient was set as follows: start with 20% B; hold for 0.1 min; linear increase to 75% B in 3 min; linear increase to 99% B in 0.5 min; at an isocratic step of 2.5 min at 99% B; return to initial conditions (20% B) in 0.5 min and holding this condition for 3 min, and equilibrated for 2 min. The injection volume was 3 μL and the flow rate 0.300 mL min^{-1}.

The instrumental parameters such as the declustering potential (DP), the focusing potential (FP), the input potential (EP), the collision energy (CE) and the cell output potential (CXP) were optimised by infusing each standard in methanolic solution (10 ng mL^{-1}), at a flow rate of 10 μL min^{-1}.

The two most intense precursor ion/product ion transitions were associated with each analyte. All analytes were detected in positive ionisation with a capillary voltage of 5500, nebuliser gas (air) at 40 psi and turbo gas (nitrogen) at 40 psi and 500 °C. With the MRM mode, the identification and quantification of ionic currents were performed for each analyte. Analyst 1.7 and Multiquant 3.0 software (Sciex) were used for instrument control and data analysis. The LC-MS/MS acquisition parameters for the selected mycotoxins are reported in Table 2. The analytical method was validated following the SANTE/11312/2021 guidelines for analytical methods on contaminants (European Commission, 2021). Linearity, limit of detection (LOD), limit of quantification (LOQ) (Table S1), matrix effect (Table S2), recovery, precision (expressed in terms of repeatability and within-laboratory reproducibility) and accuracy (Table S3) were evaluated. Briefly, the LOQs ranged between 0.01 and 0.04 μg/kg; recoveries ranged between 63% and 80% with a reproducibility below 15%.

2.7. Statistical analysis

The results were expressed as mean and standard deviation calculated on three replicates and analysed by one-way ANOVA. Tukey's multiple comparison tests computed significant differences between means at a significance level of $p \leq 0.05$. Data were processed using STATISTICA 12 for Windows (StatSoftTM, Tulsa,OK, USA) software. In the figures, the mean variability of data was indicated by the standard deviation.

3. Results

3.1. Plasma species analysis and temperature

The temporal evolution of O_3 and NO_2 has been observed by means of OAS at two distances from the SDBD source, 4 cm and 20 cm. Performing treatments in ozone regime, obtained using a duty cycle equal to 10%, no NO_2 was detected (therefore, graphs are not reported), while the concentration of O_3 was measured and increased with time. On the other hand, using the nitrogen oxide NO_x regime, obtained using a duty cycle equal to 100%, no O_3 was detected (therefore, graphs are not reported), while the concentration of NO_2 was measured and increased with time. Fig. 1 reports the concentration of O_3 and NO_2 after different treatment times at two distances from the SDBD source.

The temperature was monitored during treatments at both distances from SDBD, as shown in Fig. 2a-b, which reports the results for the O_3 and NO_x regimes, respectively. The O_3 regime absorbs a lower superficial power density (due to the 10% duty cycle), consequently, the heating of the SDBD is lower than the one observed using the nitrogen oxide NO_x regime. Moreover, the heat source is the SDBD, so the

Table 2
MS/MS detection parameters for mycotoxins.

Analyte	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	DP (V)	EP (V)	CE (V)	CXP (V)
Aflatoxin B1	3.76	313.1	285.00	107.00	7.00	32.00	11.00
			241.00	107.00	7.00	50.00	9.00
Aflatoxin B2	3.63	315.1	287.10	100.00	9.00	36.00	9.00
			258.80	100.00	9.00	40.00	11.00
Aflatoxin G1	3.60	329.1	242.90	110.00	10.00	36.00	9.00
			310.90	110.00	10.00	31.00	10.00
Aflatoxin G2	3.45	331.1	313.10	110.00	8.00	35.00	18.00
			200.00	110.00	8.00	51.00	18.00
			245.10	110.00	8.00	43.00	14.00
Ochratoxin A	4.28	404.3	358.10	81.00	9.00	21.00	15.00
			340.90	81.00	9.00	26.00	12.00
			239.10	81.00	9.00	32.00	9.00

DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision cell exit potential.

