Degradation Kinetics of Aflatoxin B_1 using UV-Plasma assisted Fluidized Bed Drying in Corn

A thesis submitted towards the partial fulfilment of the requirements for the degree of Master of Technology in Food Technology and Biochemical Engineering course affiliated to Faculty of Engineering and Technology, Jadavpur University.

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ACKNOWLEDGEMENT

I would like to express my heartfelt gratitude to the individuals who have played a pivotal role in the successful completion of my thesis. Their unwavering support, guidance, and encouragement have been instrumental in shaping this research endeavour.

First and foremost, I extend my deepest appreciation to my guide **Dr. Prasanta Kumar Biswas, Professor, Department of Food Technology and Biochemical Engineering, Jadavpur University**, whose expertise, dedication, and mentorship have been invaluable throughout this journey. Your patience, insightful feedback, and continuous support have not only enriched the quality of this work but have also contributed significantly to my personal and academic growth.

I am also grateful to our **Head of the Department, Dr. Sunita Adhikari (Nee Pramanik)** for providing me with the opportunity to pursue this research and for your constant belief in my capabilities. Your leadership and vision have created an environment conducive to academic excellence, and I am fortunate to have been a part of your department.

I extend my thanks to **Dr. Uma Ghosh, Former Professor, Department of Food Technology and Biochemical Engineering, Jadavpur University** for inspiring my interest in this field and for imparting valuable knowledge that served as the foundation for this research. Your guidance and mentorship during my earlier academic years have been instrumental in shaping my academic pursuits.

I would like to acknowledge the invaluable contributions of **Dr. Debasis Ghosh**, whose collaboration and assistance were crucial in conducting experiments and gathering data for this thesis. Your dedication and expertise were instrumental in the successful execution of this research.

Finally, I express my gratitude to **Ms. Ishita Mondal, Research Scholar, Department of Food Technology and Biochemical Engineering**, for her valuable insights, constructive criticism, and support throughout this project. Your collective wisdom and experience have enriched the depth and breadth of this work.

I would also like to thank all the non-teaching staffs, all my batch mates, and lab mates for their kind cooperation.

Last, but not the least, I wish like to express my profound gratitude and my deep feelings for my family who have been the constant source of my energy, inspiration and determination for going ahead with my academic pursuit.

I am deeply indebted to each of you for your unwavering support, encouragement, and guidance. This thesis would not have been possible without your contributions, and I am fortunate to have had the privilege of working with such exceptional individuals. Thank you for being an integral part of my academic journey.

ABSTRACT

Aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* in various agricultural food products such as wheat, peanuts, corn, walnut, red chilli etc. They are carcinogenic and leads to a variety of serious threats to human and animal health. There are about 18 different types of aflatoxins out of which aflatoxin B_1 (AFB_1) is the most potent. This study investigates the degradation kinetics of AFB_1 during the UV-Plasma assisted fluidized bed drying process in corn. In this research, a novel approach combining UV radiation and plasma technology with fluidized bed drying was employed to reduce AFB_1 levels in corn. The system utilizes a fluidized bed dryer as the drying medium, which enhances mass and heat transfer, ensuring efficient decontamination. UV radiation and plasma discharge are employed simultaneously to target AFB_1 molecules and induce degradation through photochemical and chemical reactions. The study involves systematically varying process parameters, including UV exposure time, and drying temperature, to assess their influence on AFB_1 degradation. Samples of contaminated corn are subjected to these conditions, and AFB_1 concentrations are monitored at specific time intervals using High Pressure Thin Layer Chromatography (HPTLC). The kinetics of AFB_1 degradation are analysed using mathematical model such as first-order reactions. Preliminary results demonstrate that the combined UV-Plasma assisted fluidized bed drying process effectively reduces AFB_1 levels in corn. The degradation kinetics follow first-order kinetics, and the rate of AFB_1 degradation is influenced by the applied UV exposure time, and drying temperature. These findings suggest that the proposed technology has the potential to become a promising method for mitigating AFB_1 contamination in corn, contributing to food safety and public health.

Keywords: Aflatoxin B_1, corn, UV-Plasma assisted fluidized bed drying, HPTLC, degradation kinetics.

LIST OF ABBREVIATIONS

- AFB_1 → Aflatoxin B_1
- AFB_2 → Aflatoxin B_2
- AFG_1 → Aflatoxin G_1
- AFG_2 → Aflatoxin G_2
- AFM_1 → Aflatoxin M_1
- AFM_2 → Aflatoxin M_2
- IUPAC → International Union of Pure and Applied Chemistry
- % → Percentage
- °C → Degree Centigrade
- aw → water activity
- MFO → Mixed Function Oxidase
- NADPH → Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
- DHOMST → Dihydro-O-methylsterigmatocystin parasiticus
- DNA → Deoxyribonucleic Acid
- PCR → Polymerase Chain Reaction
- CPT → Carnitine Palmitoyl Transferase
- RNA → Ribonucleic Acid
- mg → Milligram
- HCC → Hepatocellular Carcinoma
- ROS → Reactive Oxygen Species
- GIT → Gastrointestinal Tract
- BGY → Bright greenish yellow
- CAS → Controlled Atmosphere Storage
- MAP → Modified Atmosphere Packaging
- kGy → KiloGray
- Co → Cobalt
- Ir → Iridium
- Cs → Cesium
- Tm → Thulium
- USA → United States of America
- EB → Electron Beam
- FDA → Food and Drug Administration
- UV → Ultraviolet
- nm → nanometer

- cm → centimeter
- µw → microwatt
- min → minute
- hr/h → hour
- Vis → Visible
- PL → Pulsed Light
- J → Joule
- MHz → Megahertz
- GHz → Gigahertz
- Mm → millimeter
- W → Watt
- RNS → Reactive Nitrogen Species
- CD → Corona Discharge
- DBD → Dielectric Barrier Discharge
- APPJ → Atmospheric Pressure Plasma Jets
- RFP → Radio Frequency Plasma
- N_2 → Nitrogen molecule
- kpps → kilo point per second
- O_2 → Oxygen molecule
- CO_2 → Carbon Dioxide
- RH → Relative Humidity
- HVACP → High Voltage Atmospheric Cold Plasma
- CDPJ → Corona Discharge Plasma Jet
- V → Volt
- GRAS → Generally Recognized As Safe
- EOW → Electrolyzed Oxidizing Water
- NEOW → Neutral Electrolyzed Oxidizing Water
- mV → millivolt
- ORP → Oxidation-Reduction Potential
- DO → Dissolved Oxygen
- AEOW → Acidic Electrolyzed Oxidizing Water
- HR-FT-ICR-MS → High-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometer
- NMR → Nuclear Magnetic Resonance
- ACC → Available Chlorine Concentration
- HClO → Hypochlorous Acid
- ClO^- → Hypochlorite Ion
- N → Normality

- GC-MS → Gas Chromatography Mass Spectrometry
- FT-IR → Fourier Transform Infrared Spectroscopy
- TLC → Thin Layer Chromatography
- CAP → Cold Atmospheric Plasma
- SBD → Surface Barrier Discharge
- CAPP → Cold Atmospheric Pressure Plasma
- FB_1 → Fumonisins B_1
- HVACP → High Voltage Atmospheric Cold Plasma
- s → second
- g → gram
- MC → Moisture Content
- ACC → Aflatoxin B_1 Contaminated Corn
- HPTLC → High Performance Thin Layer Chromatography
- PPE → Personal Protective Equipment
- FBD → Fluidized Bed Dryer
- L → litre
- kW → kilowatt
- F → Fluorescence
- ml → millilitre
- µl → microlitre
- ° → degree
- SEM → Scanning Electron Microscope
- VAC → Volts Alternating Current
- kVA → kilovolt amperes
- Hz → Hertz
- kV → kilovolt
- SE → Secondary Electrons
- EDS → Energy-dispersive X-ray spectroscopy
- µg → microgram
- ppm → parts per million
- kg → kilogram
- Conc. → Concentration

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Chapter 1:
Introduction and Literature Review

1.1 Introduction on Aflatoxin

Secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* in various agricultural food products such as wheat, peanuts, corn, walnut, red chilli etc. are called Aflatoxins [1]. They are carcinogenic and leads to a variety of serious threats to human and animal health. They cause various complications like hepatotoxicity, teratogenicity, and immunotoxicity [2].

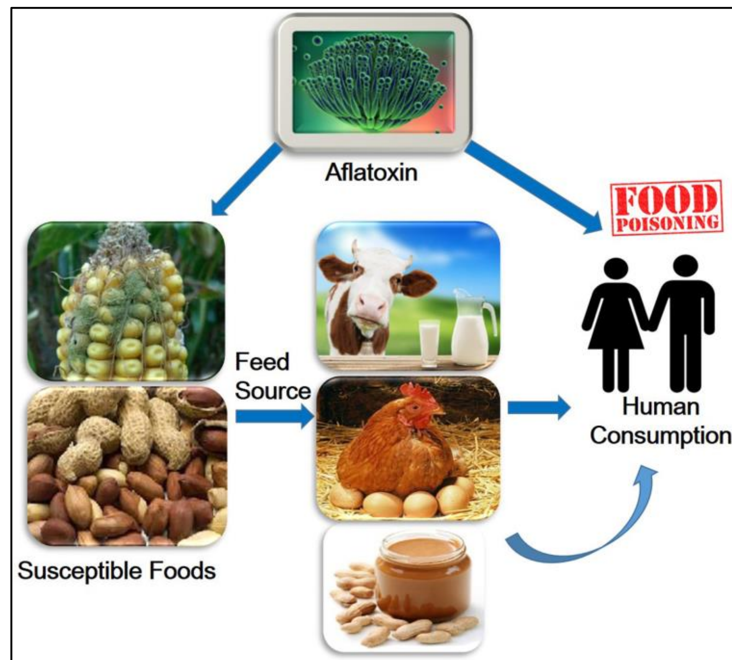


Fig 1: Overview of aflatoxin effect on humans [2]

The word aflatoxin is coined from the following set up. The first letter "A" stands for the genus *Aspergillus,* the next three letters "FLA" for the species *flavus,* and the noun "TOXIN" meaning poison [3].

Aflatoxins are difuranocoumarin molecules synthesized through the polyketide pathway. There are 18 different types of aflatoxins out of which 6 are considered important. These include AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2 respectively. These groups show difference in molecular orientation. For example, B-group aflatoxins (B_1 and B_2) have a cyclopentane ring and exhibit blue fluorescence while G-group aflatoxins (G_1 and G_2) have a lactone ring and shows yellow-green fluorescence under ultraviolet (UV) light [1].

Among these Aflatoxin B_1 is the most common and widespread and is responsible for nearly 75% of all aflatoxin contamination of food and feed [10]. IUPAC name of AFB_1 is 2,3,6α,9α-tetrahydro-4 methoxycyclopenta [c] furo [2,3:4,5] furo [2,3-h] chromene-1,11-dione [4].

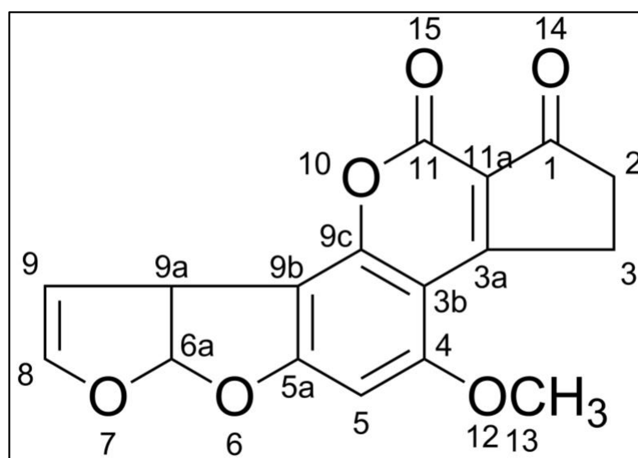


Fig 2: Molecular Structure of AFB_1 [4]

The production of aflatoxin in different agricultural food crops depends on various biotic and abiotic factors. This includes temperature, water activity (aw), substrate composition, storage time, carbon and nitrogen source, light, pH, the content of carbon dioxide (CO_2) and oxygen (O_2), loss of integrity of grains due to insects or thermal/mechanical damage, and the interaction between fungal species that grow in the same ecological zone. Out of these temperature is considered as the main factor for production of aflatoxin. The possibility of aflatoxin production in agricultural products increases in hot and humid climate. The optimal temperature for aflatoxin production ranges between 24-30°C with some alteration depending on substrate and strain. In case of shelled peanuts, corn, rice, and cottonseeds the optimal temperature for production of aflatoxin by *Aspergillus flavus* and *Aspergillus parasiticus* ranges between 20-30°C while a limited quantity of aflatoxin is produced at 10°C or 40°C [5].

1.2 Types of Aflatoxin

Table 1: Origin of aflatoxins and the products most exposed to contamination [6]

Aflatoxin	Source	Frequently Contaminated Products
❖ Difurocoumarocyclopentenone		
Aflatoxin B_1	*Section Flavi: A. flavus, A. togoensis, A. pseudotamarii, A. austwickii, A. aflatoxiformans, A. arachidicola, A. cerealis, A. mottae, A. minisclerotigenes, A. luteovirescens (formerly A. bombycis), A. novoparasiticus, A. parasiticus, A. nomius, A. pipericola, A. pseudonomius, A. pseudocaelatus, A. transmontanensis, A. sergii. Section Ochraceorosei: A. ochraceoroseus, A. rambellii. Section Nidulantes: A. miraensis, A. astellatus. A. venezuelensis, A. olivicola.*	Cereals (like rice, sorghum, wheat, barely, maize), oil seeds (like cotton seeds, rape seeds, seeds of sunflower), seeds of nuts (like pistachio, groundnut, peanuts), spices (like black and red pepper, turmeric, all spices, ginger), dairy products, meats, dried fruits, fruit juices, eggs, foods derived from these products.

Aflatoxin B_2	*Section Flavi: A. flavus, A. aflatoxiformans, A. pseudotamarii, A. cerealis, A. austwickii, A. minisclerotigenes, A. arachidicola, A. luteovirescens, A. mottae, A. novoparasiticus, A. nomius, A. pipericola, A. parasiticus, A. pseudonomius, A. pseudocaelatus, A. transmontanensis, A. sergii. Section Ochraceorosei: A. ochraceoroseus and A. rambellii.*	Cereals (like rice, sorghum, barely, wheat, corn), seeds oil (like sunflower seed, oilseed rape cotton seed), nuts (like groundnut, pistachio, peanuts), Spices (like, black and red pepper, ginger, turmeric), milk products, meats, dried fruit, eggs, fruit juices, and foodstuffs derived from such products.
Aflatoxin B_{2a}	Hydroxylated metabolite of aflatoxin B_1 is obtained by addition of water to the terminal furan double bond under acidic conditions present in the liver, stomach or soil and is produced naturally by *A. Parasiticus & A. flavus*.	NA
Aflatoxin M_1	Hepatic microsomal mixed-function oxidase (MFO) system (mainly cytochrome) produces hydroxylated metabolite of aflatoxin B_1 in mammalian liver & is formed in-vitro by liver homogenates from aflatoxin B_1 produced naturally by *A. Parasiticus A. flavus.*	Milk (human milk included) and dairy products. Meat products. Groundnut and corn moulds.
Aflatoxin M_2	Mammalian hepatic microsomal MFO produces hydroxylated metabolite of aflatoxin B_2 which is produced naturally by *A. parasiticus.*	Same as aflatoxin M_1.
Aflatoxin M_{2a}	Hydration of the acid terminal furan ring of aflatoxin M_1 to yield the hemiacetal derivative. Homogenates in the liver in-vitro.	Milk & dairy products.
Aflatoxin P_1	De-methylated aflatoxin B_1 metabolite by O-demethylase-catalysed liver microsomal oxidase.	Dairy products.
Aflatoxin Q_1	Hydroxylated metabolite of aflatoxin B_1 by microsomal enzymes in higher vertebrate and poultry liver (main monkey metabolite of aflatoxin B_1).	Assumed to be in edible parts of bovine fed on aflatoxin B_1-contaminated feed.
Aflatoxin Q_{2a}	Acid hydration of aflatoxin Q_1.	NA

Aflatoxicol (R_0)	In-vitro biotransformation of aflatoxin B_1 by a soluble cytoplasm reductase system in fish, rats and human liver produced naturally by *A. parasiticus* and *A. flavus.*	Predominantly avian goods (primary metabolite in B_1-contaminated feed fed to avian species). Dairy products. Does not accumulate in edible parts of aflatoxin B_1-infected bovine and pig feed.
Aflatoxicol M_1	Reduced metabolites of liver-catalysed aflatoxin B_1, aflatoxin R_0 or aflatoxin M_1 by soluble NADPH-dependent reductases.	Milk & dairy products.
Aflatoxicol H_1	Reduced metabolites of soluble NADPH dependent reductases catalysed by aflatoxin B_1 and aflatoxin Q_1 in the liver.	Milk & dairy products.

