

*Review Article***USE AND EFFICACY OF LOW TEMPERATURE PLASMA IN FOODS:
PROMISING INTERVENTION ON AFLATOXIN CONTROL IN MAIZE IN
KENYA – A REVIEW****Kamano HM^{1*}, Okoth M², Wambui-Kogi M² and P Kuloba¹****Hannah Mugure Kamano**

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ABSTRACT

Maize (*Zea mays var. indentata* L.) is the most important food security crop in Kenya and plays an important role in human nutrition. Mycotoxins (MTs) are fungal toxic metabolites which naturally contaminate food and feed. When ingested, inhaled or adsorbed through the skin, even in very small concentrations, are associated with various cancers, retarded growth, suppressed immunity and mutations among other complications. Aflatoxins especially aflatoxin B₁, are considered the most lethal in the group of more than three hundred known mycotoxins. In Kenya, aflatoxin contamination in maize leads to huge losses in the country's breadbasket and also in the grain reserves. One of the possible methods for control of the aflatoxin menace in maize would be through the use of plasma technology. Plasma, an electrically energized matter in form of a gas that is generated at different atmospheric pressures, has several uses. At low temperature, it makes the process of decontamination practical, inexpensive and suitable for products whereby use of heat is not desired. Non thermal plasma, a new discipline in food processing has been shown to destroy micro-organisms including spores to undetectable levels. Over the years, there has been increased concern over the rising cases of aflatoxin poisoning in Kenya due to contaminated maize. The presence of aflatoxins is promoted by various factors, among them poor storage conditions, soil type, insect activity and drought conditions before harvest. Several measures including use of hermetic storage types such as pics (Purdue Improved Cowpea Storage) bags to store maize and proper drying of maize to the right moisture content to discourage mould growth have been suggested and used to tackle the aflatoxin menace. In Mexico and Caribbean countries, nixtamalization is widely practised and has been used to reduce aflatoxin in tortilla. Nixtamalization involves cooking the maize in an alkaline solution resulting in detoxification. The traditional nixtamalization and extrusion cooking processes have been combined in making of the dough (masa) for corn tortillas and have shown better success in elimination of aflatoxin. The main challenge with the use of this method of detoxification is acidification of aflatoxin extracts, which occurs during digestion and can lead to a rebuilding of the aflatoxin molecule leading to poisoning. The application of low temperature plasma technology can bring much needed reprieve in tackling the aflatoxin menace in maize and other foods both in Kenya and even worldwide.

Key words: plasma, maize, aflatoxin, mycotoxins, food safety, food decontamination, aflatoxicosis, technology



INTRODUCTION

Aflatoxin is one of the most studied mycotoxins in the world. It is a toxic metabolite produced by aflatoxigenic fungi of the *Aspergillus* species particularly *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus pseudotamarii* and the more rare *Aspergillus nomius* [1]. Across the value chain, aflatoxins contaminate food and feed leading to devastating acute and chronic effects to huge populations [2]. These crops include maize grains, peanuts, cereals and animal feeds. Aflatoxin contamination in maize is a common occurrence in Kenya particularly in the Eastern and North Rift parts of the country. In 2010, the Kenyan government declared over 2.3 million bags of maize unfit for human consumption due to the high levels of aflatoxins [3]. The last two decades have presented a lot of advancement in technology relating to thermal sciences such as plasma technology. Low temperature plasma (LTP) is a promising intervention in food processing to improve food safety and increase shelf life of foods [4]. Plasma technology is used in production of many products and processes. In the food industry, LTP has been applied in food decontamination, enzyme inactivation, removal of toxins, food packaging applications and treatment of wastewater [5]. The main guiding factors for its increasing application are high demand for high product quality, improved productivity, environmental compatibility, precision and flexibility [6].

Plasma is the fourth state of matter formed by ionization of elements and gases among others. It comprises electrons, protons, and positive and negative ions, neutral molecules, and atoms and a variety of other particles all existing in the same environment. The purpose and behaviour of the produced plasma is influenced by the manner in which the charged and neutral particles interact. Due to these factors, their utilization in biosystems, biochemical and bioengineering processes is playing a very vital role where conventional methods could not have been possible [6]. Among other fields where useful applications have been made are medical treatment especially in sterilization, surgery, material treatment/ surface coating and waste treatment, namely decomposition of compounds containing NO_3 , NH_3 or CN_x groups as an environmental management technique. Others are catalytic reactions in chemical processes, bioprocesses in agriculture and food as a nonchemical gas phase disinfection agent, nanotechnology and biomaterials [7]. Plasma can be generated in different forms: low [non-thermal] or high temperature, high and low pressure. Hence, plasma can be created in various types that include Low Temperature Plasma (LTP) and Low-Pressure Plasma (LPP) [6].