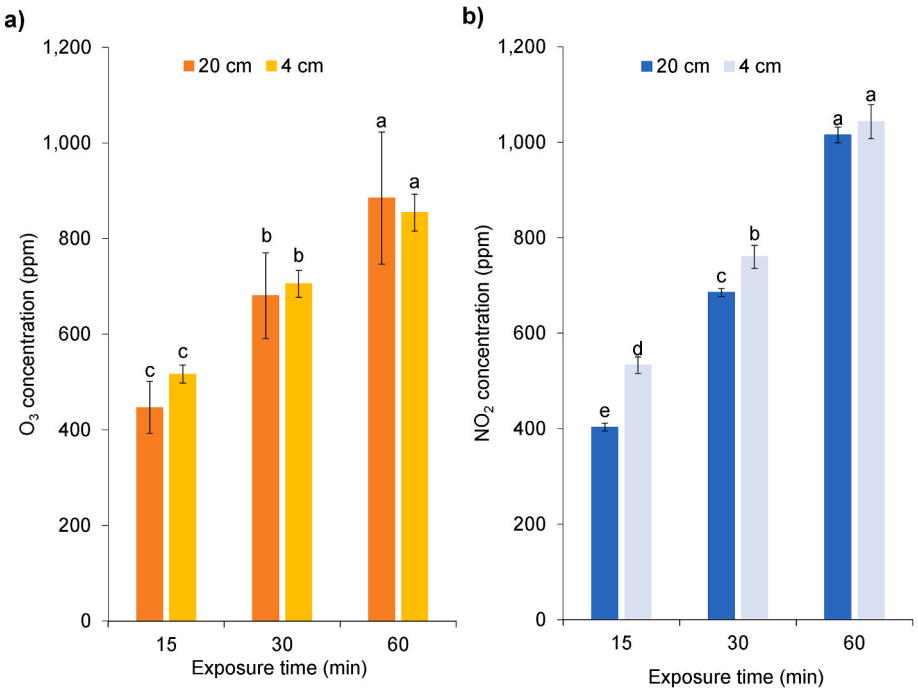

Fig. 1. Concentration of O_3 and NO_2 after different treatment times and distances from the source of CAP under O_3 regime **(a)**, and NO_x regime **(b)**. The values are the mean of three repetitions ± standard deviations. Values with different superscripted letters are significantly different ($p < 0.05$).

temperature observed at 4 cm from the plasma source is greater than that measured at 20 cm from the plasma source. The temperature of the chamber never exceeded 25 °C and 37 °C for, respectively, O_3 and NO_x regimes, at 4 cm of distance of the sample from the plasma source. Thus, the values are well below the temperature required for thermal decomposition of aflatoxins (237–306 °C) or ochratoxin A (425–490 °C) (Afsah-Hejri, Hajeb, & Ehsani, 2020). Hence, the contribution of heat is considered to be negligible for the degradation of mycotoxins.

3.2. Effect of regime and time exposure of CAP on mycotoxin degradation

The degradation of AFB1, AFB2, AFG1, AFG2, and OTA by SDBD plasma in both O_3 and NO_x regimes at 20 cm from the source was compared. As shown in Fig. 3, the mycotoxins reduction increased with time, and the degradation rate was mycotoxin and regime-dependent. In general, AFB1 and AFG1 were far more sensitive to plasma treatments than AFB2 and AFG2. In particular, the O_3 regime was more efficient for all the mycotoxins with a strong reduction of AFB1 and AFG1 (81.05 ± 5.34% and 82.25 ± 6.29%, respectively) already after 15 min of CAP exposure. It is to highlight that with the increasing exposure time, a further reduction of up to 99% for AFB1 and AFG1 was observed.

When NO_x regime CAP was produced, the reduction was significantly lower, even if was visible the same pattern. In fact, AFB1 and AFG1 were still the most sensitive, although 60' treatment determined a reduction of respectively 64.58 ± 3.32% and 63.11 ± 0.70%. Instead, AFB2 and AFG2 underwent a reduction of less than 20% (16.71 ± 3.67 and 18.80 ± 2.51%).

Regarding OTA (Fig. 3e), it was quite resistant to the cold plasma, especially when the NO_x regime was used. Indeed, this mycotoxin was not significantly reduced even after 60 min of CAP exposure. On the other hand, the O_3 regime was slightly more efficacious, with a reduction of only 37.06 ± 4.54%, after 60 min of exposure. The LC-MS analysis of the residue after cold plasma treatments in O_3 regime, was also directed towards the mass of ochratoxin B (OTB), the non-chlorinated OTA's analogue, and revealed the lack of trace.

3.3. Effect of distance from the source on mycotoxin degradation

Once demonstrated that the O_3 regime was the most effective, we evaluated the effect of the distance of the sample from the source. In Fig. 4 is depicted how the reduction percentage of each mycotoxin was affected by the distance from the plasma source (20 and 4 cm) at