❖ Difurocoumarolactone		
Aflatoxin G_1	*A. aflatoxiformans, A. flavus, A. cerealis, A. austwickii, A. minisclerotigenes, A. arachidicola, A. luteovirescens, A. mottae, A. novoparasiticus, A. nomius, A. pipericola, A. parasiticus, A. pseudonomius, A. pseudocaelatus, A. transmontanensis, A. sergii.*	Cereals (like rice, sorghum, wheat, barely, maize), oily seeds (like cotton seeds, rape seeds, sunflower seeds), nuts (like peanuts, groundnuts, pistachio nuts), spices (like ginger, black and red pepper, turmeric), milk products, meats, dried fruits, fruit juices, poultry, and feed and foods extracted from such products.
Aflatoxin G_2	*A. flavus, A. austwickii, A. aflatoxiformans, A. arachidicola, A. cerealis, A. mottae, A. minisclerotigenes, A. nomius, A. luteovirescens, A. transmontanensis, A. parasiticus, A. novoparasiticus, A. pseudocaelatus, A. pipericola, A. sergii, A. pseudonomius.*	Same as aflatoxin G_1.
Aflatoxin G_{2a}	A hydroxylated metabolite of aflatoxin G_1 obtained by catalytic addition of water to the terminal furan double bond in the presence of acidic conditions in the liver, intestine, or soil (no evidence of unique enzyme involvement) manufactured naturally by *A. flavus.*	NA

Aflatoxin GM_1	MFO produces a hydroxylated metabolite of aflatoxin G_1 in the liver of mammals. *A. parasiticus* fed aspertoxin is used as a precursor. *A. flavus* manufactures it naturally.	Dairy products and milk.
Aflatoxin GM_2	Hydroxylated mammalian liver derivative of aflatoxin G_2 by MFO in-vitro developed by Dihydro-O-methylsterigmatocystin parasiticus (DHOMST).	Dairy products and milk.
Aflatoxin GM_{2a}	Metabolite of aflatoxin G_{M1} in the mammalian liver. Hydration of the acid terminal furan ring of aflatoxin M_1 to generate hemiacetal derivative. Homogenates in the liver in-vitro.	Dairy products and milk.
Parasiticol (aflatoxin B_3)	An aflatoxin G_1 metabolite from biodegradation (hydrolysis and decarboxylation reactions) in *Rhizopus stolonifer, A. flavus, Rhizopus oryzae, Rhizopus arrhizus.*	Same as aflatoxin G_1 and B_1.

❖ Others		
Parasiticol (aflatoxin B_3)	An aflatoxin G_1 metabolite from biodegradation (hydrolysis and decarboxylation reactions) in *Rhizopus stolonifer, A. flavus, Rhizopus oryzae, Rhizopus arrhizus,* produced naturally by *A. flavus, A. parasiticus, A. nomius, A. novoparasiticus, and A. mottae.*	Same as aflatoxin G_1 and B_1.
Aspertoxin b	*A. parasiticus* and *A. flavus*	Mainly plant products that are vulnerable to contamination with *A. parasiticus* and *A. flavus*; Food products of animal origin are not considered to be important.

1.3 Mechanism of action of Aflatoxin

The numerous health effects caused by aflatoxins are the result of common or different underlined mechanisms of action that can be or not crossed in the complex net of biochemical reactions in the human body. At molecular level, a key player in aflatoxin toxicity is the epoxide derivative of aflatoxin B_1. This epoxide has the ability to bind on DNA and to disrupt transcription and translation activities, thus initiating carcinogenesis. Due to the oxidative nature of this toxic derivative it can release free radicals causing cell damage. The evolution of molecular techniques like microarrays and PCR was critical in understanding more precisely

the mechanism of action of aflatoxins, starting from molecular level, to genes, cells and organ level. Recent gene expression studies have shown that aflatoxins can cause:

a) Down regulation of carnitine palmitoyl transferase (CPT) system in mitochondrion leading to decrease of body weight gain

b) Down regulation of fatty acid metabolism pathway increasing liver weight and causing fatty liver

c) Up-regulation of cell proliferation pathway causing carcinoma, and

d) Down regulation of B cell activation lowering immunity in birds fed aflatoxin.

In addition, aflatoxins can impair protein biosynthesis by forming adducts with DNA, RNA and protein, inhibit RNA synthesis, DNA-dependent RNA polymerase activity, and cause degranulation of endoplasmic reticulum. Susceptibility to aflatoxin is higher in younger ages; it is sex-related (testosterone-dependent) and with a great inter- and intra-species variability depending on the biochemical detoxification abilities [7].

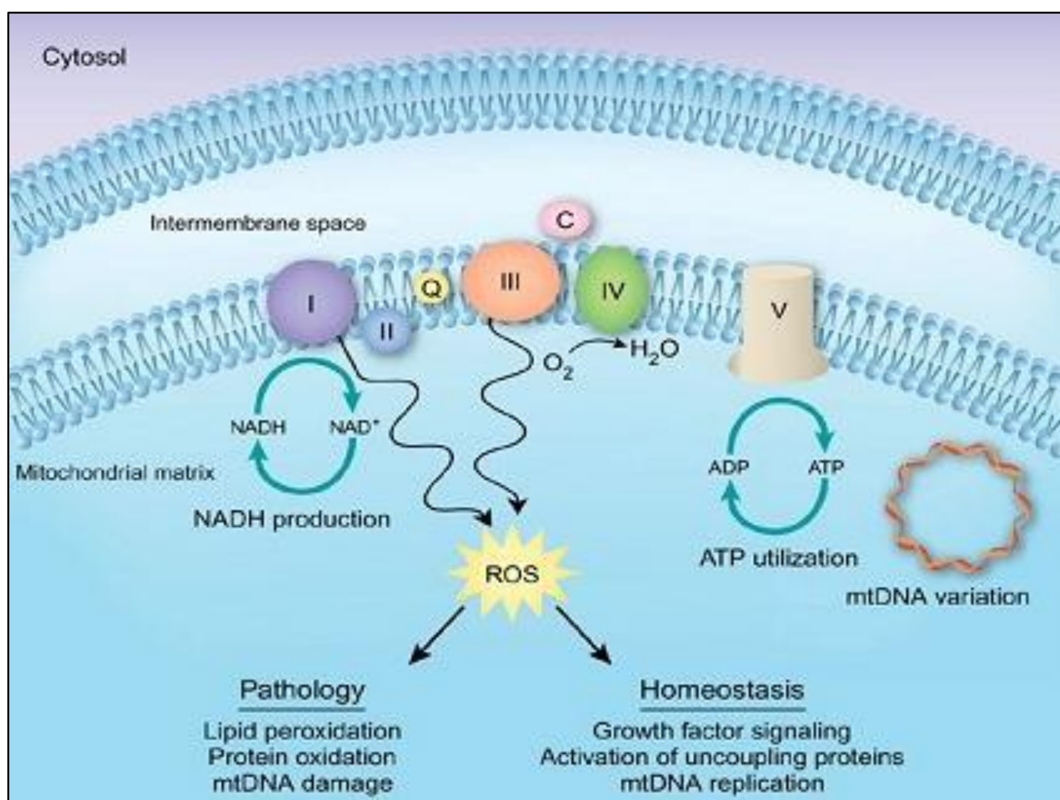


Fig 3: Mechanism of cell damage in aflatoxin toxicity [7]

1.4 Occurrence of different Aflatoxins in food and feed around the world

Table 2: Occurrence of aflatoxins in food and feed around the world [8]

Country	Food matrix	Aflatoxin	Range (µg/kg)
Turkey	Almond	AFB1	1–13
Turkey	Butter	AFM1	<0.001–0.100
Brazil	Cashew nuts	Total AFs	0.60–31.50
United States	Chilies	AFB1	<2
Costa Rica	Corn	Total AFs	24
Zimbabwe	Corn	AFB1	0.75–26.6
India	Corn	AFB1	48–383
Serbia	Corn	Total AFs	1.01–86.10
Vietnam	Corn	AFB1	1.0–34.80
Turkey	Cream cheese	AFM1	0.1–0.70
Pakistan	Dried Fruits	AFB1	0.04–9.80
Turkey	Feed	AFB1	0–5
Turkey	Figs	Total AFs	0.1–28.20
Nigeria	Ginger	Total AFs	0.11–9.52
Ethiopia	Groundnuts	Total AFs	15–11,900
Turkey	Hazelnut	AFB1	0.07–43.60
Serbia	Infant formula	AFM1	<0.03–0.02
Turkey	Lentil	AFB1	0.57–1.74
Turkey	Maize flour	AFB1	0.041–1.12
Egypt	Meat products	Total AFs	0.47–2.10
Greece	Milk	AFM1	<0.005–0.02
Iran	Milk (cow)	AFM1	0.006–0.18
Brazil	Milk (cow)	AFM1	0.05
Italy	Milk (cow/buffalo)	AFM1	0.004
Portugal	Milk (cow)	AFM1	0.005–0.07
Japan	Nuts	AFB1	0.17–2.59
Saudi Arabia	Nuts	Total AFs	1.0–110
Malawi	Nut-based foods	AFB1	0.1–40.60
Zambia	Peanuts	AB1	0.015–46.60
Taiwan	Peanut products	Total AFs	0.2–513.40
Turkey	Red-chili powder	AFB1	0.025–40.90
China	Rice	AFB1	0.03–20
India	Rice	AFB1	0.1–308
Pakistan	Rice	AFB1	0.04–21.30
China	Rice	AFB1	0.1–136.60
Tunisia	Sorghum	AFB1	0.4–25.1
Italy	Spices	AFB1	0.59–5.38
Malaysia	Spices	AFB1	0.58–4.64
Tunisia	Wheat	AFB1	0.12–18
Malaysia	Wheat	AFB1	0.55–5.07
China	Yogurt	AFM1	0.05
Iran	Yogurt	AFM1	0.006–0.021

1.5 Overview of the health risks associated with Aflatoxin consumption

High exposure to aflatoxins (above 6000 mg), through digestion, can cause severe intoxication, which results in direct liver damage and subsequent illness or death. The mechanism of aflatoxin acute toxicity is related to malfunction of the liver, which is the target organ of aflatoxin toxicity. No animals are immune to the acute toxic effects of aflatoxins.

1) Genotoxicity & Carcinogenicity- Aflatoxins as toxins are among the most carcinogenic substances known and the major cancerous hepatocellular carcinoma (HCC) risk factor. Cancer is broadly defined as any disease in which normal cells are damaged and do not undergo programmed cell death (apoptosis) as fast as they divide via mitosis. Aflatoxin B_1 is metabolized by cytochrome-P450 enzymes to the reactive intermediate AFB_1-8, 9 epoxide (AFBO) which binds to liver cell DNA, resulting in DNA adducts. DNA adducts interact with the guanine bases of liver cell DNA6. This is thought to cause mutations at the codon 249 hotspot in exon 7 of the *p53* gene, an important gene in preventing cell cycle progression, when there are DNA mutations, or signalling apoptosis [9]. These mutations seem to affect some base pair locations more than others - for example, the third base of codon 249 of the p53 gene appears to be more susceptible to aflatoxin-mediated mutations than nearby bases [10]**.** As a consequence of aflatoxin genotoxicity potential, even short-term exposure can lead to a risk of developing liver cancer. Although this mechanism of genotoxicity is well recognized and understood, non-genotoxic effects also exist and resulting cancers in the form of enzymatic necrosis from the hyphae of pathogenic Aspergilli with the production of the hyphal digestive enzymes: the primary are proteases (protein), amylases (carbohydrate), and lipases (fats) [11].

2) Neurotoxicity- The effects of aflatoxins in the nervous system are related to the action of their metabolite AFB-8, 9 epoxide and its ability to create DNA and protein adducts, to inhibit protein, RNA and DNA synthesis, to promote mitochondrial directed apoptosis of the nerve cells and to produce Oxygen Reactive Species (ROS) [12, 13, 14, 15]. Aflatoxins cause tumours in both the central and peripheral nervous system [16]. Several neurotransmitters are affected by aflatoxins in animals, such as acetylcholinesterase enzymes that affects the memory, learning and cognitive functions [17], dopamine, serotonin as well as tyrosine and tryptophan, leading to neurocognitive decline and alteration of sleep cycle, dullness, restlessness, muscle tremor, convulsions, loss of memory, epilepsy, idiocy, loss of muscle coordination, and abnormal sensations [18, 19, 20]. Liver malfunction caused by aflatoxicosis has as secondary effect, i.e. the gathering of non-detoxified ammonia in the brain. Ammonia can pass the brain barrier causing encephalopathy. Toxic encephalopathy due to aflatoxin involves multiple symptoms such as loss of balance, recent memory decline, headaches, light-headedness, spaciness/disorientation, insomnia, loss of coordination etc.

3) Mutagenicity- Aflatoxin B_1 is a potent mutagen. The mutagenic effects commence by microsomal activation of the mixed function oxidase to the epoxide form [21]. Mutagenicity studies on bacteria suggest that the possible mechanism of mutagenesis may be initiated by an aflatoxin B_1-DNA binding process, leading to the formation of single-stranded gaps as a result of inhibition of DNA polymerase activity at DNA binding sites [22]. This stimulates an error prone repair system that may induce mutation by (1) insertion of erroneous nucleotides opposite spontaneously occurring apurinic sites or (2) through errors during filling of single-stranded gaps that do not contain additional DNA lesions [23].

4) Teratogenicity- Aflatoxin B_1 is teratogenic due to its prenatal effects on certain animals. Since it is a potent inhibitor of protein synthesis in eukaryotic cells, it impairs differentiation in sensitive primordial cells [24]. Susceptibility to teratogens varies greatly during the course of gestation, although, in general, the embryo is most susceptible during the early stages of morphological differentiation. Therefore, aflatoxin B_1 is not teratogenic at all dosage levels [25]. For example, a single intraperitoneal injection of aflatoxin B_1 at 4 mg/kg body weight, administered to a hamster on the 8th day of pregnancy, caused a high proportion of malformed and dead or reabsorbed foetuses [26].

5) Hepatotoxicity- Hepatic tissues of the liver absorb toxic substances from the bloodstream and from circulation. Aflatoxins, specifically aflatoxin B_1, is eventually secreted in the liver where it is toxic to cells [27]. Aflatoxin in the liver is degraded in two phases by (1) biotransformation to a more toxic product and (2) detoxification to a less toxic and easily excretable product [28].
   In Phase 1, aflatoxin B_1 is metabolized to its reactive form by the microsomal mixed function oxidase by means of oxidation, reduction, and hydroxylation. In this phase, there is an increase in enzyme levels and the rapid metabolism of aflatoxins [29].
   In Phase 2, the reactive intermediate is detoxified into a less toxic metabolite and then excreted from the body. This process occurs through any of the mechanisms of glucuronidation, sulfation, acetylation, or reaction with glutathione, depending on the type of organism. Biotransformations are not as rapid in Phase 2 compared with Phase 1. Reactive intermediates, such as 2, 3-epoxy-aflatoxin B_1 react with macromolecules of the liver cells, resulting in fatty and pale livers, moderate to extensive necrosis, and haemorrhage.

