Potential Application of Cold Plasma Technology to control Aflatoxin contamination in Tropical Food Product

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ABSTRACT

Tropical foods, especially cereals and legumes, have been found to show elevated levels of aflatoxin contamination. This poses serious health risks to humans and animals that consume such foods. Also, these contaminated food products face frequent rejection at the international markets due to safety concerns thereby leading to food wastage, economic losses to producers, and reputational damage of the country where the aflatoxin-infested food products originate. Therefore, the need for an effective and innovative decontamination strategy other than the conventional methods to overcome these concerns has become necessary and urgent. Cold plasma technology, as a non-thermal food preservation technique, has emerged as a promising food decontamination intervention for mycotoxins inactivation thereby ensuring the safety of food and extended shelf-life. This review sought to demonstrate the potential application of cold plasma technology in causing the degradation of aflatoxin B1 in some selected foods. The application of cold plasma was observed to be effective in degrading the most toxicologically bioactive form of the aflatoxins, by rendering them less harmful than the original aflatoxin B1 molecule. Several studies confirmed the effectiveness of cold plasma treatment of various food matrices to inhibit the activities of aflatoxin-producing fungi: Aspergillus flavus and Aspergillus parasiticus. The plasma technology assisted in the disintegration of the toxigenic aflatoxin molecules (B1, B2, G1 and G2) accumulated in foodstuffs such as cereals and nuts (groundnuts). In view of its aflatoxin-control efficacy, sustainability due to low running cost, and minimal impact on the nutritive value of foods, this review proposes the adoption and utilization of cold plasma as a food preservation technique by agro-based businesses and commercial food processing companies. This will help reduce the levels of aflatoxins in foods to acceptable local and international standards to enhance food security, profitability for businesses in the food supply chain, national revenue maximization through food export, and guaranteed food safety for the global consumers.

Keywords: Cold plasma, aflatoxin-producing fungi, tropical foods

1.0. INTRODUCTION

Cereals and legumes including maize, millet, rice, as well as groundnut (peanut) and beans form part of the major diet in Africa and other parts of the world (Suleiman et al., 2017; Temba et al., 2017; Soro-Yao et al., 2014; Florkowski & Kolavalli, 2016; Ofori-Adjei, 2012). Cereals and legumes are prone to aflatoxin infestation along the agricultural chain from pre-harvest stage through the harvesting stage and post-harvesting stage (Burger et al., 2013; Lombard, 2014; Wagacha & Muthomi, 2008). Aflatoxin infestation of these common food crops has detrimental consequences on the output of the agricultural sector generally while constraining the attainability of the four food security pillars; availability, access, utilization, and stability.The consumption of cereals and legumes predisposes animals and humans to toxic aflatoxins which has serious health implications (Temba et al., 2017).

Characteristically, aflatoxins are mycotoxins: biologically active molecules, that occur in nature and produced by specific species of fungi particularly, *Aspergillus flavus* and *Aspergillus parasiticus* living in the soil and their rate of production optimized by certain environmental conditions of temperature 33◦C and relative humidity, 99% (Milani, 2013). Therefore, factors that facilitate aflatoxin contamination include high temperature, high relative humidity, bad storage conditions, and pest infestation especially in the tropical and sub-tropical regions of the world like Ghana (Burger et al., 2013; Hell & Mutegi, 2011; Kumar et al., 2008; Sowley, 2016). The four main types of aflatoxins of health and agricultural concerns include; aflatoxin B1, B2, G1, and G2. Aflatoxin B1 is the most common of the four types. *A. flavus* is known to produce only B-type aflatoxins, whereas the *A. parasiticus* produces both B- and G-type aflatoxins (Creppy, 2002; Zinedine & Mañes, 2009). The percentage composition and frequency of occurrence of each type of aflatoxins in food crops is a function of a number of factors including the type of *Aspergillus* species involved, growing and storage conditions of the crops, and other factors (Paterson & Lima, 2010). Another type of aflatoxin of interest is the M-type aflatoxins which usually do not occur on crops, but are metabolic products found in meat and milk of animals that feed on feedstock contaminated by B1 and B2 (De Ruyck et al., 2015; Iqbal et al., 2015). A study by (Tsakiris et al., 2013) found that, as animals consume aflatoxin B1 infested feedstuffs, the B1 metabolizes to form the monohydroxy product of aflatoxin M1, which is found in the meat and milk of the animals. The aflatoxin M1 is the main hydroxylated product of B1 produced through Cytochrome P450 enzymes in the liver and get expressed in the milk through the mammary gland. Aflatoxins have received global attention because of their carcinogenic, tetratogenic, and mutagenic nature (Ismail et al., 2018; Richard, 2007). Exposure to high doses of aflatoxin can cause acute health problems like aflatoxicosis, which can lead to vomiting, abdominal pain and even death when the

Exposure to high doses of aflatoxin can cause acute health problems like *aflatoxicosis,* which can lead to vomiting, abdominal pain and even death when the case becomes worse. Chronic exposure to low levels of aflatoxin over time can result in health problems namely; suppression of the immune system, delayed recovery from malnutrition diseases (kwashiorkor), liver function impairment, cancer of the liver, and reduced growth rate or stunting in children (Kalorey et al., 2001; Liu & Wu, 2010; Sherif et al., 2009; Turner et al., 2003).

Due to the health impact of aflatoxins, a number of countries have established permissible limits of aflatoxin levels in their foods. For example, for EU countries, the allowable limit is 4µg/kg while in the USA, the allowable limit is 20µg/kg for all foods (except milk). In Ghana, the permissible limit is 20µg/kg for raw groundnuts and 15µg/kg for maize. The FAO and WHO have a limit of 30µg/kg and Codex Alimentarius Commission 10µg/kg for all processed foods. Available research from many African countries and particularly Ghana found aflatoxin contamination in maize, groundnuts and sorghum and the products made from these crops to be higher than the European Union aflatoxin limit (4ppb) and that of USA (20ppb) (Bankole & Kpodo, 2005; Blankson *et al*., 2019; Kumi *et al*., 2014). It was found that aflatoxin level in approximately 40% of food commodities found in local African markets exceed allowable levels. A number of aflatoxin alert notifications in groundnut paste exported from Ghana to the EU (2004-2018) have been issued by the EU countries. Food products infested with aflatoxin in Ghana poses huge economic challenges and account for losses approximately 319,000 tons or 18% of the total yearly production of maize in the country (Omari et al., 2020). Quantifying this phenomenon, the Partnership for Aflatoxin Control in Africa (PACA) of the Africa Union Commission (AUC) estimated the economic losses of aflatoxin to Africa to be $670m yearly. (Omari et al., 2020) .

Post-harvest strategies for controlling aflatoxin in food crops involves; rapid and adequate drying after harvesting crops (Hell *et al*., 2008); cleaning and sorting of food crops (Fandohan *et al*., 2005; Whitaker & Johansson, 2005); proper storage practices (Baoua *et al*., 2014; Hell *et al*., 2008; Hell *et al*., 2014); heat treatment (Reddy & Rani, 2000); chemical treatment with various chemicals; and irradiation using gamma radiation (Iqbal *et al*., 2013; Markov *et al*., 2015). However, aflatoxins are relatively stable in solutions of neutral pH, and high-temperatures before they decompose at temperatures of 237–306°C (Pankaj *et al*., 2018). Spores of *Aspergillus spp* are resistant to UV photons due to the existence of coat on spores that is capable of absorbing UV photons. More so, the spores contain the pigment melanin in the cell structure that provides protection for the spore making them immutable to stresses from the external environment (Kim et al., 2017).

