Innovative Food Science and Emerging Technologies 108 (2026) 104394



Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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





Effect of cold atmospheric plasma treatment on patulin decomposition: A kinetic approach☆

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ARTICLE INFO

Keywords:
Cold atmospheric plasma
Patulin decomposition
Kinetic approach
Apple juices

ABSTRACT

The aim of this study was to investigate the effect of direct Cold Atmospheric Plasma (CAP) on patulin detoxification in both standard solutions and apple juices. A kinetic approach was applied to understand how the CAP process conditions' intensity may affect the patulin molecules related to toxicity. Various voltages ranging from 19 to 25 kV were applied, while the duration varied from 1 to 4 min for 5 mL aqueous standard solutions (pH = 4) or apple juices with a concentration of 100 ppb patulin. The residual patulin concentration after CAP treatment was determined by using HPLC. The concentration of generated RONS in water, such as H_2O_2, NO_3^- and NO_2^- was determined. CAP treatment led to patulin detoxification in water. Patulin decomposition efficiency reached 99 % at an applied voltage of 25 kV in a 4 min treatment period. The applied voltage significantly affects the formation of oxidizing species and decomposition of pollutants. Increasing the applied voltage and treatment time both promoted patulin decomposition. After 1 min treatment, the patulin degradation efficiency reached 16, 72 and 83 % at applied voltages of 19, 21 and 25 kV, respectively. Patulin decomposition products were detected in CAP treated samples, such as ascladiol, deoxypatulin and hydroascladiol, showing that the ring lactone was broken after CAP treatment. Patulin detoxification up to 50 % was achieved in CAP treated apple juices, while their quality remained almost unchanged. Further research is needed to understand the CAP mechanisms responsible for patulin decomposition and toxicity evaluation of the byproducts generated during processing.

Industrial relevance: Developing new strategies for controlling patulin in food products remains of utmost interest to the relevant industry. Cold atmospheric plasma is a promising technology for patulin detoxification, as the reactive species produced by CAP cause damage on patulin ring, leading to the formation of less toxic compounds. The mathematical description of patulin decomposition by CAP treatment in various process conditions is a useful tool aiming to prediction and evaluation of patulin detoxification. This tool allows for designing properly and effectively the CAP process applied, avoiding over-processing, food quality degradation as well the high energy consumption.

1. Introduction

Patulin, a neurotoxic secondary metabolite produced by fungi like *Aspergillus*, *Byssochlamys*, and *Penicillium*, poses significant health risks (Ioi et al., 2017; Puel et al., 2010). Its toxicity is well-documented, causing severe symptoms such as renal congestion, pulmonary edema, and tissue necrosis in animals (Pal et al., 2017). Fruits, vegetables, and even water sources can be contaminated, with concentrations reaching up to 1000 μg L^{-1} (Kooner et al., 2014). Patulin's strong hydrophilicity and stability facilitates its accumulation in humans through food and water consumption, leading to various acute and chronic ailments including enteritis, cramps, convulsions, and cancer (Puel et al., 2010; Saleh & Goktepe, 2019). Regulatory limits, according to World Health Organization, 1995) set on 50 μg kg^{-1} for patulin in fruit juices (Beretta

☆ This article is part of a Special issue entitled: 'EFFoST38' published in Innovative Food Science and Emerging Technologies.

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https://doi.org/10.1016/j.ifset.2025.104394
Received 18 June 2025; Received in revised form 14 November 2025; Accepted 3 December 2025
Available online 4 December 2025
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et al., 2000), highlight the need for effective detoxification methods. Moreover, fruit juices from NFC (not from concentrated juice) have already found their way to the super-market shelf, there are still major concerns that have been considered for a more sustainable production of healthy and low safety risk fruit juices (Vila-Donat et al., 2018). Consequently, many food products are susceptible to contamination with both toxin-producing microorganisms and their produced mycotoxins. The good agricultural and farming practices, post-harvest strategies (unseasonable temperature, rainfall or humidity), processing (food contaminated contact surfaces) and storage (Nan et al., 2022) are used in the agro-food industry to minimize the chance of patulin contamination. However, these strategies typically cannot guarantee the elimination of mycotoxin-producing microorganisms (Vila-Donat et al., 2018). Therefore, the relevant food industry tries to get solutions to eliminate or remove patulin using several physical, biological and chemical detoxification and decontamination techniques (Diao, Hou, et al., 2018; Vila-Donat et al., 2018). Heat treatments, such as blanching or pasteurization, for fungi and mycotoxins degradation are applied for relevant industry. Nevertheless, most mycotoxins, such as patulin are resistant in high temperatures (Abbasi et al., 2021). The application of chemical agents to decontaminate fungi and mycotoxin-infected food is another typical approach. Several synthetic chemicals, natural preservatives and other chemicals have already been used either to prevent the growth of patulin-producing molds or to react directly with the patulin for its decontamination. These techniques may negatively affect the organoleptic properties of the food and may leave the food treated with chemical residues (Liu et al., 2024). Biological methods are efficient and safer for control patulin contamination but are cost effective for the relevant industry. These methods involve applying active microorganisms that inhibit the growth of patulin-producing molds (Ngolong Ngea et al., 2020). There is a real need to find alternatives to improve the efficacy of washing treatments for fruits (Diao, Wang, et al., 2018). The effective reduction or removal of patulin is also a crucial step toward minimizing food waste for relevant industry, with direct impact on economy, society, and environment. The production loss of fruits and vegetables caused by postharvest diseases was 35–55 %, and in developing countries the loss even exceeded 55 % (Sanzani et al., 2016). Losses in this case can include both the destruction of contaminated batches leading to financial losses depending on the extent of the contamination (Sanzani et al., 2016). For the pre-mentioned reasons, it is obvious that the food industry needs to develop alternative or additive processing technologies to meet consumer expectations with safer products. The limitations associated with conventional methods of mycotoxin removal from food commodities have resulted in ongoing research on alternative mycotoxin decontamination approaches (Ioi et al., 2017). These include utilizing novel technologies, such as microwave (Hassan et al., 2018), ultraviolent (Tikekar et al., 2014), ozone (Diao, Wang, et al., 2018; Freitas-Silva & Venâncio, 2010), essential oils (Gavahian & Farahnaky, 2018), pulsed light irradiation (Wang et al., 2022), enzymes (Liu et al., 2024). Cold plasma has also recently attracted the attention of researchers around the world for both fungi growth inhibition and mycotoxin degradation with initial studies on the topic published over a decade ago (Pankaj et al., 2018). Cold atmospheric plasma (CAP) is widely known for its microbial decontamination of food products, mainly due to the interaction with reactive oxygen and nitrogen species (RONS), such as O, O_3, OH, NO_x, etc. generated in the gas phase during processing (Darvish et al., 2022). These species may lead to degradation of mycotoxins in various food products. Currently, few studies so far have also confirmed the efficacy of plasma treatments in detoxification of several food products (Moisan et al., 2002; Shi et al., 2017; Ten Bosch et al., 2017). Researchers are now focusing on optimizing the process parameters to increase the degradation efficiency along with insights into the degradation mechanism. Nevertheless, this technology has not been exploited in the food industry yet, due to the lack of data available in the literature to establish effective applications in food systems. This technology is still characterized as TRL (Technology Readiness Level) 5. Data approving its applicability and effectiveness must be produced by appropriate experiments. Within the framework of this work, the use of CAP treatment will be studied for the detoxification of patulin in aqueous standard solutions and apple juice. A kinetic approach was applied to understand how the CAP process conditions' intensity may affect the patulin molecules related to toxicity. Quality indices were also measured for the CAP treated apple juices and compared to control samples. Based on all the above, the proposed study may significantly contribute to the scientific community and the relevant industries (juice manufacturers) by producing data necessary for the potential application of CAP as new detoxification method for highly risky and perishable NFC apple juices for sustainable patulin elimination, thus safer products of superior quality, in line with the demands of consumers, food producers and EU regulations.

