

Article

Effects of Atmospheric-Pressure Cold Plasma Treatment on Deoxynivalenol Degradation, Quality Parameters, and Germination of Barley Grains

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Featured Application: Deoxynivalenol (DON), a mycotoxin, that is found predominantly in grains. Each year approximately 25% of agricultural commodities are contaminated by mycotoxins; causing financial losses to the agricultural industry and imposing harmful effects to human and livestock health. Currently, there is no effective solution for an adequate reduction of DON on grains such as barley. This study presented the efficacy of atmospheric cold plasma treatment to reduce DON levels on barley grains and provided an insight into the quality changes of barley grains after atmospheric cold plasma treatment.

Abstract: Deoxynivalenol (DON) is one of the major trichothecene mycotoxins commonly found in grains, in particular barley. This study focused on the reduction of DON concentration on barley samples using atmospheric cold plasma (ACP) treatment. The effects of moisture content, post-treatment storage, and relative humidity of air on DON degradation on barley were evaluated. Additionally, the germination and the quality parameters of barley, including protein content, β -glucan, and moisture content, were evaluated. The results showed that ACP treatment for 6 and 10 min reduced DON concentration by 48.9% and 54.4%, respectively. No significant differences were observed in the DON degradation levels by increasing the moisture content of barley from 9.5 to 15.7 g water/100 g sample and relative humidity of air from 12 to 60%. Steeping of barley grains without subsequent drying prior to ACP treatment significantly increased the degradation rate of DON by ACP due to the presence of water on the grain surface. No significant differences were observed for the tested quality parameters of barley in comparison with control samples. This study shows that ACP may offer an effective DON reduction in barley without affecting the quality attributes. However, ACP treatment parameters should be optimized to achieve a better DON reduction efficacy.

Keywords: deoxynivalenol; mycotoxins; atmospheric cold plasma; degradation; quality; grain; barley

1. Introduction

Mycotoxins are poisonous compounds produced as secondary metabolites by some species of fungi, including *Fusarium*, *Aspergillus*, *Penicillium*, and *Alternaria* [1,2]. Trichothecenes are a large group of mycotoxins sharing 9, 10 double bond and the 12, 13 epoxide group in their structure [3]. Deoxynivalenol (DON), mainly produced by *Fusarium graminearum* and *Fusarium culmorum*, is one of the major trichothecenes that contaminates cereals such as barley [4]. DON can cause vomiting, anorexia, growth retardation, immune suppression, inflammation and the necrosis of various tissues,

and diarrhea in animals [5,6]. Barley is one of the major grains that is consumed as animal feed, used to produce cereal-based products and is widely used in brewing industries. DON in grains, especially in barley, reduces grain quality and financially impacts agricultural organizations. The economic loss associated with DON was estimated at \$655 million/year in the United States from 1993 to 1996 [7].

Many strategies have been suggested for suppressing fungal growth and reducing mycotoxin formation, including pre-harvest methods (crop rotation, sowing date, tillage, use of fungicide), physical methods (i.e., dehulling, sorting, sieving, floatation, washing, steeping, milling, ultraviolet, gamma treatments, and thermal treatments), chemical methods (citric acid, acetic acid, lactic acid, NH_3 , $\text{Ca}(\text{OH})_2$, Na_2CO_3 , oxidizing agents, and reducing agents), enzymatic methods (amylases, glucanases, proteases), biological methods (fermentation, genetic engineering in plant genes for detoxification), and microbial decontamination method (prevention of ingestion by microorganism of gastrointestinal tract) [1]. Despite the progress achieved by using these methods, they have several disadvantages, including low efficacy, requirement of expensive chemicals and sophisticated equipment requirement. Furthermore, some of these methods may leave chemical residues that might cause a detrimental effect on the treated product, which may present health hazards to consumers. As a result, some of these methods are usually considered as impractical, costly, not completely effective, and time-consuming, especially for the large-scale treatment of food or feed products [1,8]. Hence, there is a need to find an effective method to control DON contamination in food and feed products.

Atmospheric cold plasma (ACP) technology is a novel technology with the potential to reduce fungi and mycotoxins' concentration [9]. The term plasma refers to an ionized state of a gas produced by increasing the internal energy of the gas. The ionization process leads to the formation of reactive species, radicals and UV light [9,10]. Reactive oxygen species (ROS) such as ozone (O_3), singlet oxygen ($^1\text{O}_2$), superoxide (O_2^-), peroxide (O_2^{-2} or H_2O_2), hydroxyl radicals (OH), and reactive nitrogen species (RNS) such as excited molecules of N_2 and nitric oxide radical (NO) are the main effective species in ACP [11]. The degradation of mycotoxins by ACP could be related to their interaction with the reactive species, UV photons, and electrons. Mycotoxin degradation efficacy of ACP is greater than that of ozone or UV treatments, due to the possible contribution of reactive species, electron, and UV photon in the ACP treatment [1]. The low temperature of ACP is an important aspect in preserving quality while improving the safety of the product. ACP may also improve the germination rate of grains and length of seedlings [12]. Previous research reported the effectiveness of ACP to reduce mycotoxins, including DON, aflatoxin and T-2 toxin [1,13,14]. However, limited information is available on the efficacy of ACP to reduce DON on barley grains. Additionally, knowledge on the effect of ACP treatment on grain quality parameters and the germination capacity of barley is important for ACP treatment optimization.

This study aimed to evaluate the effectiveness of ACP treatment on the degradation level of DON. This study also assessed the effect of ACP on barley germination and the important quality parameters of barley grains, including changes in protein and β -glucan contents and water loss.

2. Material and Methods

2.1. Barley Grains

Raw barley grains were provided by Canada Malting Co., Alberta, Canada. Barley grains were vacuum-sealed in plastic bags and transported to the laboratory at the University of Alberta and stored at 4 °C until use. The initial wet basis moisture content (MC), water activity (a_w), density, and grain dimensions of barley grains were determined (Table 1).