6) Aflatoxicosis- This is the major toxicity syndrome associated with aflatoxins and it can be subdivided into (1) primary aflatoxicosis and (2) secondary aflatoxicosis.
   Primary aflatoxicosis can be further subdivided into (a) acute and (b) chronic aflatoxicosis. Acute primary aflatoxicosis results when high to moderate concentrations of aflatoxin are consumed, usually resulting in death of the animal. It is therefore expressed as the death of the animal in a time dependent on the particular sensitivity.
   Chronic primary aflatoxicosis occurs when moderate to low concentrations of aflatoxin are consumed. The result of chronic aflatoxicosis, in most cases, is a reduction in growth rate and reproductive efficiency.
   Secondary aflatoxicosis occurs when low concentrations of aflatoxin are consumed. The biological effects associated with secondary aflatoxicosis are (1) the impairment of native resistance and (2) immunogenesis. Impairment of native resistance occurs through the reduction of phagocytic effectiveness of macrophages and nonspecific humoral substances, such as complements. Impairment of immunogenesis involves the cell-mediated immune system and therefore reduces the effectiveness of elective vaccination setups in the animals [30].

7) Reproductive Toxicity- Aflatoxins can inhibit the stereoidogenesis in mice by reduction in 3b- and 17b-hydroxysteroid dehydrogenases probably due to alterations in mitochondria, inhibition of protein synthesis/enzyme activity, alteration in plasma membrane of Leydig cell due to lipid peroxidation and/or irregular protein biosynthesis. Higher frequency in sperm count, morphology and motility was observed in the higher exposed infertile men. Spermatotoxic activity of aflatoxins support these conclusions. Possible mechanism involve disruption on sex hormone synthesis, protein synthesis inhibition, fat metabolism disruption and, possibly, direct lysis of sperm cell membrane [31, 32].

8) Impaired Growth in Children- Chronic, subclinical exposure does not lead to symptoms as severe as acute aflatoxicosis. Children are particularly affected by aflatoxin exposure, which leads to delayed development [33] and impaired growth [34]. Food borne aflatoxin exposure is common where childhood stunting and underweight are also frequent, due to a variety of possibly interacting factors such as enteric diseases, socioeconomic status, and suboptimal nutrition [35]. A cross sectional study in young children in relation to the dietary aflatoxin exposure and impaired growth revealed a striking association between exposure to aflatoxin in children and both stunting (a reflection of chronic malnutrition) and being underweight (an indicator of acute malnutrition) [36]. It was seen that children still partially breast-fed had lower exposure, almost certainly reflecting lower toxin levels in milk than in weaning and family foods. Thus, growth faltering occurs at a time of change to solid foods, when there is co-exposure to aflatoxin and a plethora of infectious hazards, such as malaria, diarrhoea and respiratory infections.

9) Intestinal Malfunctions- Intestinal cells are the first cells to be exposed to aflatoxins [37]. Rapidly dividing and activated cells and tissues with a high protein turnover are predominant in gut epithelium; consequently, the effect of aflatoxins as disruptors of protein synthesis is of great importance.
Intestinal homeostasis depends on the balance between nutrition, immune system and gut microbiota and this homeostasis is crucial for the health of the GIT, the absorption of nutrients and the functions of the immune system.

10) Immunosuppression- Aflatoxin B_1 is immunosuppressive in animals, with particularly strong effects on cell-mediated immunity and increased susceptibility to bacterial and parasitic infections [38]. Aflatoxins decrease complement activity leading to impairment of phagocytosis and reduce chemotactic ability of leucocytes. Immunosuppression caused by exposure to aflatoxins is the output of their interference with normal function of B and T-cells [39], reduction of the phagocytosis by macrophages and of the activity of Vitamin K [40]. In addition, the impairment of protein synthesis caused by dietary aflatoxin could account for the lack of humoral immunity without the necessity of B and T cell destruction. Human monocytes treated with aflatoxin B_1 resulted in impaired phagocytic and microbicidal activity and decrease in specific cytokine secretion [41]. Human exposure to aflatoxins is linked to increased prevalence of infection [42].

1.6 Control of Aflatoxin Production

The toxic effects of aflatoxins on man and animals arise from the ingestion of food and feedstuff contaminated with aflatoxins. Due to the increasing number of reports on the toxic nature of this chemical, there is a need to prevent contamination of products by aflatoxin-producing molds or to control mold growth by manipulation of their microenvironment. Prevention of mold contamination and or mold growth can be achieved by one or more of the following methods: [3]

I. Improved farm management
II. Antifungal agents
III. Genetic engineering approaches
IV. Rapid screening techniques
V. Control of environmental conditions:
    a. Moisture
    b. Temperature

c. Gaseous atmosphere

I. Improved Farm Management: It is seen that fungal attack occurs in the field; therefore, contamination of plants with aflatoxin usually occurs in the growing plant. To prevent contamination, good management practices, such as using sound, fungus-free seeds for planting, controlling insects and plant disease, and controlling irrigation practices, should be employed. In addition, harvesting plants at maturity and proper adjustment and operation of harvesting equipment to avoid damage to crops should also be employed. Good storage facilities with sound environmental conditions can be used to prevent contamination by aflatoxin-producing molds.

II. Antifungal Agents: Certain short-chain fatty acids have been shown to inhibit the growth of fungi. Propionic acid was found to be an effective fungi static agent against *Aspergillus flavus*. It is a volatile fatty acid that has been used to preserve high moisture corn without reducing its value as animal feed. The activity of propionic acid is enhanced by acid pH and certain feed ingredients. Salts of sorbic acid have also been found to inhibit growth of *A. flavus* and *A. parasiticus*. The compound dichlorvos has been found to inhibit aflatoxin biosynthesis by *A. parasiticus* even though it has no effect on fungal growth.

III. Genetic Engineering Approaches: The idea of genetic engineering to develop varieties that may be resistant to mold attack or inhibit toxin production was believed to be an ideal solution. Several laboratory studies and field work have shown varietal differences in corn with respect to resistance to *A. flavus* and aflatoxin production.
A survey of a series of corn hybrids concluded that evaluation and selection is probably more difficult for plant resistance to infection by *A. flavus* and aflatoxin production than it has been for plant resistance to diseases.

IV. Rapid Screening Techniques: Aflatoxins in contaminated seeds are generally found in only a relatively small number of kernels. In order to ensure that only high-quality food material enters the food system, rapid detection methods and removal of contaminated seeds are required. Manual, mechanical, and electronic methods have been used to exclude damaged or discoloured peanuts from food stocks and these methods have reduced the aflatoxin content to negligible levels in processed peanuts. Other techniques involve the use of low power microscopy, BGY fluorescence test, air classification & density segregation. Although these methods have low precision, they have been employed to reduce aflatoxin contamination or mold contamination in certain commodities, particularly corn.

V. Control of Environmental Conditions (Storage): Storage conditions play an important role in the physicochemical and microbiological quality of commodities. The specific fungal species that develop in a given environment depend on moisture, temperature, presence of competing microorganisms, and the nature and physiological state of the commodity. These factors influence mold metabolism and their capacity to utilize food material for growth and metabolite production.

a. Moisture: Moisture levels and temperature are the most important factors in the protection of stored grains against mold growth and aflatoxin production. It has been seen that *A. flavus* does not invade grain and oilseeds when the moisture content is in

equilibrium with a relative humidity of 70% or less. At this relative humidity, the moisture content of wheat is about 13% and approximately 7 to 10% for commodities rich in oil, such as peanuts and cottonseed. An increase in the moisture content of stored commodities, especially corn, has been found to result in a tenfold increase in aflatoxin production. This increase in moisture content at localized areas in the stored commodity is due to inadequate aeration that may cause significant variation in temperature within the stored crop. This may result in pockets of high moisture in the cooler areas of the crop (wet spot), resulting in mold growth. Therefore, there is a need for continuous and adequate aeration and a constant check on the moisture content of stored commodities and temperature of the storage environment.

b. Temperature: Temperature is one of the most critical environmental factors influencing mold growth and aflatoxin production. It was found that aflatoxin can be produced at temperatures as low as 7.5° to 10°C. Low-temperature storage can be very suitable for controlling the growth of *A. flavus*. However, temperature should be reduced to 5°C as quickly as possible throughout the product, especially perishable commodities. Modern practices of storing or enclosing commodities, especially perishables in airtight plastic containers or self-sealing plastic foils before storing under refrigerated conditions, may prove to be potentially hazardous. The airtight conditions may keep the products moist, particularly on the surface of commodities, thereby providing favourable conditions for the growth of *A. flavus*. Therefore, even though refrigeration temperatures may be an ideal means of controlling mold growth, care should be taken not to turn it into an ideal environment for aflatoxin production by maintaining temperatures preferably at 0°C. However, the use of cold temperatures for largescale storage of agricultural crops is generally not economically feasible.

a. Modified Gaseous Atmosphere: A modified atmosphere is one in which the normal composition of air has been changed or modified. This modification usually results in a reduction in the O_2 of the air, while increasing the level of CO_2 and N_2 in the atmosphere. Controlled atmosphere storage (CAS) has been used to control growth of and aflatoxin production by *A. flavus* in bulk storage of peanuts. Furthermore, CAS has been used to control insect infestation in stored grains, thereby preventing mold infection of stored products. MAP on the other hand is the enclosure of the food product in gas barrier materials which results in a reduced O_2 content of the air within the package headspace and increased CO_2 and N_2 levels. While carbon dioxide has proved to increase the lag phase and hence decrease the rate of mold growth, there is little or no data with respect to aflatoxin production in food packaged under CO_2-enriched atmospheres.

Of the potential control measures, both CAS and MAP appears to be natural and inexpensive methods of controlling mold growth and aflatoxin production in stored commodities susceptible to fungal and insect attack.

1.7 Review of the traditional methods used for Aflatoxin B_1 degradation

A) Physical Degradation

1. Degradation of aflatoxins by Irradiation: In general irradiation can be classified into two forms: ionizing (e.g. X-rays, ultraviolet rays, gamma rays, and electron beam) and non-ionizing irradiation (e.g. radio waves, microwaves, infrared waves, and visible light waves). Extensive research has been performed to apply irradiation technology for eliminating mycotoxins [43].

   a. Degradation of aflatoxins by gamma irradiation: Gamma rays are electromagnetic radiation emitted from the decay of an unstable source such as a radioactive isotope (e.g., ^{60}Co, ^{192}Ir, ^{139}Cs, and ^{70}Tm). Gamma rays are the preferred source of irradiation for food processing because of their high reactivity and penetrability. The irradiation of food up to an overall dose of 10 kGy causes no toxicological hazards and no special microbiological or nutritional problems. Gamma irradiation can facilitate radiolysis of water and generation of highly reactive free radicals like radical hydrogen, superoxide radical and hydroxyl ion, which play an important role in the destruction of aflatoxins. A study conducted examined the structure of AFB_1 radiolytic products. This analysis revealed that the double bond of the terminal furan ring was no longer in existence in most of radiolytic products due to free-radical addition reaction during gamma irradiation. The double bond in the terminal furan ring of AFB_1 is known to be associated with its toxicity. In the liver, the oxidation of the double bond in the terminal furan ring of AFB_1 by hepatic cytochrome P450 enzymes (CYPs) yields AFB_1-exo-8, 9-epoxide, which can react with the N7 atom of guanine to generate pro-mutagenic DNA adducts. Thus, the loss of the double bond of the terminal furan ring in AFB_1 after gamma irradiation treatment led to a significant reduction of its cytotoxicity in Pk15, HepG2 and SH-SY5Y cells. The efficiency of gamma irradiation-assisted degradation of aflatoxins depends on many factors, such as radiation dose, mycotoxin concentration, water content and matrix composition. Most of the studies in the literature concluded that gamma irradiation at levels ranging from 5 to 10 kGy could eliminate a significant amount of aflatoxins in food products. The efficiency of aflatoxins degradation in red chillies through gamma irradiation was studied and observed that the application of 6 kGy reduced the level of AFB_1 by more than 86%. Similarly, it was reported that the treatment of corn with gamma ray at 5 kGy resulted in a 69.8% reduction of AFB_1 level, while the dose of 10 kGy detoxified AFB_1 by 94.5%. Experiments performed also showed that gamma irradiation at 5 to 10 kGy was sufficient to eliminate 70.6 to 84.2% of AFB_1 in Brazil nuts. Other studies nevertheless showed that gamma irradiation is not very effective in aflatoxins degradation. Gamma irradiation at a dose of 10 kGy did not significantly affect the aflatoxins content in black and white pepper. In addition, gamma irradiation study was conducted with poultry feed and obtained only 18.2, 11.0, 21.1 and 13.6% reduction of AFB_1, AFB_2, AFG_1 and AFG_2, respectively, with a dose of 15 kGy. Gamma irradiation also presents high microbial inactivation capacity, thus allowing the reduction of microbial load and extension of food shelf life. The interest in the adoption of gamma irradiation in food decontamination is increasing as consumers are beginning to appreciate the benefits of irradiated food. Up to now, more than 55 countries including China, USA, Japan

and European countries have approved this technology in food processing under certain conditions. However, gamma irradiation seems to be not suitable for food with high lipid and vitamin content, mainly due to the extensive peroxidation of unsaturated bonds in the polyunsaturated fatty acids, resulting in the increase of the onset of oxidative rancidity in food.

b. Degradation of aflatoxins by electron beam irradiation: Electron beam (EB) irradiation has also shown potential for degrading aflatoxins, and this technology has the advantages of short processing time, low equipment costs, and dosage control. EB irradiation of AFB_1 in an acetonitrile solution resulted in the formation of two degradation products, $C_{14}H_{12}O_5$ and $C_{17}H_{14}O_5$. In an aqueous solution, the reduction of AFB_1 by EB irradiation led to the generation of five by-products, with a loss of the double bond in the terminal furan ring in four of the five products. Preliminary safety assessments of the degradation products conducted with the Ames test and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay, showed that the mutagenicity and cytotoxicity of EB-irradiated AFB_1 were significantly decreased. Nevertheless, the decontamination efficiency of EB irradiation is less than that of gamma irradiation. Gamma irradiation at doses of 5 and 10 kGy reduced AFB_1 content in Brazil nuts by 70.6 and 84.2%, respectively, whereas EB irradiation at the same doses resulted in AFB_1 reduction by 53.3 and 65.7%, respectively. Additionally, it was found that EB irradiation was not very effective in degrading AFB_1 in peanut meal, since an irradiation dose of 300 kGy, which was ten times of the maximum allowable dosage permitted by FDA, was required for achieving a 70% reduction of AFB_1.

c. Degradation of aflatoxins by ultraviolet irradiation: Ultraviolet (UV) irradiation has been known for decades as an effective physical method for the destruction of aflatoxins due to their photosensitivity. As a non-thermal food decontamination technology, UV irradiation offers the advantages of being practical and cost effective, and eco-friendly as it exhibits no toxic effects and no waste generation. AFB_1 absorbs UV rays at 222, 265 and 362 nm, with the absorption maxima at 362 nm. The formation of hydroxyl free radicals ($OH^{\bullet}$) initiated by UV irradiation could attack the terminal double bond at the C8-C9 position of AFB_1. The results of the Ames test and cell viability assay indicated that the mutagenicity and cytotoxicity of the photodegradation products were much lower than that of AFB_1. UV intensity and irradiation duration are key factors affecting aflatoxins elimination efficiency. UV irradiation at 800 $\mu w\ cm^{-2}$ for 30 min was sufficient to completely remove AFB_1 in peanut oil, whereas the toxin was reduced by about 79 and 85% at the intensity of 200 and 400 $\mu w\ cm^{-2}$, respectively. The treatment of peanuts with UV light at 254 nm was found to reduce AFB_1 level by 59.7% within 2 h, and by up to 99.1% after 10 h. Moreover, it was also seen that more than 80% of aflatoxins in wheat samples were eliminated when exposed to UV short wave at 254 nm for 160 min. In general, the application of moderate doses of UV irradiation does not cause extensive adverse effects on the physicochemical and sensory characteristics of food products. UV light can easily penetrate through clear or transparent liquids. Nevertheless, its penetration capacity into solid materials is limited, which results in low decontamination efficiency in food products with high content of suspended solids. Thus, opaque or granular food products need to be presented as a thin layer during UV irradiation detoxification.