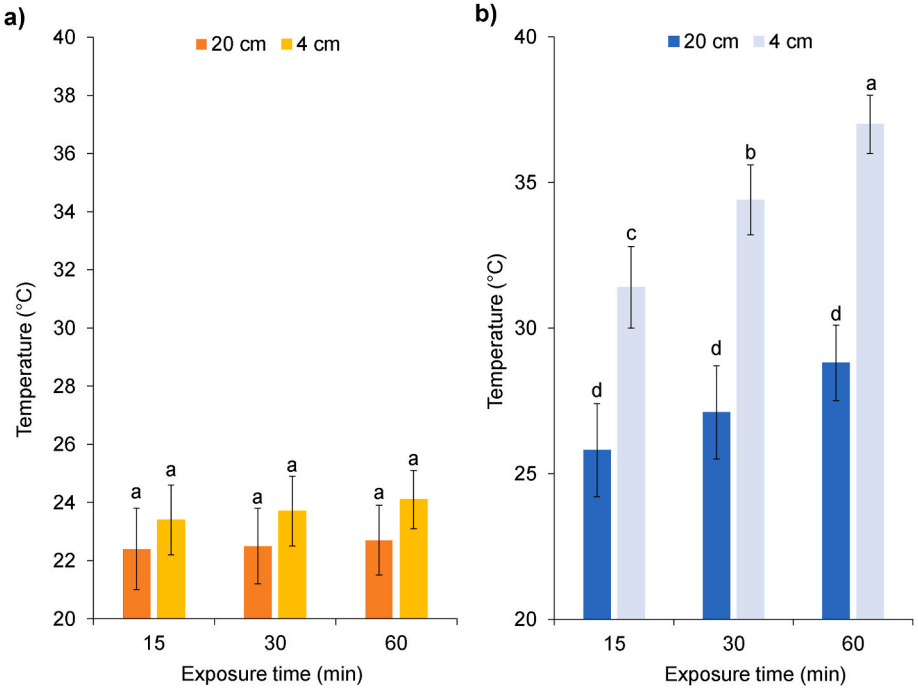

Fig. 2. Values of temperature after different treatment times and distances from the source of CAP under O_3 regime **(a)**, and NO_x regime **(b)**. The values are the mean of three repetitions ± standard deviations. Values with different superscripted letters are significantly different ($p < 0.05$).

different exposure times (30 and 60 min) when O_3 regime was applied. In the case of AFB1 and AFG1 (Fig. 4a,c), the most sensitive mycotoxins to CAP, the distance from the plasma source did not affect their reduction. However, in the case of AFB2 and AFG2 (Fig. 4b,d), the reduction significantly differed after 60 min, being more degraded at a minor distance from the source (4 cm), as expected (Puligundla, Lee, & Mok, 2020).

On the other hand, the effect of the distance was considerable for OTA, which exhibited the highest resistance, as described above. Exposing the OTA to CAP for 30 min determined an 18.14 ± 3.23% reduction when positioned at 20 cm from the source; the percentage was 51.88 ± 4.13% when the samples were positioned at 4 cm. Extending the exposure time to 60 min, the distance from the source had an even more pronounced effect on reducing OTA levels. In this case, the reduction percentage significantly increased from 37.06 ± 4.54% to 67.90 ± 6.85%, decreasing the source-to-sample distance from 20 cm to 4 cm, respectively.

On the contrary, the mycotoxins exposed to plasma treatment under NO_x regime were not influenced by the distance from the source, as evidenced in Table 3.

3.4. Evaluation of matrix effect on mycotoxins degradation by CAP treatment

By subjecting pure mycotoxins to CAP treatment, it was possible to fine-tune specific parameters such as the regime (O_3 regime), distance from the plasma source (4 cm), and exposure time (30 and 60 min). In view of the application of cold plasma for detoxifying treatments on foods, we have chosen pistachio kernels among low-moisture foods to evaluate the matrix effect. Uncontaminated whole and chopped pistachio kernels were artificially contaminated by spotting a mixture of AFs and OTA (final concentration of 100 μg/kg). The results obtained after exposition to 30 and 60 min are reported in Fig. 5.

After 30 min treatment, the percentage of mycotoxin reduction did not show statistical significance, except for AFG1 (10.27 ± 0.52%) in chopped pistachio. However, significant variations were observed after 60 min of treatment. In particular, OTA exhibited a reduction of 23.31 ± 3.76% in chopped pistachio, while it was 8.63 ± 8.93 in whole pistachios. Similarly, AFB1 showed a reduction of 19.91 ± 2.48 in chopped pistachio, compared to 5.08 ± 5.92 in whole pistachios.

Regarding the certified control material, the initial quantification of all mycotoxins was within the uncertainty range indicated by the supplier's data sheet. The treatments of the certified sample proved to be more effective and time depending, resulting in a 20% reduction already at 30 min, and reaching a degradation of around 30% for OTA, AFB2 and AFG1, and of 20% for AFB1 after 60 min of treatment (Fig. 5).

4. Discussion

All the investigated mycotoxins showed considerable chemical degradation when exposed as pure molecules to CAP treatments, depending on regimes of plasma (O_3 and NO_x), treatment time and distance from the plasma source. The comparison of our results with the literature data is not always immediate or possible because each device utilised for CAP treatments presents a unique combination of plasma source and application parameters, such as processing voltage, discharge frequency, and distance of electrode from the substrate, making the resulting effectiveness and exposure times quite different. As previously described (Molina-Hernandez et al., 2022), the SDBD device used in this investigation implies that the substrate in the treatment chamber is substantially exposed to only long-lived species. Ions, electrons and short-lived species are confined in the mesh holes or within a distance from the source of less than 1 mm due to their slow diffusion rate (Hasan & Walsh, 2016; Simoncelli et al., 2019). Over time, two main long-lived species accumulate within the system: *i*) O_3 is accumulated when a low superficial power density is applied (duty cycle 10%), resulting in maximum ozone production and no detectable presence of nitrogen oxide (O_3 regime); *ii*) NO_2 is accumulated when a high superficial power density is applied (duty cycle 100%), maximising the production of nitrogen oxide and leading to an almost total reduction in ozone presence, being NO_x responsible for ozone degradation mechanisms (NO_x regime) (Chirokov, Gutsol, & Fridman, 2005). It can be assumed that these reactive species play an important role in the mycotoxin degradation, and, based on our results, it could be supposed