Table 1. Physicochemical specifications of barley grains.

Crop Year	MC (g Water/100 g Sample)	a_w	Grain Density (g/cm ³)	Grain Dimensions	
				Length (mm)	Width (mm)
2015	10.8 ± 1.7	0.450 ± 0.003	1.19 ± 0.09	9.32 ± 0.97	3.88 ± 0.02

Moisture content is a measurement of the total amount of water contained in the sample. Water activity provides information about the energy status of water in foods and indicates the available water for the growth of microorganisms and reactions in foods. Water activity is calculated as the ratio of the vapor pressure of water in a food sample to the vapor pressure of pure water. The MC of the barley grains was determined by the AACC Method (44–19.01, AACC International). The water activity (a_w) was determined using the a_w meter (4TE, patent number 5816704, Aqualab, Pullman, WA, USA). Grain density was determined by dividing the grain mass by the volume. The volume was measured by the liquid displacement method. Two grams of barley grain was added to a 15 mL falcon tube containing water. The rise in the level of the water in the tube was used to determine the volume [15].

2.2. Mycotoxin Standards

HPLC grade acetonitrile (ACN) and methanol (Fisher Chemical, Geel, Belgium), and standard solutions of deoxynivalenol (1 mg/mL acetonitrile Sigma Aldrich, Mississauga, ON, Canada) were used. ACN was used to dilute deoxynivalenol and produce 50 µg/mL DON solution.

2.3. Sample Preparation

Barley grains (11–12 grains, 0.495–0.505 g) were individually spiked using 200 µL of DON (50 µg/mL) in a plastic cup with a diameter of 3.89 cm and height of 1.14 cm. The samples were then dried at room temperature (~23 °C) for 10 min, to ensure that the ACN solution was evaporated completely.

2.4. ACP Treatment

A dielectric barrier discharge (DBD) atmospheric cold plasma (ACP) system (PG 100-3D, Advanced Plasma Solutions, Malvern, USA) was used to treat the barley grains in an acrylic chamber (Figure 1). The airtight chamber (8 × 8 × 10 cm inner dimensions) had inlets and outlets for the transmission of humid air. Quartz glass on the side of the chamber was used to perform emission spectroscopy measurements. The upper electrode of the DBD unit was connected to an alternating high voltage (0–34 kV) plasma generator with 1 A current and approximately 300 W power. The high voltage electrode was made of solid copper (2.5 cm diameter) covered by a dielectric (1 cm thick) and with 1 mm thickness of quartz disk at the bottom. The mycotoxin spiked barley grains (11–12 grains) inside the plastic cups were placed on the top of the ground electrode. The ground electrode was covered with a glass slide of 1 mm thickness. The gap between the electrodes was 5 mm and the gap between the surface of the barley grains and the high voltage electrode was ~2 mm. The selected ACP treatment times were 0, 2, 4, 6, 8, and 10 min. The standard output frequency of DBD system was 3500 Hz, duty cycle was 70%, and the output pulse width was 10 µs.

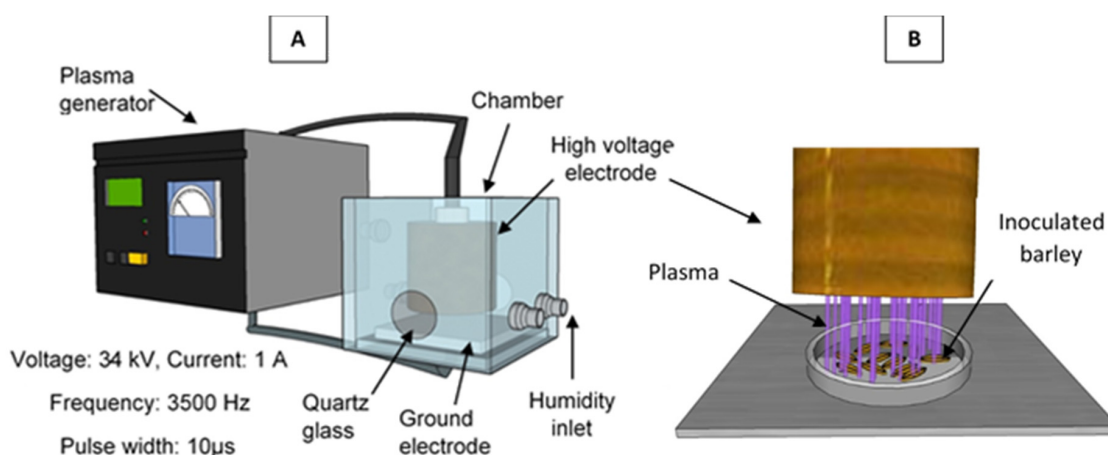


Figure 1. (A): Schematic diagram of the experimental setup used for dielectric barrier discharge (DBD) atmospheric cold plasma (ACP) treatment. (B): Close-up diagram presenting the treatment of barley grains inside the ACP chamber.

2.5. Extraction and Quantification of DON

DON extraction was based on the method described by Lim et al. [16] with modification. Treated barley grains (0.5 g) were transferred to 8 mL glass culture tubes and then 2 mL ACN-water in a proportion of 84:16 was added. The mixture was vortexed at 3000 rpm for 10 min. After thorough mixing of the samples with extraction solvent, 1 mL of the supernatant of the extract was passed through a 0.2-µm PTFE syringe filter, and transferred into glass culture tubes and dried under a constant flow of N₂ gas. The residue was redissolved in 1 mL water, with acetonitrile (85:15 v/v) used as the mobile phase, vortexed for 1 min, and injected (25 µL) into the HPLC for analysis.

