SHELF-LIFE STUDY OF PEANUTS BY DEGRADING AFLATOXIN B1 USING UV ASSISTED FLUIDIZED BED DRYING

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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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of my Master of Technology in Food Technology and Biochemical Engineering.

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

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ABSTRACT:

Aflatoxins (AFTs) are group of secondary metabolites produced by filamentous fungi such as *Aspergillus flavus, Aspergillus. parasiticus* etc. AFTs contaminate foods, feeds, other raw ingredients used to produce them and that pose a significant threat to human health. These toxins are hydroxylated metabolites form of AFB1 and AFB2 are known as difuranocoumarin compounds. Naturally, these AFs have carcinogenic, teratogenic and mutagenic effects and caused several metabolic disorders. This study investigates the degradation of AFBI during the UV-Plasma assisted fluidized bed drying process in peanuts. In this research, a novel approach combining UV radiation and plasma technology with fluidized bed drying was employed to reduce micro-organisms and level of aflatoxin in peanuts. The system utilizes a fluidized bed dryer as the drying medium, which enhances mass and heat transfer, ensuring efficient decontamination. The study involves systematically varying process parameters like UV exposure time to assess the influence on AFBI degradation. Samples of contaminated peanuts are subjected to this UV treatment, and AFBI concentrations are monitored at specific time intervals using High Pressure Thin Layer Chromatography (HPTLC) to determine the shelf life of raw peanuts. In this study, chemical quality of peanuts and degraded byproducts after treatment was also analyzed.

Keywords: Aflatoxin Bl, peanuts, UV-Plasma assisted fluidized bed drying, HPTLC, degradation, shelf life

Chapter 1:
Introduction and Literature Review

1.1 Introduction on Aflatoxin

The fungi *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius* and *Aspergillus tamari* produced aflatoxins are naturally secondary metabolites bisfuranocoumarin compounds [1, 2]. In agriculture *A. flavus* is a mutual contaminant and *A. pseudotamari A. bombycis, A. ochraceus*, and *A. nomius* are normally aflatoxin-producing species. *A. minisclerotigenes and A. arachidicola* are two another newly defined aflatoxigenic species [3]. Aflatoxins - B1, B2, G1 and G2 are four common contaminants of food products. Aflatoxins biosynthetically arise through polyhydroxy anthraquinone intermediates are acetate-derived decaketides. *A. flavus and A. parasiticus* species are found universally in the soil and air and grow at temperatures between 22 and 35°C [4, 5]. Aflatoxins classified as teratogenic, genotoxic, carcinogenic and invisible poisons by the World Health Organization (WHO).

Aflatoxins belong to a class of compounds that are very similar in chemical structure, all having a difuran ring and coumarin, the former being the basic toxic structure, while the latter being associated with carcinogenesis. Some studies indicate the acute or chronic effects of aflatoxins exposure on skin, liver, heart, and lung, with liver cancer being one of the most common and deadly detrimental effects of aflatoxins [8]. There are four major aflatoxins (Fig. 1): aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2), while aflatoxin M (AFM), which frequently poses a risk to milk safety, is the hydroxylated metabolites of AFB through the P450 system [7]. Of the known aflatoxins, AFB1 is the most toxic and widely distributed aflatoxin, and it is regarded as group 1 carcinogen by the International Agency for Research on Cancer (IARC)[8]. The oxidative stress caused by AFB1 is well-documented to be vital to the genotoxicity of the aflatoxins. In terms of aflatoxins biosynthesis, it is an intricate process involving many enzyme reactions and is affected by numerous biotic and abiotic factors, such as oxidative stress, developmental stage, and temperature. Indeed, aflatoxins production appears to be physiologically dependent on energy status. Chemically, aflatoxins are relatively stable in neutral solutions, and their pure product is a colorless crystal with high-temperature resistance, with decomposition temperatures of 237–306 ◦C. Due to the chemical stability of aflatoxins, it is difficult to degrade once they are contaminated. Unfortunately, many crops are prone to fungal invasion and accordingly contaminated with aflatoxins, especially in tropical and subtropical regions of the world, where crops are the most susceptible to aflatoxins derived from *A. flavus* and *A. parasiticus* during harvesting, storage, and processing. Under this scenario, more than 120 countries have established legislation and regulation on the acceptance level of aflatoxins in food and feedstuff [9].

1.2 Sources and Types of Aflatoxin

A number of airborne conidia and propagules that infect plants like cotton created by *A. flavus* [10]. During harvest in the agriculture form, in storage conditions, and during processing grains can be infected by *A. flavus, A. parasiticus* and are commonly isolated from corn, cottonseed, peanuts, and tree nuts. *Aspergillus flavus* can grow at temperatures ranging between 12 and 48°C and consisted of mycelium, conidia, or sclerotia [11]. AFB1, AFB2 produces by *A. flavus* but AFG1, and AFG2, AFB1, and AFB2 are produces by A. parasiticus and A. nomius fungal isolates [12]. The hydroxylated metabolites which is known as AFM1 and AFM2 produced by AFB1 and AFB2. AFB2 and AFG2 are manufactured at one-tenth to one-third of the amount of AFB1 and AFG1, correspondingly. And in largest quantities of AFB1 is produced in several strains. After classification by the International Agency for Research on Cancer (IARC) in 1987 (Category 1A) the aflatoxin B1 is as carcinogen, and AFM1 is a potentially carcinogenic substance with a toxicity range of AFB1 > AFG1 > AFB2 > AFG2 according to Category 2B. Aflatoxin detected in food in majority that ultimately harms to human and animal health among the mycotoxins affecting food and feed [13, 14]. Under the culture conditions most of the species produced major mycotoxin known as aflatoxin B1. AFB1 and AFB2 are named because of their strong blue fluorescence under UV light, whereas AFG1 and AFG2 fluoresces greenish yellow [15]. The B-toxins are categorized by the fusion of a cyclopentenone ring to the lactone ring of the coumarin structure, while G-toxins contained and additional fused lactone ring. In human other metabolites of AFB1 include Aflatoxin Q1 (AFQ1), aflatoxicol (AFL), AFM1, AFB2 and AFB1–2, 2-dihydrodiol. Both un-metabolized (B1, B2, G1, G2) as well as metabolized forms (aflatoxicol, M1 and M2) of aflatoxins get excreted in urine, stool and milk.

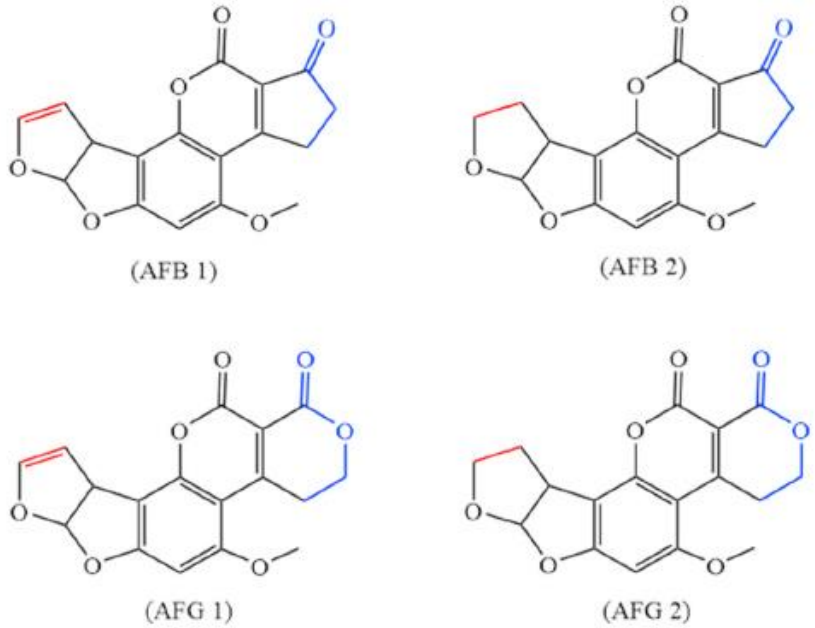


Figure 1: Structures of different aflatoxins [16]

1.3 Conditions for Production of Aflatoxin

Aflatoxins are usually associated with drought stress often occurs in various crop in the agriculture field before harvest. During the rainy seasons the poor storage conditions can increase the aflatoxins concentration. And these conditions developed chiefly in humid and hot regions where humidity and high temperature are optimal for growth of molds and for production toxin [17]. Several factors provide an ideal environment which promotes the growth of fungi. The principal climatic circumstances such as erratic rainfall, drought, more temperature between 20 and 35°C and more humidity (40–89%), provides a suitable environment for the molds growth and aflatoxins production. In proper dried and stored foods the molds cannot grow properly [18].