While there has been some progress in the interaction of plasmas with organic materials, the study of plasma-living tissue interaction is an almost unexplored field [8]. Two areas where interaction between plasma and living tissue have been exploited are categorized as destructive and non-destructive; destructive sterilization of medical devices, surgery etcetera and non-destructive treatment of wheat and oat seeds to enhance their germination and early growth [7]. Low temperature plasma did not harm the living cells of the seed and thus implied that this can also be employed in other foods to improve bioavailability or even biosafety such as in the case of aflatoxin in maize. Low temperature plasma has also been used for the treatment of wool fabric in



which the wool characteristics of wettability were changed [9]. Low temperature plasma is usually free of complicated magnetic fields and ultraviolet ray emissions are negligible; thus, it can be used in the field of food processing [10].

METHODOLOGY

A systematic approach was used in compiling the studies undertaken on aflatoxin decontamination of maize grain using low temperature plasma. Full text peer reviewed articles were sought from ISI web of Science and Scopus indexed publications. The review focused on the existing and current methods used in the detoxification of contaminated maize with special emphasis on use of low plasma technology. In the case of plasma related studies, the time frame ranged from 2007 to 2021. The articles were selected after examining their title, abstract and keywords. The keywords used in the search were “maize,” “corn,” “low temperature plasma,” “cold plasma,” “fungi” and “aflatoxin.” The criteria for inclusion of the articles were: relevance of the article to the topic of study, time frame of review and if the abstract is written in English language. There was no limit on the geographical location. The exclusion criteria used were: plasma studies not relevant to the topic of study, studies not falling within the review time frame and abstracts not written in English. Consequently, the authors selected 9 studies that reported successful aflatoxin decontamination (above 50%) in maize grain and other related food matrices using low temperature plasma (Table 1).

RESULTS

Current methods used in reduction of aflatoxins in contaminated maize grain

Several types of methods classified as physical, microbial, chemical and enzymatic are used in reduction of aflatoxin content of maize [11]. Physical methods used include hand sorting to remove visibly infected grain, washing, dehulling, polishing and even classification by colour-based fluorescence under UV light rays. The challenge with these methods is they have to be applied mainly at an industrial level using optical density sorting equipment [11]. Chemical methods used include ozone treatment, alkali, use of mycotoxin binders and even use of organic and inorganic acids [12]. These have a challenge of the possibility of the subsequent products formed after treatment rebuilding back in the body once the treated maize is consumed leading to poisoning. They may also change the organoleptic properties of the food. Microbial methods involve use of dominating microflora that out compete the aflatoxigenic fungi. Enzymatic methods also generally employ the use of enzymes that interfere with the metabolism of the fungi making it impossible for the pathogenic fungi to thrive [12]. These methods are not only expensive and tedious but also time consuming making their implementation quite challenging.

Application of low temperature plasma science in controlling aflatoxicosis

The microbiology and safety of grains, seeds, nuts and their products remain very important due to their extensive use as human food and in livestock feeds. The fungal attack in cereal grains is caused by field fungi, which attack grains at high moistures or storage fungi, which attack grains stored at relatively low moisture [11]. *Alternaria*, *Cladosporium* and *Fusarium* are typical examples of field fungi whilst *Eurotium*,