Prolong thermal treatment also causes damage to food leading to undesirable changes in food quality, such as loss of heat-labile nutritional components,

changes in the physical texture of food, and changes in the organoleptic characteristics of food (Hernandez *et al*., 2019; Pereira & Vicente, 2010). As a result of their chemical stability, high-temperature resistance, and UV photo-insensitivity, degrading aflatoxins in contaminated foods is very challenging. Since mycotoxins have been found to have resistance against thermal processing, a shift of focus towards the adoption of non-thermal technologies such as cold plasma technology is generating interest as an alternative to the traditional aflatoxin-control strategies. (Gavahian & Cullen, 2020). This review, therefore, explores the potential application of cold plasma technology to inactivate the aflatoxin-product fungi as well as degrading aflatoxin compounds in foods.

2.0. COLD PLASMA TECHNOLOGY

Cold plasma technology is an emerging non-thermal food processing approach which has wide range of applications in the food industry particularly for decontamination of foods and food packages (Afshari & Hosseini, 2014; Mahendran et al., 2017). Due to its efficient antimicrobial efficiency and low working temperature (<60°C), cold plasma technology is gaining acceptance for food preservation in fruits and vegetables (Ramazzina et al., 2015), meat and poultry (Kim et al., 2013), dairy (Gurol et al., 2012), beverages (Liao et al., 2018; Muhammad et al., 2019; Xiang et al., 2018), cereals (Butscher et al., 2016), and spices (Hertwig et al., 2015). The plasma technology principle is governed by partial ionization of positive and negative ions, free radicals, charged particles in the form of electrons and photons, and gas-containing neutral molecules. Plasma is observed to interact with microbial cells and inactivate microorganisms of all kinds (Mendes-Oliveira et al., 2019; Misra et al., 2011; Umair et al., 2022). Inference made by (Hojnik et al., 2017) suggests that cold plasma technology, which has powerful degradative effect on mycotoxins relative to the traditional decontamination interventions, is more effective and fast in achieving results. Investigations have proven that cold plasma treatment reduces aflatoxin level by 93 percent after 8 minutes of exposure to cold atmospheric pressure plasma generated with ambient air (Hojnik et al., 2019). Cold plasma aflatoxin degradation power is derived from the Reactive Oxygen Specifies (ROS) and Reactive Nitrogen Species (RNS) which react with the aflatoxin double bonds causing oxidative damage to the bond resulting in the formation of molecules that are less toxic than the initial aflatoxins. Cold plasma technique, relative to the traditional physical, chemical and biological decontamination techniques in the food industry, has no adverse effect on product quality and sensory characteristics of the food (Barba et al., 2017; Sainz-García et al., 2019). Thus a number of investigations have been triggered to study the effect and mechanism of decontamination of aflatoxins using cold plasm (Nguyen et al., 2022; Shi, Ileleji, et al., 2017; Wielogorska et al., 2019).

However, the viability of use of cold plasma technology to decontaminate aflatoxin in indigenous African foods particularly Ghana has not been explored. This review therefore seeks to examine the potential use of cold plasma to inactivate the aflatoxins-producing fungi and also to cause degradation of aflatoxins in various indigenous Ghanaian foods (cereals, legumes as well as milk and milk products) and recommend the adoption of the technology to minimize the negative health impact and economic losses due to aflatoxins in foods.

2.1. Efficacy of Cold Plasma in Controlling Fungal Spores and Aflatoxins in Foods

A good number of physical, chemical, and biological interventions have been used to decontaminate aflatoxin-producing fungi as well as degrade aflatoxins in food but with limited efficacy. For example, physical strategy involving cleaning and sorting grains to reduce aflatoxin contamination in grains is ineffective (Shi et al., 2014). Aflatoxins are also chemically stable in neutral solutions, and resist high-temperature stresses (Pankaj et al., 2018). The spores of aflatoxin-producing fungi, *A. flavus* and *A. parasiticu*s are known to be resistant to UV photons due to the ability of the spore coat to absorb UV photons. The spores also contain the pigment melanin in the cell structure that provides protection for the spore making them non-responsive to stresses from the external environment (Kim et al., 2017). Due to chemical stability, high-temperature resistance, and UV photo-insensitivity nature of aflatoxins and the aflatoxin-producing fungi, decontamination of the aflatoxin-producing fungi and degrading aflatoxins produced in contaminated foods is very challenging and therefore requires the adoption of cold plasma technique. Cold plasma treatment has been proven to decontaminate aflatoxigenic fungi (*A. flavus* and *A. parasiticus*) using the atmospheric pressure fluidized bed plasma (Dasan et al., 2016) and degrade aflatoxin level by 93 percent after 8 minutes (Hojnik et al., 2019) ; or by the use of high voyage atmospheric cold plasma (Nguyen et al., 2022; Nikmaram & Keener, 2022). Cold plasma technique, leaves no adverse effect on the quality and sensory characteristics of the food (Barba et al., 2017; López et al., 2019). The negative effects of the technique, such as toxicity, are still under evaluation, but there are already studies demonstrating that there is no negative impact (Dzimitrowicz et al., 2022; Pohl et al., 2022). Factors of cold plasma accounting for its efficacy in decontamination of aflatoxin-producing fungi as well as the degradation of the aflatoxins produced in foodstuffs include; the plasma source (Okyere et al., 2022; Sifuentes-Nieves et al., 2021; Wang et al., 2023; Yan et al., 2019), the input power (Devi et al., 2017; Liao et al., 2017), and input voltage and frequency(Cheng et al., 2017; Dasan et al., 2016; Deng et al., 2007), type of gas used and their composition, and the reaction species in the plasma (Amini & Ghoranneviss, 2016). Zhi et al 2023

explored the application of dielectric barrier discharge cold plasma to reduce aflatoxin in food Figure 1 illustrates the experimental equipment used in their study. This consists of dielectric barrier plate, high voltage electrode, high voltage electric field generator and controller.