d. Degradation of aflatoxins by photocatalysis: Several recent studies have demonstrated that the use of UV–visible irradiation in combination with semiconducting photocatalysts can increases the efficiency of aflatoxins degradation in liquid matrix. During the photocatalytic degradation process, the photo-generated valence band holes (h^+), hydroxyl free radicals ($OH^•$) and superoxide radical ($O_2^{•-}$) are capable of directly oxidizing AFB_1. The most widely used photocatalyst is TiO_2, as it is highly active under UV irradiation, non-toxic, highly efficient, and has long-term photostability. Evaluation of photocatalytic degradation of AFB_1 in methanol by activated carbon supported TiO_2 catalyst (AC/TiO_2) under UV–Vis light was also done. The degradation rate reached 95% within 120 min by UV–Vis irradiation in the presence of AC/TiO_2 (6 mg mL^{-1}), whereas only 50% of AFB_1 was degraded by UV–Vis irradiation alone. Approximately 60.4% removal of AFB_1 was achieved within 120 min by employing this TiO_2/UV system, which was higher than that of UV photolysis alone (35.1%). In a study, AFB_1 and AFB_2 in Sudanese peanut oil were almost completely removed by immobilized TiO_2 within 4 min using UV–Vis light. Additionally, the physicochemical characteristics of peanut oil, including fatty acids composition, free fatty acids content, peroxide value, saponification value, acid value, iodine value, moisture and volatile matters as well as the refractive index did not significantly change during the photocatalytic treatment. The major disadvantage of TiO_2 photocatalyst is its restriction to UV irradiation, which accounts for only 4% of the sunlight spectrum. Thus, efforts have been devoted to developing visible-light-driven photocatalysts for aflatoxins reduction.

e. Degradation of aflatoxins by pulsed light: Another non-thermal technology called pulsed light (PL) has also been employed for the decontamination of aflatoxins in food and feed. Pulsed light is an FDA-approved technology for the rapid and efficient surface decontamination of food products with an upper limit fluence of 12 J cm^{-2}. This novel technology creates short, high-intensity flashes of broadband emission light (100–1100 nm) including ultraviolet, visible and infrared rays. The intensity of the emitted light is about 20, 000 times more intense than direct sunlight at sea-level. It was reported that eight flashes of PL (light flux of 1 J cm^{-2} during one 300 ms flash) degraded 92.7% of AFB_1 in water. PL treatment of rough rice for 80 s reduced AFB_1 and AFB_2 by 75.0 and 39.2% respectively, while a treatment time of 15 s reduced AFB_1 and AFB_2 in rice bran by 90.3 and 86.7% respectively. Using a brine shrimp lethality assay and the Ames fluctuation assay, further demonstrated that PL treatment inactivated the cytotoxicity and mutagenicity of AFB_1 and AFB_2. Similarly, it was observed a 91% reduction in aflatoxins in dehulled peanuts after PL treatment (0.4 J cm^{-1} per pulse). Additionally, it was shown that PL treatment did not significantly affect the chemical qualities such as peroxide value, fatty acid content and acidity value of oil extracted from peanuts, although slight changes in peanut kernel colour were noted. However, despite the progress mentioned above, the breakdown products of aflatoxins after PL treatment are still under investigation. Characterization of the possible photodegradation pathways of aflatoxins under PL treatment will provide deep insight into the degradation mechanism and kinetics of this technology. There is also a need for designing cost-effective PL equipment capable of generating high UV output in order to apply this emerging technology at an industrial scale.

f. Degradation of aflatoxins by microwave heating: Microwaves are electromagnetic waves in frequencies ranging from 300 MHz to 300 GHz with wavelengths from 1 m to 1 mm. The frequency of a domestic microwave is 2450 MHz, while industrial microwave systems generally use either 915 or 2450 MHz. Microwave heating is a unique volumetric heating method, which transforms electromagnetic field energy into thermal energy through the polarization effect of the electromagnetic radiation. Microwaving has been widely applied in the drying, heating, cooking and extraction of food. Some reports also documented the use of microwave heating in degrading aflatoxins in food materials. Evaluation of the effect of microwave treatment during alkaline-cooking of aflatoxin-contaminated corn was done. The results showed that AFB_1 and AFB_2 were reduced by 36 and 58%, respectively, when the contaminated corn was microwave-heated at a power output of 1650 W for 5.5 min. Moreover, microwave cooking of peanut and peanut products, respectively, resulted in a 50 to 60% reduction of the level of AFB_1, while AFB_2 content decreased to non-detectable limits. Overall, microwave heating demonstrated moderate success in reducing aflatoxins levels in food commodities. Microwave manufacturers are able to customize equipment to specific applications and food product types. However, it has always remained a challenge to address the non-uniform temperature distribution during microwave heating, which may lead to the formation of cold and hot spots in the treated food. Aflatoxins in cold spots cannot be effectively detoxified, while overheating in hot spots may cause nutritional losses and quality deterioration. More studies are also required to optimize the process parameters to increase the degradation efficiency along with structure elucidation and safety evaluation of the degradation products.

2. Degradation of aflatoxins by cold plasma: Plasma is a highly energized ionized gas, generally known as the fourth state of matter, consisting of electrons, ions, UV irradiation and reactive neutral species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). In general, plasma can be loosely categorized according to its temperature into thermal and cold (also referred to as non-thermal) plasma. The generation of cold plasma can be achieved by means of electrical discharges in gases at atmospheric pressure and a temperature range from 30 to 60°C. Moreover, cold plasma can be described by the system used to generate it, for example, corona discharge (CD), dielectric barrier discharge (DBD), atmospheric pressure plasma jets (APPJ), and radio frequency plasma (RFP). This rather new technology has been shown to enable the rapid detoxification of aflatoxins at ambient temperature and pressure conditions. DBD N_2-plasma was used to degrade aflatoxins inoculated on dehulled hazelnuts. The results showed that approximately 70% of AFB_1 and total aflatoxins were detoxified following 12 min exposure to a 1150 W plasma treatment. Moreover, it was demonstrated that AFB_1 and AFG_1 were more sensitive to N_2-plasma treatment compared to AFB_2 and AFG_2, respectively. Another study using N_2-plasma found that the performance of plasma in degrading AFB_1 was connected to processing time and plasma frequency. According to the results, 15 min of N_2-plasma treatment at 1.5 kpps reduced AFB_1 by more than 90% and the effects of carrier gas type (air and modified atmosphere gas containing 65% O_2, 30% CO_2, and 5% N_2) and relative humidity (RH of 5, 40 and 80%) on AFB_1 degradation induced by high voltage atmospheric cold plasma (HVACP). The best result was observed while operating the plasma in a 40% RH modified atmosphere gas wherein 88.3% of AFB_1 in corn was eliminated within 5 min. In the case of peanut samples, 5 min of agitated APPJ treatment decreased total aflatoxins levels by 38% without negatively affecting

chemical qualities of peanut oil. Evaluation of the effect of corona discharge plasma jet (CDPJ) treatment on the degradation of AFB_1 on glass slides and in spiked food samples. Following CDPJ treatment for 30 min, the AFB_1 concentration on slides was decreased by 95%, whereas AFB_1 levels in rice and wheat were only reduced by 56.6 and 45.7%, respectively. The authors suggested that this disparity could be due to chemical interaction of AFB_1 with the food matrix resisting the degradation of the toxin. In summary, the degradation efficiency of aflatoxins by cold plasma is highly dependent on the plasma system used, the operating parameters applied (e.g., working gas, moisture, and energy input), exposure time, and type of food products. Cold plasma has been presented as a viable technology for aflatoxin decontamination in food and feed. However, this novel technology is still in the early stages of evaluation. Further research is needed to optimize plasma process conditions for different food materials. Moreover, the potential negative impacts of plasma treatment on the nutritional value and organoleptic properties of food products need to be addressed and regulated at the forefront. Finally, new plasma generating equipment, tailored for the food industry, that are easy to operate, cost effective compared with conventional approaches, and guarantee safety by adequate insulation, grounding and shielding are needed.

B) Chemical Degradation

1. Degradation of aflatoxins with ozone: Ozone is a powerful oxidizing agent with a redox potential of 2.07 V, capable of detoxifying a wide variety of emerging contaminants in food commodities. Normally, ozone can be produced by several methods such as ultraviolet irradiation, electrical discharge in oxygen and electrolysis of water. Spontaneous decomposition without forming hazardous residues on treated products makes ozone a promising alternative in food processing industry. Studies on mycotoxins degradation by ozone accelerated after it was granted Generally Recognized As Safe (GRAS) status for use in food and water. Ozonation has already been successfully employed for the removal of AFB_1 in laboratory-scale trials in food, such as corn, wheat, red pepper and peanuts. The results of sub-chronic toxicity studies on rats indicated that ozone treatment of aflatoxin-contaminated peanuts could significantly reduce hepatotoxicity and nephrotoxicity of AFB_1. The ozonolysis mechanism of AFB_1 involves an electrophilic attack on the double bond in difuran ring moiety, leading to the formation of a primary ozonide followed by rearrangement into molozonide derivatives such as aldehydes, ketones and organic acids. The ozonolysis efficiency of AFB_1 depends not only on ozone concentration and exposure time but also on the moisture content of the food matrix. Successful commercial application of this technology requires the development of equipment that can efficiently eliminate aflatoxins from food and feed at high-capacity and continuous mode.

2. Degradation of aflatoxins with electrolyzed oxidizing water: Electrolyzed oxidizing water (EOW) is generated by the passage of a dilute salt solution (~1% NaCl) through an electrolytic chamber, where the anode and cathode are separated by a membrane. The two major types of EOW are neutral electrolyzed oxidizing water (NEOW), which has a pH of 5.0–6.5, an oxidation–reduction potential (ORP) of 800–900 mV and high dissolved oxygen (DO); and acidic electrolyzed oxidizing water (AEOW), which has a pH < 3.0, a high ORP >1000 mV and high DO. EOW has gained immense popularity in recent years as a novel broad-spectrum disinfectant in the food, medical and agricultural industries. EOW returns to ordinary water after use and poses no threat to

humans and the environment. Chlorine and high ORP are known to be the main contributors to the sanitization effect of EOW. Apart from antibacterial and antifungal properties, EOW has shown promising potential for aflatoxin detoxification in agricultural products. A 85% reduction was observed in AFB_1 level in peanuts after soaking for 15 min in AEOW. This treatment did not significantly affect the appearance and nutrient contents of peanuts. The AFB_1 degradation product following AEOW treatment was identified as AFB_1-8-chloro-9-hydroxy by high-resolution Fourier transform ion cyclotron resonance mass spectrometer (HR-FT-ICR-MS) and 1H nuclear magnetic resonance (1H NMR), and was determined to be devoid of mutagenicity and cytotoxicity. In addition, the authors proposed that the high level of available chlorine concentration (ACC) in EOW was the major factor responsible for AFB_1 degradation, while ACC in the form of HClO was probably more efficient than ACC derived from the ionic form of ClO^- in detoxifying AFB_1.

3. <u>Degradation of aflatoxins with organic acids:</u> Organic acids are considered as food-grade additives by most food-safety control authorities worldwide. The degradation of aflatoxins in rice, pepper and soybeans by soaking in organic acids has been studied and proved effective in laboratory experiments. In a study, soaking of contaminated soybeans in 1.0 N citric acid, lactic acid, and tartaric acid for 18 h at room temperature reduced AFB_1 by 94.1, 92.7, and 95.1%, respectively. The degradation efficiency depends on temperature, moisture content, treatment time, and acid concentration. Acidulation by citric acid reduced AFB_1 by 97% within 96 h at room temperature, while 98% reduction was achieved by boiling AFB_1 for 20 min in citric acid. The effect of extrusion-cooking process was evaluated with addition of citric acid on the degradation of aflatoxins in sorghum. The result showed that the degradation rate increased with the increase of moisture content and acid concentration. Citric acid and lactic acid hydrolyzed the double bond of difuran ring moiety in AFB_1, resulting in the formation of AFB_1-8-hydroxy as a major degradation product, which exhibited reduced toxicity on HeLa cells. The high level of organic acids in fruit juices makes them natural and readily available sources for the degradation of AFB_1 in contaminated food.

4. <u>Degradation of aflatoxins with plant extracts:</u> Natural plant extracts have been widely used as food additives and pharmaceuticals since ancient times for their anti-microbial, anti-inflammation, anti-oxidation, and immune boosting activities. The potential use of natural plant extracts in mycotoxin detoxification has received much attention in recent years. Aqueous extracts of 31 medicinal plants were investigated for their ability to detoxify AFB_1. They found that among the different plant extracts, the leaf extract of Adhatoda vasica Nees showed the highest AFB_1-detoxifying activity with a degradation efficiency as high as 98% after incubation at 37°C for 24 h. The same group of researchers also demonstrated that pre-feeding rats with spray-dried formulation of Adhatoda vasica Nees leaf extract could counteract the hepatotoxicity induced upon subsequent exposure to AFB_1. Plant extracts are very complex mixtures, and their components varies with plant species and chemotype, phonological stage, tissue, and method of extraction. There has been little in-depth research to identify active compounds in plant extracts responsible for the observed detoxification of aflatoxins. A preliminary study showed that partially purified alkaloids from Adhatoda vasica Nees leaf extract exhibited strong AFB_1 detoxification activity. It should also be noted that aflatoxin degradation with plant extracts could be the result of various compounds working a multi-step process. Further studies are needed to provide deeper insight into

the action model as well as the potential interactions of natural plant extracts with food and feed matrices.

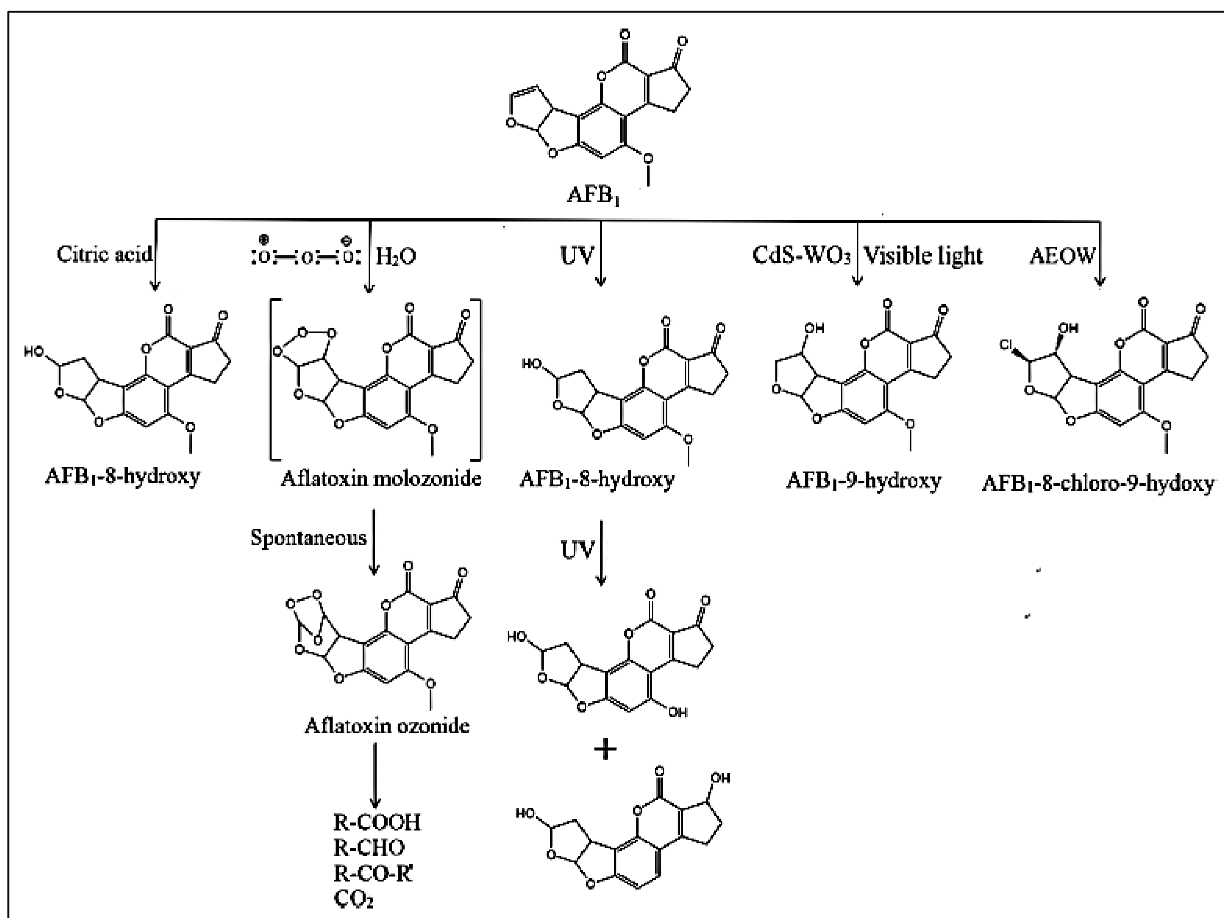


Fig 4: Degradation pathways of AFB_1 by several physical and chemical treatments [43]

C) Biological Degradation

Scientists have come to favour the use of biological methods for the degradation of aflatoxins. This method takes the form of microbial or enzymatic transformation of aflatoxins into non-toxic or less toxic metabolites. A number of microorganisms from various sources such as soil, water, animal excreta and even contaminated food materials have been reported to be capable of degrading aflatoxins. To gain further insights into microbial degradation, researchers are now focusing on the identification of functional genes and purification of aflatoxin-degrading enzymes. The use of microbes as well as isolated enzymes for the removal of aflatoxins in food and feed is an efficient, specific, and environmentally friendly decontamination strategy.