2. Materials and methods

2.1. Raw materials

Patulin standard solution (>99 % purity) was provided by physicochemical laboratory of Institute of Technology of Agricultural Products ELGO-DEMETER. From the initial stock of 200 ppm patulin in ethyl acetate, one milliliter was taken and evaporated with N_2 gas. Twenty milliliters of ethanol was added to create a 10 ppm patulin solution. One milliliter from this solution is evaporated again with N_2 gas and then 10 mL of acidified water (pH = 4) was added, resulting in 1 ppm of patulin solution and then stored at −20 °C until further analysis. The standard was diluted with acidified water (pH = 4) to 100 ppb, as the working solution before its application. Apple juice was also prepared using selected red-yellow Greek apples (*Lasithiou cv*), with similar weight and ripeness degree. Since the obtained apple juice was cloudy, a clarification procedure was followed before the experiments. Twenty grams of juice was centrifuged (3000 rpm for 10 min) with 300 μL of enzyme and 20 ± 0.2 g of deionized water. Then, the mixture was heated in a water bath at 37–40 °C for 2 h. Subsequently, centrifugation was performed at 3000 rpm for 10 min, to perform the enzymatic treatment. Finally, the supernatant was collected and then stored at −20 °C until further analysis. From the 1 ppm patulin solution, one milliliter was evaporated with N_2 gas and then 10 mL of clarified apple juice was added, resulting in apple juice with 100 ppb patulin concentration.

2.2. Experimental design

Based on the experimental set up, direct CAP treatments were carried out by a pin-jet CAP device using plain air as gas phase. Various combinations of CAP voltages (19–25 kV) and times (1–4 min) was tested for 5 mL sample. CAP treatments were performed either on aqueous standard solutions of patulin 100 ppb or on clarified apple juices with patulin concentration of 100 ppb. The first step was to study the effect of direct CAP treatment on mycotoxins detoxification in aqueous standard solutions and to understand how the reactive nitrogen and oxygen species (RONS) produced by CAP affect the mycotoxins concentration. A full kinetic approach of mycotoxin degradation by applying CAP treatment in different intensity conditions was demonstrated. The CAP physicochemical characteristics, such as the hydrogen peroxide (H_2O_2) content, pH value as well as nitrate anions (NO_3^-) and nitrite anions (NO_2^-) concentrations were determined on standard solutions of patulin. In addition, the concentration of patulin was measured by High Performance Liquid Chromatography (HPLC) for all studied conditions. Degradation mechanism was further confirmed by high-resolution (HR) ion mobility mass spectrometry (IM-MS). In the second part, the effect of direct CAP treatment direct for the patulin degradation in apple juice was studied. Apart from the mycotoxins analysis, other quality indices were also studied in the process conditions applied for the apple juices such as: a) total soluble solids, b) color (CIELAB method), c) total phenolic compounds, d) total antioxidant activity and e) pH-value. All

the obtained data was described using appropriate equations. Based on these models, the optimal process conditions was selected, based on criteria such as the maximum initial patulin concentration reduction and simultaneously the less effect on quality parameters of the apple juices, the minimum necessary process time and energy required. Finally, for comparison purposes, in order to determine which of the reactive species produced by plasma had the greatest effect on the degradation of patulin, three different artificials solutions were prepared: (a) a standard 100 ppb patulin solution with pH = 4 and a peroxide concentration of 100 ppm, (b) a standard 100 ppb patulin solution with pH = 4 and a nitrate concentration of 100 ppm and (c) a standard 100 ppb patulin solution with pH = 4 and a peroxide concentration of 100 ppm and a nitrate concentration of 100 ppm. In addition, heat treatment was also tested for the degradation of patulin. More specifically, a standard patulin solution of 100 ppb with pH = 4 was heated for 5 min at 100 °C. During CAP treatment and especially in more intense CAP conditions, an increase in temperature was observed due to the treatment (up to 75 °C). For all the above samples, the patulin concentration was also determined and compared with CAP treated sample (in more intense condition).

2.3. Cold atmospheric plasma set-up

The experimental set-up for the plasma processing of oils comprises a non-thermal (cold) plasma reactor with a pin-to-liquid dielectric barrier discharge (DBD) configuration. The high-voltage (HV) pin electrode is facing the sample that is placed inside a cylindrical quartz vessel of 70 mm diameter. The latter is positioned on top of a grounded plane electrode with a total electrode gap of 30 mm. A fixed 5 mL quantity of sample is inserted in the container leading to a narrow 2 mm discharge gap between the pin and the sample surface. Both the sample substrate and quartz bottom of the container serve as dielectric barriers, whilst the discharge is ignited in ambient air. The plasma discharges are driven by sinusoidal HV signals using a PVM500 power supply (Information Unlimited) at fixed frequency (20 kHz). The HV waveforms were recorded via a Testec HV probe (TT-HVP 2739) and a Rigol DS2202A digital oscilloscope. Plasma treatment was studied at different high voltage values and processing times. The temperature was recorded before and immediately after each treatment, with the highest recorded temperature being 75 °C in the most intense condition using a portable digital thermometer (Jenway Series 2000, UK). All experiments were performed in triplicate.