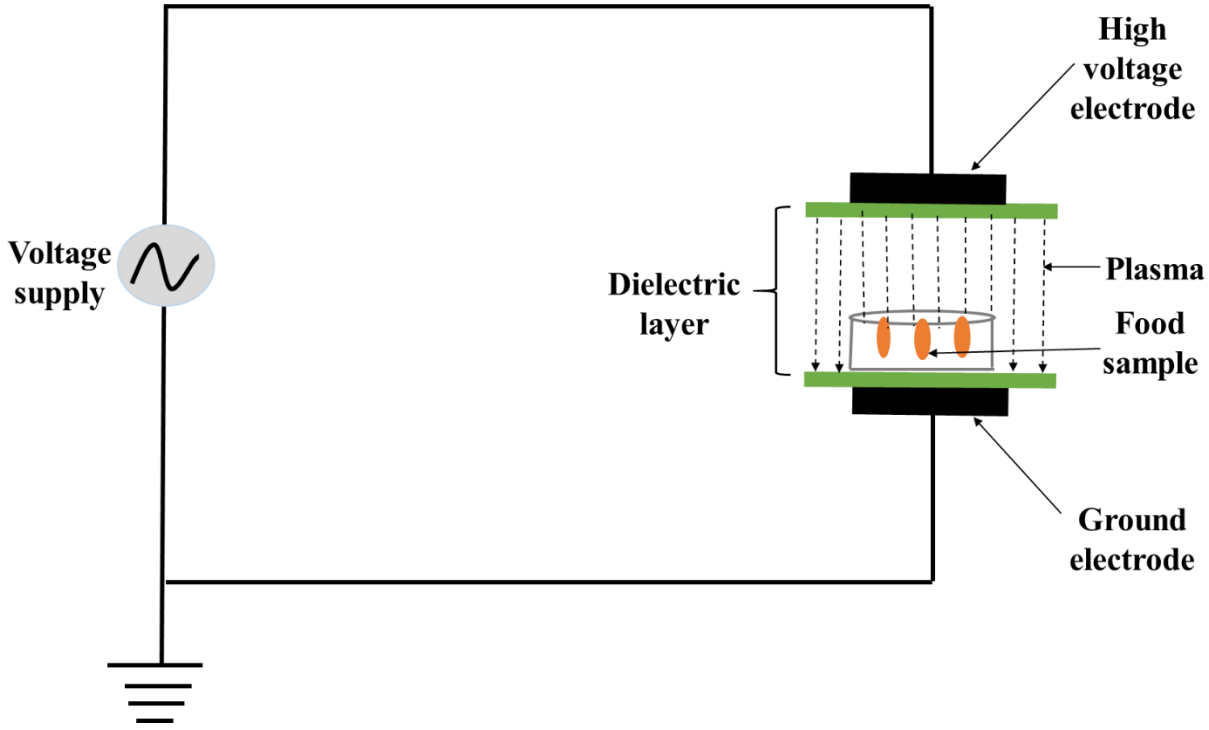

Figure 1: The schematic of the experimental equipment for DBD cold plasma system. (Zhi et al., 2023)

2.2. Cold Plasma Inactivation of Aflatoxin-producing Fungi in Foods

Table 1 provides a summary of the advancement of cold plasma application in some selected topical foods. A study undertaken by Suhem et al. (2013) focusing on inactivation of *A. flavus* on malt extract agar media and in a brown rice cereal bar, adopted the cold plasma jet technique. Argon was used for the treatment and two power levels, 20W and 40W were considered, with the treatment time set from 5 to 25 min, the frequency range of 50-600 kHz, and the voltage of the plasma generator maintained at constant at 10kV. The extremely high treatment condition (40W, 25 min) was observed to cause complete inactivation of the *A. flavus* in the agar. The results further indicate that the treatment condition at 40W for 20min at 25°C, 100% RH extended the brown rice cereal bar with a shelf-life of 20 days. But the treatment resulted in the production of undesirable or objectionable aroma perceived by a sensory testing panelist. The formation of the aroma may be occasioned by lipid oxidation caused by the

interaction of the free radicals with lipid component of the brown rice cereal bar during the plasma treatment. The study also revealed that the spores and vesicles of *A. flavus* were also damaged during the plasma treatment. The implication of the finding is that cold plasma is capable of extending the shelf-life of cereal such as rice by causing inactivation of aflatoxin B-type producing fungus, *A. flavus*.

In a later study, Devi et al. (2017) examined the effect of cold plasma treatment, on the growth of *Aspergillus parasiticus* and *Aspergillus flavus* as well as aflatoxins production. By infesting the fungal species onto the groundnuts artificially and subjecting the inoculated samples to air plasma treatment at 40W and 60W power levels across various time intervals revealed significant inactivation of *A. flavus* and *A. parasiticus* by 99.3% and 97.9% individually. The study also revealed total damage to the fungal spore membrane resulting from electroporation and etching effect exacted by the reactive species of plasma. The finding of the research suggests that cold plasma treatment can cause a complete inactivation of aflatoxin-producing fungi in legumes like groundnuts. Therefore, it has the prospect of being used to decontaminate aflatoxins in legumes.

2.3. Cold Plasma Degradation of Aflatoxins in Foods

Cold plasma is still in its technologically nascent state, but gaining a great deal of potential within the food industry for aflatoxin decontamination (Wielogorska et al., 2019). Several investigations have revealed the aflatoxin degradative effects of cold plasma while impacting minimally on the nutritional characteristics or organoleptic properties (Wang et al., 2016) as shown in Table 2. The aflatoxin occurring in food matrix is subjected to degradation by reactive species including O, O_3, OH, NO, and NO_2 generated by the cold plasma. The generated reactive species bombard chemical bonds of the aflatoxin molecules causing degradation or changing them into other harmless products (Misra et al., 2019). This preservation treatment ensures high decontamination of mycotoxins at relatively low temperature of 5–30◦C without altering the quality characteristics relative to the existing conventional/traditional approaches (Mir et al., 2021; Wielogorska et al., 2019). This section examines the findings from the use of cold plasma technology to decontaminate aflatoxins in various food crops including cereals grains, legumes etc. so as to establish the potentials of use of the technology to reduce the contamination levels of aflatoxins in indigenous Ghanaian foods to acceptable standards. This has the benefit of reducing the economic losses of foods due to high aflatoxin levels in food and also ensures that food is toxicologically safe for consumption. Shi, Ileleji, et al. (2017) investigated the effectiveness of high voltage atmospheric cold plasma treatment in breaking down aflatoxins in corn (maize). By subjecting the samples to air treatment at

Table 1: Cold plasma treatment in controlling aflatoxins-producing fungi in selected foods

Type of food matrix	Source of Cold plasma	Treatment conditions	Type of aflatoxin-production fungi	Significant findings	References
Brown rice cereals	Radio-frequency atmospheric cold plasma jet	Pressure: 1 atm Gas: Argon Power: 40W Flow rate: 10 L/min, Freq: 50–600 kHz Voltage:10 kV (max)	*A. flavus*	40W for 20 min cuased an inhibition in the growth of *A. flavus* for 20 days stored contantly at 25°C and 100% RH	(Suhem et al., 2013)
Rice	Dielectric barrier discharge (DBD) cold plasma	Power: 220 V-50 Hz. operating voltage: 28– 169 kV, frequency: 50–200 Hz. Time: 35 mm during treatment. Grain mass: 20.0 g dielectric barrier at 70– 100 kV for 5–60 min, temperature of 20 ± 2 ◦C humidity of 56 ± 1%. Gas: (0%O_2 + 70%N_2 + 30% CO_2, 25%O_2 + 45%N_2 + 30% CO_2, 45%O_2 + 25%N_2 + 30% CO_2 and 65% O_2 + 5%N_2 + 30% CO_2)	*A. parasiticus (BNCC 144221)* *A. parasiticus*	- The degradation rate of AFB_1 (aflatoxin B_1) reached the maximum at the voltage of 100 kV. In addition, AFs was reduced from 161.05 µg/kg to 70.33 µg/kg, with the largest degradation rate of 56.37%. - in general, the treatment reduced 1.08%–55.34% of AFB1 and 4.23%–56.37% of AFs (total aflatoxin)	(Zhi et al., 2023)

Groundnuts	Glow discharge plasma	frequency 13.56 MHz. Power: 40 W and 60 W. Voltage: 1500 V 1950 V. Humidity (RH) of air used: 45.3 ± 0.3% Grain mass:10.0 g	*Aspergillus parasiticus* *Aspergillus flavus*	- The inactivation of A. *flavus* and A. *parasiticus* is effective at higher power level (60 W) than 40 W; the optimum time for inhibition at 60 W was 6 min earlier than 40 W treated samples. - There is 97.9 % and 99.3% reduction in the growth of A. *parasiticus* and A. *flavus* respectively, when treated at 60 W powers	(Devi et al., 2017)
Peanut kernels	plasma jet	Frequency: 13.7 A and 12.5 MHz Power: 180 or 200 W flow rate: 30 standard liters per minute (slm) Time: 2.5, 3.5, 5, 7.5, 10 min mass: 15 ± 1 g storage: 25 ◦C and 85–90% relative humidity (RH) for 5 days	*Aspergillus flavus*	-The plasma treatments carried out at 180 and 200 W 3.5 min exhibited reductions of 4.08 and 4.64 log CFU/g, respectively	(Lin et al., 2022)

to air treatment at varying relative humidity (5, 40, 80% RH), treatment time (1, 2, 5, 10, 20, and 30 min), the aflatoxin content in the corn sample was reduced tremendously by 62% and 82% at 1 and 10 min treatment respectively at a constant RH 40% air. The degradation kinetics of aflatoxin of the cold plasma treatment followed a logistic model. Higher degradation of aflatoxin was observed with increasing relative humidity (40%, 80%).