The concentration of mycotoxin was determined by HPLC (Shimadzu Scientific Instruments, Inc. MD, USA). The chromatography was performed on a reverse phased Agilent Zorbax SB-C18 250 mm × 3 mm, 5 µm column in isocratic elution. The injection volume was 25 µL at the flow rate of 0.5 mL/min, using water and ACN (85:15 v/v) as the mobile phase. DON was determined at the wavelength of 218 nm, using a photodiode array (PDA) detector. The limit of HPLC detection of DON was 0.1 µg/mL. The recovery of the whole procedure was 76.6% and the precision was within +/− 5%.

2.6. Cold Plasma Diagnostics

The optical emission spectrum of ACP discharge in the treatment chamber was acquired using a spectrophotometer (Black comet C-25, StellarNet Inc., Tampa, FL, USA), coupled to an optical fiber with a resolution of 0.5 nm, signal to noise ratio of 1000:1, in the wavelength range of 180 to 850 nm. The light emitted by excited plasma species between the electrodes was captured using optical fiber (F600-UVVIS-SR, StellarNet, Inc., Tampa, FL, USA). One end of optical fiber was connected to the spectrophotometer and the other end with the collimating lens kept 25 mm away from the side quartz window incorporated to the center of the sidewall of the plasma chamber. The set integration time was 550 ms and the set values of the number of scans to average was 3 to obtain uniform spectra using SpectraWiz software (StellarNet Inc., Tampa, FL, USA). The ACP system was operated for 1 min, then the spectra were acquired.

2.7. Measurements of Ozone, Nitrous Gas, and Hydrogen Peroxide Concentration

Concentrations of ozone (O₃), nitrous gas, and hydrogen peroxide (H₂O₂) inside the treatment chamber were measured at 60–80, 360–380, and 600–620 s during ACP treatment using Dräger short-term detector tubes (Dräger Safety AG & CO, Lubeck, Germany). Ozone 10/a Dräger tube (CH 21001), a nitrous fume 50/a Dräger tube (81 01 921) and a hydrogen peroxide 0.1/a Dräger tube (81 01041) were used to measure the gas concentration by Dräger accuro gas detector pump, following

the manufacturer's instructions. Smaller volumes of gas were collected to ensure that ozone, and hydrogen peroxide content falls within the measurement range of Dräger detector tubes i.e., 20 mL for ozone, and 20 mL for nitrous gases and hydrogen peroxide were collected into the tubes.

2.8. Effect of Moisture Content of Barley Grains and Environmental RH

The effects of wet basis moisture content (MC) of barley and relative humidity (RH) of air inside the chamber on DON degradation efficacy of ACP were determined. Barley grains were kept for 10 h and 17 h in a controlled humidity chamber (BTL-433, ESPEC North America Inc., Hudsonville, MI, USA), with 95% relative humidity (RH) at 25 °C, to increase the MC of barley from 9.5 g water/100 g sample to 15 and 16 g water/100 g sample, respectively. Then, DON was inoculated on the grains and air-dried for 10 min to allow the evaporation of ACN from inoculum and then treated by ACP. To understand the effect of environmental RH, a humidifier was connected to the chamber inside it, to increase the RH before ACP treatments.

2.9. Post-Treatment Storage of Barley Grains on DON Degradation by ACP

Some of the reactive species in ACP have a long shelf life and are able to react with the sample for extended times. We stored the 6 min ACP-treated barley grains for 10 min inside the air-tight plasma chamber and also for 24 h inside sealed plastic cups outside the chamber at room temperature, to evaluate the effect of storage on further degradation of DON.

2.10. Effect of Steeping of Barley Grains on DON Degradation by ACP

Steeping is one of the crucial steps in producing malt from barley. The amount of moisture content of substrates is an important factor determining the efficacy of the ACP process. The effect of steeping on DON degradation by ACP was assessed to determine if in this process step we could apply ACP, which is relevant to the brewing industry. For this purpose, the barley grains were steeped for 21 h before ACP treatment. The moisture content of the grains was increased from 9.5 to 43.9 (g water/100 g sample) after steeping. Previous reports remarked on the importance of the presence of water in increasing the antimicrobial efficacy of ACP treatment [17–19]. Moreover, to assess the effect of water on the surface of the steeped grain on DON degradation by ACP, two approaches were used. In the first approach, pure ACN was substituted with ACN/water with a ratio of 20:80 (v/v) to prepare the DON inoculum on steeped grain. In the second approach, samples with or without a drying step after inoculation were used for the ACP treatment of steeped grains. The drying step of inoculum was skipped to see the effect of water on DON degradation.

2.11. Quality Parameters of Barley Grains after ACP

To assess the effect of ACP on quality parameters of barley, the moisture, protein, and β -glucan content were measured after ACP treatment of barley grains for a defined period of time. Moisture, protein, and β -glucan content are the important quality indicators of barley in the brewing industry. Protein content was determined by combustion nitrogen analysis [20]. Protein content was calculated by multiplying the nitrogen content (% N) by 6.25 as the conversion factor. β -glucan content was determined using the megazyme mixed-linkage β -glucan assay procedure [21]. The moisture content of the barley sample was determined by the oven method [22].

2.12. Germination of Barley Grains after ACP

For assessing the effect of ACP on germination of barley grains, ACP treatment was applied to seeds for 0, 1, 6, 10 min. Each treatment contained three batches of grains, where ACP treatment was applied independently to each batch. Then, the individual grains were transferred to germination pouches (17.8 × 16.5 cm, Mega International, Minneapolis, MN, United States) on light shelves containing 100 mL of deionized water. Five growth pouches, each containing two grains, were used for each

treatment ($n = 10$) and every one of the batch. Altogether, there were 30 plants per each ACP treatment. Barley plants were grown at room temperature with supplemental lighting ($200 \mu\text{mol m}^{-2}\text{s}^{-1}$ at the top of the growth pouch, SunBlaster T5HO, 54W, 0900304), maintaining a photoperiod of 16 h/8 h light/dark cycles. After 1 week of germination, the shoot length and number of roots were measured manually. The roots were scanned using an optical scanner STD 4800 and analyzed for root length, surface area, average diameter, and volume, using WinRHIZO software (Regent Instruments Inc., Quebec City, QC, Canada).