2.4. Analytical methods

2.4.1. Determination of reactive oxygen and nitrogen species in samples

The CAP characteristics including the concentration of reactive species (RONS) concentrations will be recorded. The determination of peroxides H_2O_2 in patulin solutions is based on the spectrophotometric method as described by Eisenberg (1943) using the titanium sulfate reagent ($Ti(SO_4)_2$). The absorbance was measured at 410 nm against distilled water. The concentration of H_2O_2 was calculated using a standard curve, by preparing standard solutions in the range of 0–25 mg/L. The existence of NO_2^- in samples was evaluated based on the protocol described by Merino (2009). The concentration in NO_2^- was calculated through a calibration curve prepared by standard nitrite solutions in the range of 1–50 mg/L. The absorbance was measured at 540 nm against distilled water. The concentration in NO_3^- was determined according to the method described by Robarge et al. (1983). The absorbance was measured at 410 nm against distilled water. A calibration curve was prepared by standard nitrate solutions in the range of 1–50 mg/L NO_3^-. For all measurements, it was used a UV–Vis Spectrophotometer (Lovibond XD 7500, Tintometer GmbHXX). All measurements were performed in triplicate.

2.4.2. pH-value determination

The pH value of all samples was measured using an ORION pH-meter (ORION ion analyzer model EA 940, USA). All measurements were performed at samples of 3 mL at a constant temperature of 20.0 ± 1.0 °C.

2.4.3. Determination of Patulin by high performance liquid chromatography in samples

The patulin concentration for all samples was identified by using a Shimadzu Nexera LC-40 HPLC system equipped with SPD-M40 Detector and a SIL-40C autosampler injector. HPLC was also equipped with an Agilent Eclipse Plus C18 column. A diode array detector was programmed to detect the signal at wavelengths 276 nm. Mobile phase A contained 0.095 % *v*/v $HClO_4$. Mobile phase B contained acetonitrile. The method was calibrated by a standard solution of patulin (5–100 ppb) used for calibration curves. For untreated and CAP treated apple juices, an extraction method was performed before HPLC analysis based on the method proposed by MacDonald et al. (2000). All samples were analyzed in triplicate.

2.4.4. Determination of degradation products by high-resolution (HR) ion mobility mass spectrometry (IM-MS) in samples

For the confirmation of the degradation products, CAP treated samples were analyzed on Acquity I-class UPLC separation system coupled to a Vion IMS QTOF mass spectrometer (Waters, Wilmslow, UK) equipped with an electrospray ionization (ESI) interface. Samples were injected (1 μL) and chromatographically separated using a reversed-phase C18 BEH Acquity column (2.1 × 100 mm2, 1.7 μm particle size) (Waters,Milford, MA, USA). Gradient elution was set according to Righetti et al. (2020). Mass spectrometry data were collected in negative electrospray mode over the mass range of *m/z* 100–1100. Source settings were maintained by using a capillary voltage of 2.5 kV, a source temperature of 120 °C, a desolvation temperature of 500 °C, and a desolvation gas flow of 1000 L h − 1. A TOF analyzer was operated in sensitivity mode, and data were acquired using HDMSE, which is a data-independent approach (DIA) coupled with ion mobility. The optimized ion mobility settings included a nitrogen flow rate of 90 mL min^{-1} (3.2 mbar), a wave velocity of 650 m s − 1, and a wave height of 40 V. The TOF was also calibrated prior to data acquisition and covered the mass range from m/z 151 to 1013. TOF and CCS calibrations were performed for both positive- and negative-ion mode. Data acquisition was conducted using a UNIFI 1.8 (Waters, Wilmslow, UK).

2.4.5. Determination of quality characteristics of apple juices

The total soluble solids (Brix) of apple juices were measured using a digital Refractometer (°Brix- KERN Digital Refractometer, KERN & SOHN GmbH, Germany). Color quantification of apple juices was determined based on CIE Lab coordinates (L: lightness, a: redness, b: yellowness) using a colorimeter HunterLab (Reston Virginia, USA). For each sample the chroma (Eq.1), hue angle (Eq.2) and the total color change ΔE (L_0, a_0, b_0 are the L, a and b values before CAP treatment) (Eq. 3), were calculated:

$$Chroma = \sqrt{a^2 + b^2} \quad (1)$$

$$Hue\ angle = \tan^{-1}\left(\frac{b}{a}\right) \quad (2)$$

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (3)$$

2.4.6. Determination of total phenolic compounds concentration and antioxidant activity of apple juices

After CAP processing of each sample, the total phenolic compounds (mg caffeic acid/100 g d.m), and antioxidant activity (mg Trolox equilevant/100 g d.m) were determined for untreated and CAP treated apple

juices. The solid-liquid extraction method was performed using 10 mL of methanol:water mixture (80:20 *v*/v) and mixed with 1 g of juice. The mixture was incubated in an ultrasonic bath for 1 h. Then the mixture was filtered and stored in −20 °C until further analysis. The total phenolic compounds content was determined using a spectrophotometric technique based on Folin-Ciocalteu method (Waterhouse, 2002). Antioxidant activity was assessed using the DPPH following Brand-Williams et al. (1995) and ABTS assays, with the ABTS radical cation decolorization assay performed according to Biskup et al. (2013). All measurements repeated twice.

2.5. Statistical analysis

One-way or factorial analysis of variance (ANOVA) was used to evaluate the statistically significant differences and the interactions between untreated and CAP treated samples for all studied indices. The experimental data were analyzed using Statistica 7 (Stat Soft, Tulsa, OK, USA). The comparisons of means were calculated using Duncan's test at the 5 % level of significance ($p \leq 0.05$).

3. Results and discussion

3.1. Effect of CAP treatment on aqueous patulin solutions – Physicochemical characteristics of solutions

The CAP characteristics of the aqueous solution of patulin such as hydrogen peroxide (H_2O_2) contents, as well as nitrate anions (NO_3^-) (Fig. 1) were measured directly after CAP experiments. Peroxides and nitrate ions (RONS) produced by CAP treatment maintain their activity for a longer duration compared to other radicals produced during CAP treatment. During CAP treatment in aqueous solution of patulin, it was observed that more intense CAP conditions resulted in increased RONS concentrations (Fig. 1). For 1 min treatment time, the concentration of H_2O_2 was 15.30, 30.11, 37.48, and 42.43 ppm for 19, 21, 23, and 25 kV, respectively. The corresponding concentration of NO_3^- was 38.3, 60.6, 70.7, and 78.7 ppm for 19, 21, 23, and 25 kV, respectively. In order to evaluate and describe the effect of CAP treatment on RONS concentration in aqueous patulin solutions, experimental data was fitted to empirical equations. The increase of RONS concentrations of samples due to CAP treatment as a function of time followed by a zero-order kinetic model (Fig. 3). The constant rates of the concentration increase of peroxides and nitrate ions were calculated (Table 1). Increasing the voltage applied, resulted in higher rates. From 19 to 25 kV, a 4-fold increase of the rate of peroxide concentration and a 2.5-fold increase of the rate of nitrate ion concentrations was measured.