1.4 Biochemical Transformation of Aflatoxin

Aflatoxins biotransformation is interconnected closely with their toxic and carcinogenic effects. Therefore, in species sensitivities to aflatoxin B1 (AFB1) - induced carcinogenesis the biotransformation pathways of aflatoxin are hazardous [19]. To the reactive AFB1–8, 9-epoxide requires microsomal oxidation of AFB1 to utilize its hepatocarcinogenic effects. AFBO serves as a critical pathway for AFB1 detoxification may be conjugated enzymatically with GSH (Figure 2). To form the primary AFB1-DNA adduct, 8, 9-dihydro-8- (N7-guanyl)-9-hydroxyaflatoxin B1 (AFB1-N7-Gua) when the epoxide reacts with DNA. And it can break down into the apurinic (AP) site or the AFB1-formamidopyrimidine (FAPY) adduct are the two secondary lesions. AFB1-N7-Gua adducts causes G to T mutations has been observed in Escherichia coli [20]. In blocked replication AFB1-FABY also resulted. In single-stranded DNA blocks replication the dominant species whereas the AFB1-FABY form present normally in double-stranded DNA is mutagenic [21].

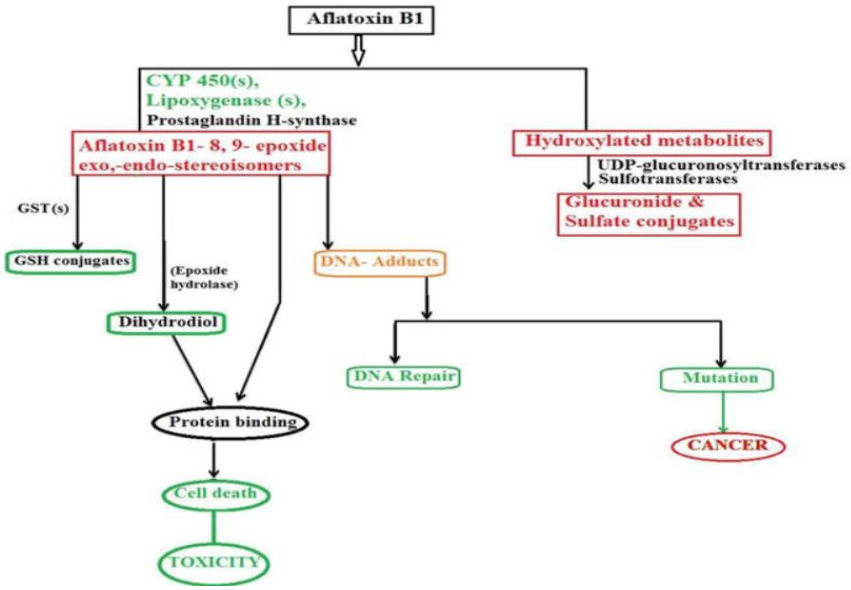


Figure 2 :Biochemical transformation of aflatoxin [22]

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 Bl. 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:

- Down regulation of carnitine palmitoyl transferase (CPT) system in mitochondrion leading to decrease of body weight gain
- Down regulation of fatty acid metabolism pathway increasing liver weight and causing fatty liver
- Up-regulation of cell proliferation pathway causing carcinoma, and
- 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.

1.5 Health Consequences Related to Aflatoxin

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 Bl 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. 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. As a consequence of aflatoxin genotoxicity potential, even shortterm 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) [23].

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) [24,25]. Aflatoxins cause tumours in both the central and peripheral nervous system. Several neurotransmitters are affected by aflatoxins in animals, such as acetylcholinesterase enzymes that affects the memory, learning and cognitive functions [26], 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 [27,28]. 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 Bl is a potent mutagen. The mutagenic effects commence by microsomal activation of the mixed function oxidase to the epoxide form [29]. Mutagenicity studies on bacteria suggest that the possible mechanism of mutagenesis may be initiated by an aflatoxin BI-DNA binding process, leading to the formation of singlestranded gaps as a result of inhibition of DNA polymerase activity at DNA binding sites [30]. 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 [31].

4) Teratogenicity- Aflatoxin Bl 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 [32]. 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 Bl is not teratogenic at all dosage levels [33]. For example, a single intraperitoneal injection of aflatoxin Bl 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 [34].

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. Aflatoxin in the liver is degraded in two phases by (l) biotransformation to a more toxic product and (2) detoxification to a less toxic and easily excretable product [35]. In Phase 1, aflatoxin Bl 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 [36].

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 Bl 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 [37].

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 [38].

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 and impaired growth. 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. 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) [39]. 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. 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 Bl 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, 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 Bl 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 Permitted Limit of Aflatoxin

Food and Drug Administration (FDA) permitted an entire quantity of 0.5 g/kg or 50 ng/l in milk and 20 ng/g in livestock feed in US. The permitted levels of aflatoxin M1 in milk, milk products and baby food are 0.005 mg/kg in European countries. Various regulations for permitted levels of aflatoxin in livestock feed sets by other countries. For example the permitted levels of aflatoxin from 0.05 to 0.5 μg/kg setup by European Union (EU). The environmental factors like weather conditions are effective the determining acceptable levels of aflatoxin. In tropical countries the permitted levels of this toxin are more compared to cold countries.

1.7 Controlling Aflatoxin level by UV Treatment

Aflatoxin production can be controlled by destroying or oxidizing fungal cell structure, interrupting cell components and cell metabolism, and ultimately leading to fungal death.

UV radiation directly damages DNA, inhibiting its transcription and replication; furan ring is photodegraded by UV radiation. UV is widely applicable to most types of microorganisms, with low cost and simple operation.

Specific action mechanisms of UV radiation are:

(i) Loss of membrane integrity. Fungal cells can resist harsh external environment because of their membrane integrity. Once the integrity of the membrane is lost, the fungal cell is vulnerable to be attacked. Hence, extensive studies have been conducted on this main prerequisite for inactivation. The cause of membrane breakdown may be the etching by various radicals from plasma, or the accumulation of charged particles on the membrane surface [43]. The etching is regarded as the key inactivation mode of CPT, which can not only enhance the diffusion of the second RS, such as NO, OH, H2O2 and O3 in the cell but can also break the bonds, causing morphological changes [44]. Another potential mechanism is electrostatic interference, which can damage the membrane structure when the electrostatic force on the membrane surface is higher than the tensile strength of the membrane [45]. Scientists demonstrated that atmospheric pressure fluidized bed plasma has a fatal effect on the membrane integrity of *A. flavus* and *A. parasiticus* spores. In the case of cell membrane rupture, leakage of intracellular contents occurs and other cellular components are attacked by plasma penetration.

(ii) DNA damage. Previous studies elucidated that DNA damage directly induced by UV photons, and this is considered as the ultimate cause of fungal death. UV photons induce the dimerization of thymine bases of DNA strand, alters the bonds between DNA bases, and changes DNA conformation, which eventually leads to DNA strand breakdown [46]. Under the premise of fungal spore surface etching, it is easier for the UV photons to contact the DNA, as observed by ˇSimonˇcicov´a et al. (2018), showing DNA fragmentation after CAPP for 30 s.

(iii) Proteins oxidation. Proteins play a vital role in the chemical signal exchange and metabolite transport among cells [47]. If proteins are destroyed, it will indirectly bring about the death of fungal cells. Proteins may subject to oxidative damage caused by ROS that penetrate through the cell membrane, changing the conformation and three-dimensional structure of proteins and cytosolic enzymes, and in turn affecting cell vitality. Kim et al. (2014) reported that *A. flavus* was effectively inhibited by CPT, which might be due to RS oxidized glycoproteins in the fungal cell, thereby leading to the inhibition of fungal growth.