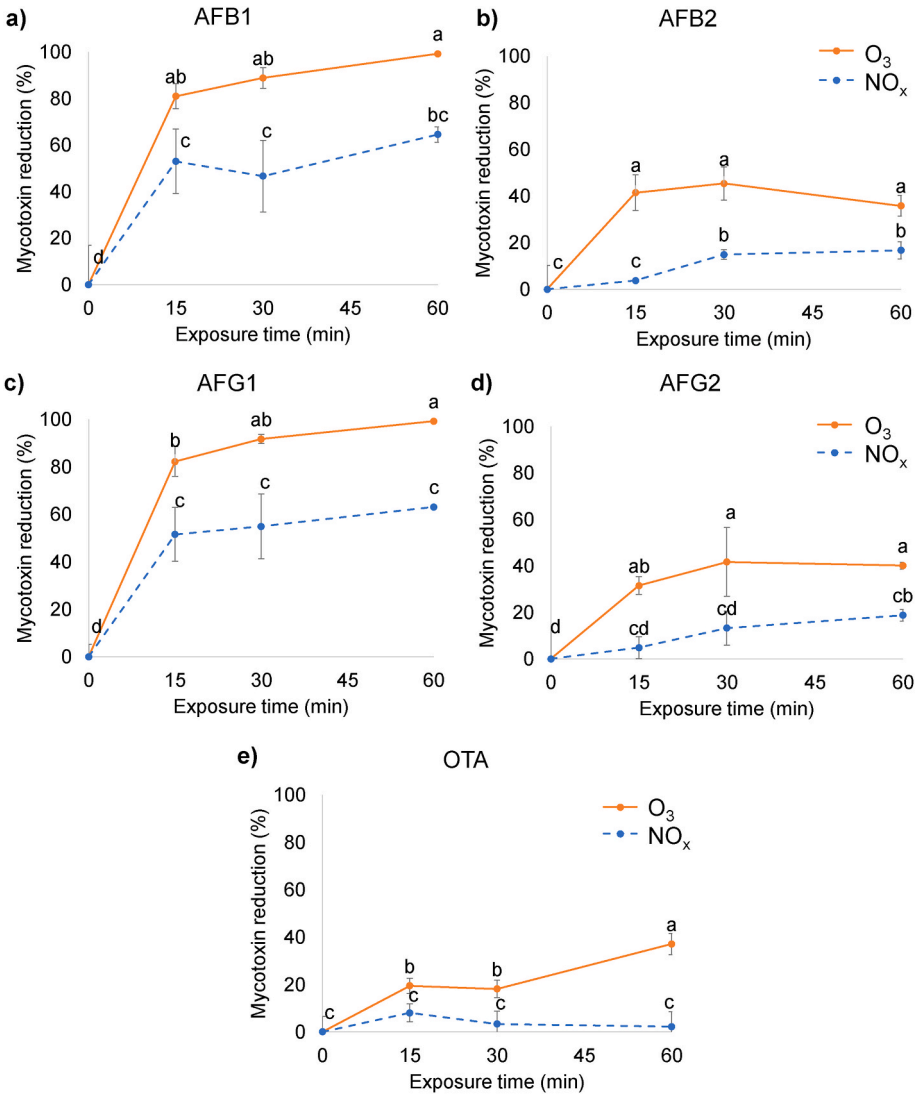

Fig. 3. Percentage reduction of standard aflatoxins B1 (AFB1) **(a)**, B2 (AFB2) **(b)**, G1 (AFG1) **(c)**, G2 (AFG2) **(d)** and ochratoxin A (OTA) **(e)**, by exposition to CAP treatment for different time (0–60 min) and under two operation regimes (O_3 and NO_x). Values are presented as means ± standard deviations (n = 3). Values with different superscripted letters are significantly different ($p < 0.05$).

that ozone has greater efficacy, although significant reductions were also observed for treatments in NO_x regime, characterised by the absence of ozone (Fig. 3). Although the ozone effect on mycotoxins degradation is known (Karaca, Velioglu, & Nas, 2010; Pankaj, Shi, & Keener, 2018; Sujayasree et al., 2022), no study evidenced the role of nitrogen oxide or more in general reactive nitrogen species (RNS) in the mycotoxins detoxification.

Studies about AFB1 decontamination by cold plasma performed in nitrogen gas (Sakudo, Toyokawa, Misawa, & Imanishi, 2017; Shi, Ileleji, Stroshine, Keener, & Jensen, 2017) accounted for the major role of reactive oxygen species in the degradation process, explaining the lower response of the treatment carried out in pure nitrogen gas than in air, with the lower concentration of ozone and other ROS (Shi, Ileleji, et al., 2017). Different degradation pathways of AFB1 have been depicted, revealing the formation of multiple degradation products. It has been hypothesised that these products are formed through the reaction of AFB1 with ozone (O_3), hydrogen peroxide (H_2O_2), and other reactive oxygen species (ROS), primarily free radicals (Wu, Cheng, & Sun, 2021).