The pH values and nitrite anions (NO_2^-) concentration were also determined in each sample (Table 2). Nitrite ions (NO_2^-) are a category of RONS that do not retain their activity for long periods compared to other radicals produced during CAP treatment. They (NO_2^-) are converted in NO_3^-. The concentration of NO_2^- was significantly lower (<1 ppm) compared to the concentrations of nitrate ions and peroxides across all plasma treatment conditions studied (Table 2). As was expected, the pH of the samples decreased as the treatment time and applied voltage increased. The production of RONS was expected to reduce the pH of the sample. Higher concentration of peroxides and nitrate ions led to greater pH reduction in the samples. Compared to the existing literature, the results of the present study show similar trends. Previous studies applying plasma at voltages up to 40 kV for treatment durations of up to 1 h in aqueous solutions containing toxins, reported H_2O_2 concentrations of up to 130 mg/L, nitrate concentrations of approximately 50 mg/L, and very low nitrite concentrations (Nguyen et al., 2025; Ni et al., 2024; Triantaphyllidou & Aggelopoulos, 2025). These findings are in good agreement with the concentrations range observed in the current work.

Generally, the composition and concentration of RONS depend on the CAP processing conditions such as the type of gas and its composition, the voltage applied and the distance from the material (Domonkos et al., 2021). Katsaros et al. (2023), found that CAP treatment of water

Table 1
Constant rate of concentration increase k (min^{-1}) for aqueous patulin solutions (100 ppb) after CAP processing for voltages ranged from 19 to 25 kV.

Constant rate of concentration increase k (min^{-1})		
Voltage applied (kV)	k_{H2O2}	k_{NO3-}
19.0	10.68 ± 0.53^a	30.34 ± 0.84^a
21.0	28.85 ± 0.67^b	45.40 ± 2.84^b
23.0	36.22 ± 1.98^c	64.60 ± 5.46^c
25.0	42.16 ± 1.96^d	78.32 ± 6.42^d

± represents standard error as calculated by linear regression. Different superscript numbers indicate significantly different means ($p < 0.05$) between different voltages applied.

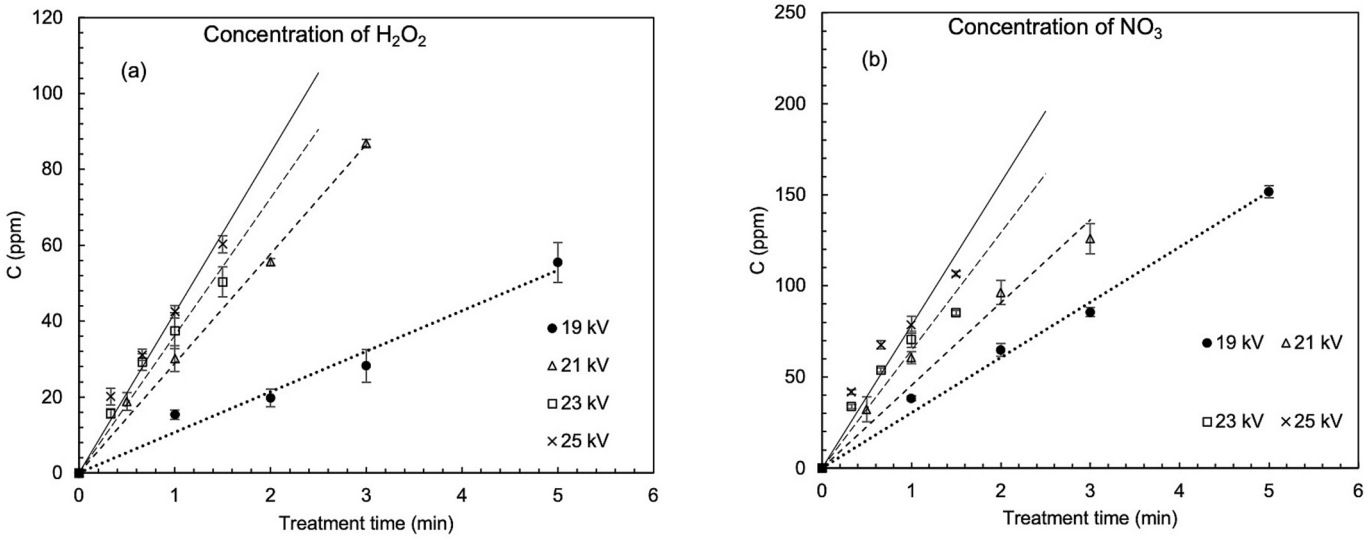

Fig. 1. (a) Concentration of H_2O_2 and (b) NO_3^- for aqueous patulin solutions (100 ppb) after CAP processing for voltages ranged from 19 to 25 kV and treatment times from 20 s to 5 min. Dashed lines represent the fit of linear equation to experimental data. Error bars represent standard deviation from multiple treatments and measurements.

Table 2
Concentration of NO_2^- and pH values for aqueous patulin solutions (100 ppb) after CAP processing for voltages ranged from 19 to 25 kV and treatment times from 20 s to 5 min.

Voltage applied (kV)	Treatment time (min)	NO_2^- (ppm)	pH
19.0	1.0	1.42 ± 0.05	3.85 ± 0.02[a]
	2.0	0.19 ± 0.02	3.10 ± 0.01[b]
	3.0	0.16 ± 0.03	3.00 ± 0.02[c]
	5.0	0.18 ± 0.02	2.95 ± 0.03[d]
21.0	0.5	0.44 ± 0.12	3.86 ± 0.01[a]
	1.0	0.16 ± 0.05	3.70 ± 0.00[b]
	2.0	0.58 ± 0.15	3.16 ± 0.02[c]
	3.0	0.15 ± 0.04	3.03 ± 0.01[d]
23.0	0.3	0.17 ± 0.01	4.00 ± 0.02[a]
	0.6	0.72 ± 0.15	3.62 ± 0.01[b]
	1.0	0.45 ± 0.09	3.58 ± 0.03[c]
	1.5	0.19 ± 0.06	3.51 ± 0.01[d]
25.0	0.3	0.65 ± 0.11	3.34 ± 0.00[a]
	0.6	0.18 ± 0.02	3.08 ± 0.01[b]
	1.0	0.45 ± 0.17	3.05 ± 0.02[c]
	1.5	0.44 ± 0.08	3.01 ± 0.00[d]

± represents standard deviation from multiple treatments and measurements. Different superscript numbers indicate significantly different means ($p < 0.05$) between different voltages applied.

led to the production of various reactive species, either primary that are formed directly from plasma such as ozone (O_3), hydroxyl radicals (•OH) and atomic nitrogen (N), singlet oxygen ($^1O_2$), superoxide ion (O_2^-) or secondary that arise from the further interaction of the primary species with the environment such as hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), nitric oxide (NO*), nitrate (NO_3^-) and nitrite (NO_2^-) ions. Primary species from the gas phase, such as ozone (O_3) and hydroxyl radicals (•OH), are transported into the water, where secondary species such as hydrogen peroxide (H_2O_2) and nitrate ions (NO_3^-) are formed, by processes such as diffusion, absorption and chemical transport (Perinban et al., 2019). The reactive oxygen species (e.g. OH, O, O_2^-, H_2O_2) influence the oxidative reactions in the liquid, while the reactive nitrogen species contribute to the conversion of nitrogen compounds (production of nitrate ions and ammonia) (Adamovich et al., 2022). The more stable reactive species (O_3, NO_3^-, H_2O_2) penetrate deeper, while short-lived species (e.g. O_2^-, $ONOO^-$) remain near the surface, with penetration being affected by factors such as air gap, voltage and treatment duration (Perinban et al., 2019).