(iv) Lipid peroxidation. The accumulation of lipid peroxide is likely to cause cell death because it obstructs cell function. Membrane lipid peroxidation changes the fluidity and permeability of the membrane, causing changes in cell structure and function. At the same time, with the intracellular invasion by ROS, lipid undergoes a peroxidative chain reaction, forming a series of harmful lipid peroxidation products, with the most typical being malondialdehyde [48]. This intracellular lipid peroxidation may damage other cell components and disturb their functions.

(v) The occurrence of apoptosis. Research has indicated that cell apoptosis is one of the main pathways for decontaminating microorganisms. During CPT, RS penetrate fungal cells, react with various cell components, break the state of intracellular equilibrium and thus promote apoptosis of cells [49]. Niedzwiedz et al. (2019) proposed that one

of the mechanisms that plasma treatment might inactivate fungal cells was apoptosis or necrosis [50], and this view has been supported by some research results.

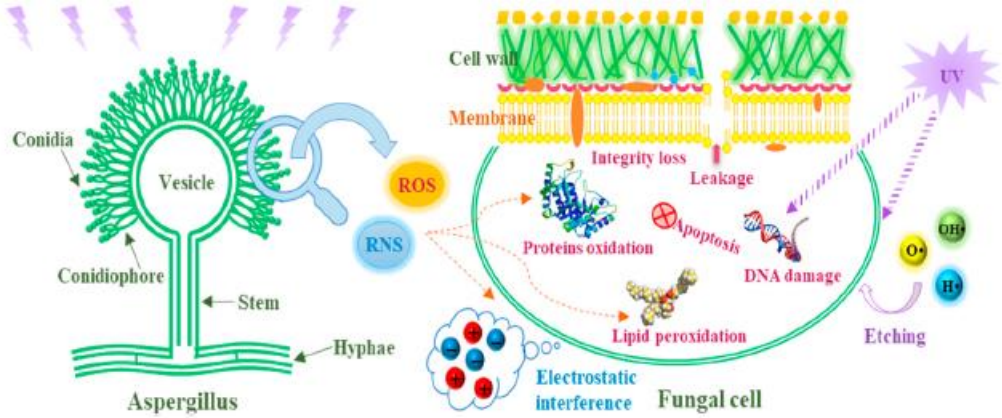


Fig 3: Mode of action of UV radiation for fungal cell degradation [51]

1.8 Degraded By-Products after UV Treatment

Two aflatoxin degradation products, product 1 ($C_{16}H_{16}O_6$) and product 2 ($C_{17}H_{14}O_7$) have been shown to be major ozonolysis products of the treatment of AFB1 by aqueous ozone. Four other new major degradation products were identified, indicating that the degradation of AFB1 by HVACP treatment involves new pathways and reactive oxygen gas species other than ozone. Based on the structure of the six degradation products of AFB1 produced by HVACP treatment, two degradation pathways were proposed as shown in Figure 6 and Figure 7. The first pathway is from AFB1 to degradation products $C_{17}H_{15}O_7$ (m/z 331.0821), $C_{16}H_{17}O_6$ (m/z 305.1028), and $C_{19}H_{19}O_8$ (m/z 375.1056). [52] The second pathway is from AFB1 to degradation products $C_{14}H_{13}O_5$ (m/z 261.0755), $C_{14}H_{11}O_6$ (m/z 275.0549), and $C_{17}H_{13}O_7$ (m/z 329.0666). [53] The first degradation pathway involves primarily addition reactions in which a water molecule (H_2O), hydrogen molecule (H) or aldehyde group (CHO) is added to AFB1. The first branch reaction starts with the addition of water molecules (hydration reaction) to the C8-C9 double bond at the furan ring of AFB1 to form the degradation product $C_{17}H_{15}O_7$ (m/z 331.0821). The methoxy group (-OCH_3) of AFB1 was cleaved to form the intermediate product $C_{16}H_{13}O_6$ (m/z 301.0712), and the carbonyl groups of the intermediate product $C_{16}H_{13}O_6$ were further hydrogenated to form the degradation product $C_{16}H_{17}O_6$ (m/z). [52] The second pathway mainly involves epoxidation and oxidation reactions. The first branch is the formation of degradation product $C_{17}H_{13}O_7$ (m/z 329.0666) through epoxidation of the terminal double bond of AFB1. The epoxidation reaction could be attributed to the hydroperoxyl radical (HO_2•) which is generated during treatment. [53] Its concentration increases when the air relative humidity is higher. HO_2• is one type of peroxyl radical that reacts with double bonds and leads to epoxide formation. In the second branch, the furofuran ring of AFB1 is cleaved and the degradation product $C_{14}H_{11}O_5$ (m/z 261.0755) is formed. Further oxidation of this product leads to formation of another degradation product, $C_{14}H_{11}O_6$ (m/z 275.0549). [54]
305.1028). The second branch of the reaction is the addition of an aldehyde group (CHO) to form the intermediate product, $C_{19}H_{15}O_8$ (m/z 371.0767). Next the carbonyl groups in the lactone ring

and cyclopentanone of this intermediate product were hydrogenated to form the degradation product, C19H19O8 (m/z 375.1056). From the first degradation pathway of AFB1, the crucial reactive agents are the hydrogen atom (H) and the hydroxyl radical (OH•), which were generated in the HVACP system by the breaking down of water molecules. These two species are responsible for hydration and hydrogenation to form new degradation products. Another reactive agent generated is the aldehyde (CHO) radical, which is formed in the HVACP system when carbon dioxide (CO2) is present in the gas used. [55]