In this study, only the two long-lived molecules O_3 and NO_2 were quantified (Fig. 1), but, also based on the mentioned literature, we hypothesise the participation of other reactive species that can contribute to the mycotoxins degradation. In fact, although not coming directly from the plasma source, radical species and other reactive molecules could be present in the treatment chamber as a result of reactions with water molecules present in the air, like H_2O_2 and $OH^{\bullet}$ generated by O_3 decomposition in humid air (Shi, Cooper, Stroshine, Ileleji, & Keener, 2017), and HNO_3, formed by the addition of OH to NO_2 (Moiseev et al., 2014).

Our results showed that the AFs degradation was regime, exposure time and structure dependent, with AFB2 and AFG2 the most resistant to the treatment. In this context, Park et al. (2007) reported a 100% degradation of AFB1 on glass cover slides after treatment with microwave-argon plasma device at the power of 1000 W by only 5s. Sakudo et al. (2017) observed a reduction of pure AFB1 from 200 ppb to 12 ppb in 15 min, under nitrogen gas plasma generated by a plasma apparatus using a short high voltage pulse from a static induction thyristor power supply at 1.5 kpps (kilo pulse per second). As reported by Shi, Cooper, et al. (2017), pure AFB1 placed on a glass cover was degraded by 76% in 5 min of HVACP (high voltage atmospheric cold plasma) direct treatment, in air having 40% relative humidity, at 90 kV, 50 kHz, 200 W. Hojnik et al. (2019) demonstrated that an indirect exposure to cold atmospheric pressure plasma of AFs on glass coverslips, placed at a distance of 5 mm from the plasma generation region (7–10 kV, 40 Hz), caused the almost complete degradation within 8 min. In a

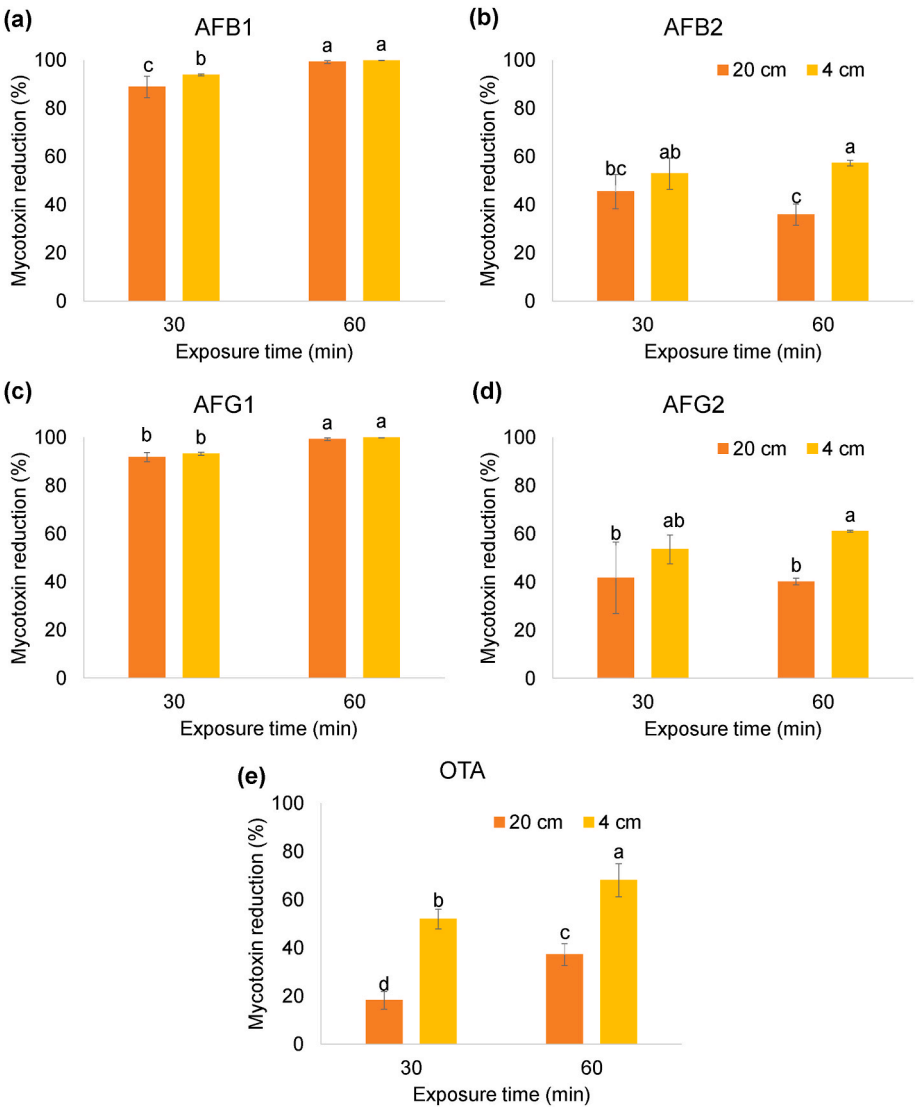

Fig. 4. Percentage reduction of standard aflatoxins B1 (AFB1) **(a)**, B2 (AFB2) **(b)**, G1 (AFG1) **(c)**, G2 (AFG2) **(d)** and ochratoxin A (OTA) **(e)**, subjected to CAP treatment, under O_3 regime, at different distance from the plasma source (20 and 4 cm) and different exposure times (30 and 60 min). Values are presented as means ± standard deviations (n = 3). Values with different superscripted letters are significantly different ($p < 0.05$).