1. Microbial degradation of aflatoxins: A number of Pseudomonas strains, such as *P. putida* 12–3, *P. aeruginosa* N17-1, and *P. anguilliseptica* have been reported to be capable of degrading aflatoxins. The abilities of *P. putida* MTCC1274 and *P. putida* MTCC2445 to remove AFB_1 from culture medium were investigated, and the results showed that both strains of *P. putida* degraded more than 90% of AFB_1 during an incubation period for 24 h. Gas chromatography mass spectrometry (GC–MS) and Fourier transform infrared spectroscopy (FT-IR) analyses revealed that the lactone ring

of AFB_1 was modified by *P. putida*, yielding three non-fluorescent compounds (AFD_1, $C_{16}H_{14}O_5$; AFD_2, $C_{17}H_{14}O_6$; and AFD_3, $C_8H_4O_3$). Moreover, the in vitro cytotoxicity test on HeLa cells showed that all these metabolites are less toxic when compared with AFB_1. A preliminary study investigated the mycotoxin degradation ability of thirty-two Rhodococcus strains. The results showed that 59% of the investigated Rhodococcus strains could degrade more than 90% of AFB_1 and remove its genotoxicity. Thin layer chromatography (TLC) analysis confirmed the cleavage of the lactone group of AFB_1 by *R. erythropolis* ATCC 4277. The potential use of *R. pyridinivorans* K408 to detoxify AFB_1 was evaluated in corn-based whole stillage, and found that the AFB_1 level in solid and liquid phases of the whole stillage was eliminated by 63 and 75%, respectively. Microbial consortium can be constructed using bacteria, fungi or both. There are complex and intricate networks of communication in microbial communities, which play decisive role in the adaptation of microbes in the presence of xenobiotic compounds. A thermophilic microbial consortium TADC7 was developed with stable and efficient AFB_1 degradation activity. Cell-free supernatant of TADC7 consortium showed AFB_1 degradation rate of more than 95% in a wide temperature range from 50 to 90°C. According to 16S rDNA sequencing analysis, Geobacillus and Tepidimicrobium may play major roles in AFB_1 degradation by TADC7 consortium.

2. Enzymatic degradation of aflatoxins: Important technological advances in the field of molecular biology have allowed scientists to isolate and identify aflatoxin-degrading enzymes from microbes. Biodegradation based on cell-free enzyme preparations avoids the shortcoming of applying whole microorganisms, which aparts from their degradation activity, may impair the organoleptic properties of food commodities and produce undesirable compounds. Previous studies have documented that aflatoxins could be degraded by laccases, peroxidases, oxidases and reductases. While these aflatoxin-degrading enzymes are effective, there are practical obstacles to their application in food and feed industry. One of the main problems is the low production yields of these enzymes in their native hosts. Thus, it would be economically unfeasible to produce them using standard fermentation or culture techniques. However, production of these enzymes using recombinant DNA technologies could address this issue. On the other hand, modern food and feed processing tends to be sophisticated and precise, and generally requires high temperature, extreme pH, and the use of solvents. However, the stability and catalytic efficiency of wild-type enzymes under stress conditions is less than satisfactory. The recently developed enzyme engineering technologies like random mutagenesis and directed evolution will open the gate to the improvement of properties of aflatoxin-degrading enzymes. Biological agents to be used in practice as animal feed additives must rapidly degrade mycotoxins into non-toxic metabolites, under different oxygen conditions and in a complex environment. Up to now, there is still lack of scientific methods standardized to investigate the activity of mycotoxin-degrading enzymes in the digestive systems of animals. In vitro models mimicking the digestive process in the gastric-intestinal tract have been proposed to assess the effects of different feed enzymes (phytase, xylanase, and β-glucanase) on release of trace elements in feedstuffs. Thus, it is possible to develop in vitro models to evaluate the efficiency of aflatoxin-degrading enzymes in reducing the bioavailability of aflatoxins in the digestive tract of animals.

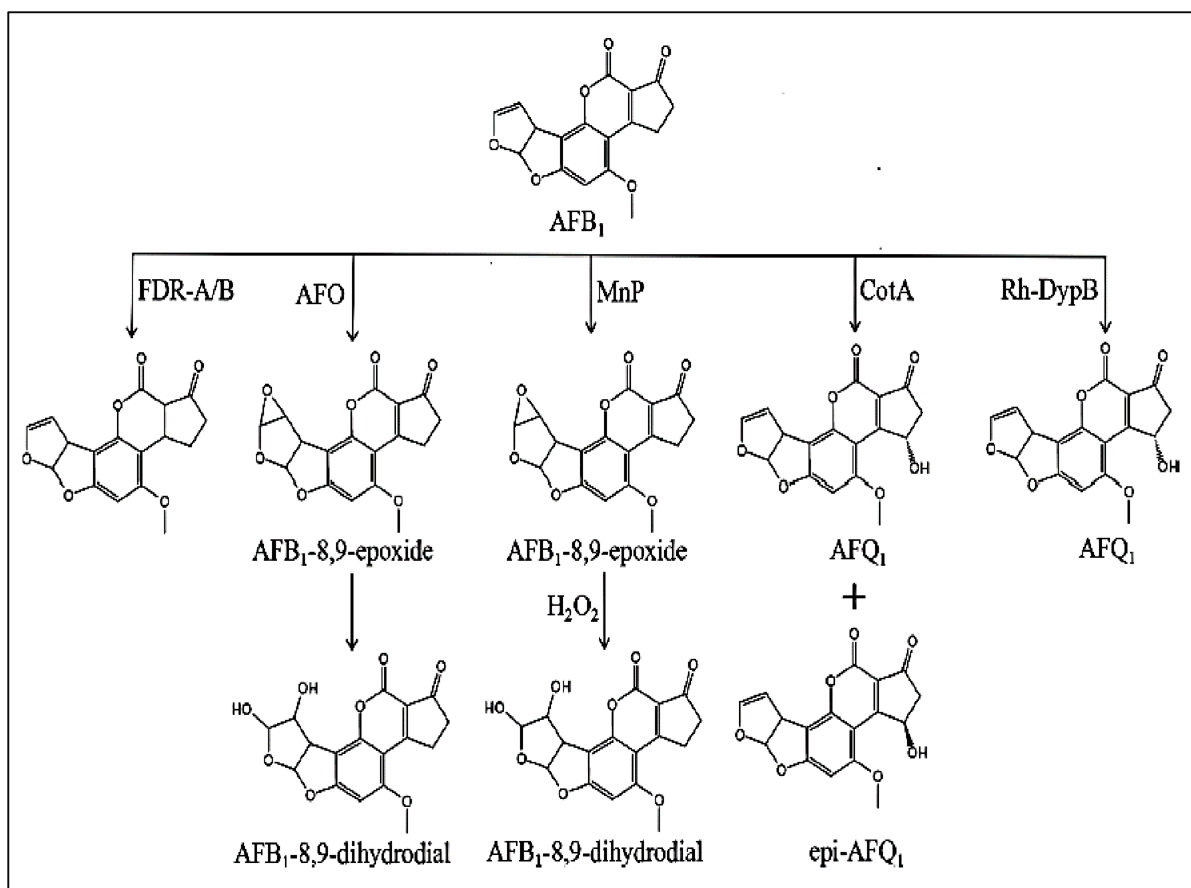


Fig 5: Degradation pathways of AFB_1 by aflatoxin-degrading enzymes [43]

1.8 Discussion of previous studies on the degradation of aflatoxin in corn

- The study on "Cold atmospheric pressure plasma-assisted removal of aflatoxin B_1 from contaminated corn kernels" highlights the use of CAP treatment generated with an air SBD (Surface Barrier Discharge) system intended for the removal of AFB_1 from the contaminated corn kernels surface, where 100% removal is achieved. This indicates that an indirect plasma exposure system could potentially represent an efficient plasma setup for the treatment of the grains in real-case scenarios. Contradictory to the findings presented in several previous studies, exposures to CAP in this study did not induce any significant chemical or morphological modifications of the corn kernel surface, which fits into the guidelines of the food industry that require the development of novel food processing methods with minimal influence on the treated food matrix. Although this study has demonstrated that CAP treatment is an effective and safe approach, additional tests should be performed to prove CAP as an entirely acceptable method for the treatment of the food. The additional examination of the approach will be mainly based on the treatment of the more significant number of test samples, with an emphasis on the evaluation of the nutritional and organoleptic properties of the treated food [44].

- Another study on the "Efficacy and Safety Evaluation of Ozonation to Degrade Aflatoxin in Corn" demonstrates the effectiveness and permanency of ozone treatment in reducing AFB_1 levels in corn. Although additional studies are necessary to elaborate the safety of

the ozonation process, this study shows that the ozonation procedure reduces the mutagenic potential of AFB_1-contaminated corn. On the other hand, the less inhibitory effect of the hexane extract from treated contaminated corn as compared to the untreated same corn suggested that the ozonation process might have caused formation of fat-soluble reaction products that have relatively low mutagenic potential. It is hypothesized that the ozonation decontamination process might have produced oxidation products such as, oxidized linoleic acid, malonaldehyde, and acrolein that have been found to be mutagenic in the *Salmonella* mutagenicity assay. It is therefore necessary to pursue further study on this aspect to determine the distribution of the ozone-aflatoxin reaction products, and to characterize and identify these reaction products [45].

- The research on "A holistic study to understand the detoxification of mycotoxins in maize and impact on its molecular integrity using cold atmospheric plasma treatment" have described one important intervention that has the potential to be utilised as a strategy to mitigate mycotoxin contamination in food and feed. For the first time, a holistic study to understand the breakdown of mycotoxins in terms of reducing toxicity as well as the broader effects of CAPP (Cold Atmospheric Pressure Plasma) treatment on the molecular integrity of maize has been undertaken. The reduction of the levels of mycotoxins present was assessed by investigating a number of factors including the nature of gases used, plasma exposure time, by-products formation and their toxicity, efficiency in the matrix as well as global influence of the treatment on the matrix. Results generated show that optimized CAPP is capable of significant reduction of two highly important mycotoxins which are prevalent maize contaminants i.e. AFB_1 and FB_1 (Fumonisins B_1) following just 10 min of treatment. As such, CAPP has the potential to aid or replace solutions currently employed in post-harvest decontamination of mycotoxins in grains where both number of toxins and their levels can be very high. However, further extensive research is required to fully elucidate and model CAPP reactive species interaction with complex food matrices which would facilitate prediction of treatment efficiency and by-products formation, which is a substantial challenge due to intricate characteristics of natural toxins which vary both in structure and biological activity. Despite the fact that performed toxicity assessment of the treated maize samples showed no increase in cellular toxicity in HepG2 cell line after CAPP treatment, possible increased toxicity should still be investigated in other cell assays or in vivo to fully confirm CAPP safety, especially in produce destined for human consumption. What is more, detected significant differences in small molecular mass constituents of the matrix must be studied in more detail to determine if an alteration of the nutritional value occurs as a result of the treatment to balance the trade-off between safety and sustenance, especially in matrices reach in antioxidants and lipids. Finally, further assessment of CAPP application in large volumes of naturally contaminated samples is a crucial next step on the road for upscaling and potential implementation [46].

- The study on "Reduction of Aflatoxin in Corn by High Voltage Atmospheric Cold Plasma" states generation of reactive gas species was characterized with optical emission spectroscopy and measured with dragger tubes. Generation of reactive gas species was influenced by gas type and relative humidity. Higher concentration of ozone and NOx were generated during HVACP (High voltage atmospheric cold plasma) treatment in MA65 than in air and with lower relative humidity. The concentration of ozone and NOx increased initially with increasing HVACP treatment time, reached a peak and stabilized after 10 min HVACP treatment. Aflatoxin could be rapidly degraded by HVACP treatment. Aflatoxin in corn was degraded by 62% and 82% after 1 and 10 min, respectively, using HVACP treatment in RH 40% air. First order Weibull and Logistic models were applied to fit the

degradation kinetics of aflatoxin by HVACP treatment and the Logistic model was found to be the best model. Complete degradation of aflatoxin in corn was not achieved by HVACP treatment, which seems to be caused by the inability of reactive species generated by HVACP to penetrate into corn kernels. MA65 as the working gas was slightly more effective for HVACP treatment in degrading aflatoxin in corn than air because of higher concentrations of ozone and NOx species are generated by MA65. HVACP treatment with humid air and MA65 at relative humidity 40% and 80% was more effective than dry gas (5% RH). Direct or indirect HVACP treatment was equally effective in the degradation of aflatoxin in corn. Stirring the corn sample during HVACP treatment and post-treatment storage could increase aflatoxin degradation by HVACP treatment [47].

- Another study on "Development and Optimization of Cold Plasma Pre-treatment for Drying on Corn Kernels" states that cold plasma pre-treatment technology was employed to corn kernels drying, and it was found that plasma pre-treatment can shorten drying time and increase drying rate. The effects of these parameters were optimized using Design-Expert to obtain the desired drying rate and drying time of corn kernels. Response surface regression illustrated that cold plasma pre-treatment time, plasma pre-treatment power, and drying temperature were significantly correlated with drying rate and drying time. No significant difference was found between experimental and predicted values. The optimal conditions were temperature 52.5°C, plasma pre-treatment power of 500W, and plasma pre-treatment time 50 s, and the corresponding drying rate and drying time were 3.6163 (g/g h^{-1}) and 1.29 hr, respectively. The results show that cold plasma pre-treatment can significantly reduce the drying time and improve the drying efficiency [48].

- The study on "Effect of ozone treatment on aflatoxin B_1 and safety evaluation of ozonized corn" illustrates that ozone can effectively degrade AFB_1 in corn and the degradation rate improves with the increase of the ozone concentration and treatment time. The moisture content (MC) of corn has a negative correlation with the degradation rate of AFB_1 in corn as the degradation rate for AFB_1 Contaminated Corn (ACC) with 13.47% MC is higher than that with 20.37% MC. The toxicity of the untreated and ozone-treated ACC was evaluated using HepG2. Untreated ACC obviously inhibits the growth of HepG2 with the survival rate, morphology, and cell apoptosis considered. Meanwhile, ozonized ACC does not affect the HepG2 growth, the toxicity disappears for the degradation products of ozonized ACC [49].