Aspergillus and *Penicillium* are storage fungi. *Aspergillus spp.* is associated with the aflatoxin poisoning. Aflatoxins are heat resistant and their detoxification from food is not possible by use of normal food processing temperatures. Methods for their detection are also expensive and complicated thus making them unavailable to many. Aflatoxin B1, B2, G1 and G2 are produced by some strains of *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* [12] with aflatoxin B1 being the most common. Use of Atmospheric Pressure Plasma (APP) also commonly referred to as Non-Thermal Plasma (NTP) in the food industry has opened up doors for improvement in the area of food safety. It involves the use of a neutral ionized gas that is ionised by use of high electric current resulting in an environment comprising many reactive species. These include positive ions, negative ions, electrons, photons and molecules (excited or non-excited) at or near room temperature. There are also changes in pressure that give different forms of low- or high-pressure plasma. In the fresh and processed foods industry, plasma has been used in inactivation of microbes albeit at an experimental level. Uses of plasma activated water [13] and plasma packaging have also been implemented in the food industry. The limitation for full upscaling of the plasma technology has been the limited knowledge on the effects of the NTP on the chemical and nutritional characteristics of the food after treatment with plasma. Therefore, more studies to investigate the safety and cost implications of the use of this technology are needed in order to open more avenues for its uptake in many food applications. Several studies have reported the inactivation of fungi and lowering of aflatoxin in grains and nuts [16–19]. A study on the effect of low temperature plasma on *Aspergillus flavus* and *Aspergillus parasiticus* showed a decrease of 5.48 and 5.20 log₁₀ CFU/g respectively after 5 min plasma treatment [20].

Microbial inactivation mechanism of plasma

The use of plasma as a sterilization method was first patented in 1968 and the plasma made from oxygen was first applied in 1989 [17]. Since then, several studies have been carried out to assess the use of plasma for microbial inactivation. The result was that interacting plasma agent with biological matter contributed to lethal action. Plasma treatment could effectively inactivate a wide range of microorganisms including spores and viruses. Effect of plasma on different microorganisms can be completely selective, meaning that it can damage pathogenic microorganisms without damaging host or it can activate different pathways in different organisms [18]. The reactive species in plasma have been known to cause the oxidative effects on the outer surface of microbial cells. Nitrogen and oxygen gas plasma are good sources of reactive oxygen-based and nitrogen-based species such as O., O₂, O₃, OH, NO., NO₂. Chemical rate constant of atomic oxygen for oxidation at room temperature is higher than that of molecular oxygen [19]. Cold plasma species destroy fungi through various pathways finally resulting in their inactivation as summarised in Figure 1. These pathways include destruction of cellular protein, fragmentation and release of DNA, deformation of mycelial tips and accumulation of body lipids [4]. The reactive species in the plasma environment change the DNA of the microorganism and therefore prevent the cells from replicating. The role of UV photons in inactivation of microorganisms when they are subjected to plasma was reviewed in detail by Boudam [21]. Many studies have found that reactive species had the most important role in inactivation of microorganisms whereas the role of UV photons in plasma was minor [22]. However,



results from these studies demonstrated that more research needed to be done over the role of UV photons in plasma. Contribution of each of the above-mentioned mechanisms in inactivation of microorganisms depends on plasma characteristics and type of microorganism. The duration required for inactivation of the microorganism is dependent on the type of device producing the plasma, gas pressure, gas composition, voltage, and the distance of the microbe from the discharge glow. The types of microorganisms include Gram-positive, Gram-negative and spores [23].

The efficacy of different gas compositions and temperatures was studied using *Bacillus spp.* Spores [24]. They found that oxygen-based plasma was more efficient than pure argon plasma. Another study compared the efficiency of exposure of the substrate to plasma [25]. Findings showed that the amount of heat energy transferred to a substrate was less in remote exposure as compared to direct exposure. Many of the short-lived reactive species in the plasma environment did not reach the substrate which made the treatment very inefficient in microbial inactivation.

Mycotoxin degradation mechanism of plasma

This being an emerging area of research, the mechanism of degradation of mycotoxins by use of plasma technology is not fully understood and not much literature is available. However, more recent studies have shown that plasma can indeed lower and, in some cases; completely destroy mycotoxins in different foods as shown in Table 1. A recent study by Shi *et al.* [26] showed a 62 and 82% decrease in aflatoxin in corn with 1 and 10 min treatment respectively at 40% humidity. Air and a modified high oxygen mixture (65% O₂, 30% CO₂, 5% N₂) was used with a combination of different relative humidity levels (5, 40 and 80%). Degradation pathways are associated with molecular structure, the nature of the plasma and most importantly, the interaction of the toxin molecules with the activated plasma species [27]. The release of O* and OH* free radicals during the treatment is highly associated with the degradation of the mycotoxins during plasma treatment [26]. Several studies investigating the effect of cold plasma on aflatoxin B1 (AFB1), have shown a breakdown at C8 and C9 double bond of the dihydrofuran rings [28,29]. The loss of the double bonds at the terminal furan ring is associated with the reduced toxicity and carcinogenicity of AFB1 as it is very characteristic of these two functions. However, more studies are needed in order to give more insights on cold plasma degradation of mycotoxins. Plasma has also been applied in other food applications such as hazel nuts, pistachio, peanuts, date palm fruits and rice extracts and showed promising results (Table 1).