Table 3
Percentage reduction of standard mycotoxins subjected to CAP treatment, in NO_x regime, at different distances from the plasma source and exposure times.

Input *d* (cm)	*t* (min)	Mycotoxin reduction (%)				
		AFB1	AFB2	AFG1	AFG2	OTA
20	30	46.71 ± 15.42	14.93 ± 2.08	54.93 ± 13.63	13.26 ± 7.30	3.33 ± 5.45
	60	64.58 ± 3.32	16.71 ± 3.67	63.11 ± 0.70	18.80 ± 2.51	2.21 ± 6.30
4	30	47.82 ± 7.68	15.21 ± 0.88	45.46 ± 5.65	15.57 ± 5.84	3.65 ± 7.91
	60	56.68 ± 0.48	15.18 ± 0.56	49.38 ± 6.02	8.70 ± 1.11	2.06 ± 1.61

Input *d*-distance from the source; *t*-exposure time. Values are presented as means ± standard deviations (n = 3). *Statistically significant at $p < 0.05$ level.

more recent study (Puligundla et al., 2020), a corona discharge plasma jet (CDPJ) was used to degrade AFB1 on glass slides, and a 95% of degradation in 30 min at the optimised 1.50 A current and 15 mm sample-to-electrode distance was reached.

As known, the mycotoxin degradation is related to the chemical structure of the mycotoxins molecules. The higher response of AFB1 and AFG1 respect to AFB2 and AFG2 lies in the double bond C8═C9 of the terminal furan ring of the furofuran moiety, only present in AFB1 and AFG1, the same structural characteristic responsible for their high toxicity (Wogan, Edwards, & Newberne, 1971). In fact, mechanistic studies carried out on AFB1 CAP degradation (Shi, Cooper, et al., 2017; S. Q. Wang et al., 2015) demonstrated that all the identified degradation products lost their C8═C9 double bond in accompaniment to further modifications of the furofuran ring, lactone ring, cyclopentanone and methoxyl group. In this work, the breakdown products after plasma treatments were not investigated, but these studies are desirable for the future, particularly to identify AFB1 degradation products formed by the action of NO_2 radical. As the interaction of NO_2 with an activated carbon surface consists in the reduction of NO_2 to NO by the transfer of an oxygen atom to chemical groups containing only one oxygen (Jeguirim, Belhachemi, Limousy, & Bennici, 2018), we expect that the cyclopentanone ring and the ether functionality may be involved in the NO_x regime degradation process (Fig. 6).

Conversely, regarding the regime effect, our results showed a different behaviour compared to the literature reports. In the study of Hojnik et al. (2019), two plasma powers were used, termed low (ROS

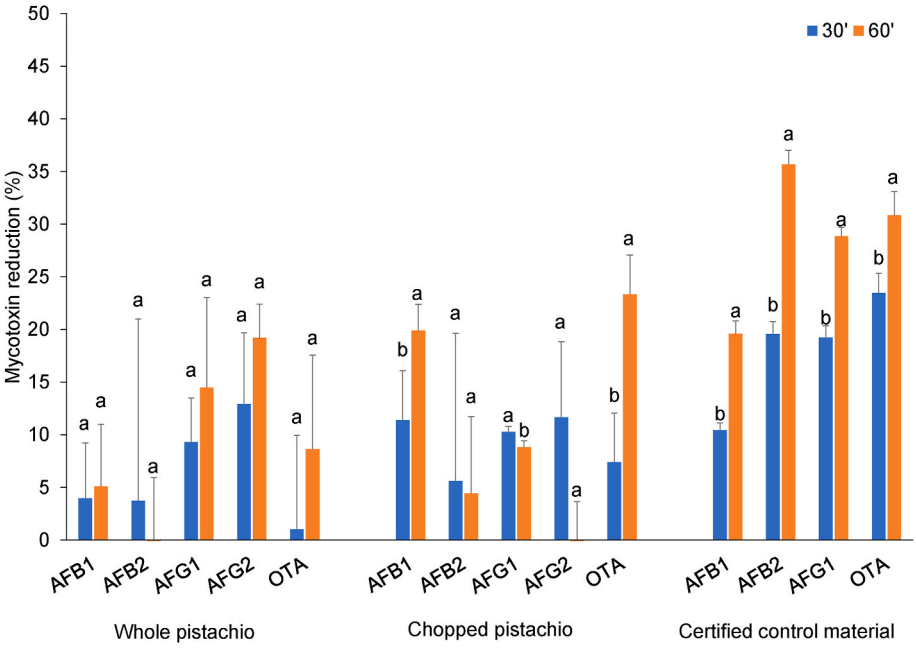

Fig. 5. Percentage reduction of aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2) and ochratoxin A (OTA) in artificially contaminated whole and chopped pistachio and certified control material, subjected to CAP treatment, under O_3 regime, at 4 cm distance from the plasma source, at 30 and 60 min exposure time. Values with different superscripted letters are significantly different ($p < 0.05$).