Similar investigation by Devi et al. (2017) examined the effect of cold plasma treatment on the growth of *Aspergillus parasiticus* and *Aspergillus flavus* as well as aflatoxins productions as an alternative to chemical free and thermal treatments. By artificially infesting the fungal species onto the groundnuts and the subjecting it to air plasma treatment at 40W and 60W energy levels across various time intervals revealed that the treatments of the inoculated samples at 40W 15 min and 60W 12min plasma induced 70% and 90% degradation in aflatoxin B1 level. The outcome of this study confirms the capacity of cold plasma treatments to cause degradation of aflatoxins in food rendering them less toxic. Also, Basaran et al. (2008), in applying low pressure cold plasma (LPCP) equipped with air gases and sulfur hexafluoride (SF_6), investigated the effectiveness of plasma treatment against aflatoxins produced by *A. parasiticus* inoculated on a wide range of nut samples using air gases for 20 min. The air plasma treatment found a 50% degradation effect in total aflatoxins (AFB1, AFB2, AFG1, and AFG2). The study also observed a 20% reduction in the total aflatoxin content when the gas was changed to sulfur hexafluoride (SF_6) for the same treatment time of 20 min. The study portrays the potential use of cold plasma to degrade aflatoxins in nuts.

Siciliano et al. (2016) investigated aflatoxins degradation in food foodstuffs, such as nuts using cold atmospheric pressure plasma by optimizing the cold atmospheric pressure plasma conditions to reduce the presence of aflatoxins on dehulled hazelnuts. The impacts of different gases (N_2, 0.1% O_2 and 1% O_2, 21% O_2), power (400, 700, 1000, 1150 W) and exposure time (1, 2, 4, and 12 min) were optimized. The results showed that hazelnuts at cold plasma condition of (1000 W, 12 min) portrayed a significant reduction in the load of total aflatoxins and AFB1 to over 70%. Aflatoxins B1 and G1 were observed to be more responsive to plasma treatments relative to aflatoxins B2 and G2. Aflatoxin B1 responded to cold plasma treatment more than G1. The use of cold atmospheric plasma promises to be an ideal method for aflatoxin decontamination in foods and also has marginal impact on the nutritional properties of the food.

Sakudo et al. (2017) inoculated aflatoxin B1 to a cover glass and subjected the inoculated glass to cold plasma treatment fitted with nitrogen gas and using a short high voltage pulse from a static induction thyristor power supply at 1.5 kpps (kilo pulse per second). The finding with respect to enzyme-linked immunosorbent assay showed that a 20 mL sample

a 200 ppb solution of AFB1 was effectively degraded by more than 90% of its original value (less than one tenth of the initial concentration level) within 15min. The results of high-performance liquid chromatography provided information of the degradation of AFB1 by plasma treatment yielding small fragments, purported to come from the original stock of the AFBI. Additionally, the biological activity of the AFBI was measured using a cell-based assay of HepG2 and it revealed that the AFA1 lost its cytotoxic activity signaling the potential of cold plasma treatment to render the cytotoxic capacity of AFB1 less effective.

Table 1: Cold plasma treatment in degrading aflatoxins in selected foods

Type of aflatoxin	Cold plasma treatment	Type of food matrix	% Reduction of aflatoxin	References
Aflatoxin B1	Helium gas with 0.5 and 0.75% of oxygen, exposure time 10 min, frequency 20kHz	Corn	65%	(Wielogorska et al., 2019)
Aflatoxins	Treatment time 1min, humidity 40%, modified atmosphere 65% oxygen 30% CO_2/5% N_2	Corn	62%	(Shi, Ileleji, et al., 2017)
Aflatoxin	Treatment time 10 min, humidity 40%, modified atmosphere 65% oxygen 30% CO_2/5% N_2	Corn	82%	(Shi, Ileleji, et al., 2017)
A1, B1, G1 and G2	Low pressure cold plasma gas : air and sulfur hexafluoride (SF_6) Time : 5 min	Hazelnuts, peanuts, and pistachio nuts	50% reduction in total aflatoxins (AFB1, AFB2, AFG1, and AFG2) for 20mins, -20% reduction in total aflatoxin was observed after 20 min SF6 plasma treatmen	(Basaran et al., 2008)
Aflatoxin B1	Air plasma treatment at 40Wand 60W for 12mins	Groundnuts	-70% for 40W -90% for 60W	(Devi et al., 2017)
A1, B1, G1 and G2	Treatment was done using different gases at varying concentration, time, and power of the plasma device	Hazelnuts	>70% total aflatoxins	(Siciliano et al., 2016)

2.4. Mechanism of Action of Cold Plasma effect on Aflatoxins Degradation

Based on the mechanism of cold plasma induced degradation of aflatoxins, Wu et al. (2021) observed two main studies focused on the chemical partway for the degradation with respect to AFB1 including the pathway proposed by Wang et al. (2016) and that of Shi, Ileleji, et al. (2017) as shown in Figure 2. Wang et al. (2016), in studying the effectiveness of low-temperature radiofrequency plasma (LTRFP) in AFB1 removal as a contaminant made the observation that the rate of degradation AFB1 was about 88.3% reduction after cold plasma treatment at 300W for 10min. The results showed AFB1 breaking down into five major products classified under two pathways: first pathway resulting in 3 of the products (Products A, B, and C), and the second pathway giving rise to the other 2 products (Products D, and E). All the AFB1 degradation products did not retain their C=C double bonds on the terminal furan ring. Free radicals from cold plasma such as O•, H• and OH• were implicated for initiating the reactions leading to the formation of the degradation products. The conclusion is that the degradation of AFB_1 is by free radical reaction mechanism involving reactive species from cold plasma. In another study, Shi, Cooper, et al. (2017) investigated the efficacy of use of high voltage atmospheric cold plasma (HVACP) to cause degradation of pure AFB1 and found that the treatment with HVACP had the capacity to induce the degradation of AFB1 resulting in AFB1 losing 76% of its initial concentration after 5 min of treatment time. The study also found six main degradation products and classified the mechanism of their formation from AFBI into two main reaction pathways on the basis of the molecular structure. The first reaction pathway of the degradation is observed to be addition reaction involving the addition of small molecules in two separate ways; one with addition of water (H2O) to form product 1 followed by hydrogen (H2) to form product 2, and the other with addition of aldehyde (CHO) to form product 3. The second pathway is observed to include two separate processes; one involving epoxidation reaction initiated by hydroperoxyl radical (HO_2•) forming product 4, and the other involving oxidation reaction through the initiative of HO•, H_2O_2, and O_3 to from product 5 followed by product 6. As illustrated in Figure 3. The central point in this study is that ROS are perceived to be the main cause of AFB1 degradation and that all the products are formed with the initial break down of C_8=C_9 double bonds on the furan ring, followed by reaction with reactive species (RS) from cold plasma and consequently the formation of degradation products with lower toxicities level than AFB1.