1.9 Research Aim and Objective

The aim of the present study is to investigate the effectiveness of UV-Plasma assisted fluidized bed drying in reducing AFB_1 levels in corn and to determine the degradation kinetics of this process.

The objectives of the study were:

- AFB_1 Degradation: The primary focus of the study is to understand the kinetics of aflatoxin B_1 degradation during UV-Plasma assisted fluidized bed drying. This involves assessing the rate at which aflatoxin B_1 breaks down under different drying conditions.

- Drying Process Optimization: The study explores various drying parameters, including plasma treatment duration, and temperature, to determine the optimal conditions for aflatoxin B_1 degradation while preserving the quality of corn.

- Kinetic Modeling: Develop mathematical models that describe the degradation kinetics of aflatoxin B_1 during the drying process. This will involve determining reaction rate constants and activation energies associated with the degradation.

- Quality Assessment: Assessing the quality of dried corn after treatment, including changes in colour, texture, and sensory attributes, to ensure that the drying process does not adversely affect corn quality.

- Analytical Techniques: Appropriate analytical technique, such as HPTLC (High Performance Thin Layer Chromatography) was employed to quantitatively measure aflatoxin B_1 levels.

- Practical Applications: The practical applications and feasibility of implementing UV-Plasma assisted drying as a potential method for aflatoxin reduction in corn at an industrial scale was explored.

Overall, this study aims to contribute to the understanding of the kinetics of aflatoxin B_1 degradation during UV-Plasma assisted fluidized bed drying and to provide valuable insights into the feasibility of this technology as a means to enhance food safety in corn processing.

Chapter 2:
Materials and Methods

2.1 Selection and Preparation of Corn Samples Contaminated with AFB_1

Contamination of corn samples with Aflatoxin B_1 can be a serious health concern, as Aflatoxin B_1 is a potent carcinogen produced by *Aspergillus flavus* and *Aspergillus parasiticus* that grow on different food crops, including corn. To ensure safety, careful selection and preparation of corn samples for testing are crucial. Below are the steps to select and prepare corn samples contaminated with Aflatoxin B_1:

- Source of Contaminated Corn Samples: Corn samples that are suspected of being contaminated with Aflatoxin B_1 were collected from the local market. These samples can be from specific batches or regions known to be at risk for mold growth and aflatoxin contamination.

- Safety Measures: When handling potentially contaminated samples, it's essential to follow safety protocols. Appropriate Personal Protective Equipment (PPE), such as gloves, lab coats, and masks, were used to avoid direct contact with the samples.

- Sample Size: The sample size should remain more or less same depending on the batch.

- Sample Storage: The corn samples were stored in clean and airtight containers, preferably in a cool and dark environment at around 4°C to prevent further mold growth and potential changes in aflatoxin levels.

- Handling and testing aflatoxin-contaminated samples require expertise, and so it is advisable to involve trained professionals or utilize certified testing laboratories to ensure accurate and reliable results. The goal is to safeguard human and animal health by identifying and mitigating the risks associated with aflatoxin exposure.

2.2 Design and set-up of UV-Plasma assisted Fluidized Bed Drying System

- For generation of UV-assisted plasma inside Fluidized Bed Dryer, a Reliance Enterprise industry-scale stainless steel Fluidized Bed Dryer was used for treatment of granular materials.
- UV-assisted plasma was generated using a Philips 75W, 500V UV light.
- Temperature and time were controlled from the control panel.
- The UV light was placed inside the FBD chamber keeping in mind factors such as accessibility for maintenance and alignment with the FBD.
- All possible outlets were locked & covered properly to avoid exit of plasma activated hot air and entry of visible light.
- The UV light and the FBD both were connected to the main power supply.
- Temperature and time for each set of treatment was set every time from the control panel.
- During the experiment the in-built FBD light was switched off and the motor was switched on.
- Samples were repeatedly conveyed to the treatment zone by means of an aeration gas flow supplied from a compressor with a 900 L/min air yield, 8 bar working pressure, 200 L tank volume and 5.5 kW engine power integrated into the system so as to ease the fluidization process [50].

- A stainless steel sieve was designed for homogeneous distribution of air released from the compressor and placed just at the bottom of the reactor.
- Following the plasma treatment, the samples were taken into sterile conical flasks and sample preparation was done.
- Certain safety measures should be kept in mind like ensuring that the material being dried is compatible with the UV-plasma and the FBD environment. UV-blocking shields and other proper protective equipment should also be used.

Fig 1: Philips UV light

Fig 2: Reliance Enterprise industry-scale FBD

Fig 3: FBD control panel

2.3 Experimental procedures for AFB_1 degradation kinetics study

Studying the degradation kinetics of Aflatoxin B_1 (AFB_1) typically involves a series of controlled experiments to observe how the toxin breaks down over time under different conditions. Here's an outline of the experimental procedures for conducting an AFB_1 degradation kinetics study in corn:

a) Experimental set-up: For generation of UV-assisted plasma inside Fluidized Bed Dryer, a Reliance Enterprise industry-scale stainless steel Fluidized Bed Dryer was used for the purpose. UV-assisted plasma was generated using a Philips 75W, 500V UV light.

b) Pre-Treatment process: Six to seven corn kernels weighing nearly 1.5-3g were used for each set of treatment. The kernels were washed thoroughly and then treated for degradation of aflatoxin at three different temperatures and four different time intervals respectively.

c) Drying Process: Samples (≈ 3 g) were treated for 5, 10, 15, and 20 min at 30, 40 and 50°C. The FBD was preheated until the treatment temperature (30, 40 or 50°C) was achieved and after the treatment time the heater was turned off and air passed through each sample to accelerate cooling. The temperature and time values used were defined after doing ample literature survey. The treated corn samples were taken in conical flasks for sample preparation. Each time and temperature study was replicated, and means and standard deviations were calculated.

d) Sample Preparation: Treated corn kernels were dipped in 5ml of solvent containing Petroleum Benzene (60-80°C) and Acetonitrile in the ratio of 98:2 [51]. The kernels dipped in solvent was kept overnight and then vortexed the next day. Volume of solvent in each conical flask was checked and if required solvent was added to make up the volume. A micropipette was used to draw 1ml of sample from each conical flask into separate Eppendorf tubes for HPTLC analysis.

2.4 Analysis of AFB_1 concentration of corn after treatment using HPTLC

Previously prepared sample of UV-Plasma treated corn taken in Eppendorf tubes were used for this purpose.

- Pre-washing and Pre-marking step→ At first TLC plate (Silica gel 60 F_{254}, 20*10 cm) was taken and was washed with methanol to remove dirt and other particles and then heated on the plate heater to remove excess moisture. Then a ruler was used to mark a distance of 8cm on each side of the plate.

- Selection of steps in visionCATS software→ For creating a new method in the CAMAG provided visionCATS software all the steps required in the experiment were selected. Linomat 5 was selected in the application step, Chamber was selected in the development stage, Derivatizer was selected in derivatization step and Scanner 4 was selected for data acquisition step.

- Plate calculation→ After finishing step definition, the sequence table that is Track Assignments identified by tracking number and dependent on the plate layout parameters

and position of the plates provided by the visionCATS software was entered by clicking on it.

- A 20*10 twin-trough-chamber was used for this purpose. It was first rinsed with distilled water and then a filter paper was added on one side of the chamber so that saturation does not occur when TLC plate is added on the other side. 20ml of 92:8 Chloroform:Acetone [51] mixture was then poured into it. The trough was then tilted at an angle of 45° for equal distribution of solvent in both the chambers.

- The TLC plate was set on the Linomat 5 applicator. Sample and standard AFB_1 solution was drawn from the Eppendorf tubes using a syringe which was then applied on the plate in the form of spray amounting to nearly 4.2 µl. After application of each sample the syringe was washed with methanol.

- After completing application of all the samples, the plate was put into the twin-trough-chamber at an angle of 45° and was kept for 5-10 mins or until the solvent reached the pre-marked distance.

- After the solvent reached the pre-marked distance the plate was removed from the chamber and then air dried for 2-3 mins.

- Photo documentation with digital camera→ After the plate is dried, it was put inside the digital camera chamber for taking pictures of the plate both under UV and fluorescent light and this data was collected by visionCATS software.

- Detection of band, scanning, and quantification→ After observing and collecting the images of the band, the plate was removed from the digital camera chamber and put inside Scanner 4 for scanning of the chromatogram. First scanning was done under a light sensitive source using a deuterium lamp at 254 nm wavelength and the chromatograms were detected. Then the range having sharp peaks was selected, which was again scanned under a UV source using both deuterium and tungsten lamp at a range of 254-450 nm wavelength. This detected the absorbance which was used for further quantification.

Fig 4: CAMAG Linomat 5 Applicator

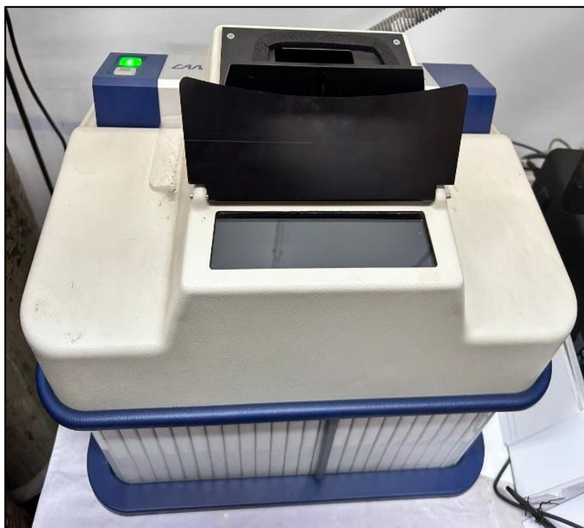


Fig 5: Digital camera

Fig 6: CAMAG Scanner 4

2.5 SEM Analysis of Outer Corn Shell

Corn kernels treated with UV-Plasma were subjected to Scanning Electron Microscopy for study of morphological modifications.

- The corn kernels were first treated with UV-Plasma at 40°C for 20 mins.
- After treatment the outer corn shell was removed using a scalpel and dissected into smaller portions.
- Then these were vacuum dried to remove any small amount of excess moisture to reduce outgassing from possible organic contaminations and residual water in the sample [44].
- Corn being a non-conductive material can trap incident beam electrons on the surface and result in shinning spots in SEM images. Also this built-up charge can cause overheating and deterioration of the sample.
- So deposition of a layer of gold provides high-quality microscopic images due to its high electrical conductivity and small grain size.
- Hitachi MCIOOO Ion Sputter was used for gold coating the samples.
- After gold coating was finished SEM analysis was performed using Hitachi SU3800 SEM with EDS Facility having Single Phase Voltage of 100-240 VAC, Power of 1.5kVA, & Frequency of 50/60Hz.
- The operating voltage was 20kV.
- Microscopic images were taken at 1500 SE and 3000 SE respectively.

Fig 7: Hitachi MCIOOO Ion Sputter

Fig 8: Hitachi SU3800 SEM with EDS Facility

2.6 Degradation kinetics theory of AFB_1

Degradation kinetics of AFB_1 under UV-Plasma assisted fluidized bed drying is expected to follow First-Order Reaction Kinetics [52].

A first-order reaction is a chemical reaction in which the rate varies based on the changes in the concentration of only one of the reactants. The differential rate expression for a first-order reaction can be written as:

$$\text{Rate} = \frac{-d[A]}{dt} = k[A]$$

- 'k' is the rate constant of the first-order reaction, whose unit is s^{-1}/min^{-1}.
- '[A]' denotes the concentration of the first-order reactant 'A'.
- $\frac{-d[A]}{dt}$ denotes the change in the concentration of the first-order reactant 'A' in the time interval 'dt'.

To obtain the integral form of the rate expression for a first-order reaction, the differential rate law for the first-order reaction must be rearranged as follows:

$$\frac{-d[A]}{dt} = k[A]$$
$$\Rightarrow \frac{d[A]}{[A]} = -kdt$$

Integrating both sides of the equation, the following expression is obtained:

$$\int_{[A]_0}^{[A]} \frac{d[A]}{[A]} = -\int_{t_0}^{t} kdt$$

which can also be rewritten as:

$$\int_{[A]_0}^{[A]} \frac{1}{[A]} d[A] = -\int_{t_0}^{t} kdt$$

Since,

$$\int \frac{1}{x} = ln(x)$$

The equation can be rewritten as follows:

$$\ln[A] - \ln[A]_0 = -kt$$
$$\ln[A] = -kt + \ln[A]_0 \text{ (or) } \ln[A] = \ln[A]_0 - kt$$

Raising each side of the equation to the exponent 'e' (since $e^{\ln(x)} = x$), the equation is transformed as follows:

$$e^{ln[A]} = e^{ln[A]_0 - kt}$$

Therefore,

$$[A] = [A]_0 e^{-kt}$$

This expression is the integrated form of the first-order rate law.

The concentration v/s time graph for a first-order reaction is as follows:

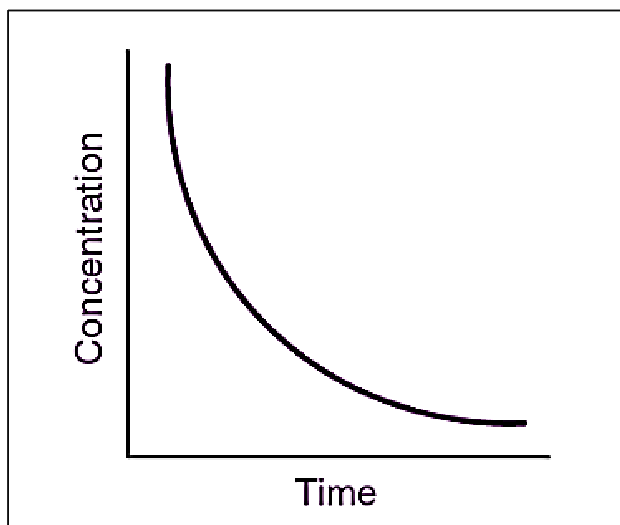


Fig 9: Concentration v/s time graph for first-order kinetics [52]

For first-order reactions, the equation $\ln[A] = -kt + \ln[A]_0$ is similar to that of a straight line ($y = mx + c$) with slope -k. This line can be graphically plotted as follows:

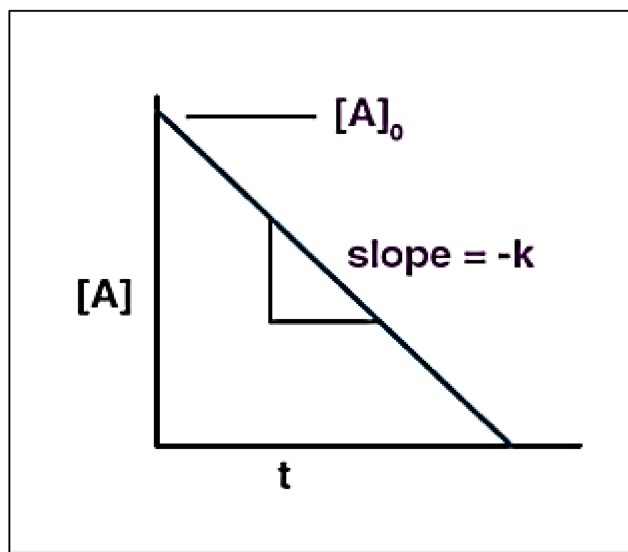


Fig 10: Graph showing similarity between first-order kinetics equation to that of a straight line [52]

Thus, the graph for ln[A] v/s t for a first-order reaction is a straight line with slope -k.

Chapter 3:
Results and Discussion

3.1 Representation of AFB_1 concentration profile at different operating temperatures and time

Weight of the corn samples after treatment with UV-Plasma assisted Fluidized Bed Drying at three different temperature and four different time combinations were noted down for further calculation.