2.13. Statistical Analysis

All the experiments were performed in triplicate. Values from all experiments were expressed as the mean $\pm$ standard deviation (SD). Significant differences ($p < 0.05$) were determined using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (IBM SPSS v.21, Armonk, NY, USA).

3. Results and Discussion

3.1. Effect of ACP Treatment on Deoxynivalenol Degradation on Barley Grains

A significant ($p < 0.05$) decline in the DON concentrations on barley grains by ACP treatment was generally observed (Figure 2).

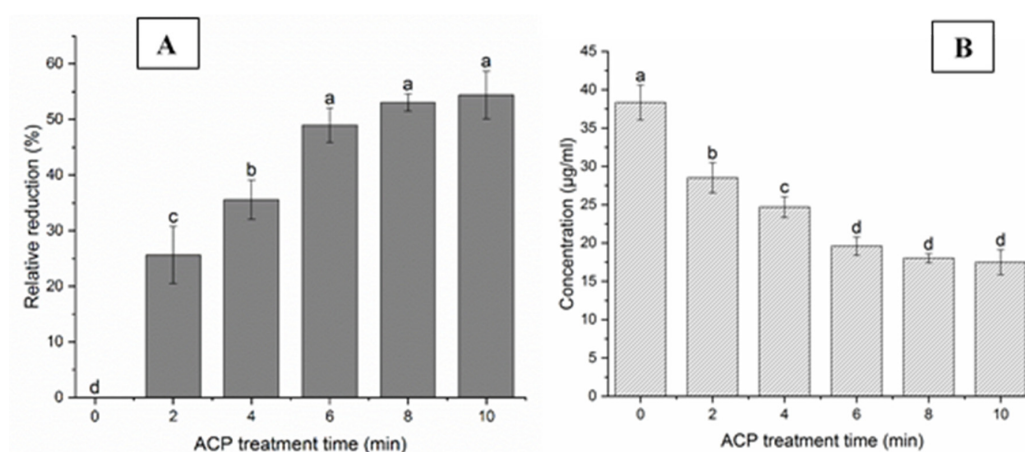


Figure 2. Effect of ACP treatment on (A) relative deoxynivalenol reduction % and (B) deoxynivalenol concentration ($\mu\text{g/mL}$) on barley. Data are shown as least square means $\pm$ standard deviations. Values with different letters in each figure are significantly different ($p < 0.05$, $n = 3$).

A significant decrease in DON concentration within 6 min ACP treatment was observed, but thereafter, the reduction rate was not significant ($p \geq 0.05$). The degradation rates after 6 and 10 min ACP treatments were 48.9% and 54.4%, respectively. The generated ozone in plasma probably had a significant effect on DON degradation [23,24]. In general, for a long treatment time, with the production of more reactive species, an increase in the DON degradation rate should be observed. However, during extended treatment times, the generated ozone can be depleted by other reactive species by the following destruction cycles (Equations (1)–(9)) [25,26].





where the reactive species $HO_2\cdot$, $\cdot OH$, $\cdot H$, $\cdot O$, $\cdot N$, $NO\cdot$, $NO_2\cdot$ are hydroperoxyl radical, hydroxyl radical, atomic hydrogen radical, atomic oxygen radical, atomic nitrogen radical, nitrogen oxide, and nitrogen dioxide radical, respectively. The constant concentration of ozone in the environment may be attributed to the simultaneous ozone generation and depletion, due to its reactions with other reactive species in longer treatment times. This could be the possible reason for small increases in degradation rates of DON after 6 min ACP treatment. The constant concentration of ozone in longer treatment times was observed in previous studies [27,28]. Zhuang et al. [27] reported no further increase in ozone concentration after 180 s of in-package cold plasma treatment, attributed to the quenching of ozone by reactions with N_2 , water, and/or new reactive species formed in plasma during long treatment times. It is worth mentioning that the ozone generation and depletion reactions are complicated and are dependent on various factors, i.e., not only treatment time, but also temperature, gas composition, voltage, etc. [26].

Chen et al. [29] reported 22.5 and 34.6% degradation in DON level on raw and germinating barley, respectively, by using plasma-activated water (PAW), treated for 5 min. They did not observe a significant ($p \geq 0.05$) decline in DON concentration in the treated samples between 5 and 20 min using PAW. In our recent study on pure DON on a cover-glass, formation of C = O group and epoxy ring in the ACP treated sample was observed, which could be due to the reaction between ROS with DON [30]. We achieved a degradation level of 39.8% in pure DON after 15 min ACP on cover-glass, in comparison to the 48.9% degradation of DON, inoculated on barley after ACP treatment for 6 min. This highlights the importance of the type and nature of the substrate on DON degradation.

Abramson et al. [31] applied 80 °C heat to DON on barley, and the concentration was reduced from 18.4 to 14.7 µg/g DON barley, 1 day after the thermal treatment. DON concentration was reduced by 58%, 5 days after the treatment. Wang et al. [26] reported the use of ozone treatment to wheat samples, to reduce DON concentration. Treatment with 75 mg/L ozone for 90 min reduced DON concentration by 53.5% on wheat kernels. Santos Alexandre et al. [24] reduced DON concentration by 32% after 240 min ozone treatment. It is assumed that a combination of different degrading mechanisms such as chemical reaction with reactive species generated in ACP such as O_3 , O , OH , NO_x , decomposition after collision with electrons and ions [14], and UV light are responsible for cleavage of toxin molecule by ACP treatment. This combination could be the reason for obtaining a greater degradation rate by ACP in comparison to ozone treatment. In the current study, 54.4% reduction was achieved in 10 min of ACP treatment, which could be further increased by modifying the process factors in ACP system i.e., voltage and frequency, type of gas medium, RH of the air, hurdle treatment, etc. However, further studies are necessary to evaluate the toxicity of the intermediate and final products formed during ACP processing of DON.