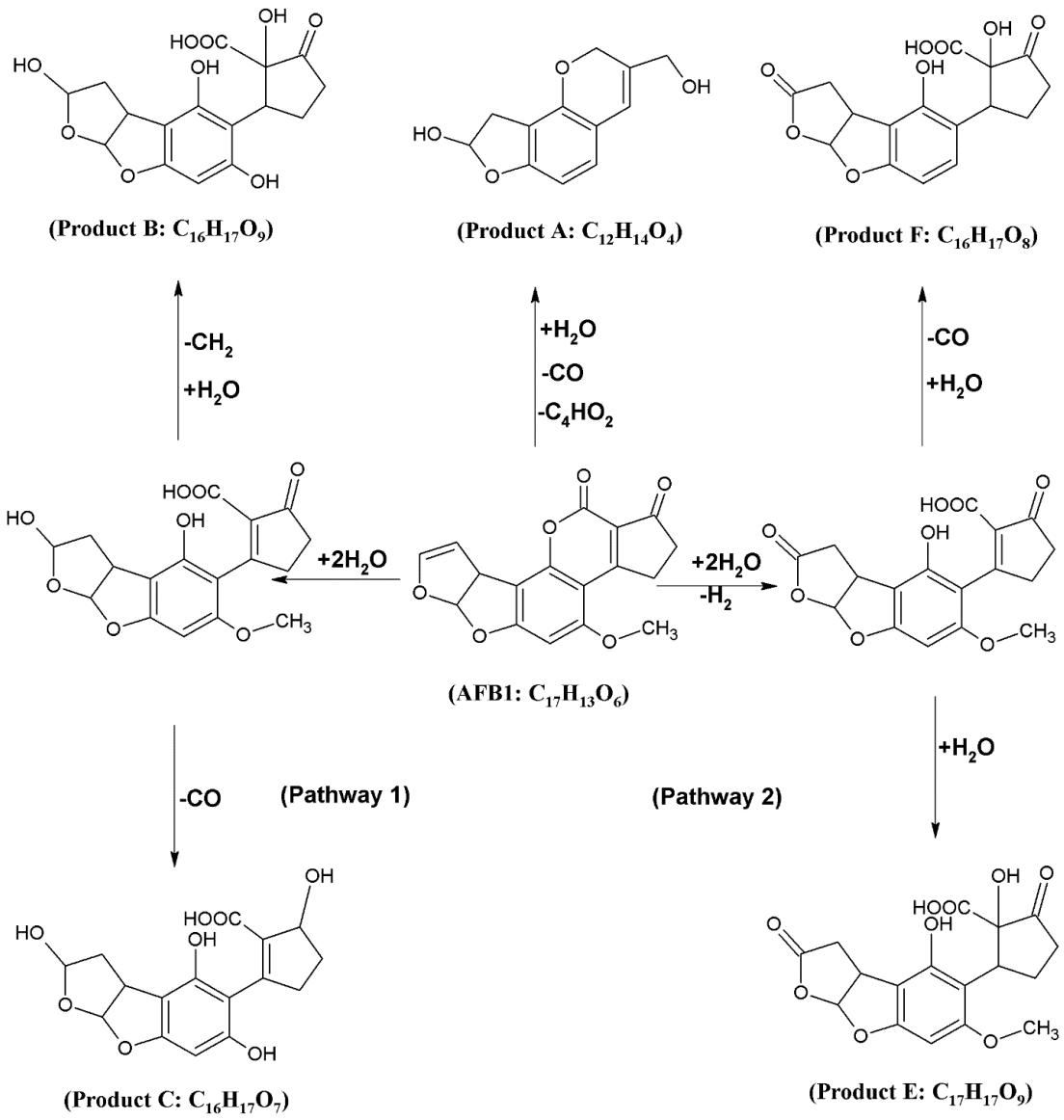

Figure 2: Pathways of degradation of aflatoxin B1 as proposed by Wang et al. (2016)

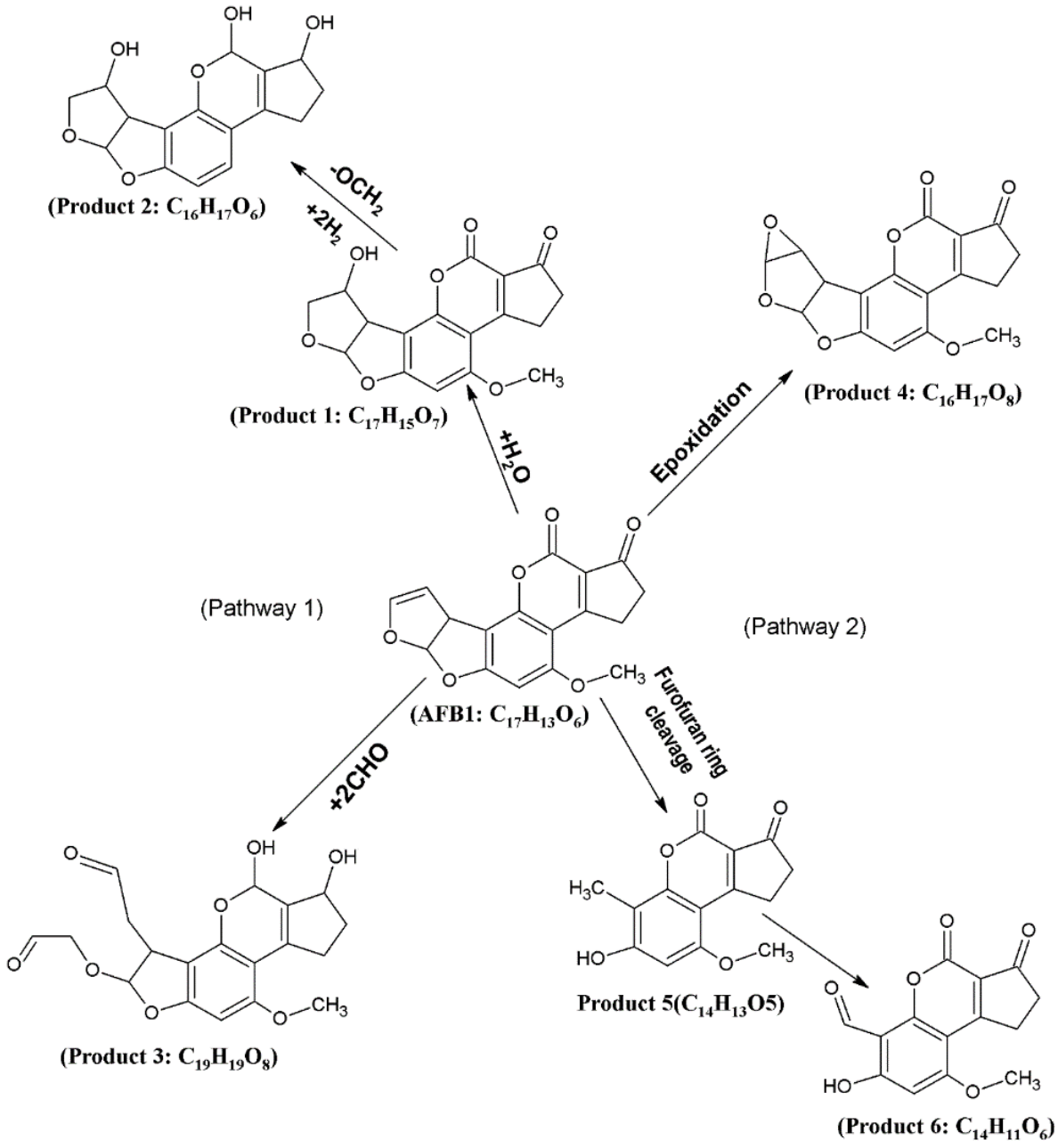

Figure 3: Pathways of aflatoxin degradation as proposed by Shi, Cooper, et al. (2017)