Table 1: Weight of treated corn samples at different temperature and time

Temperature (°C)	Time (min)	Weight (g)
30	5	2.275
	10	2.255
	15	2.183
	20	2.290
40	5	2.168
	10	2.139
	15	1.976
	20	2.143
50	5	2.314
	10	2.085
	15	1.813
	20	1.984

Weight of the corn samples taken as Control (untreated) = 2.252g

Concentration of AFB_1 in UV-Plasma treated corn samples were analyzed using HPTLC. The resulting chromatogram is as follows:

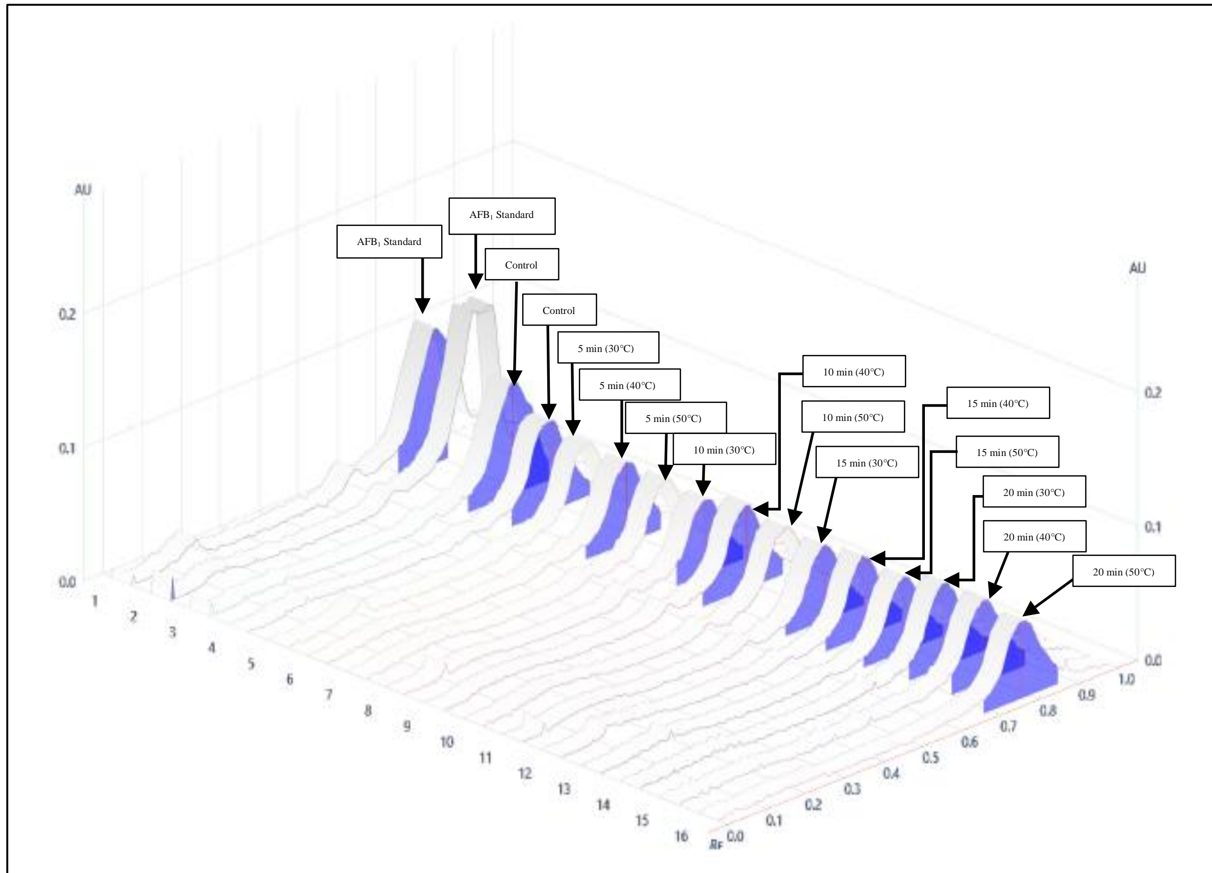


Fig 1: Chromatogram showing AFB_1 concentration of treated corn samples at different temperature and time

The above chromatogram clearly depicts the fact that concentration of AFB_1 in UV-Plasma treated corn samples decreased linearly both with increasing time and temperature as compared to the untreated ones. The concentration values obtained from CAMAG HPTLC were expressed in µg/ml. These values were then equated with the weight of the samples taken after treatment and finally the concentration of AFB_1 was calculated in µg/kg or ppm (parts per million). The concentrations in ppm is given in the table below:

Table 2: Concentration of AFB_1 in treated corn samples in ppm

Temperature (°C)	Time (min)	Concentration of AFB_1 (ppm)
30	5	3923.5
	10	3524.9
	15	3328.1

	20	3131.3
40	5	4226.2
	10	4083
	15	4068.8
	20	3962.1
50	5	4197.7
	10	4187.9
	15	4178.1
	20	4168.3

Concentration of AFB_1 in Control (untreated corn samples) = 5659.4 ppm

3.2 Degradation of AFB_1 in UV-Plasma assisted Fluidized Bed Drying

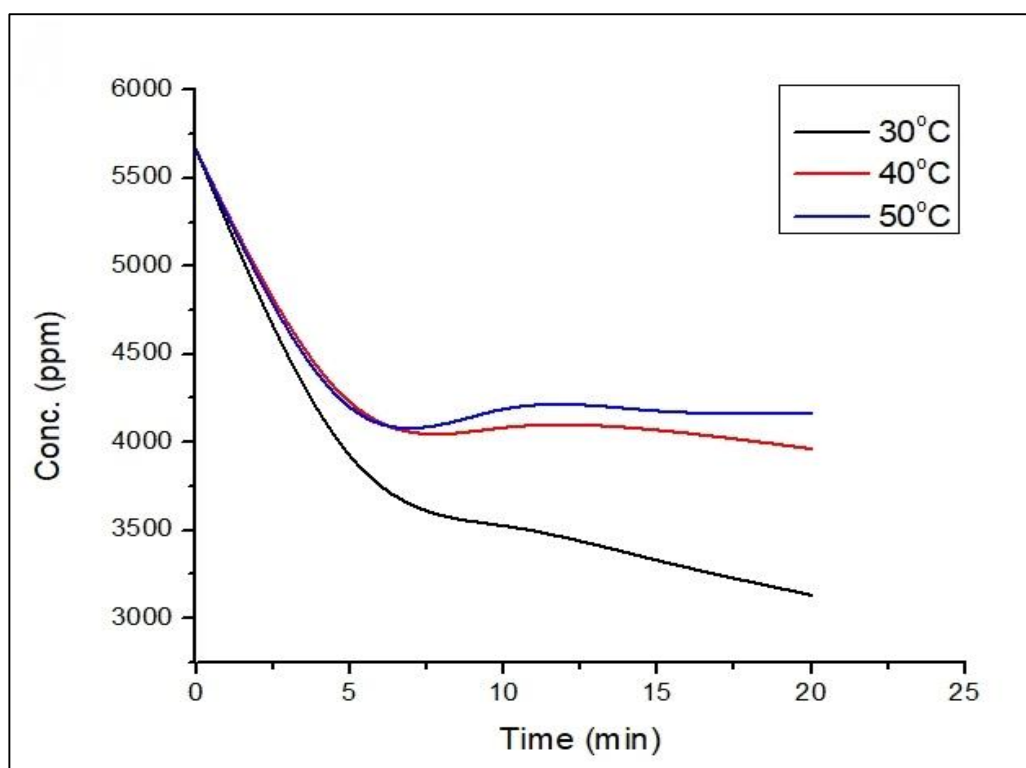
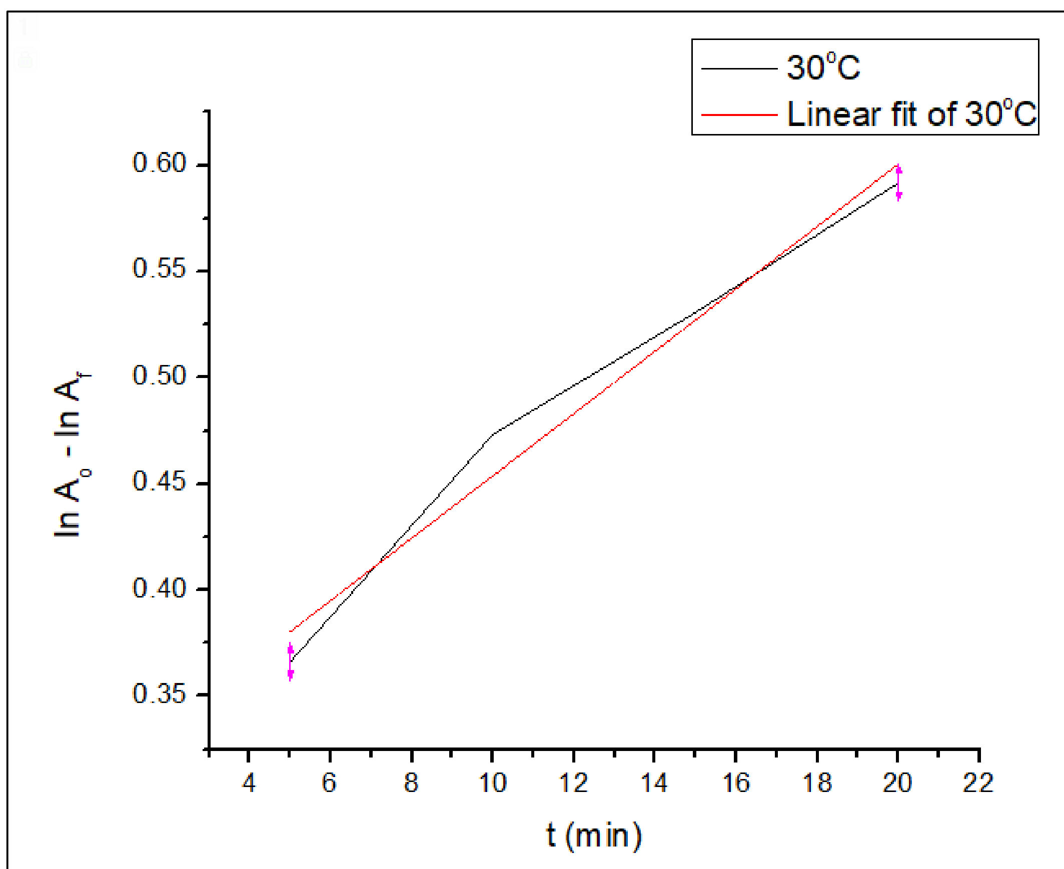
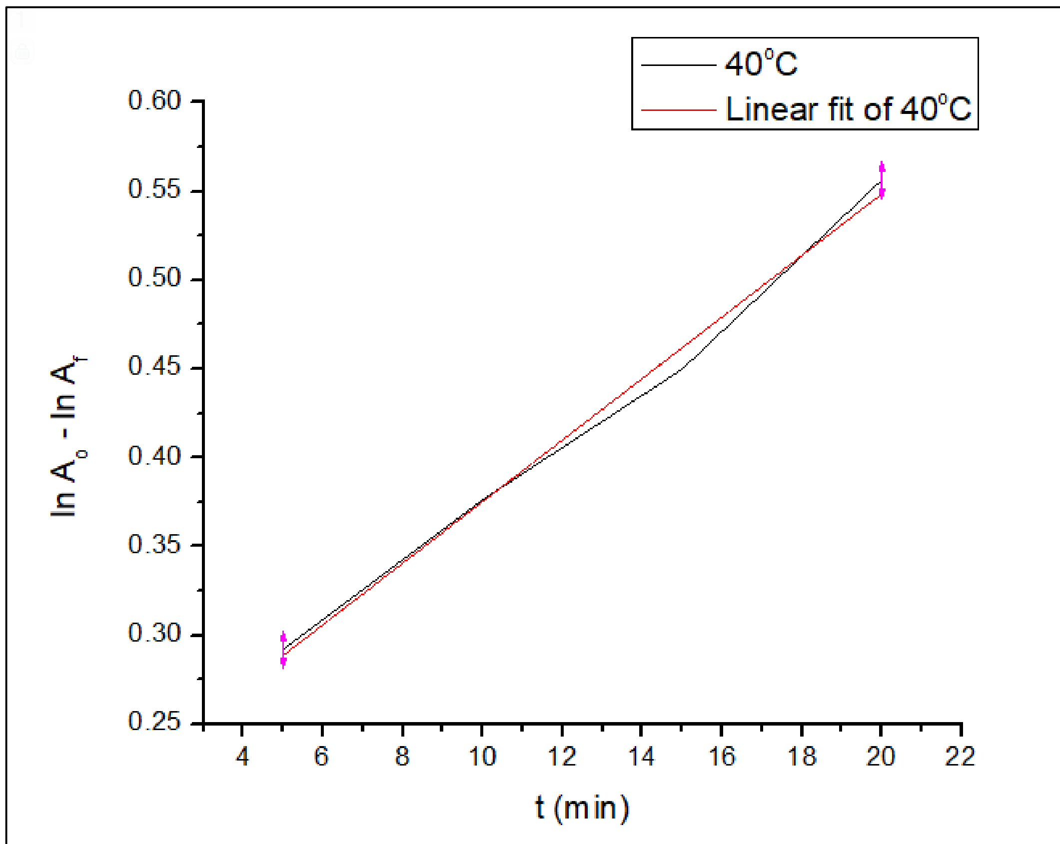


Fig 2: Degradation of AFB_1 by UV-Plasma assisted Fluidized Bed Drying as a function of initial AFB_1 quantity at different temperature and time

The residual percentage variations of AFB_1 as a function of initial AFB_1 quantity and treatment time and temperature are illustrated in the above figure. According to the figure, it could be seen that no changes in AFB_1 content in control were recorded during the entire experimental period, but the residual percentages of the three treatment groups decreased markedly along with the exposure time and temperature and the degradation rate reached 70% after treatment. No significant difference in the residual percentage at each time interval was observed among the three treatment groups, implying that the initial AFB_1 quantity in the selected regime had no effect on AFB_1 degradation. In contrast to the initial AFB_1 quantity, the treatment time and temperature obviously influenced AFB_1 degradation. The residual percentages declined markedly along with the increase in temperature in the same time intervals.

3.3 Degradation Kinetics of AFB_1 UV-Plasma assisted Fluidized Bed Drying





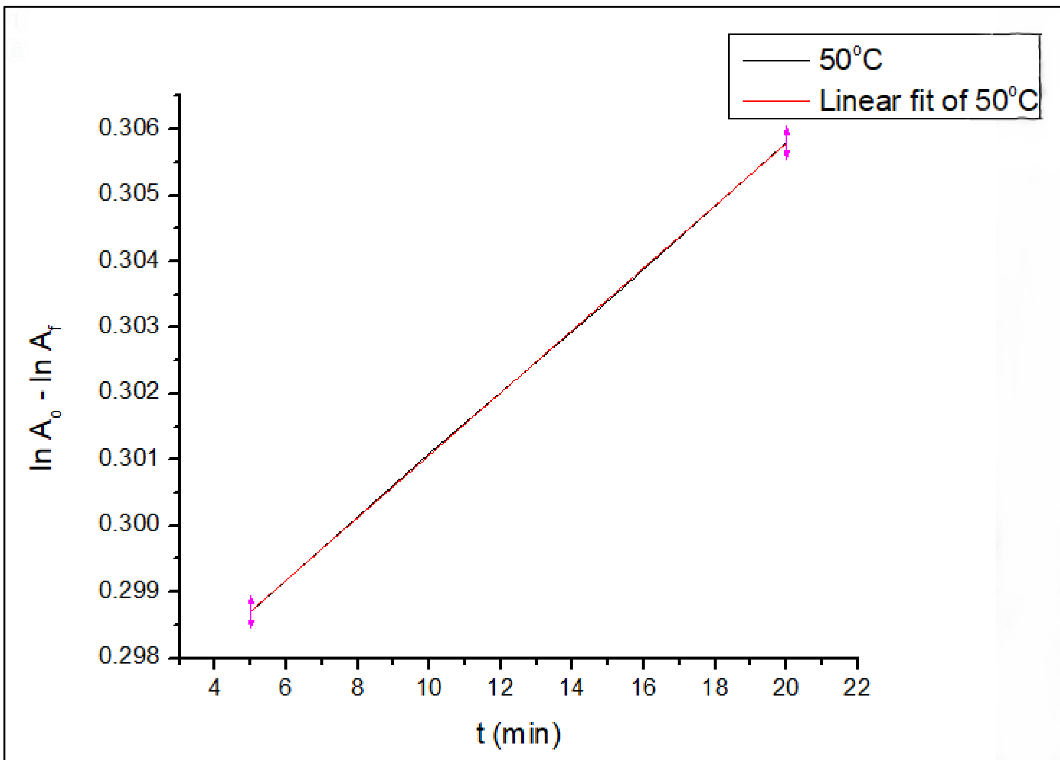


Fig 3: Kinetics of AFB_1 degradation at different temperature and time

To explore the degradation behaviour of AFB_1 by UV-Plasma assisted Fluidized Bed Drying, the degradation kinetic was studied. The plot of **$\ln(A_0)$-$\ln(A_f)$** [52] against exposure time as a

function of initial AFB_1 quantity and treatment temperature is given in the above figure. The linear relationship between $\ln(A_0)$-$\ln(A_f)$ and exposure duration indicated that the degradation followed a first-order reaction (**$R^2 > 0.99$**), given by the equation $A_f = A_0e^{-kt}$, where A_0 and A_f were the quantity of remaining AFB_1 at time 0 and t, and k was the first-order rate constant. The half-life $t_{1/2}$ of AFB_1, which refers to the time required for its concentration to fall to half of its initial value, was calculated according to the equation t1/2 = 0.693/k. **First-order rate constant (k)** was calculated from the intercepts and was found to be in the range of **0.2-0.3 min^{-1}**.