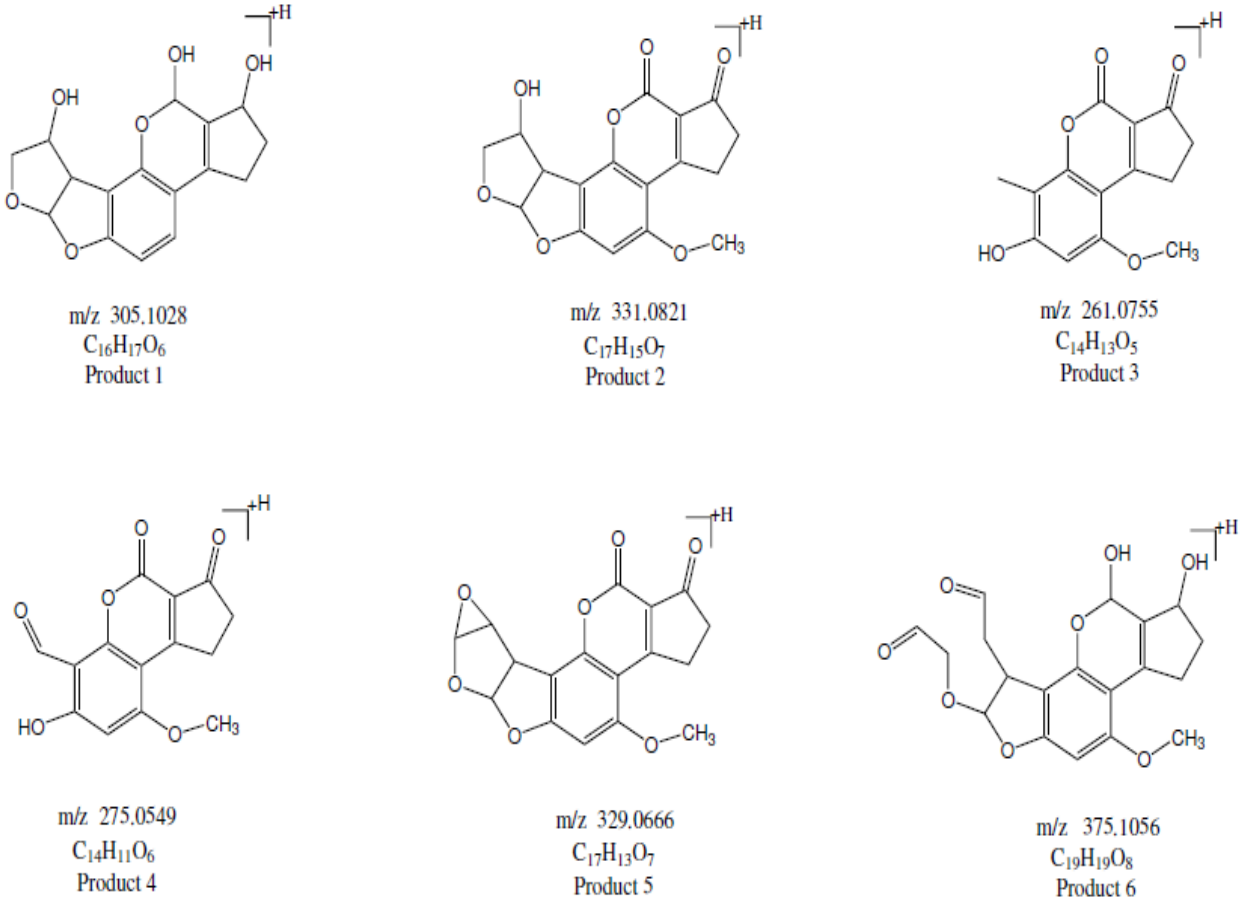
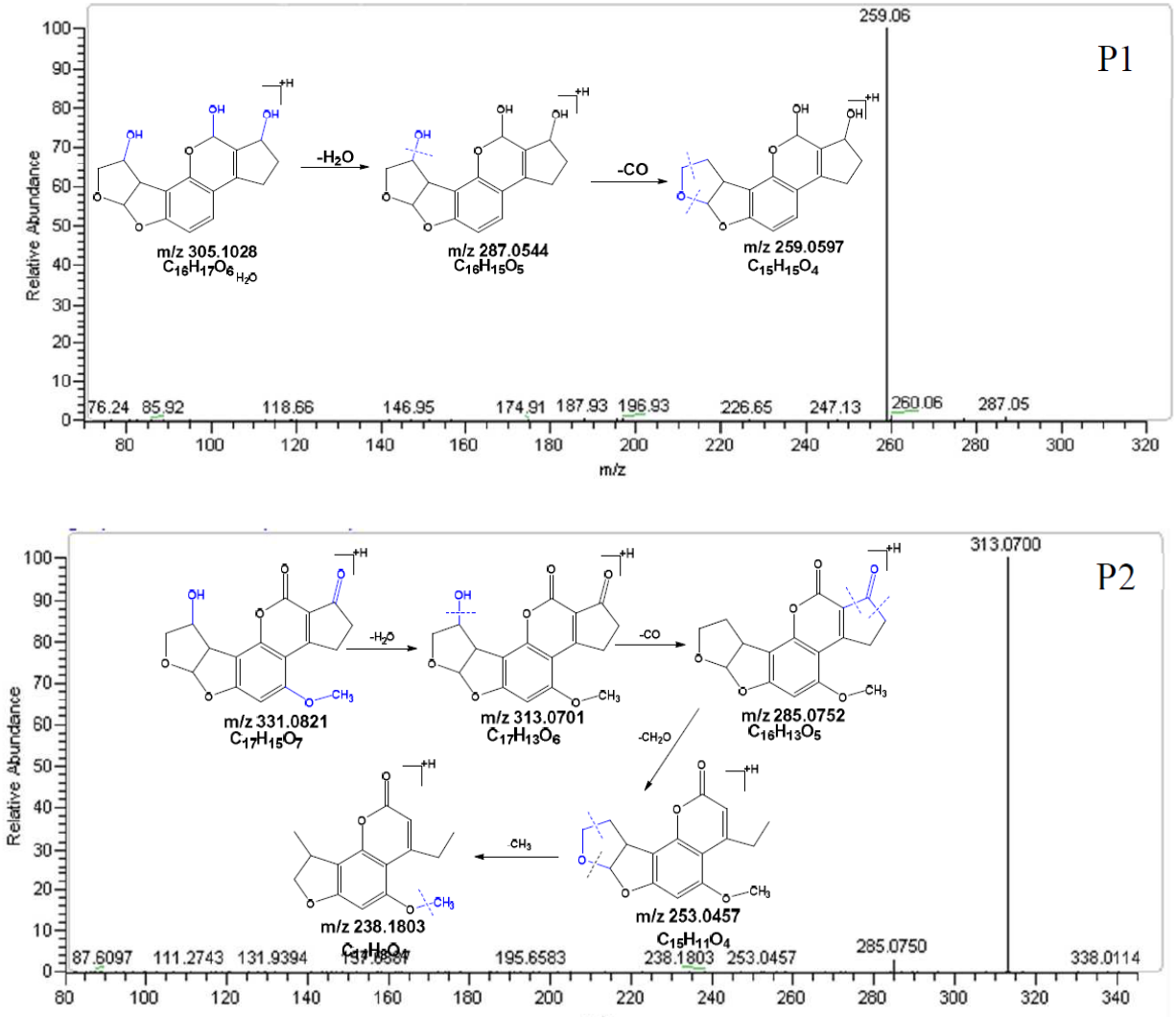


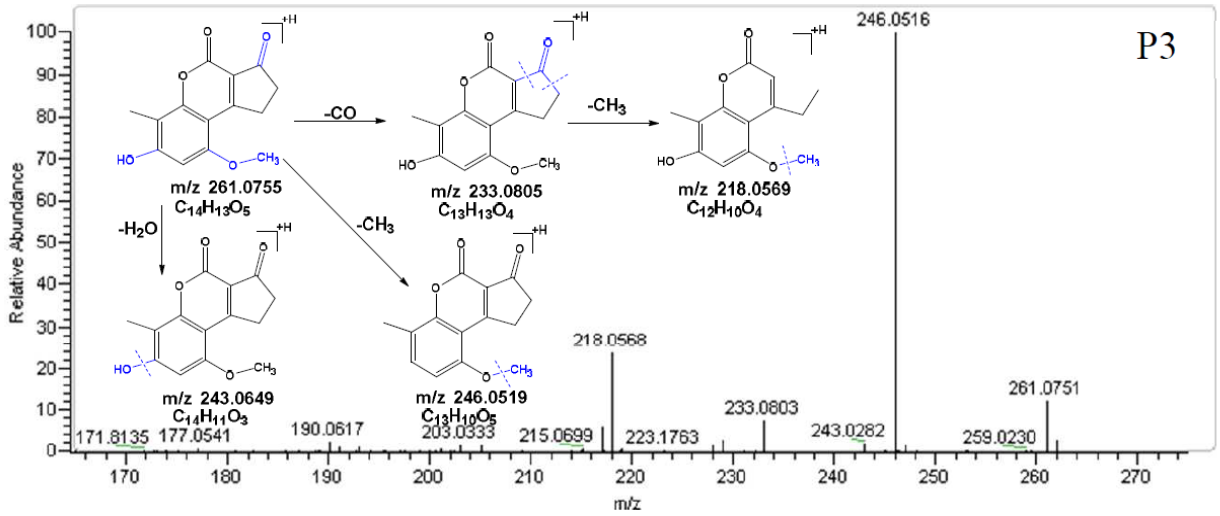
Fig 4. Proposed structures of degraded products of AFB1 after treatment

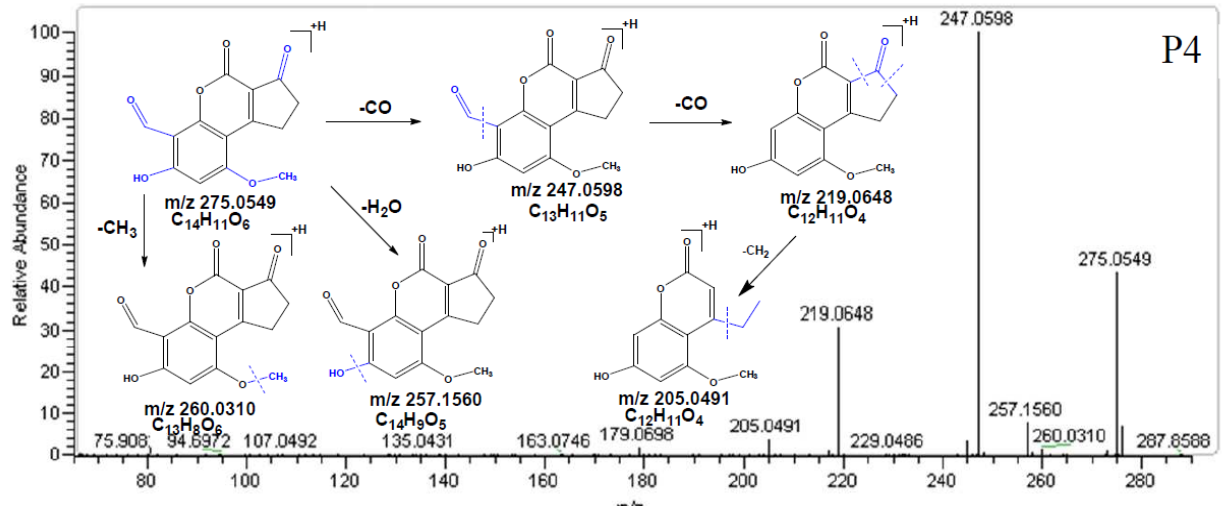
Since the discovery of aflatoxin in the early 1960s, the toxicology of aflatoxins have been widely studied.The relationship of structure-to bioactivity (toxicity, carcinogenicity and mutagenicity) of aflatoxins has been elucidated. AFB1 has an optimal molecular structure for acute toxicity, mutagenicity, and carcinogenicity. Any changes in the furofuran ring, the lactone ring, the cyclopantenone, or the methoxyl structure would result in a marked reduction in biological activity.The furofuran moiety of the aflatoxin structure is essential for toxic and carcinogenic activities, and the double bond in the terminal furan ring is a notably important determinant of toxic and carcinogenic potency. All of the six proposed major degradation products of AFB1 lost their double bond, and the degradation products were different from AFB1 by further modification