Potential application in food

Non-thermal plasma (NTP) has found use in the food industry in several applications including decontamination of food products. The most recent studies on destruction of fungi and aflatoxin degradation in maize using cold plasma were systematically reviewed (Table 1).

In 2007, Park *et al.* [31] studied the effect of low temperature plasma on aflatoxin B1 among other mycotoxins. The study concluded that the mycotoxins and their cytotoxicity were completely degraded after 5 seconds of treatment. Basaran *et al.* [17] later in 2008 carried out a similar study on hazel nuts, pea nuts and pistachio nuts



contaminated with aflatoxins (B1, B2, G1 and G2) and applying low temperature plasma. The total aflatoxins were reduced by 50% after 20 minutes of air plasma treatment. In 2015, another study by Wang *et al.* [39] found that 88.3% of aflatoxin B1 was degraded from a bearing plate after 10 minutes of treatment with low temperature radio frequency plasma. Sicilaino *et al.* [32] in 2017 concluded that up to 70% detoxification of aflatoxin B1 was achieved using a combination of gases [nitrogen and oxygen] in generating the plasma. The efficacy of destruction was better on aflatoxin B1 and G1 as opposed to B2 and G2. In 2017, Ten Bosch *et al.* [33] found that pure mycotoxins were completely degraded after 60 seconds exposure to low temperature plasma. Shi *et al.* [16] also carried out a study on degradation of aflatoxins in maize by use of low temperature plasma. There was a 62 -82% decrease in the aflatoxin content after exposure for 1 and 10 minutes respectively. Another subsequent study by Sen *et al.* [18] revealed a reduction in the level of aflatoxin B1 of between 72-73%. Over 95% of aflatoxin B1 was destroyed after 30 minutes of exposure to low temperature plasma according to a study by Puligundla *et al.* [34] in 2019. In 2021, Nishimiwe *et al.* [35] carried out a study on the effect of low temperature plasma on the cytotoxicity of aflatoxin. The findings were that there was significant reduction in the cytotoxicity of aflatoxin B1, and thus showing the potential of plasma as a possible safe decontamination method.

A discussion on the future prospects of atmospheric pressure plasma

Atmospheric Pressure Plasma (APP) has shown a promising future in decontamination of foods and feed. A combination of APP and other non-thermal methods of decontamination could be the breakthrough the world has been waiting for. In this case, synergistic effects may be considerable, however, scaling up this technology remains a challenge to be solved. One of the constraints of experimental work on APP is that treatment must not have negative impact on the organoleptic and nutritional properties of food. Nevertheless, there have been limited investigations on this aspect of treatment. At room temperature, the activated species of cold plasma selectively destroy the pathogen without causing any chemical residues [20]. However, more studies should investigate the effect of NTP on the nutritional, chemical as well as shelf life of food and feed. Most importantly, risk assessment of the process is necessary to ascertain the food products are free of toxic residues in future studies. The estimated costs and safety of the gas used in the plasma treatment should also be investigated [38]. Non-thermal plasma is an emerging technology for reducing microbial population on the surface of fresh and processed foods. Various reactive species of plasma interact with biological cell to cause changes on cell wall and morphology of the microorganisms that lead to death. Because of the limited information about the nutritional and chemical changes in food products treated with this technology, especially, sensitive food which has high content of lipid and vitamins, additional issues concerning food quality and safety must be considered [39].

Non-thermal plasma is a promising technology that has the potential to destroy fungi and also detoxify food and feed by degrading the toxins produced. The method could present a more sustainable and cheaper method for decontamination of food and feed. For scaling up of this technology to be possible, several concerns need to be addressed. Cold plasma systems should be tailor made to handle food and feed in bulk either in



batch or continuous systems which should be explored in future studies. Since mycotoxins that are formed on grains such as cereals [maize] are found on the surfaces, they can easily be destroyed by use of cold plasma while ensuring the nutritional integrity of the food or feed [4].