In our unpublished previous study, thermal treatment at 80 °C for 25 min had no significant effect on DON degradation [30]. As the temperature in our cold plasma system was less than 40 °C [32], the increase in temperature during plasma treatment did not have any effect on DON degradation.

3.2. Cold Plasma Diagnostics

Optical emission spectroscopy (OES) is widely used for plasma diagnostics. OES enables an understanding of the degradation mechanisms and helps to optimize the decontamination process. Excited states are produced mostly by collisions with energetic electrons, which can be measured by OES. Dominant plasma reactive species were detected by OES in the UV/VIS wavelength (180 to

850 nm) region (Figure 3). It was evident that the emission was in the near UV region (300–400 nm), that can explain the purple color of the ACP photons. In the spectrum, N₂ spectra were dominant, as the peaks in the wavelength region of 310–450 nm are the vibrational band of neutral and ionic emission of nitrogen molecule [32]. The peak in 296 nm corresponds to OH as one of the reactive species [33]. Some of the reactive oxygen species such as atomic O could not be identified, probably due to the particle collisions with N₂ and O₂, resulting in the quenching of O (³P) and O (⁵P) energy [34] and production of ozone and nitrogen oxide species. The results demonstrate the fact that the major reactive species in ACP were ROS and RNS.

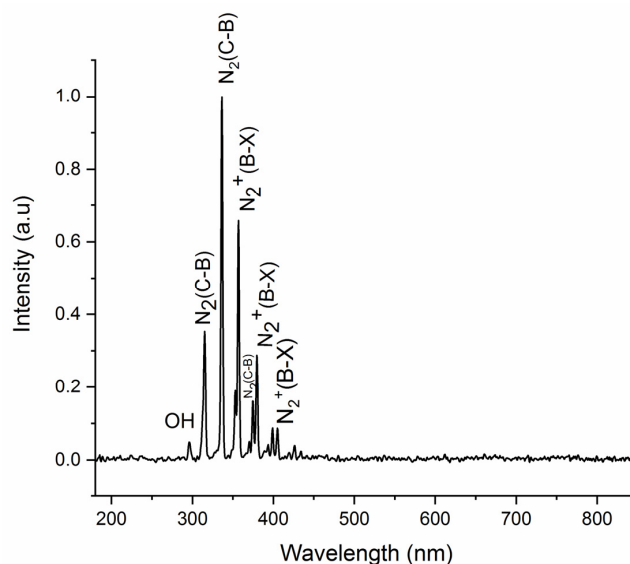


Figure 3. Optical emission spectra of DBD ACP operating in air at 34 kV, 3.5 kHz, 5 mm gap.

3.3. Ozone, Nitrous Gas, and Hydrogen Peroxide Concentration in DBD ACP

Concentrations of ozone, nitrous gases and hydrogen peroxide as the major reactive species in ACP were measured inside the plasma chamber in different time scales (Table 2).

Table 2. Ozone, nitrous oxides and hydrogen peroxide concentration during ACP treatment.

ACP Treatment Time(s)	O ₃ (ppm)	H ₂ O ₂ (ppm)	NO _x (ppm)
60–80	600	100	400
360–380	675	150	470
600–620	675	200	480

In DBD ACP, the electromagnetic field splits the O–O bond in oxygen molecule and the resulting oxygen free radical reacts with another oxygen molecule to form ozone [35]. Hydrogen peroxide is derived from the reaction of plasma radicals ([•]OH, O₂^{•−}), with water molecules (H₂O) and molecular oxygen (O₂) [36]. With increase in ACP treatment time, the concentrations of all the reactive species would be increased. Ozone was the most prevalent reactive species with 600 ppm concentration after 1 min ACP. The concentrations of nitrous gases and H₂O₂ were increased in 6 and 10 min ACP treatments, in comparison to 1 min ACP. H₂O₂ had the lowest concentration compared to ozone and nitrous gases. The concentration of H₂O₂ was doubled and reached 200 ppm after 10 min, compared to that after 1 min ACP treatment. This could be due to the generation of H₂O₂ by a number of reactions involving several reactive species, during plasma treatment. In the presence of water vapor present in the environment, H₂O₂ could be generated by several reactions (Equations (10)–(14)) [37,38].





It is believed that reactions (12) and (13) could be the major reactions involving hydrogen peroxide formation [38]. Hydrogen peroxide could be primarily formed by recombination of hydroperoxyl radicals (Equation (13)) at high oxygen concentrations and low local OH radical concentration [38]. The generation of hydroperoxyl radicals from ozone depletion (Equation (2)) may also have indirectly contributed to H_2O_2 generation. The concentration of ozone was the same after 6 and 10 min treatments. Degradation rates of DON after 6 and 10 min ACP were not significantly different ($p \geq 0.05$). As discussed in Section 3.1, the ozone species possibly played a major role in the degradation of DON and hence, at the same concentration of ozone; after 6 and 10 min ACP treatments, the degradation rates of DON were similar. In previous studies, ozone was identified as the primary contributor for the degradation of mycotoxins [1,23,39]. Between 6 and 10 min ACP treatments, the concentration of ozone stayed the same, possibly due to the interaction of ozone with other reactive species present in ACP, to produce new compounds such as NO_2 and $HO_2\cdot$ [38]. Further studies are required to achieve a better insight regarding the role of each of the reactive species in the degradation of each mycotoxin and their possible synergistic effects to degrade mycotoxins.