3.2. Kinetic approach of the effect of CAP treatment on aqueous patulin solutions – Degradation of patulin

The potential use of cold atmospheric plasma (CAP) as technology for the degradation of patulin in standard solutions was studied. The aim was to monitor how the reactive nitrogen and oxygen species (RONS) produced during CAP treatment, affected the patulin structure, leading to its degradation. The residual concentration of patulin was measured for all studied CAP conditions and are presented in Fig. 2.

As the intensity of CAP conditions increased, the reduction of patulin concentration also increased. For 1 min treatment time, the residual patulin concentration was 75.0, 29.1, 19.3, and 10.2 ppb for voltages of 19, 21, 23, and 25 kV, respectively. For voltages >21 kV and treatment times >1 min, the residual patulin concentration was <20 ppb, achieving approximately 80 % reduction (near-complete degradation). The obtained results showed that CAP treatment led to an amount of patulin less than the EU recommended amounts (50 μg kg^{-1}), which can be characterized as safe regarding the consumer's health. The experimental data were fitted to an exponential model from which the reduction rate constants of patulin degradation were calculated (Table 3). More intense voltage applied led to higher rates of patulin degradation. Constant rate of patulin degradation was doubled when treated from 21 to 25 kV. The dependence of patulin degradation rate constants on voltage was mathematically described by a linear equation (Eq. 4):

Table 3
Constant rate of patulin degradation k (min^{-1}), empirical constant a (min^{-1} kV^{-1}) and constant rate of patulin degradation k_{ref} (min^{-1}) at reference voltage equal to 20 kV for aqueous patulin solutions (100 ppb) after CAP processing for voltages ranged from 19 to 25 kV.

Voltage applied (kV)	Rate constants of patulin degradation k (min^{-1})
19.0	0.390 ± 0.019[a]
21.0	1.013 ± 0.062[b]
23.0	1.463 ± 0.165[c]
25.0	2.011 ± 0.233[d]
a (min^{-1} kV^{-1})	0.266 ± 0.011
k_{ref} (min^{-1})	1.219 ± 0.025

± represents standard error as calculated by linear regression. Different superscript numbers indicate significantly different means ($p < 0.05$) between different voltages applied.

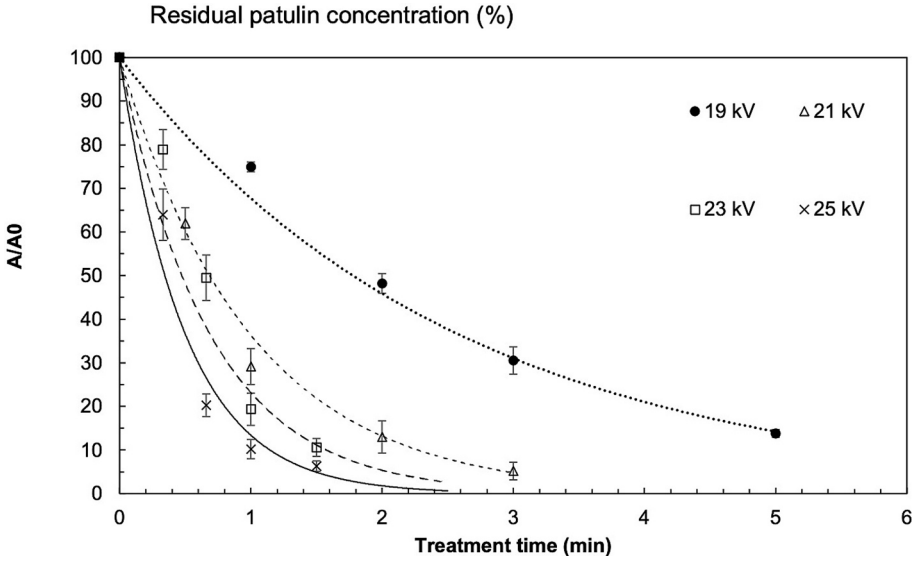

Fig. 2. Residual patulin concentration for aqueous patulin solutions (100 ppb) after CAP processing for voltages ranged from 19 to 25 kV and treatment times from 20 s to 5 min. Dashed lines represent the fit of exponential equation to experimental data. Error bars represent standard deviation from multiple treatments and measurements.

$$k = k_{ref} + a \bullet (V - V_{ref}) \quad (4)$$

where k is the rate constant at applied voltage; k_{ref} is the rate constant at V_{ref} (=21 kV); V is the applied voltage and a is empirical constant. The constant a was calculated to be 0.266 min^{-1} kV^{-1}, and the patulin degradation rate constant for the reference voltage was 1.219 min^{-1}. This model was selected since patulin degradation under CAP treatment followed an exponential decay behavior vs time. Furthermore, the experimental data were very ell fitted to this model justifying its use.