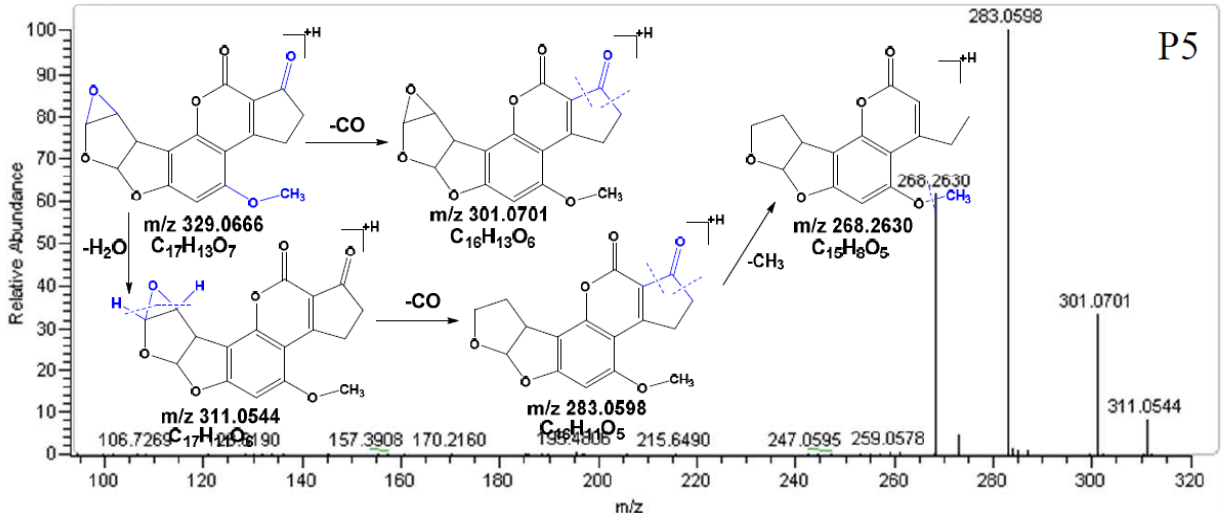
of the furofuran ring (products 1-6), lactone ring (products 1, 6), cyclopantenone (products 1, 6), or the methoxyl structure (product 1).
288 Therefore, on the basis of the-relationships between chemical structure and biological activity reported in the literature, the authors predict that the toxicity, carcinogenicity and mutagenicity of the degradation products will be substantially less than those attributes of AFB1. [56]

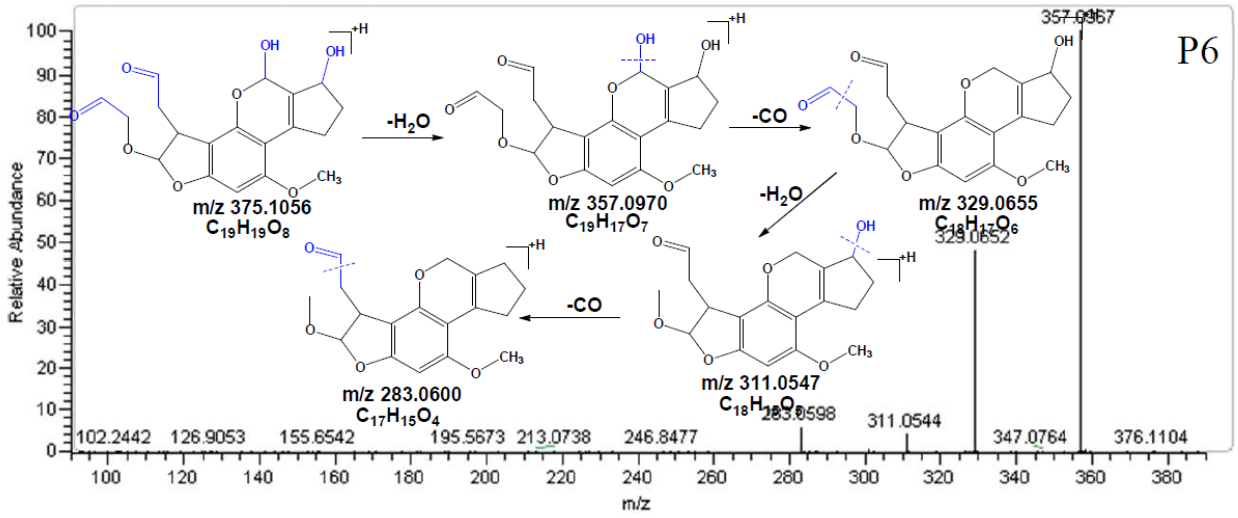
Proposed pathways for the formation of degraded products: [57]




P3
m/z 261.0755
C14H13O5
-CO
m/z 233.0805
C13H13O4
-CH3
m/z 218.0569
C12H10O4
-H2O
m/z 243.0649
C14H11O3
-CH3
m/z 246.0519
C13H10O5
246.0516
218.0568
261.0751
233.0803
243.0282
259.0230
171.8135
177.0541
190.0617
203.0333
215.0699
223.1763
Relative Abundance
m/z


P4
m/z 275.0549
C14H11O6
-CO
m/z 247.0598
C13H11O5
-CO
m/z 219.0648
C12H11O4
-CH3
-H2O
-CH2
m/z 260.0310
C13H8O6
m/z 257.1560
C14H9O5
m/z 205.0491
C12H11O4
247.0598
275.0549
219.0648
257.1560
260.0310
287.8588
205.0491
229.0486
75.908
94.6972
107.0492
135.0431
163.0746
179.0698
Relative Abundance


P5
m/z 329.0666
C17H13O7
-CO
m/z 301.0701
C16H13O6
-H2O
-CO
-CH3
m/z 311.0544
C17H11O6
m/z 283.0598
C16H11O5
m/z 268.2630
C15H8O5
283.0598
301.0701
311.0544
268.2630
247.0595
259.0578
157.3908
170.2160
215.6490
Relative Abundance
m/z



1.8 Research Aim and Objective:

The aim of the present study is to investigate the effectiveness of UV-Plasma assisted fluidized bed drying in reducing AFBI levels in peanuts and to determine its shelf life and presence of degraded products after the treatment

The objectives of the study were:

- AFBI Degradation: The primary focus of the study is to understand the kinetics of aflatoxin Bl degradation during UV-Plasma assisted fluidized bed drying. This involves assessing the rate at which aflatoxin Bl breaks down under different conditions.
- Shelf-life estimation of peanuts: Peanuts were exposed to UV treatment at certain intervals and the level of aflatoxin and micro-organisms in treated and untreated peanuts were quantified using HPTLC.
- Chemical Quality Analysis: Free Fatty Acid content of peanut oil extracted from treated and untreated peanut oils were quantified.
- Degraded Products: Presence of degraded products resulting from UV treatment of peanuts were studied using LC-MS
- Practical Applications: The practical applications and feasibility of implementing UV assisted drying as a potential method for aflatoxin reduction in peanuts at an industrial scale was explored.