3.4. Effect of MC of Barley Grains and Environmental RH on DON Degradation by ACP

Environmental relative humidity and the moisture content of the substrate are important factors which can influence the decontamination by ACP [8]. The effects of RH of the air and MC of barley grains on the degradation of mycotoxin were evaluated (Table 3). Increasing the MC of the barley grains from 9.5 to 15.76 g water/100 g sample reduced the degradation level from 54.4% to 47.0% by ACP; however, this reduction was not significant ($p \geq 0.05$). Generally, the presence of water in the system increases the production of ROS [19], which can contribute to a greater degradation rate. However, due to the drying step after the inoculation of DON, the water contents of the grains were similar to each other before ACP treatment. Although, the reduction in DON degradation rate by increasing the water content was not significant ($p \geq 0.05$), the possible changes in the surface of the grains due to the presence of higher water content could change the reaction rate of the reactive species with the toxin and affect the degradation rate. To know the optimum moisture content to achieve highest DON degradation, barley with different water contents should be treated with ACP to draw a conclusion.

Table 3. Effect of moisture content (MC) of barley and relative humidity (RH) of the surrounding air on deoxynivalenol (DON) degradation by ACP.

Plasma Time (min)	MC (g Water/100 g Sample)	DON Reduction (%)
0	9.5 ± 0.0	0 ^b
6	9.5 ± 0.3	54.4 ± 2.2 ^a
6	14.9 ± 0.3	49.8 ± 5.9 ^a
6	15.7 ± 0.2	47.0 ± 5.9 ^a
RH (%)		
0	12	0 ^b
6	12	62.9 ± 3.3 ^a
6	60	65.7 ± 3.0 ^a

Values with different letters in the same column are significantly different ($p < 0.05$, $n = 3$).

The effect of environmental RH on DON degradation by ACP was assessed. Increasing the RH from 12% to 60% increased the degradation rate of DON, but it was not significant ($p \geq 0.05$). A greater RH could be attributed to an increase in the concentration of OH radicals due to the presence of water molecules and lead to greater oxidation [40]. On the other hand, high RH could reduce the surface resistance of the dielectric material, so it would reduce the number of micro-discharges, [41]. A high RH may weaken the plasma due to the loss of the electron energy in electron-molecule collisions. Poorer transmissibility of UV radiation, and protective water film around the mycotoxin, could be the other reasons for reduced ACP efficiency [19]. It is recommended to assess the degradation of DON at different percentages of RH and determine an optimum RH under ACP to achieve the highest decontamination rate.

3.5. Effect of Post-Treatment Storage of Barley Grains on DON Degradation by ACP

Storage after ACP treatment affected the degradation of DON on barley (Table 4). An 8.1% reduction in DON was observed when the inoculated barley grains were stored for 24 h without ACP treatment. This could be due to the natural degradation of DON by oxidation, in contact with the air. DON degradation after 6 min ACP was 45%; when the 6 min ACP-treated DON was stored for 24 h at room temperature, the degradation rate was further increased to 53.6%. This increase in degradation was likely due to the degradation of DON during storage without much contribution from ACP. Degradation of DON increased when we stored the 6 min ACP treated grains for 10 min inside the air-tight treatment chamber after treatment, but the result was not significantly different ($p \geq 0.05$) from the reduction in DON after 6 min ACP treatment followed by 24 h storage. Some of the reactive species, such as ozone in ACP, have longer half-lives and they could have extended interactions with the substrate even when the ACP system was not functioning; however, this should be justified by comparing the result with 6 min ACP followed by 10 min storage outside the treatment chamber at room temperature. No previous study has reported the effect of post-treatment storage on mycotoxins stability. The effect of post-treatment storage on the inactivation of bacteria was evaluated by Yadav et al. [33], and Klockow and Keener [42], and the similar observation of higher reductions in the cell count of bacteria in post-treatment storage was observed.

Table 4. Effect of post-treatment storage on DON degradation on barley by ACP.

Treatment	DON Degradation Rate (%)
No treatment, no storage	0 ^d
0 min ACP, 24 h storage	8.1 ± 2.7 ^c
6 min ACP, no storage	45.0 ± 0.9 ^b
6 min ACP, 24 h storage	53.6 ± 1.8 ^a
6 min ACP, 10 min storage inside treatment chamber	52.0 ± 1.6 ^a

Data are shown as least square means ± standard deviations. Values with different letters are significantly different ($p < 0.05$, $n = 3$).

3.6. Effect of Steeping the Barley Grains on DON Degradation by ACP

One of the major applications of barley is in the brewing industry. Steeping is one of the crucial steps to produce malt for the beer preparation. If barley is contaminated with DON, it can be present in the final product [43]. On the other hand, the presence of moisture is a determining factor for the ACP treatment efficacy. Increasing the moisture content of the substrate was anticipated to increase the generation of ROS (e.g., hydroxyl radicals) [17–19], which could then increase the DON degradation rate. An increase in hydroxyl radicals could further increase the generation of ozone, as ozonation is catalyzed by free radicals such as hydroxyl ion [43]. The uptake of ozone by the product would then increase at higher moisture contents, since ozone is water-soluble [44]. This could increase the contact between ozone and DON and consequently improve its degradation rate. However, the degradation of DON by 6 min ACP treatment on steeped and dried barley grains was 36.3%

(Sample B) (Table 5). This DON degradation in steeped barley grains was lower compared to that in barley grains without steeping (48.9%), as reported in Section 3.1 (Figure 2). During the steeping process, water will be absorbed and the grains will swell, which can influence the surface properties and topography of the grain surfaces. The topology of the substrate surface can influence the efficiency of DON degradation by ACP treatment. The fissures and grooves on the rough surfaces of the grains could protect DON from plasma generated species [19]. The effect of the surface characteristics of the substrate was determined in previous studies for the inactivation of bacteria by ACP [45–47]. The greater inactivation of bacteria via ACP treatment was observed on smooth surfaces. Moreover, ACP treatment could modify surface topology and change surface properties. Park et al. [12] observed changes on the surface of plasma-treated barley grains compared to the untreated samples. The changes on the surface of the grains after ACP treatment could be due to the chemical reactions of the reactive species on surfaces or energy exposure by ACP.