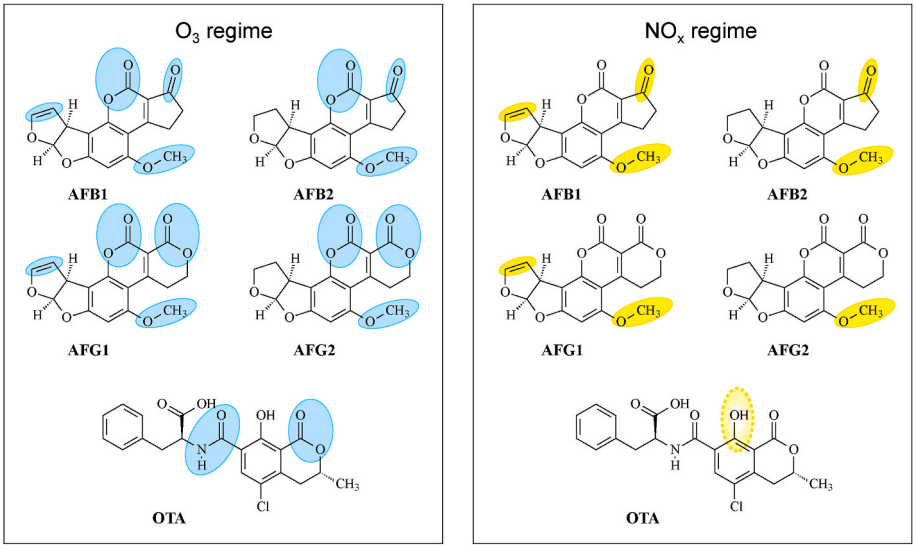

Fig. 6. Chemical structures of aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2) and ochratoxin A (OTA). Functional chemical groups probably involved in the action by CAP treatment under O_3 (blue circles) and NO_x (yellow circles) regimes are highlighted. The dashed circle indicates a potentiality impaired by steric hindrance.

dominated mode) and high (RNS dominated mode) power. In contrast with our findings, no significant differences between the low and high power treatments emerged for AFs, while a higher decontamination was observed for trichothecenes, HT-2 and T-2 after CAP treatment under high power conditions. Although achieved in different ways, the two operating conditions are comparable to our two regimes, O_3 and NO_x regime, respectively, and in our study for all AFs O_3 regime showed to be more effective.

Regarding OTA, scarce are the studies about the reduction by atmospheric cold plasma in food matrices, as date palm fruit (Ouf, Basher, & Mohamed, 2015), barley (Durek, Schlüter, Roscher, Durek, & Fröhling, 2018), roasted coffee (Casas-Junco et al., 2019) and rice grain (Guo et al., 2023). Only in one case the mycotoxin was treated as a pure molecule, but in aqueous solution (Wielogorska et al., 2019). In this case, 50% reduction in mycotoxins was achieved after a 2.2 min exposure to a plasma jet using helium as the gas effluent; however, when the gas was changed in helium with 0.75% oxygen, the time required to achieve a 50% reduction increased to 30 min.

The detoxification mechanism for OTA has not been elucidated yet. However, biodegradation and alkaline or acid treatments account for the hydrolysis of the amide bond resulting in L-phenylalanine and ochratoxin alpha, the latter showing no toxicity (L. Wang et al., 2022). In addition, as biodegradation process, the opening of the lactone ring of OTA was also hypothesised, but the resulting open hydrolysed form seems to be as toxic as OTA (Abrunhosa, Paterson, & Venâncio, 2010). The application of ozone to degrade OTA has proven more effective

towards OTA in solution than dry OTA and towards OTA in corn samples with higher moisture content than low-moisture ones (Qi et al., 2016). Previously, the attack by ozone of the chlorinated ring of OTA, resulting in free chlorine, had also been considered (Tiwari et al., 2010).

With our operative conditions, we can hypothesise that in O_3 regime, with the active role of the air humidity, predominantly the hydrolysis of the amide bond takes place (Fig. 6). Moreover, the absence of trace of the non-chlorinated analogue OTB after treatments, suggests the C–Cl bond non-involvement. On the other hand, studies about the toxicity of OTA and OTB related the lower toxicity of OTB to its more rapid elimination rather than the absence of the chlorine compared to OTA (Mally et al., 2005). Concerning the NO_x regime, the high resistance of OTA towards degradation could be envisaged in the absence of functionalities in the mycotoxin chemical structure capable of oxidising by reduction of NO_2. However, the only potentially oxidisable group, the hydroxyl on the benzene ring, is challenging to reach due to steric hindrance (Qi et al., 2016) (Fig. 6).