Chapter 2:
Materials and Methods

2.1 Selection of Peanut Samples Contaminated with AFBI

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

- Source of Contaminated Peanut Samples: Peanut samples that are suspected of being contaminated with Aflatoxin Bl were collected from the local market for degradation study. These samples can be from specific batches or regions known to be at risk for mold growth and aflatoxin contamination. Fresh packaged peanut samples were collected for shelf life study process.

- 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 4^0 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.

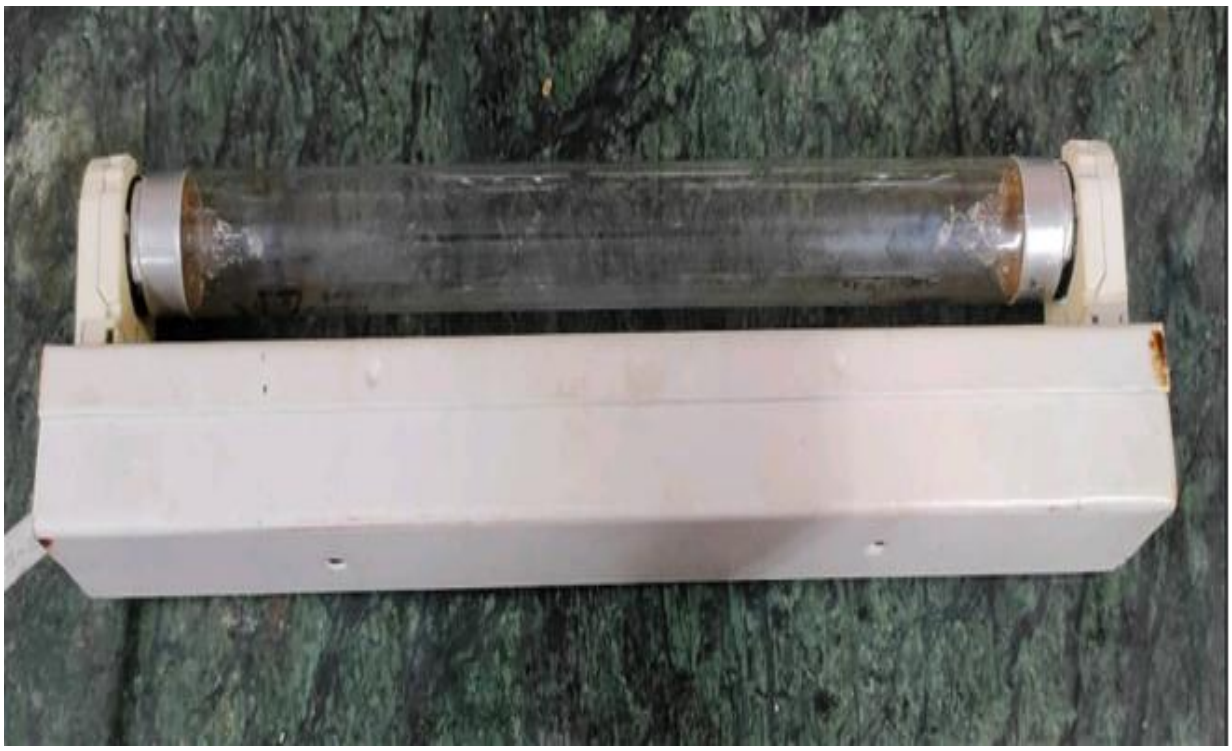


Fig 6. Philips UV Light

Fig 7. Control Panel of Fluidized Bed Drier

Fig 8. Fluidized Bed Drier

2.3 Experimental procedures for AFBI degradation kinetics study

Studying the degradation kinetics of Aflatoxin Bl (AFB1 typically involves a series of controlled experiments to observe how the toxin breaks down over time under different exposure conditions. Here's an outline of the experimental procedures for conducting an AFBI 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: 10 peanuts weighing nearly 6-7g were used for each set of treatment. The peanuts were washed thoroughly and then treated for degradation of aflatoxin at five different time intervals respectively.

c) Drying Process: Samples (6-7 g) were treated for 5, 15, 20, 25 and 30 mins at 40^0 C. The FBD was preheated until the treatment temperature (40 ^{0}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 peanuts 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 peanut kernels were dipped in 5ml of solvent containing Petroleum Benzene (60-80^0 C) and Acetonitrile in the ratio of 98:2 [58]. The peanuts 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 1 ml of sample from each conical flask into separate Eppendorf tubes for HPTLC analysis.

2.4 Experimental procedures for Shelf-Life Study of Peanuts

Shelf- life study of peanuts were studied by exposing the peanuts to UV radiation at a certain time interval and measuring the level of aflatoxin in treated and untreated peanut samples. Here's an outline of the experimental procedures for conducting an AFBI 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: One set of peanuts were UV treated on a fixed interval and stored in an airtight container. 10 samples from that treated peanut set were taken for analysis. The kernels were washed thoroughly and then treated for degradation of aflatoxin at five different time intervals respectively. On the other hand one set was kept untreated.

c. Treatment Process: Samples (6-7 g) were treated for 30 mins at 40^0 C. The FBD was preheated until the treatment temperature (40 ^{0}C) was achieved and after the treatment time the heater was turned off and air passed through each sample to accelerate cooling. The treatment was given thoroughly at time intervals and the peanuts were stored in an air tight container till further aflatoxin analysis and next treatment.

d. Sample Preparation: Treated peanut kernels were dipped in 5ml of solvent containing Petroleum Benzene (60-80^0 C) and Acetonitrile in the ratio of 98:2 [58]. The peanuts 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 1 ml of sample from each conical flask into separate Eppendorf tubes for HPTLC analysis.

Fig 9: Sample Preparation of Peanuts for detection of Aflatoxin Level

Fig 10: Storage of peanuts

2.5 Inoculation of Peanuts and Microbiological Study

Microbiological Study was done by inoculating fresh sterilized peanuts with Aspergillus species (which was separately prepared) and the level of micro-organisms present in treated and untreated peanuts were understood by measuring the optical density of the media.

1. Peanut kernels were collected from the local market and stored at 4℃
2. Moisture of the peanut samples were adjusted to 15 % before inoculation with *Aspergillus flavus* as the optimum moisture content for AFT production on peanuts is 15%–30% [59]
3. Aspergillus strains were taken and proliferated on Czapek Dox Agar media (Agar- 15g/L, Sucrose- 30 g/L, Sodium nitrate- 2g/L, Dipotassium phosphate- 1g/L, Magnesium sulphate- 0.5g/L, Potassium chloride- 0.5 g/L, Ferrous sulphate- 0.01g/l .)
4. The strains were incubated at 30℃ for 5-7 days
5. The greyish green portion of the growth indicated the presence of *Aspergillus flavus*.

Fig 11. Growth of Aspergillus species on Czapek Dox Agar media

6. The presence of Aspergillus flavus was confirmed by streaking the greyish green portion on Aspergillus Differential Medium Base (formation of reddish orange color on the reverse side of the colonies)