Table 5. Effect of solvent type and treatment conditions (drying time and ACP treatment time) on degradation of DON on steeped barley grains by ACP.

Treatment	Solvent (ACN:Water)	Treatment Description	Reduction (%)
A	100:0	10 min drying + 0 min ACP	0 ^e
B	100:0	10 min drying + 6 min ACP	36.3 ± 5.4 ^c
C	100:0	0 min drying + 6 min ACP	66.3 ± 2.3 ^a
D	20:80	10 min drying + 0 min ACP	15.7 ± 4.6 ^d
E	20:80	10 min drying + 6 min ACP	53.6 ± 1.0 ^b
F	20:80	0 min drying + 6 min ACP	65.6 ± 7.5 ^a

All the samples were steeped barley grains. Values with different letters in the same column are significantly different ($p < 0.05$, $n = 3$).

The drying step after mycotoxin inoculation on barley grains before ACP treatment had a significant impact ($p < 0.05$) on the degradation of DON. Since the drying step was used before ACP treatment, the effect of greater moisture on the surface of barley grains due to steeping, on DON degradation during ACP treatment, was negligible. Hence, we believe that the reduction in degradation rate in steeped barley compared to the unsteeped grains could be due to the changes in surface properties of the steeped grains. In 10 min drying of the barley grains inoculated with DON in pure ACN, the solvent was completely evaporated. However, due to the higher boiling point of water compared to ACN, the water in ACN/Water (20:80 v/v) was not evaporated, but the ACN could be evaporated completely within 10 min. This means that the 10 min drying step would have allowed the ACN to evaporate, but some amount of water was still there. Consequently, we observed significantly ($p < 0.05$) higher degradation rates of DON in treatment E compared to B, due to the presence of water. Comparison of the treatment C (with 100% ACN, 0% water) with treatment F (20 % ACN, 80% water) suggests the greater impact of ACN compared to water on the degradation of DON by ACP. The considerable effect of ACN on DON degradation under ACP could be observed by comparing treatments B and C. The degradation rate of DON increased from 36.3 to 66.3% by skipping the drying step and hence the presence of ACN. A greater degradation rate of DON in treatment F (no drying step) was observed in comparison to E (10 min drying), since the amounts of water and ACN were higher in treatment F, which contributed to the higher degradation of DON. Overall, the results of this experiment suggest the importance of drying step and hence the presence of water and ACN on the surface of the barley grains to increase the degradation rate by ACP; it seems that water contributes to the formation of greater amounts of ROS.

3.7. Effect of ACP Treatment on Quality Parameters of Barley Grains

Moisture content, protein, and β -glucan content of barley were evaluated after 6 and 10 min ACP treatments (Table 6). ACP treatment could result in drying of the substrate [48]; however, based on the process parameters i.e., voltage, frequency, treatment time, the gap between the electrodes, etc.,

the drying effect could be negligible. ACP generates ionized molecules in a strong electric field. Water molecules orient themselves in the direction of the electric field and the evaporation of water molecules can occur, resulting in drying effect of product by ACP [49]. However, in our study, the moisture content of the grains was slightly reduced after ACP treatment, which was not significant ($p \geq 0.05$). The protein and β -glucan were not affected significantly ($p \geq 0.05$) after ACP treatment. This could be due to the fact that although ACP treatment influenced the surface of the substrate, it could not penetrate the inner parts of the grains, leaving them unaffected.

Table 6. Effect of ACP treatment on quality parameters of barley grains.

Treatment	N ₂ (%)	Protein (%)	Carbon (%)	β -Glucan (%)	MC (g Water/100 g Sample)
Control	1.71 $\pm$ 0.02 ^a	10.68 $\pm$ 0.15 ^a	44.1 $\pm$ 0.2 ^a	3.96 $\pm$ 0.14 ^a	9.7 $\pm$ 0.1 ^a
6 min ACP	1.62 $\pm$ 0.05 ^a	10.39 $\pm$ 0.33 ^a	44.07 $\pm$ 0.21 ^a	3.98 $\pm$ 0.08 ^a	9.6 $\pm$ 0.0 ^a
10 min ACP	1.64 $\pm$ 0.05 ^a	10.26 $\pm$ 0.29 ^a	43.93 $\pm$ 0.55 ^a	4.23 $\pm$ 0.25 ^a	9.4 $\pm$ 0.2 ^a

Values with different letters in the same column are significantly different ($p < 0.05$, $n = 3$).

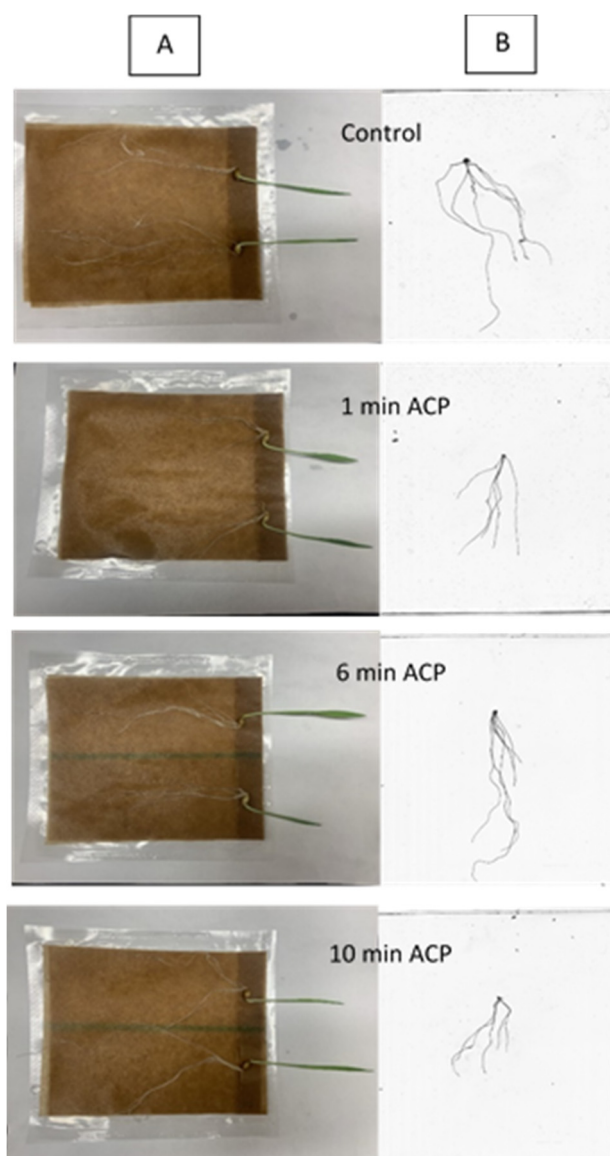
3.8. Effect of ACP Treatment on Germination of Barley Grains