3.4 Effect of UV-Plasma assisted treatment on colour and texture of the sample

The impact of UV assisted plasma on the colour of agricultural produce has always been a topic of interest in many studies. It is important to preserve the colour of corn during the post-harvest operations as it is the direct evaluation factor for consumers [53]. The colour of the plasma treated samples are dependent on treatment conditions and the biological natures of the sample. No change in colour was observed in the UV-Plasma treated corn.

Mere change of crunchiness of corn samples with UV-plasma treatment was observed due to cell damage on the upper surface. The extent of tissue breakdown by plasma depends on the specific tissue structures. Waxy layers on the surface of corn are more likely to suffer cell leakage and texture deterioration. Although the effects of plasma treatment on tissue structure and texture have been widely investigated, the structural breakdown is not the only pathway to texture change. Respiration and related enzymes that can be influenced by plasma also play their parts [54].

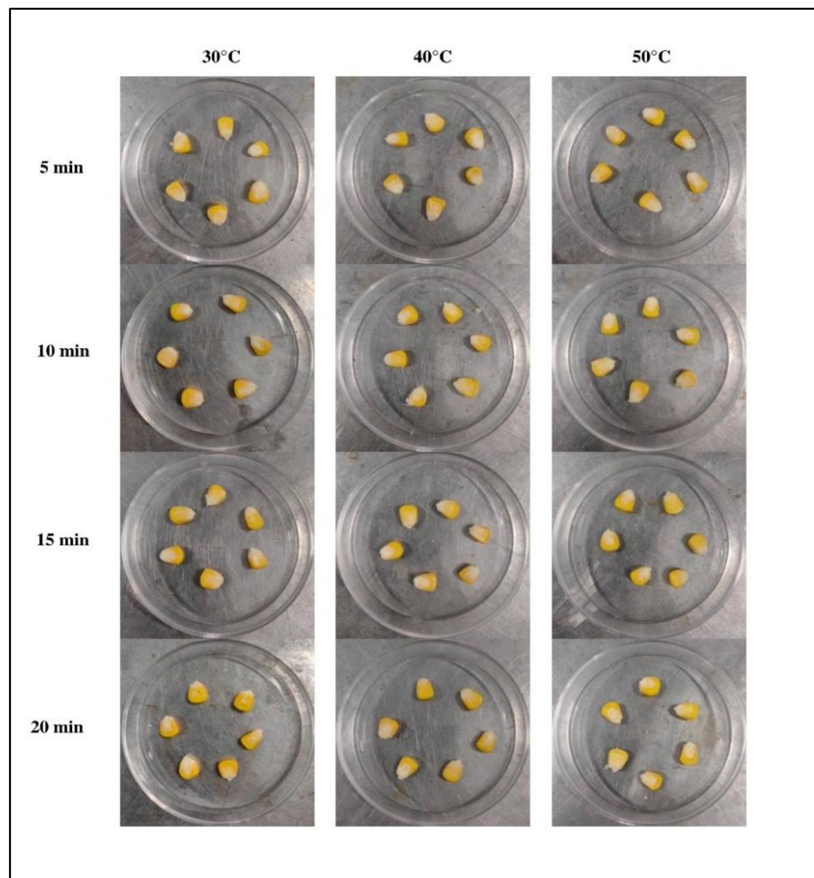


Fig 4: Corn colour unaffected and texture merely affected by UV-Plasma assisted treatment

3.5 Discussion of the SEM images with respect to the experiment

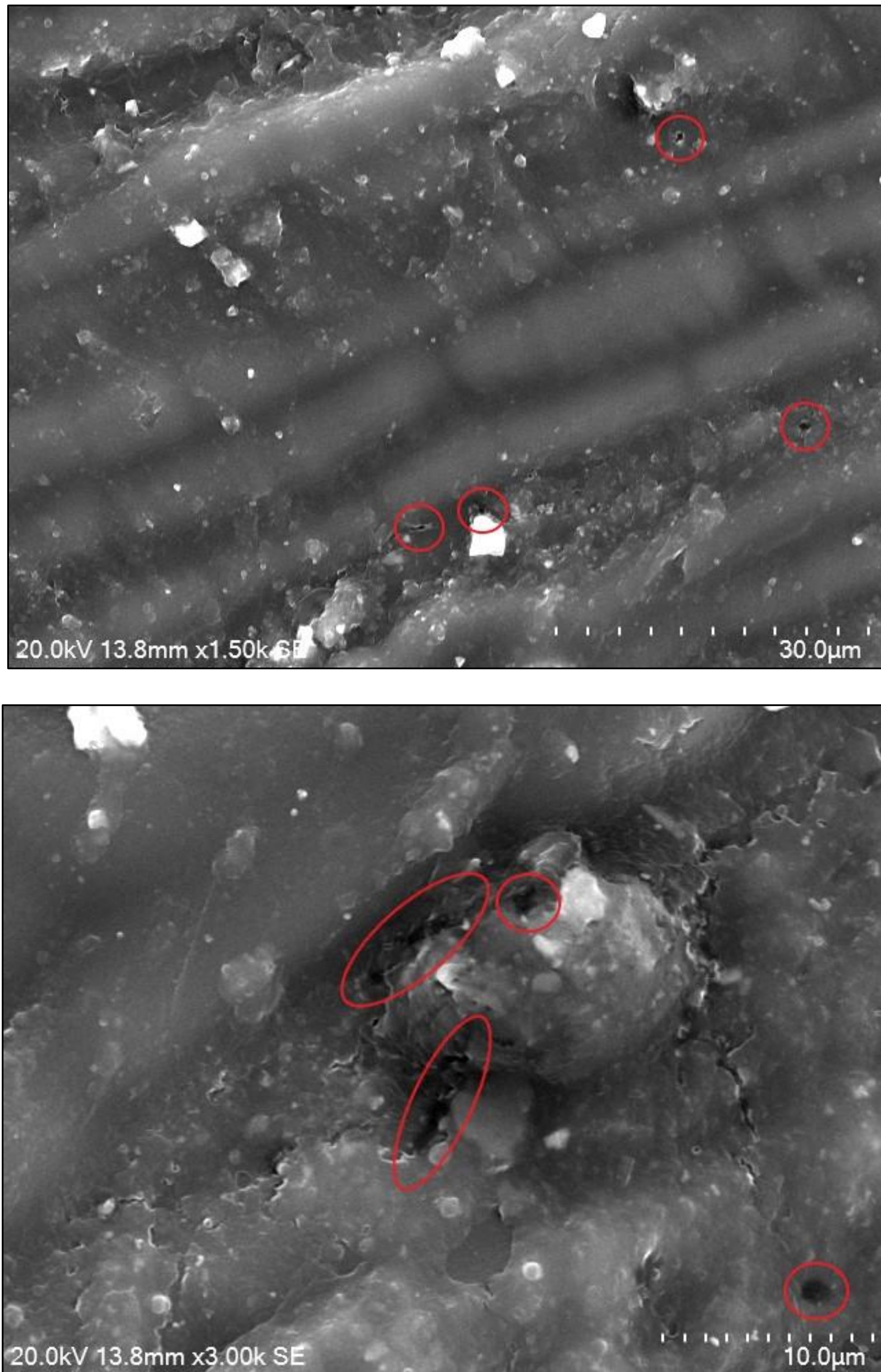


Fig 5: SEM images of outer corn shell after UV-Plasma assisted treatment

The morphological study using SEM revealed the drying effect of the UV-plasma assisted treatment and because of it cracks were observed on the surface. This is because of the reactivity of oxygen along with the UV exposure on the surface of corn. It was also observed

that the cracks were homogeneous over the surface. So it supports the fact that the UV-plasma assisted treatment over the surface intensifies the drying rate which actually helps in inactivation of molds responsible for production of aflatoxin. Cracks over the surface of corn indicates drying i.e. loss of moisture which in turn helps in decreasing the contamination possibilities by *Aspergillus flavus* and *Aspergillus parasiticus* and thereby intensifies food security. The below schematic diagram shows the proposed interaction of UV-Plasma assisted treatment with the surface of corn.

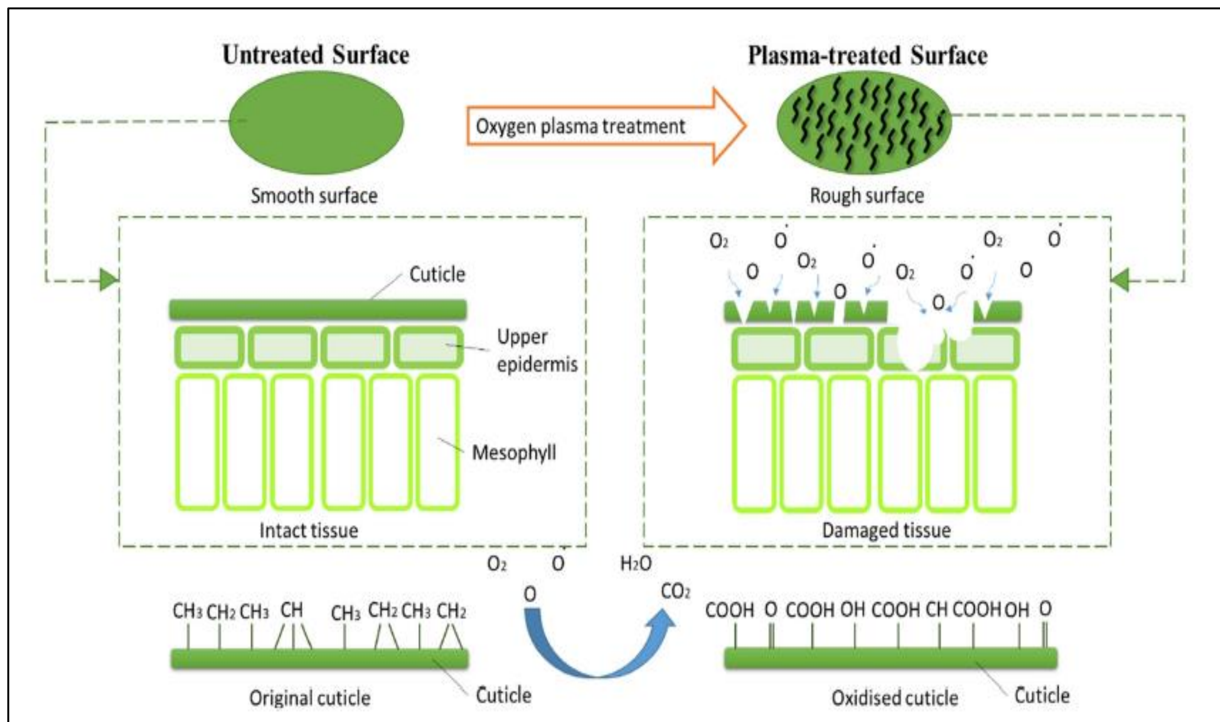


Fig 6: Interaction of UV-Plasma assisted treatment with the surface of corn [54]

Chapter 4:
Conclusion and Future Scope

Conclusion

The degradation kinetics of AFB_1 using UV-Plasma assisted fluidized bed drying in corn hold promise as an innovative approach for mycotoxin reduction in agricultural products. This study demonstrates the feasibility of UV-Plasma assisted drying for aflatoxin B_1 degradation in a fluidized bed, but several key conclusions, future research directions, and implications can be drawn from the findings:

i. The study has shown that UV-Plasma assisted fluidized bed drying can effectively degrade aflatoxin B_1 in corn with a degradation rate of **70%**, making it a viable technique for mycotoxin reduction. The degradation kinetics also showed that the residual percentages of AFB_1 declined markedly along with the increase in temperature in the same time intervals.
ii. The degradation kinetics data obtained in this study can be used to develop predictive models for aflatoxin B_1 degradation under various treatment conditions, aiding in process control and optimization. The linear relationship between $\ln(A_0)$-$\ln(A_f)$ and exposure duration indicated that the degradation followed a first-order reaction **($R^2 > 0.99$)** with **rate constant (k)** in the range of **0.2-0.3 min^{-1}**.
iii. The morphological study using SEM revealed the drying effect of the UV-plasma assisted treatment and because of it cracks were observed on the surface. This supports the fact that the UV-plasma assisted treatment over the surface intensifies the drying rate which actually helps in inactivation of molds responsible for production of aflatoxin thus proving it to be a surface phenomenon.
iv. The UV-Plasma assisted treatment also proved to be effective in maintaining the colour and texture of the agricultural products.
v. The successful implementation of UV-Plasma assisted drying can lead to improved food safety and quality as it causes drying i.e. loss of moisture which in turn helps in decreasing the contamination possibilities by *Aspergillus flavus* and *Aspergillus parasiticus* and thereby intensifies food security. This technology can be integrated into quality control and assurance processes in the food industry, ensuring that aflatoxin B_1 levels meet regulatory standards.
vi. Therefore, UV-Plasma assisted fluidized bed drying can potentially offer a rapid and energy-efficient means of mycotoxin reduction compared to traditional methods like heat treatment or chemical detoxification. It can contribute to enhancing food safety and reducing economic losses associated with mycotoxin-contaminated crops. Additionally, this technique aligns with the growing demand for sustainable and eco-friendly food processing methods.

In conclusion, the study on the degradation kinetics of aflatoxin B_1 using UV-Plasma assisted fluidized bed drying in corn demonstrates the feasibility and potential of this technology for mycotoxin reduction in agricultural products.

Future Scope of the Study

Investigating the influence of different process parameters like treatment temperature and time on the degradation kinetics can help identify optimal conditions for aflatoxin B_1 reduction. Further research should focus on the identification of degradation by-products to ensure that the process does not generate harmful compounds. Evaluating the cost-effectiveness and scalability of UV-Plasma assisted drying for aflatoxin B_1 reduction on an industrial scale is essential. The study has significant implications for process intensification in the food and agriculture industries. UV-Plasma assisted drying can potentially offer a rapid and energy-efficient means of mycotoxin reduction compared to traditional methods like heat treatment or chemical detoxification. It can contribute to enhancing food safety and reducing economic losses associated with mycotoxin-contaminated crops. Assessing the environmental impact of UV-Plasma assisted drying, such as energy consumption and waste generation, should be a part of future studies to ensure its sustainability and compliance with environmental regulations. Further research and development efforts, along with an emphasis on process optimization and environmental considerations, are crucial to harness the full potential of this innovative technique in enhancing food safety and quality while contributing to process intensification in the food and agricultural sectors.

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