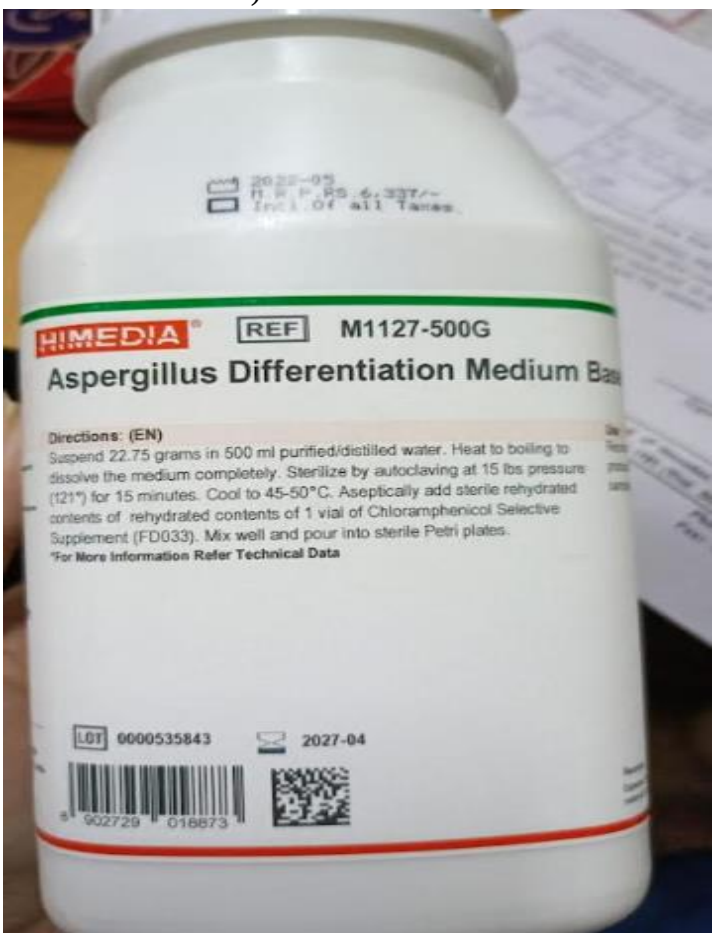


Fig 12: Aspergillus Differential Medium Base

Fig 13: Growth of A. flavus on differential media

7. Peanuts were taken at Erlenmeyer flasks with 100mL distilled water, autoclaved at 121.1 ℃, 15 psi, 15 minutes.
8. Sterilized flasks were inoculated with 25ml of spore suspension and then incubated at 30℃ for 10 days.
9. 20 Peanuts were taken from the inoculated suspension from where 10 peanuts were given UV treatment and 10 peanuts were kept untreated.
10. After treatment, both the untreated and treated peanuts were put into separate conical flasks containing sterilized Czapek Dox Agar media and were incubated at 30℃ for 5 days. The flasks were continuously agitated for uniform distribution of fungal cells and nutrients.
11. After 5 days of incubation, the microbial growth was estimated by measuring the optical density of both the media using a spectrophotometer.

2.6 Analysis of AFB1 concentration of peanuts 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 F254, 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 vision CATS software: For creating a new method in the CAMAG provided vision CATS 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 vision CATS 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^{0} for equal distribution of solvent in both the chambers.

- The TLC plate was set on the Linomat 5 applicator. Sample and standard AFBI 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 Pl. 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-troughchamber at an angle of 45 ^{0} and was kept for 5-10 mins or until the solvent reached the premarked 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 14: CAMAG Linomat 5 applicator

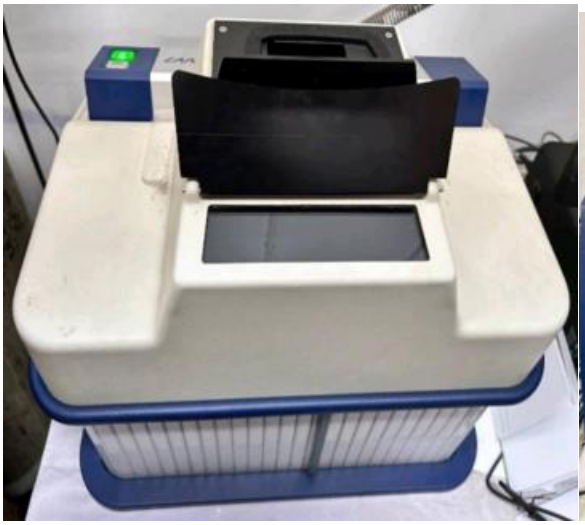


Fig 15: Digital camera

Fig 16: Scanner

2.7 Degradation Kinetics of AFB1

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

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-l/min[1].
- ' [A]' denotes the concentration of the first-order reactant 'A'.

____ denotes the change in the concentration of the first-order reactant 'A' in the time dt 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:

$$-d^{l};^{41} - k[A]\, d$$

$$\underline{d[Al} \quad \text{-kdt}$$

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

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

which can also be rewritten as:

$$\frac{1}{[A]}d[A] = -k\,dt$$

The equation can be rewritten as follows:

$$\int_{[A]_0}^{[A]} \frac{1}{[A]}d[A] = -\int_0^t k\,dt$$

$$\ln[A] - \ln[A]_0 = -kt$$

$$\ln[A] = -kt + \ln[A]_0$$

Raising each side of the equation to the exponent 'e' (since eIn(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.

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

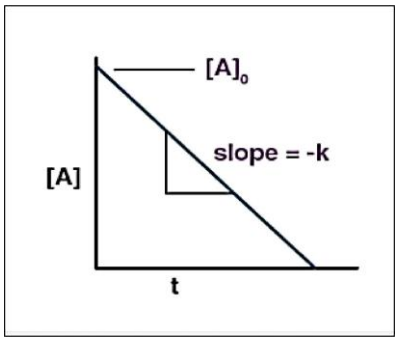


Fig 17: Graph showing similarity between first-order kinetics equation to that of a straight line

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

2.8 Analysis of Degraded By-products Post treatment using LC-MS

1. UV-Treated peanut kernels were dipped in 5ml of solvent containing Petroleum Benzene ($60\text{-}80^0$ C) and Acetonitrile in the ratio of 98:2 [58]. The peanuts dipped in solvent was kept overnight and then vortexed the next day.
2. A micropipette was used to draw 1 ml of sample from each conical flask into separate Eppendorf tubes for LC-MS analysis.

2.9 Free Fatty Acid Analysis of Peanut oil

Amount of Free Fatty acid of the peanut oil extracted from both treated and untreated peanuts were quantified to analyze the chemical quality of the UV treated peanuts.

1. Treated and control peanut oil was extracted via Soxhlet extraction.
2. Weigh the amount of peanut oil in the conical flask
3. Add 50 mL of neutral ethyl alcohol
4. Heat the mixture for about 15 minutes in water bath
5. Titrate hot against standard sodium hydroxide solution to a pink end point using phenolphthalein as an indicator.
6. Substract titre value of blank alcohol.