3.0. CONCLUSION

The high level of aflatoxin contamination of indigenous Ghanaian foods especially cereals and legumes has led to food wastage and economic losses to producers. This calls for the urgent need to adopt an appropriate aflatoxin control strategy alternative to the traditional aflatoxin-control methods. Cold

plasma technology has been found to have the potential to inactivate both the aflatoxin-producing fungi as well as degrade aflatoxin compounds in contaminated foods to acceptable standards. The potential of cold plasma in controlling aflatoxins is grounded on four factors. First, the effectiveness of the technology to inactivate aflatoxins-producing fungi specifically *A. flavus*, and *A. parasiticus* in various foods similar to indigenous Ghanaian foods. Second, the capacity of the technology to cause the degradation of the aflatoxins molecules or convert them to other forms with a weakened capacity to induce *aflatoxicosis* and its associated adverse health conditions in humans and animals. Third, the application of the technology has minimal effect on the nutritive value of the foods subjected to treatment. Fourth, the technique is also sustainable due to its minimum energy requirements for operation and its low investment cost. Therefore, micro, small and medium-sized agro-based businesses as well as large food processing companies particularly in aflatoxin endemic countries can adopt the cold plasma technology to reduce the levels of aflatoxins in foods and food products to acceptable local and international standards. This will help reduce the huge economic losses caused by aflatoxin contamination. It will also ensure availability of food (food security), reduction in food wastage, and ensure food safety for the population. The risk of rejection of food and food products from African countries at the international markets will be reduced.

REFERENCES

Afshari, R., & Hosseini, H. (2014). Non-thermal plasma as a new food preservation method, its present and future prospect. Archives of Advances in Biosciences, 5(1).

Amini, M., & Ghoranneviss, M. (2016). Effects of cold plasma treatment on antioxidants activity, phenolic contents and shelf life of fresh and dried walnut (Juglans regia L.) cultivars during storage. Lwt, 73, 178-184.

Bankole, S., & Kpodo, K. (2005). Mycotoxin contamination in food systems in West and Central Africa. Reducing impact of Mycotoxin in Tropical Agriculture with emphasis on health and trade in Africa, Acra, Ghana, 13-16.

Baoua, I., Amadou, L., Ousmane, B., Baributsa, D., & Murdock, L. (2014). PICS bags for post-harvest storage of maize grain in West Africa. Journal of Stored Products Research, 58, 20-28.

Barba, F. J., Mariutti, L. R., Bragagnolo, N., Mercadante, A. Z., Barbosa-Canovas, G. V., & Orlien, V. (2017). Bioaccessibility of bioactive compounds from fruits and vegetables after thermal and nonthermal

processing. Trends in Food Science & Technology, 67, 195-206.

Basaran, P., Basaran-Akgul, N., & Oksuz, L. (2008). Elimination of Aspergillus parasiticus from nut surface with low pressure cold plasma (LPCP) treatment. Food Microbiology, 25(4), 626-632.

Blankson, G., Mills-Robertson, F., & Ofosu, I. (2019). Survey of occurrence levels of aflatoxins in selected locally processed cereal-based foods for human consumption from Ghana. Food Control, 95, 170-175.

Burger, H., Shephard, G., Louw, W., Rheeder, J., & Gelderblom, W. (2013). The mycotoxin distribution in maize milling fractions under experimental conditions. International Journal of Food Microbiology, 165(1), 57-64.

Butscher, D., Zimmermann, D., Schuppler, M., & von Rohr, P. R. (2016). Plasma inactivation of bacterial endospores on wheat grains and polymeric model substrates in a dielectric barrier discharge. Food Control, 60, 636-645.

Cheng, X., Dong, P., Huang, Z., Zhang, Y., Chen, Y., Nie, X., & Zhang, X. (2017). Green synthesis of plasmonic Ag nanoparticles anchored TiO2 nanorod arrays using cold plasma for visible-light-driven photocatalytic reduction of CO2. Journal of CO2 Utilization, 20, 200-207.

Creppy, E. E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology letters, 127(1-3), 19-28.

Dasan, B. G., Boyaci, I. H., & Mutlu, M. (2016). Inactivation of aflatoxigenic fungi (Aspergillus spp.) on granular food model, maize, in an atmospheric pressure fluidized bed plasma system. Food Control, 70, 1-8.

De Ruyck, K., De Boevre, M., Huybrechts, I., & De Saeger, S. (2015). Dietary mycotoxins, co-exposure, and carcinogenesis in humans: Short review. Mutation Research/Reviews in Mutation Research, 766, 32-41.

Deng, S., Ruan, R., Mok, C. K., Huang, G., Lin, X., & Chen, P. (2007). Inactivation of Escherichia coli on almonds using nonthermal plasma. Journal of food science, 72(2), M62-M66.

Devi, Y., Thirumdas, R., Sarangapani, C., Deshmukh, R., & Annapure, U. (2017). Influence of cold plasma on fungal growth and aflatoxins production on groundnuts. Food Control, 77, 187-191.

Dzimitrowicz, A., Pohl, P., Caban, M., Jamroz, P., Cyganowski, P., Bykowski, M., Klimczak, A., & Bielawska-Pohl, A. (2022). How does direct current atmospheric pressure glow discharge application influence on physicochemical, nutritional, microbiological, and cytotoxic properties of orange juice? Food Chemistry, 377, 131903.

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W., & Wingfield, M. (2005). Natural occurrence of Fusarium and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. International Journal of Food Microbiology, 99(2), 173-183.

Gavahian, M., & Cullen, P. (2020). Cold plasma as an emerging technique for mycotoxin-free food: Efficacy, mechanisms, and trends. Food Reviews International, 36(2), 193-214.

Gurol, C., Ekinci, F., Aslan, N., & Korachi, M. (2012). Low temperature plasma for decontamination of E. coli in milk. International Journal of Food Microbiology, 157(1), 1-5.

Hell, K., Fandohan, P., Ranajit Bandyopadhyay, R. B., Kiewnick, S., Sikora, R., & Cotty, P. J. (2008). Pre-and postharvest management of aflatoxin in maize: an African perspective. In Mycotoxins: Detection methods, management, public health and agricultural trade (pp. 219-229). CABI Wallingford UK.

Hell, K., & Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. African Journal of Microbiology Research.

Hell, K., Ognakossan, K. E., & Lamboni, Y. (2014). PICS hermetic storage bags ineffective in controlling infestations of Prostephanus truncatus and Dinoderus spp. in traditional cassava chips. Journal of Stored Products Research, 58, 53-58.

Hernandez, C., Alamilla-Ortiz, Z. L., Escalante, A. E., Navarro-Díaz, M., Carrillo-Reyes, J., Moreno-Andrade, I., & Valdez-Vazquez, I. (2019). Heat-shock treatment applied to inocula for H2 production decreases microbial diversities, interspecific interactions and performance using cellulose as substrate. International Journal of Hydrogen Energy, 44(26), 13126-13134.