The effect of ACP on germination parameters of barley was time-dependent (Table 7). The grains treated for 6 min by ACP had the highest germination percentage (93.3%). ACP treatment for 1 and 10 min decreased some of the germination parameters such as root length and root surface area, shoot length, and the number of roots compared to the untreated control. However, the 6 min ACP treatment improved some of the germination parameters, but not significantly ($p \geq 0.05$) (i.e., the average root diameter, root volume, and the germination percentage) (Figure 4). Root growth parameters are important in exploiting the soil and uptake of the minerals, especially for less mobile nutrients. Root growth affects vigorous shoot growth and crop yield, especially in low nutrient soils [49]. ACP treatment can affect the root growth and, as a result, the ability of plants to explore soil and uptake water and nutrients will be changed [50]. Previous research reported that ACP treatment impacted the germination rate and germination parameters of several grain crops [12,13,46]. A number of theories reported that the changes in the germination rate of the ACP treated grains could be related to: (1) change in water absorption of the grains, (2) change in their surface characteristics as a result of chemical reaction of the reactive species with the seed surface, (3) or the physical energy deposition of plasma exposure on grains, (4) changes in the biological reactions of the grains, (5) change in the internal functional metabolites such as gamma-aminobutyric acid, and 1,1-diphenyl-2-picrylhydrazyl activity [12], (6) alterations in protein structure, and (7) stimulation of the natural signal (i.e., stimulation of growth factor, opening the calcium channel) [51]. The attack of oxygen radicals and bombardment by low energy ions may induce seed coat erosion and contribute to a change in germination [51]. Previous studies reported that the germination parameters were influenced based on the plasma treatment time and optimum plasma time should be considered for each plant species [51–53]. In this study, we determined that the ACP treatment for 6 min was able to reduce almost half of the DON on the barley grains and it did not show any significant ($p \geq 0.05$) adverse effects on germination parameters of barley. However, to fully understand the biological effects of ACP species on germination, the characteristics of each type of plasma, and the mechanisms between plasma species and each biomolecule should be studied. This could be quite challenging due to the inconstant state of ACP, diversity of target species in the plant, and the complexity of biomolecular response. A systematic approach for investigating the simple biomolecules and complex tissues to necessary to be able to realize the biological responses between plasma species and biomolecules. Overall, these results lay the foundation for future research that can optimize ACP treatment based on DON degradation, germination rate, and quality analysis.

Table 7. Comparison of germination parameters of barley grains as a function of ACP treatment time.

Treatment	Average Root Length (cm)	Average Root Surface Area (cm ²)	Average Root Diameter (mm)	Root Volume (cm ³)	Shoot Length (cm)	Number of Roots	Germination Percentage (%)
Control	44.2 ± 17.8 ^a	6.37 ± 2.52 ^a	0.462 ± 0.037 ^a	0.073 ± 0.029 ^{ab}	6.76 ± 1.72 ^a	5.67 ± 0.64 ^a	80
1 min ACP	33.7 ± 19.4 ^{ab}	4.88 ± 2.50 ^b	0.495 ± 0.11 ^a	0.058 ± 0.026 ^b	6.40 ± 1.92 ^a	5.16 ± 1.49 ^a	83.3
6 min ACP	42.2 ± 15.7 ^{ab}	6.35 ± 2.23 ^a	0.489 ± 0.060 ^a	0.078 ± 0.029 ^a	7.36 ± 1.62 ^a	5.46 ± 0.84 ^a	93.3
10 min ACP	32.6 ± 20.0 ^b	4.84 ± 2.50 ^b	0.498 ± 0.066 ^a	0.058 ± 0.027 ^b	6.26 ± 2.07 ^a	5.15 ± 1.26 ^a	90

The values indicate the average of the germinated grains for each parameter. Values with different letters in the same column are significantly different ($p < 0.05$, $n = 3$).

**Figure 4.** Effect of ACP treatment on seedling growth of barley after 7 days of germination. (A): photo images. (B): scanned root images using WinRHIZO software.

4. Conclusions

In this study, the potential of ACP treatment for the degradation of DON on barley grains and the effects on selected grain quality parameters, germination, and seedling growth parameters were evaluated. ACP treatment was able to reduce 48% of DON concentration on barley grains in 6 min. The degradation rate was further increased by the addition of water to the surface of by steeping without subsequent drying of the grains before ACP treatment. ACP treatment produced ROS and

RNS, however, ozone exhibited the highest concentration. The storage of barley grains after 6 min ACP treatment further increased the degradation rate of DON. Protein, β -glucan and moisture content of the barley grains were not significantly affected by ACP treatment. The effect of ACP on germination parameters of barley grains was time-dependent, and 6 min ACP treatment showed the best results. Overall, ACP treatment showed promising results on degrading DON, without significant adverse effects on the quality parameters of barley grains. Future research is needed to optimize and coordinate critical process factors required to achieve required DON degradation levels, while maintaining barley quality. Cost-efficiency and other practical challenges will also need to be addressed once this laboratory technique is validated, scaled-up and adapted for commercial applications.

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