The produced RONS affect either the biomolecules of food, occurring the oxidation of proteins, carbohydrates and lipids, or their various physicochemical properties, such as pH and their antioxidant capacity, due to interactions with their components (Dharini et al., 2023; Domonkos et al., 2021). Several studies focused on the use of CAP for mycotoxins degradation. Ott et al. (2021) observed that the presence of mycotoxins alters the composition of RONS, reducing the production of O_3, NO_3, N_2O_4, as they are probably consumed in degradation reactions, while the production of NO_2 increases and accelerates, perhaps due to the breakdown of nitrogenous compounds of mycotoxins. Wang et al. (2023) showed that RONS produced by plasma are strong oxidizing agents and can degrade mycotoxins. More specifically, the combination of reactive species (ROS and RNS) has a synergistic effect, degrading mycotoxins through reactions such as oxidation, addition, ring cleavage (e.g. patulin) and side chain removal. According to literature, there are several studies where CAP treatment has been used for the elimination of patulin. Shirazi et al. (2025) used CAP treatment (17–23 kV, 2.5–10 min) with dielectric barrier discharge (DBD) on fresh cut apple slice contaminated with patulin. It was observed that after CAP treatment at 23 kV for 2.5 and 8.5 min resulted in complete degradation of patulin for 14 days storage under refrigeration. At 20 kV (10 min), patulin remained below the permissible limits up to the 14th day of storage. Moreover, a low-temperature plasma treatment was performed on aqueous solutions of patulin by Xue et al., 2021. In 30 min of treatment, patulin degradation reached 72 % at 12 kV and 96 % at 18 kV due to the produced $^1O_2$, •OH, $O_2•^-$ and hydrated electrons formed by the plasma. The degradation effect of dielectric barrier discharge (DBD) plasma (50 kV, 1. 5 A for 3 min) on patulin was also investigated by Chen et al. (2022). They observed that the degradation rate of patulin solution was up to 90.92 % after CAP treatment, that might be attributed due to four free radicals produced by plasma with order $e^- > O_2^- > \cdot OH > H_2O_2$. They also concluded that the degradation of patulin was mainly due to the short-lived species that attacked the lactone ring and unsaturated double bonds of patulin. CAP treatment has also been used to achieve degradation of other mycotoxins in foods, such as aflatoxin B1 (Hojnik et al., 2017; Sakudo et al., 2017; Sen et al., 2019; Shi et al., 2017; Siciliano et al., 2016), ochratoxin (Casas-Junco et al., 2019; Wang et al., 2024) and DON (Chen et al., 2022). Surface barrier discharge (SBD) plasma treatment (10–20 W) was applied in a mixture of acetonitrile and deionized water contaminated with aflatoxin B1 and it was achieved >80 % degradation of aflatoxin B1 in 15 s (Hojnik et al., 2017). Other novel technologies have already been applied in several studies to degrade patulin. Liu et al. (2024) studied patulin detoxification using enzymes, such as esterase, peroxidase, lipase A, and lipase M. All enzymes can degrade patulin with a rate up to 97.8 %, producing other species such as desoxypatulinic acid and hydroascladiol with lower toxicity than patulin. The quality and volatile compounds did not alter significantly. Diao, Hou, et al. (2018) used ozone detoxification equipment (7–12 mg/L ozone, 0–30 min) for patulin degradation in apple juice. Ozone application resulted in efficient patulin degradation with no change in quality of juices. The high pressure (HPP) assisted degradation of patulin in model juices was determined by Hao et al. (2016) that they found that degradation rates of patulin after HPP ranged from 0.04 to 0.19 ppb/s.

3.3. Proposed mechanism of the degradation of patulin by CAP treatment

The proposed mechanism is based on the breakdown of the lactone ring and the double bond (Xue et al., 2021). Initially, ozone interacts with the conjugated double bonds of patulin, causing opening of the galactone ring with formation of ascladiol, dehydroascladiol and hydroxypatulin. Following the complete breakdown of the structure, the reaction leads to intermediate products, such as 1,4-dioxan-2-ol and glyoxylic acid. 1,4-dioxan-2-ol is further oxidized, giving other products such as ethers and alcohols (tripropylene glycol monomethyl ether and 1-(2-methoxy-1-methylethoxy)-2-propanol.) Finally, these products undergo further oxidation, leading to the formation of acetic acid, formic acid, carbon dioxide (CO_2) and water (H_2O). The degradation of patulin relies on reactive species produced by plasma, such as O_2,•OH, $O_2^-•$ and hydrated electrons.

CAP-treated solutions were further analyzed by HR-IMS in order to confirm patulin degradation and investigate the degradation mechanism. Since no standard of possible degradation forms was available, and since ionization intensities couldn't be directly compared, a qualitative analysis was performed. Accurate masses and CCS values were collected for patulin and degradation forms. The analysis confirmed a rapid patulin disappearance upon treatment, consistently with the HPLC-UV kinetic data, and the formation of several minor peaks. Among them, ascladiol, hydroascladiol and deoxypatulinic acid were annotated with good accuracy (Table 4). While the formation of such forms is consistent with the literature (Xue et al., 2021; Nicolau-Lapeña et al., 2023 and should be regarded as a confirmation of the CAP detoxification, it should be noted that no linearity was observed in their abundance in the tested samples. This could be ascribed by further interconversion occurring in the solutions and/or the inherent instability of the degradation forms. The theoretical CCS values (CCS_{theo}) were compared to the observed values (CCS_{obs}) with an excellent agreement (< 0.5 %). A very good mass error accuracy and isotope similarity were also observed. Unfortunately, lower MW compounds, likely originated from the backbone breakdown, were not observed in the analysis, due to the *m/z* calibration range of the QT of system. A complete elucidation of patulin degradation pathways would require further studies involving the identification of multiple transient intermediates. The current results provide clear mechanistic insight into the main reactions involved. The identification of the primary degradation products supports the proposed degradation mechanisms under the conditions investigated.

3.4. Degradation of patulin using artificial RONS solutions – Comparison with CAP treated patulin samples

For comparison and understanding purposes, to determine which of the reactive species produced due to plasma had the greatest effect on the degradation of patulin, four different artificial solutions of 100 ppb patulin with pH = 4 were prepared: (a) with 100 ppm peroxide concentration, (b) with 100 ppm nitrate concentration, (c) with both 100 ppm peroxide and nitrate concentrations and (d) heated for 5 min at 100 °C. For all the above samples, the concentration of patulin was determined (Table 5). The artificial solutions of standard concentrations of peroxides and nitrate ions were insufficient to cause patulin degradation in the respective solutions. The artificial solution of both compounds led to a maximum 20 % reduction in patulin. The reactive species produced by CAP interacted synergistically, resulting in new products that promote further patulin degradation. During CAP treatment, numerous additional compounds are generated, triggering reactions capable of breaking down patulin. Therefore, other compounds produced by CAP play a key role in patulin decomposition reactions. Thermal treatment at 100 °C for 5 min had no significant effect on patulin degradation. This indicates that the temperature increase occurring during plasma treatment could not cause a reduction in its concentration.

Table 4
Patulin degradation products after CAP treatment (applied voltage of 25 kV in a 4 min treatment period).

m/z	ID	rt (min)	adducts	formula	mass error (ppm)	CCS_{obs}	CCS_{theo}	Isotope similarity
155.0351	ascladiol	2.52	M-H-	C7H8O4	−0.21	129.4	129.3	98.7
155.0351	deoxypatulin	2.48	M-H-	C7H8O4	−0.32	130.8	130.3	99.1
157.0504	hydroascladiol	2.65	M-H-	C7H10O4	−0.02	129.6	129.8	97.7
153.0193	patulin	2.89	M-H-	C7H6O4	−0.01	123.58	123.58	99.9

Table 5
The concentration of patulin (ppb) in artificial solutions of 100 ppb patulin with pH = 4 (a) with 100 ppm peroxide concentration, (b) with 100 ppm nitrate concentration, (c) with both 100 ppm peroxide and nitrate concentrations and (d) heated for 5 min at 100 °C.