Hertwig, C., Reineke, K., Ehlbeck, J., Erdoğdu, B., Rauh, C., & Schlüter, O. (2015). Impact of remote plasma treatment on natural microbial load and quality parameters of selected herbs and spices.

Journal of Food Engineering, 167, 12-17.

Hojnik, J., Ruzzier, M., & Konečnik Ruzzier, M. (2019). Transition towards sustainability: Adoption of eco-products among consumers. Sustainability, 11(16), 4308.

Hojnik, N., Cvelbar, U., Tavčar-Kalcher, G., Walsh, J. L., & Križaj, I. (2017). Mycotoxin decontamination of food: Cold atmospheric pressure plasma versus "classic" decontamination. Toxins, 9(5), 151.

Iqbal, S. Z., Bhatti, I. A., Asi, M. R., Zuber, M., Shahid, M., & Parveen, I. (2013). Effect of γ irradiation on fungal load and aflatoxins reduction in red chillies. Radiation Physics and Chemistry, 82, 80-84.

Iqbal, S. Z., Jinap, S., Pirouz, A., & Faizal, A. A. (2015). Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: A review. Trends in Food Science & Technology, 46(1), 110-119.

Ismail, A., Gonçalves, B. L., de Neeff, D. V., Ponzilacqua, B., Coppa, C. F., Hintzsche, H., Sajid, M., Cruz, A. G., Corassin, C. H., & Oliveira, C. A. (2018). Aflatoxin in foodstuffs: Occurrence and recent advances in decontamination. Food Research International, 113, 74-85.

Ji, S. H., Ki, S. H., Ahn, J. H., Shin, J. H., Hong, E. J., Kim, Y. J., & Choi, E. H. (2018). Inactivation of Escherichia coli and Staphylococcus aureus on contaminated perilla leaves by Dielectric Barrier Discharge (DBD) plasma treatment. Archives of biochemistry and biophysics, 643, 32-41.

Kalorey, D., Kurkure, N., Sakhare, P., Warke, S., & Ali, M. (2001). Effect of growell on performance, organ weight and serum trace element profile of broilers. Asian-Australasian Journal of Animal Sciences, 14(5), 677-679.

Kim, H.-J., Yong, H. I., Park, S., Kim, K., Bae, Y. S., Choe, W., Oh, M. H., & Jo, C. (2013). Effect of inactivating Salmonella Typhimurium in raw chicken breast and pork loin using an atmospheric pressure plasma jet. Journal of Animal Science and Technology, 55(6), 545-549.

Kim, J. E., Oh, Y. J., Won, M. Y., Lee, K.-S., & Min, S. C. (2017). Microbial decontamination of onion powder using microwave-powered cold plasma treatments. Food Microbiology, 62, 112-123.

Kumar, V., Basu, M., & Rajendran, T. (2008). Mycotoxin research and mycoflora in some commercially important agricultural commodities. Crop protection, 27(6), 891-905.

Kumi, J., Mitchell, N., Asare, G., Dotse, E., Kwaa, F., Phillips, T., & Ankrah, N. (2014). Aflatoxins and fumonisins contamination of home-made food (weanimix) from cereal-legume blends for children. Ghana medical journal, 48(3), 121-126.

Liao, X., Li, J., Muhammad, A. I., Suo, Y., Chen, S., Ye, X., Liu, D., & Ding, T. (2018). Application of a dielectric barrier discharge atmospheric cold plasma (Dbd-Acp) for Eshcerichia coli inactivation in apple juice. Journal of food science, 83(2), 401-408.

Liao, X., Xiang, Q., Liu, D., Chen, S., Ye, X., & Ding, T. (2017). Lethal and sublethal effect of a dielectric barrier discharge atmospheric cold plasma on Staphylococcus aureus. Journal of Food Protection, 80(6), 928-932.

Lin, C.-M., Patel, A. K., Chiu, Y.-C., Hou, C.-Y., Kuo, C.-H., Dong, C.-D., & Chen, H.-L. (2022). The application of novel rotary plasma jets to inhibit the aflatoxin-producing Aspergillus flavus and the spoilage fungus, Aspergillus niger on peanuts. Innovative Food Science & Emerging Technologies, 78, 102994.

Liu, Y., & Wu, F. (2010). Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. Environmental health perspectives, 118(6), 818-824.

Lombard, M. J. (2014). Mycotoxin exposure and infant and young child growth in Africa: what do we know? Annals of nutrition and metabolism, 64(Suppl. 2), 42-52.

López, M., Calvo, T., Prieto, M., Múgica-Vidal, R., Muro-Fraguas, I., Alba-Elías, F., & Alvarez-Ordóñez, A. (2019). A review on non-thermal atmospheric plasma for food preservation: Mode of action, determinants of effectiveness, and applications. Frontiers in microbiology, 10, 622.

Mahendran, R., Abirami, C. K., & Alagusundaram, K. (2017). Cold plasma technology: An emerging non-thermal processing of foods—a review. Engineering interventions in agricultural processing, 33-55.

Markov, K., Mihaljević, B., Domijan, A.-M., Pleadin, J., Delaš, F., & Frece, J. (2015). Inactivation of aflatoxigenic fungi and the reduction of aflatoxin B1 in vitro and in situ using gamma irradiation. Food Control, 54, 79-85.

Mendes-Oliveira, G., Jensen, J. L., Keener, K. M., & Campanella, O. H. (2019). Modeling the inactivation of Bacillus subtilis spores during cold plasma sterilization. Innovative Food Science & Emerging Technologies, 52, 334-342.

Mir, S. A., Dar, B., Shah, M. A., Sofi, S. A., Hamdani, A. M., Oliveira, C. A., Moosavi, M. H., Khaneghah, A. M., & Sant'Ana, A. S. (2021). Application of new technologies in decontamination of mycotoxins in cereal grains: Challenges, and perspectives. Food and chemical toxicology, 148, 111976.

Misra, N., Tiwari, B., Raghavarao, K., & Cullen, P. (2011). Nonthermal plasma inactivation of food-borne pathogens. Food Engineering Reviews, 3, 159-170.

Misra, N., Yadav, B., Roopesh, M., & Jo, C. (2019). Cold plasma for effective fungal and mycotoxin control in foods: mechanisms, inactivation effects, and applications. Comprehensive reviews in food science and food safety, 18(1), 106-120.

Mošovská, S., Medvecká, V., Gregová, M., Tomeková, J., Valík, Ľ., Mikulajová, A., & Zahoranová, A. (2019). Plasma inactivation of Aspergillus flavus on hazelnut surface in a diffuse barrier discharge using different working gases. Food Control, 104, 256-261.

Muhammad, A. I., Chen, W., Liao, X., Xiang, Q., Liu, D., Ye, X., & Ding, T. (2019). Effects of plasma-activated water and blanching on microbial and physicochemical properties of tiger nuts. Food and Bioprocess Technology, 12, 1721-1732.

Nguyen, T., Palmer, J., Phan, N., Shi, H., Keener, K., & Flint, S. (2022). Control of aflatoxin M1 in skim milk by high voltage atmospheric cold plasma. Food chemistry, 386, 132814.

Nikmaram, N., & Keener, K. M. (2022). Degradation of Aflatoxin M1 in skim and whole milk using high voltage atmospheric cold plasma (HVACP) and quality assessment. Food Research International, 162, 112009.

Okyere, A. Y., Rajendran, S., & Annor, G. A. (2022). Cold plasma technologies: Their effect on starch properties and industrial scale-up for starchmodification. Current Research in Food Science.