In order to evaluate the matrix effect, among the low-moisture foods pistachio kernels have been chosen. To simulate better a real situation of point distribution of the mycotoxins, we artificially contaminated whole and chopped pistachio kernels, by spotting a mixture of AFs and OTA molecular standards. The optimised conditions were applied: O_3 regime, 30 and 60 min, 4 cm distance from the plasma source. The standard deviations of the results (Fig. 5) were quite high due to the inhomogeneity of the contamination as well as to an uneven contact between plasma and food (Rao et al., 2023). As expected, the mycotoxins in food were reduced to a much lesser extent than pure molecules. A slightly better response was observed for chopped pistachio kernels than whole ones, most likely due to a larger surface area of the food exposed to plasma. A 20% AFB1 reduction and an approximately 10% AFG1 degradation were achieved. Moreover, it is noteworthy the 23% reduction of OTA. To our best knowledge, this is the first investigation of OTA degradation in pistachio food matrix using cold plasma. Recently, Makari, Hojjati, Shahbazi, and Askari (2021) accounted for a direct cold plasma treatment of artificially AFB1 contaminated pistachio powder. Samples with a thickness of 3 mm, placed at 3 mm of distance from the electrodes, exposed for 180 s, resulted in 52% reduction of the mycotoxin, compared to 65% reduction of the pure molecule under the same conditions. In parallel, the decontamination effect on pistachio kernels inoculated with *Aspergillus flavus*, consisting in a decrease by 4 logs in the spore population after 180 s of the treatment, was also observed, providing a further example of the detoxifying action of cold plasma towards toxigenic fungi and towards their toxins (Basaran et al., 2008; Esmaeili, Hosseinzadeh Samani, Nazari, Rostami, & Nemati, 2023; Ghorashi, Tasouji, & Kargarian, 2020). In roasted coffee a complete inhibition of *Aspergillus westerdijikiae*, *Aspergillus steynii*, *Aspergillus versicolor*, and *Aspergillus niger* spores was achieved (4 log reduction) after 6 min of treatment with cold plasma, while a 50% reduction of OTA was observed after 30 min (Casas-Junco et al., 2019). In rice grain the microbial activities of *Aspergillus niger*, *Rhizopus oryzae*, *Penicillium verrucosum* and *Fusarium graminearum* were significantly inhibited by 8 min of cold plasma treatment, and OTA and DON were reduced of 56% and 61%, respectively (Guo et al., 2023). In general, in all these studies, the authors observed that quality characteristics were only slightly affected by the treatment, concluding that cold plasma is an efficient decontamination technology for food.

Studies about the impact of cold plasma treatments on the oxidation of the pistachio kernels, using the same device as the present investigation, have been reported (Foligni et al., 2022). No significant changes in the whole composition of lipids were observed when the O_3 regime operative conditions were applied for 30 min at a distance of 16.5 cm from the plasma source. However, further studies are needed to evaluate the lipid oxidation varying the exposure time and the sample to plasma source distance.

The treatment applied to the certified control material clearly illustrates the influence of the sample's condition on treatment effectiveness. Our artificial contamination tests provide insights into the efficacy of CAP treatment on ready-to-consume products, specifically whole or chopped pistachios, exhibiting sporadic natural contamination with a notable concentration of mycotoxins, even when co-occurring. In contrast, results observed for the certified product emphasise the high effectiveness of CAP treatment on processed matrices rendered nearly homogeneous. This is a crucial aspect to take into account when considering the potential applications of cold plasma technology for food decontamination.

5. Conclusions

The SDBD device used as a cold atmospheric plasma source has the ability to operate in two distinct regimes: the O_3 regime and the NO_x regime. In the O_3 regime, the device is optimised to maximise the accumulation of ozone (O_3), while in the NO_x regime, the device is designed to maximise the accumulation of nitrogen dioxide (NO_2) in the treatment chamber. The study carried out on pure mycotoxins assessed their more extensive degradation when O_3 regime is working. At the optimised 4 cm distance from the source, and 60 min of exposure time, AFB1 and AFG1 99%, AFB2 and AFG2 60% and OTA 70% reductions were observed, respectively. The CAP treatment applied on artificially contaminated pistachios, at the same optimised conditions, resulted in interesting mycotoxins degradation: AFB1, 5%, 20% and 20% for whole, chopped and certified slurry pistachios, respectively; OTA, 9%, 23% and 31% for whole, chopped and certified slurry pistachios, respectively.

In our opinion, the investigation to evaluate the pistachio food matrix effect on mycotoxins degradation was very important. The artificial contamination of whole and chopped pistachio kernels was deliberately punctiform and uneven, so that the subsequent treatment would simulate a truly contaminated food as closely as possible. The observed percentage of reduction, although limited, was quite satisfactory, indicating CAP as effective in reducing the levels of mycotoxins present. This observation holds significant importance as it highlights the potential of plasma applications. In fact, by reducing mycotoxin content and eliminating toxigenic fungi, cold plasma demonstrates to be a promising technology to contribute to the overall control and mitigation of fungal contamination in food products.

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CRediT authorship contribution statement

Jessica Laika: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Eduardo Viteritti:** Formal analysis, Investigation, Validation. **Junior Bernardo Molina-Hernandez:** Data curation. **Manuel Sergi:** Methodology. **Lilia Neri:** Visualization. **Romolo Laurita:** Formal analysis, Investigation. **Silvia Tappi:** Data curation. **Antonella Ricci:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Clemencia Chaves-López:** Conceptualization, Project administration, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2024.110286.

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