Samples	Patulin concentration (ppb)
Aqueous standard solution of 100 ppb patulin with pH = 4 and H_2O_2 concentration of 100 ppm	100.13 ± 1.25
Aqueous standard solution of 100 ppb patulin with pH = 4 and NO_3^- concentration of 100 ppm	100.16 ± 2.12
Aqueous standard solution of 100 ppb patulin with pH = 4 and H_2O_2 and NO_3^- concentration of 100 ppm	82.37 ± 3.25
Aqueous standard solution of 100 ppb patulin with pH = 4 that thermal treated for 5 min in 100 °C	99.65 ± 1.45

± represent standard deviation from multiple treatments and measurements.

3.5. Effect of CAP on apple juice – Degradation of patulin, evaluation of quality indices and bioactive compounds concentration

The effect of direct use of cold plasma technology on patulin that was inoculated in apple juice was also studied. Apart from patulin degradation in apple juices, selected quality and nutritional indices were also determined. The concentration of patulin in the apple juice samples was significantly reduced after CAP treatment ($p < 0.05$). More intense plasma conditions led to a greater reduction in patulin concentration compared to the untreated ones (Fig. 3). Under the most intense plasma condition (25 kV & 1 min), a reduction of up to 50 % in patulin concentration was observed. In literature, plasma treatment (DBD) was also applied on corn (gas: ambient air with modified atmosphere 65 % O_2 / 30 % CO_2 / 5 % N_2, power: 200 W, frequency: 50 Hz, voltage: 90 kV, treatment time: 1–30 min) and resulted in a reduction of aflatoxins by 62 % after 1 min and 82 % after 10 min of treatment at 40 % humidity (Shi et al., 2017). CAP treatment also led to a reduction of total aflatoxin by >70 % when applied for 12 min with a combination of N_2 and O_2 gases in peeled hazel nuts based on Siciliano et al. (2016). A reduction of OTA by 50 % after 30 min of CAP treatment with DBD plasma (power: 30 W, voltage: 850 kV with He gas) on coffee was also observed by Casas-Junco et al. (2019). Sen et al. (2019) performed CAP treatment on hazelnuts (power: 655 W, frequency: 25 kHz, time: 8.5 min) leading to a degradation of AFB1 by 72 % and of total AFs (AFB1 and AFB2) by 70 %, without a significant effect on their organoleptic characteristics. According to Chen et al. (2022), DBD cold plasma with 50 kV and gas mixture (CO_2, N_2, O_2, air) achieved 83.99 % degradation of DON in 8 min. Based on the data received from the study of Wang et al. (2024) observed that CAP treatment at 30 kV resulted in 62 % and full degradation of OTA after 4- and 10-min CAP processing, respectively, without altering the quality of raisins. They also observed that OTA was converted to non-toxic compounds.

Regarding the quality parameters, no significant differences ($p > 0.05$) were observed between total soluble solids and pH-value of the untreated and plasma-treated apple juices. Plasma treatment did not significantly affect ($p > 0.05$) the color parameters, even under the most intense condition. The untreated apple juice had a color value of 12.59 and a hue angle of 105.4°, corresponding to a yellow color. After plasma treatment, the color and hue angle of the samples ranged from 11.86 to 12.00 and from 108.85 to 110.53, respectively, without showing significant differences from each other. The total color difference (ΔE) compared to the untreated apple juice ranged from 1.08 to 1.72 for the plasma-treated samples, values that are considered negligible (only $\Delta E > 4.0$ is perceptible). Plasma treatment under mild conditions (<21 kV) did not significantly affect ($p > 0.05$) the concentration of phenolic compounds in the apple juice samples. More intense plasma treatment conditions (>23 kV) led to a slight reduction in the concentration of phenolic compounds (<5 % reduction). The DPPH assay indicated that there were no significant differences in the antioxidant capacity between

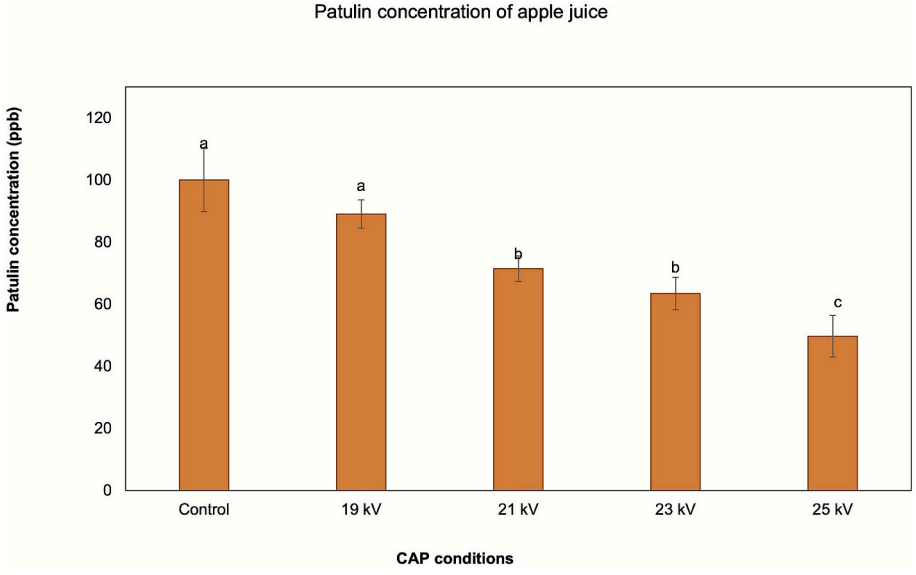

Fig. 3. Residual patulin concentration (%) in untreated and CAP treated apple juices at voltages ranged from 19 to 25 kV. Error bars represent standard deviation from multiple treatments and measurements. Different superscript numbers indicate significantly different means ($p < 0.05$) between different voltages applied.