Omari, R., Tetteh, E., Baah-Tuahene, S., Karbo, R., Adams, A., & Asante, I. (2020). Aflatoxins and their management in Ghana: A situational analysis.

Pankaj, S., Shi, H., & Keener, K. M. (2018). A review of novel physical and chemical decontamination technologies for aflatoxin in food. Trends in Food Science & Technology, 71, 73-83.

Paterson, R. R., & Lima, N. (2010). Toxicology of mycotoxins. Molecular, Clinical and Environmental Toxicology: Volume 2: Clinical Toxicology, 31-63.

Pereira, R. N., & Vicente, A. A. (2010). Environmental impact of novel thermal and non-thermal technologies in food processing. Food Research International, 43(7), 1936-1943.

Pohl, P., Dzimitrowicz, A., Cyganowski, P., & Jamroz, P. (2022). Do we need cold plasma treated fruit and vegetable juices? A case study of positive and negative changes occurred in these daily beverages. Food Chemistry, 375, 131831.

Ramazzina, I., Berardinelli, A., Rizzi, F., Tappi, S., Ragni, L., Sacchetti, G., & Rocculi, P. (2015). Effect of cold plasma treatment on physico-chemical parameters and antioxidant activity of minimally processed kiwifruit. Postharvest Biology and Technology, 107, 55-65.

Reddy, U., & Rani, P. (2000). Effect of processing on detoxification of aflatoxins in maize. Indian Journal of Nutrition and Dietetics, 37(2), 59-63.

Richard, J. L. (2007). Some major mycotoxins and their mycotoxicoses—An overview. International Journal of Food Microbiology, 119(1-2), 3-10.

Sainz-García, E., López-Alfaro, I., Múgica-Vidal, R., López, R., Escribano-Viana, R., Portu, J., Alba-Elías, F., & González-Arenzana, L. (2019). Effect of the atmospheric pressure cold plasma treatment on Tempranillo red wine quality in batch and flow systems. Beverages, 5(3), 50.

Sakudo, A., Toyokawa, Y., Misawa, T., & Imanishi, Y. (2017). Degradation and detoxification of aflatoxin B1 using nitrogen gas plasma generated by a static

induction thyristor as a pulsed power supply. Food Control, 73, 619-626.

Sherif, S. O., Salama, E. E., & Abdel-Wahhab, M. A. (2009). Mycotoxins and child health: The need for health risk assessment. International journal of hygiene and environmental health, 212(4), 347-368.

Shi, H., Cooper, B., Stroshine, R. L., Ileleji, K. E., & Keener, K. M. (2017). Structures of degradation products and degradation pathways of aflatoxin B1 by high-voltage atmospheric cold plasma (HVACP) treatment. Journal of Agricultural and Food Chemistry, 65(30), 6222-6230.

Shi, H., Ileleji, K., Stroshine, R. L., Keener, K., & Jensen, J. L. (2017). Reduction of aflatoxin in corn by high voltage atmospheric cold plasma. Food and Bioprocess Technology, 10, 1042-1052.

Shi, H., Stroshine, R. L., & Ileleji, K. (2014). Aflatoxin reduction in corn by cleaning and sorting. 2014 Montreal, Quebec Canada July 13–July 16, 2014,

Siciliano, I., Spadaro, D., Prelle, A., Vallauri, D., Cavallero, M. C., Garibaldi, A., & Gullino, M. L. (2016). Use of cold atmospheric plasma to detoxify hazelnuts from aflatoxins. Toxins, 8(5), 125.

Sifuentes-Nieves, I., Mendez-Montealvo, G., Flores-Silva, P. C., Nieto-Pérez, M., Neira-Velazquez, G., Rodriguez-Fernandez, O., Hernández-Hernández, E., & Velazquez, G. (2021). Dielectric barrier discharge and radio-frequency plasma effect on structural properties of starches with different amylose content. Innovative Food Science & Emerging Technologies, 68, 102630.

Sowley, E. N. K. (2016). Aflatoxins: A silent threat in developing countries. African Journal of Biotechnology, 15(35), 1864-1870.

Suhem, K., Matan, N., Nisoa, M., & Matan, N. (2013). Inhibition of Aspergillus flavus on agar media and brown rice cereal bars using cold atmospheric plasma treatment. International Journal of Food Microbiology, 161(2), 107-111.

Temba, M. C., Njobeh, P., & Kayitesi, E. (2017). Storage stability of maize-groundnut composite flours and an assessment of aflatoxin B1 and ochratoxin A contamination in flours and porridges. Food Control, 71, 178-186.

Tsakiris, I. N., Tzatzarakis, M. N., Alegakis, A. K., Vlachou, M. I., Renieri, E. A., & Tsatsakis, A. M. (2013). Risk assessment scenarios of children's exposure to aflatoxin M1 residues in different milk types from the Greek market. Food and chemical toxicology, 56, 261-265.

Turner, P. C., Moore, S. E., Hall, A. J., Prentice, A. M., & Wild, C. P. (2003). Modification of immune function through exposure to dietary aflatoxin in Gambian children. Environmental health perspectives, 111(2), 217-220.

Umair, M., Jabbar, S., Ayub, Z., Muhammad Aadil, R., Abid, M., Zhang, J., & Liqing, Z. (2022). Recent advances in plasma technology: Influence of atmospheric cold plasma on spore inactivation. Food Reviews International, 38(sup1), 789-811.

Wagacha, J., & Muthomi, J. (2008). Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. International Journal of Food Microbiology, 124(1), 1-12.

Wang, C., Zhu, Y., & Wang, J. (2016). Comparative study on the heat stability of goat milk and cow milk. Indian Journal of Animal Research, 50(4), 610-613.

Wang, J., Xing, C., Xia, J., Chen, H., Zhang, J., & Yan, W. (2023). Degradation of carbendazim in aqueous solution by dielectric barrier discharge cold plasma: Identification and toxicity of degradation products. Food chemistry, 403, 134329.

Whitaker, T. B., & Johansson, A. S. (2005). Sampling uncertainties for the detection of chemical agents in complex food matrices. Journal of Food Protection, 68(6), 1306-1313.

Wielogorska, E., Ahmed, Y., Meneely, J., Graham, W. G., Elliott, C. T., & Gilmore, B. F. (2019). A holistic study to understand the detoxification of mycotoxins in maize and impact on its molecular integrity using cold atmospheric plasma treatment. Food chemistry, 301, 125281.

Wu, Y., Cheng, J.-H., & Sun, D.-W. (2021). Blocking and degradation of aflatoxins by cold plasma treatments: Applications and mechanisms. Trends in Food Science & Technology, 109, 647-661.

Xiang, Q., Liu, X., Li, J., Liu, S., Zhang, H., & Bai, Y. (2018). Effects of dielectric barrier discharge plasma on the inactivation of Zygosaccharomyces rouxii and quality of apple juice. Food chemistry, 254, 201-207.

Yan, D., Lin, L., Xu, W., Nourmohammadi, N., Sherman, J. H., & Keidar, M. (2019). Cold plasma-based control of the activation of pancreatic adenocarcinoma cells. Journal of Physics D: Applied Physics, 52(44), 445202.

Zhi, A., Shi, S., Li, Q., Shen, F., He, X., Fang, Y., Hu, Q., & Jiang, X. (2023). Aflatoxins degradation and quality evaluation in naturally contaminated rice by dielectric barrier discharge cold plasma. Innovative Food Science & Emerging Technologies, 103426.

Zinedine, A., & Mañes, J. (2009). Occurrence and legislation of mycotoxins in food and feed from Morocco. Food Control, 20(4), 334-344.