CONCLUSION

The effectiveness of NTP depends on various factors. The type of food under treatment also influences the conditions and duration of the exercise. Some of the limitations that have been identified whilst using APP for food sterilization are pertaining the volume and size of the food sample being treated. Treatment of bulky and irregularly shaped foods is not fully achieved. Secondly, microbial inactivation occurs on the surface of the food being treated since plasma reactive species are limited to penetrate into foods [40]. Uniformity of the substrate affects the effectiveness of the plasma treatment. The effect of cold plasmas on flours has not also received the deserved attention. The suitability of combining plasma treatment to the other conventional methods should be explored. The limitation for full upscaling of the plasma technology has been the limited knowledge on the effects of the NTP on the chemical and nutritional value of the food after treatment with plasma. Therefore, more studies to investigate the safety and cost implications of the use of this technology are needed in order to open up more avenues for its uptake in many food applications.



Table 1: Summary of most recent studies related degradation of aflatoxins in food matrices

| Matrix | Type of aflatoxin | Plasma type | Process settings | Study conclusions | Reference | Year |
|------------------------------------|--|---|---|--|-----------|------|
| N/A | Aflatoxin B1, deoxynivalenol and nivalenol | Low temperature plasma generated using microwave energy | Argon gas at a flow rate of 100L/min for 1-10s | The mycotoxins were completely degraded after 5s exposure to plasma | [31] | 2007 |
| Hazelnuts, peanuts, pistachio nuts | Aflatoxins B1, B2, G1,G2 | Low temperature plasma at low pressure generated using a dielectric barrier discharge (DBD) | Air at 300W ionization power, voltage of 20kV for 5-20 minutes | Upto 50% reduction in the level of total aflatoxins after exposure of between 5-20 minutes | [17] | 2008 |
| Bearing plate | Aflatoxin B1 | Low temperature plasma – radio frequency plasma | Plasma at 15 pascals pressure, 100-300W power, exposure time ranged between 2-10 minutes | Up to 88.3% of aflatoxin B1 degraded after 10 minutes of exposure to plasma | [39] | 2015 |
| Hazel nuts | Aflatoxins B1, B2, G1,G2 | Low temperature plasma generated using a (DBD) | A mixture of pure nitrogen and oxygen at ionization power of 0.4-2 kW, exposure time ranging 1,2,4,12 minutes | Up to 70% reduction in the level of aflatoxins with more effectiveness in decreasing the level of Aflatoxins B1 and G1 as opposed to B2 and G2 | [32] | 2016 |
| Maize | Aflatoxins B1, B2, G1,G2 | Low temperature plasma generated using a (DBD) | Air and modified atmosphere exposed to 50Hz of ionization power, 90 kV voltage | Aflatoxins reduced by 62-80% for 1 and 10 minutes treatment respectively: a much greater reduction | [16] | 2017 |



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|---------------------------|----------------------------|---|--|---|------|------|
| | | | for 1-30 minutes | observed in more humid conditions (80% RH) than in the dry air (5%RH). | | |
| Glass cover slip | Aflatoxin B1 | Low temperature plasma generated using a static induction thyristor | Nitrogen gas at 0.5 atmosphere for 0-30 min | The aflatoxin reduction was up to 90% after exposure of 15 minutes | [40] | 2017 |
| Hazel nuts | Aflatoxins B1, B2, G1,G2 | Low temperature plasma generated at low pressure | Dry air exposed to 655W ionization power, voltage of 13.56 kHz, pressure < 0.25mbar for 30 minutes | A reduction in the level of aflatoxin B1 of between 72-73% | [18] | 2019 |
| Wheat, rice, glass slides | Aflatoxin B1 | Low temperature plasma generated using corona discharge plasma jet | Air at 20kV voltage, ionization power of 58kHz for 5,10,15,20,25,30 minute intervals | Over 95% of aflatoxin B1 destroyed after 30 minutes of exposure | [34] | 2019 |
| N/A | Aflatoxin B1, Hep G2 cells | High voltage low temperature plasma generated using a DBD | Plasma generated at 85kV and exposure time of 0,2,5,10, 20 minutes | There was significant reduction in the cytotoxicity of aflatoxin B1, showing potential plasma as possible safe decontamination method | [35] | 2021 |



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