the untreated apple juice and the CAP-treated samples across all treatment conditions. Regarding the ABTS assay, no significant changes were observed at the low-intensity CAP treatment conditions (19 kV) compared to the untreated one. In contrast, under more intense CAP conditions (>21 kV), an increase of approximately 13 % in antioxidant capacity was detected (from 951 to 1079 mg Trolox equivalent/100 mL apple juice) (Fig. 4). This increase may be associated with structural effects of CAP on plant-based matrices. It has been reported that CAP treatment can induce microstructural modifications, such as the formation of cracks and depressions on the surface of plant tissues, which enhance the release of intracellular compounds and increase extraction efficiency. Additionally, CAP has been shown to increase the surface hydrophilicity of plant matrices by partially degrading the cuticular layer, thereby promoting the diffusion of internal hydrophilic molecules—such as antioxidant compounds—toward the surrounding medium. These effects arise from the action of plasma-generated reactive oxygen and nitrogen species, which can modify surface functional groups (Heydari et al., 2023). Given that the apple juice in this study was treated in its unfiltered form, such mechanisms may explain the slight enhancement in antioxidant capacity observed under high-intensity plasma exposure. Similar observations have been reported in the literature. Giannoglou et al. (2021) showed that CAP treatment (Surface Dielectric Barrier Discharge plasma – SDBD, 6 kV, 42 kHz and 10 min) of strawberries resulted in an increase of approximately 20.9 % in total phenolic content and 16.5 % in antioxidant activity, further supporting the notion that plasma-induced microstructural modifications can enhance the extraction of bioactive compounds. Bao et al. (2020) observed that High-Voltage Atmospheric Cold Plasma (HVACP) treatment (60 kV for 15 min) enhanced the extraction of phenolics and anthocyanins from grape pomace, with significant increases at 5 and 15 min of plasma exposure. Katsaros et al. (2023) showed that nitrogen Dielectric Barrier Discharge – DBD plasma (5–15 W, 6 kHz) increased the TPC of green tea leaves by inducing surface erosion that facilitated solvent penetration, whereas air plasma led to phenolic degradation due to oxygen radicals. Generally, CAP treatment selectively targets reactive mycotoxins while leaving major juice quality components, such as sugars, organic acids, and pigments, largely unaffected due to their lower reactivity. The mild treatment conditions applied in this study were not sufficient to induce significant degradation of these quality-related compounds. This selective reactivity explains why CAP can effectively reduce mycotoxin levels without compromising the overall quality of apple juice.

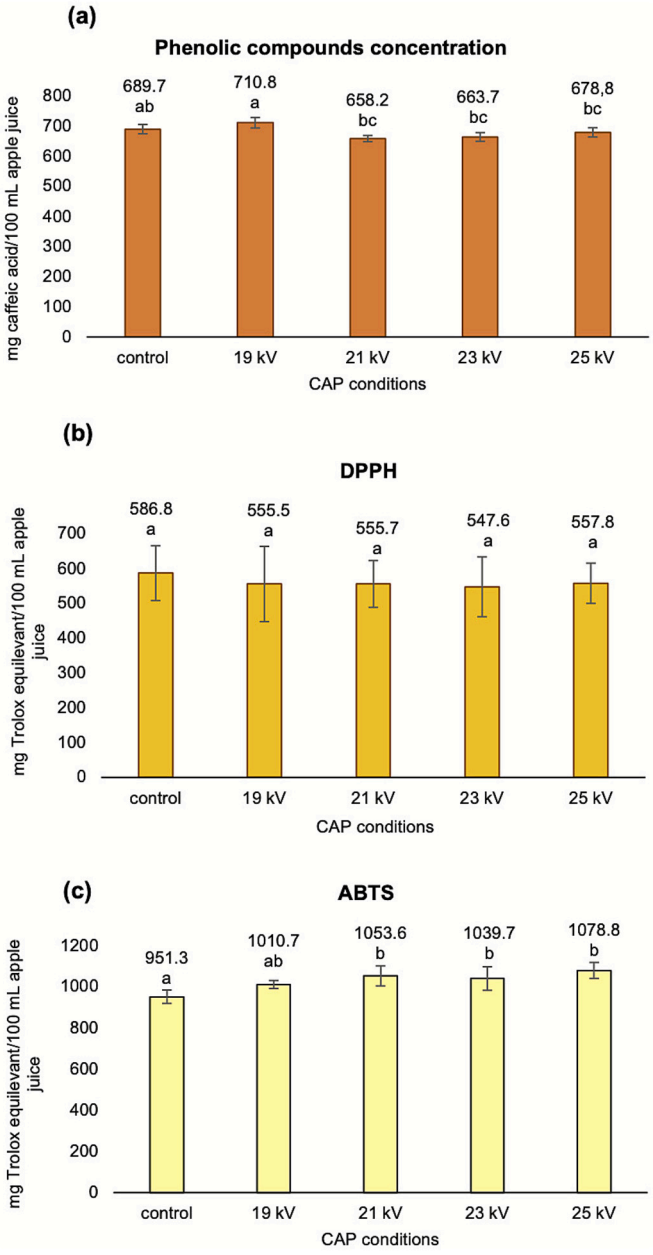

Fig. 4. (a) Total phenolic compounds concentration (mg caffeic acid/100 mL apple juice), (b) antioxidant activity based on DPPH method (mg Trolox equivalent/100 mL apple juice) and (c) antioxidant activity based on ABTS method (mg Trolox equivalent/100 mL apple juice) in untreated and CAP treated apple juices at voltages ranged from 19 to 25 kV. Error bars represent standard deviation from multiple treatments and measurements. Different superscript numbers indicate significantly different means ($p < 0.05$) between different voltages applied.

4. Conclusions

The study demonstrated that cold plasma technology can degrade patulin in apple juice even under mild conditions, making it a promising innovative method for mycotoxin removal. Regulatory limits in fruit juices (50 μg kg^{-1}) were achieved after CAP treatment either on aqueous standard solutions or apple juices that were inoculated with patulin. Meanwhile, the apple juice's quality attributes, such as color, brix, pH, antioxidant capacity, and phenolic content, remain almost unchanged. The reactive oxygen and nitrogen species, H_2O_2 and NO_3^-, generated by the plasma emerged as critical, as they act synergistically in the degradation of patulin. Analysis of patulin degradation products in aqueous standard solutions and apple juice led to other molecules with probably less toxicity, confirmed the action of CAP, while further research is required to understand the mechanisms governing the process and evaluate the toxicity of the resulting by-products. Future applications include the use of cold plasma on whole fruits before processing them into juices is proposed, as well as evaluating its effectiveness in degrading other mycotoxins in different foods. CAP technology is emerging as a promising and environmentally friendly strategy for improving food safety, providing new perspectives for the food industry and the scientific community.

CRediT authorship contribution statement

Varvara Andreou: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Aikaterini Lamprou:** Writing – original draft, Validation, Software, Data curation, Conceptualization. **Pantelis Natskoulis:** Writing – review & editing, Supervision, Resources, Methodology, Formal analysis. **Chiara Dall'Asta:** Software, Formal analysis. **Panagiotis Dimitrakellis:** Writing – review & editing, Investigation. **Vasilis Valdramidis:** Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis, Conceptualization. **George Katsaros:** Writing – review & editing,

Visualization, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Funding

The present work was supported by FunShield4Med project financed from the European Union's Horizon Europe Research and Innovation Programme (Grant Agreement 101079173).

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.

Acknowledgements

No acknowledgements.

Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

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