Fig 18: Grounded peanuts for Soxlet extraction

Fig 19: Soxlet Extraction process

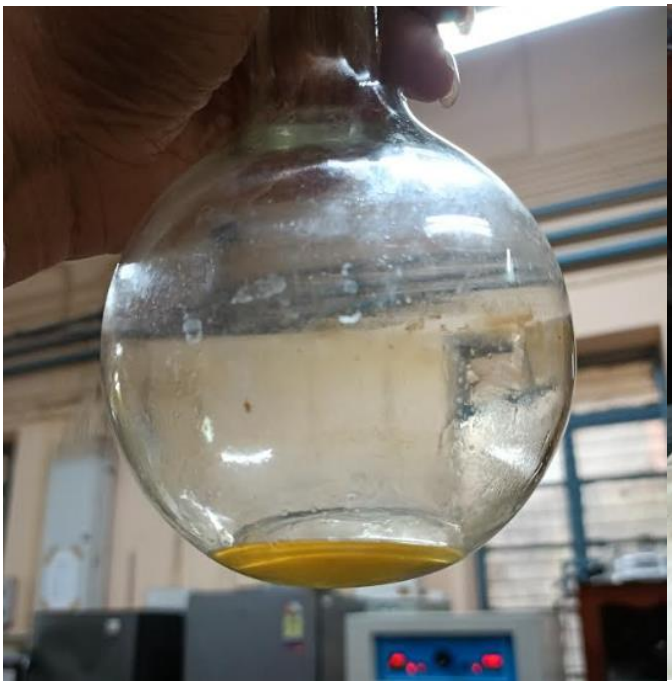


Fig 20: Extracted peanut oil

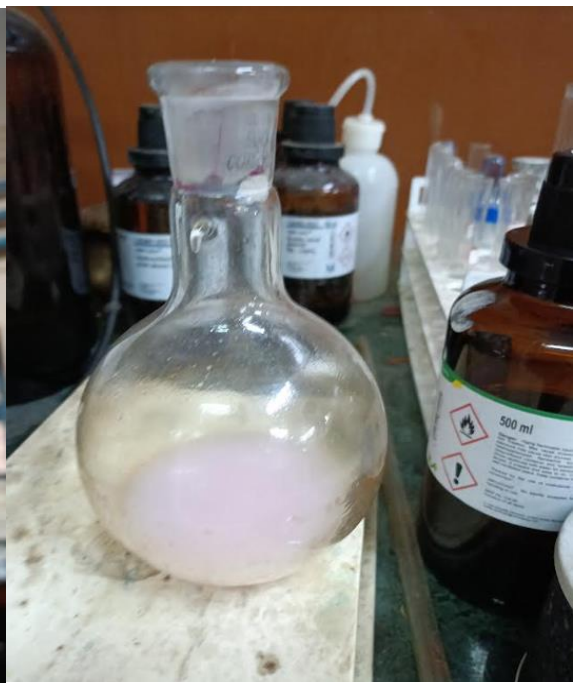


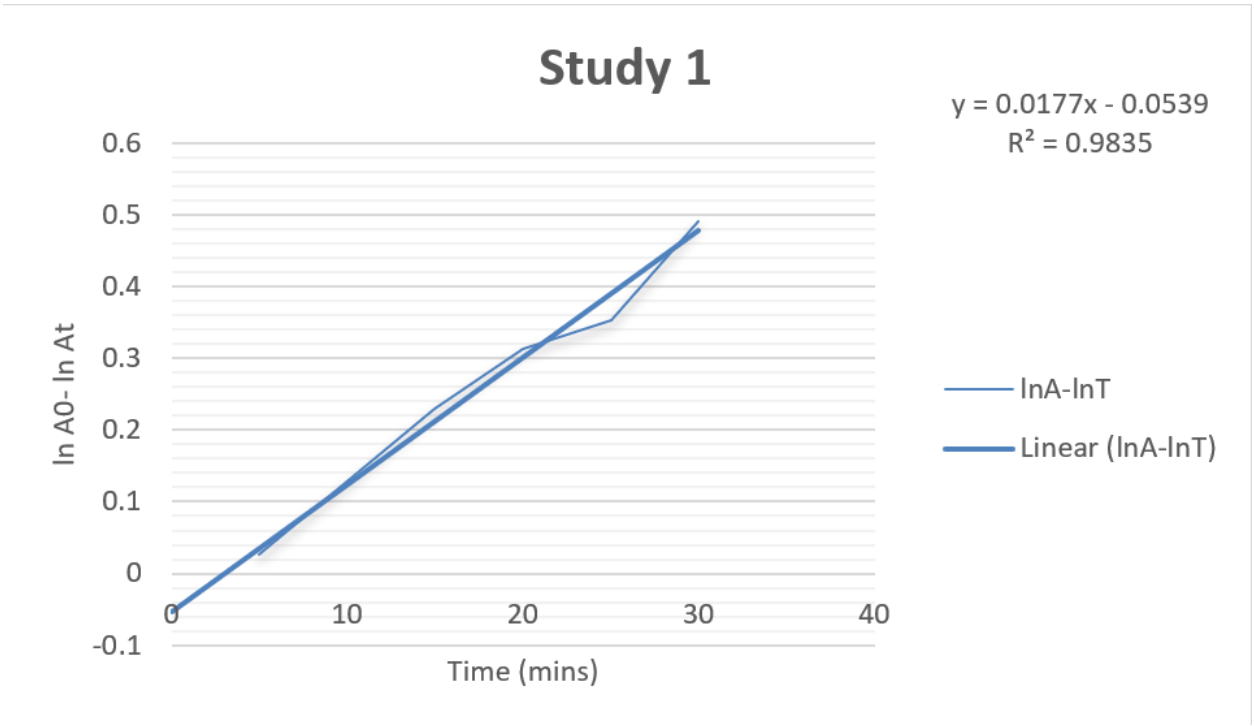
Fig 21: Reaching of end point during titration while estimating FFA

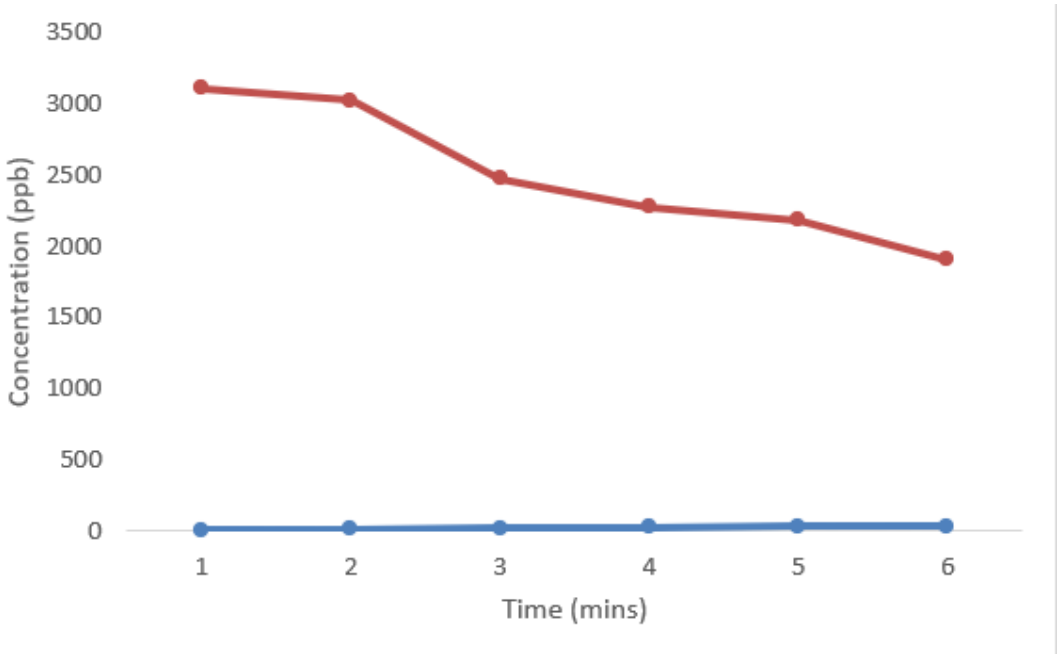
Chapter 3:
Results and Discussion

3.1 Degradation Kinetics of AFB1 (at 40 ℃)

Study 1:

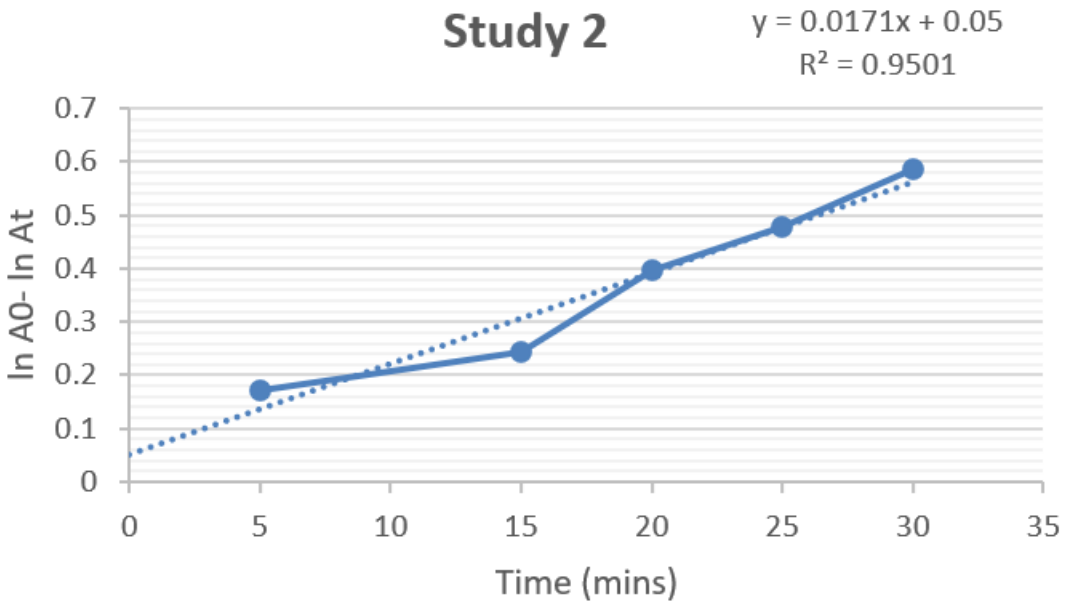
Temperature	Time of Exposure (mins)	Weight of peanuts (g)	Concentration of AFB1 (ppm)
40℃	0 (untreated & control)	5.833	3.103
	5	5.924	3.021
	15	5.894	2.468
	20	5.811	2.269
	25	6.258	2.178
	30	6.564	1.897

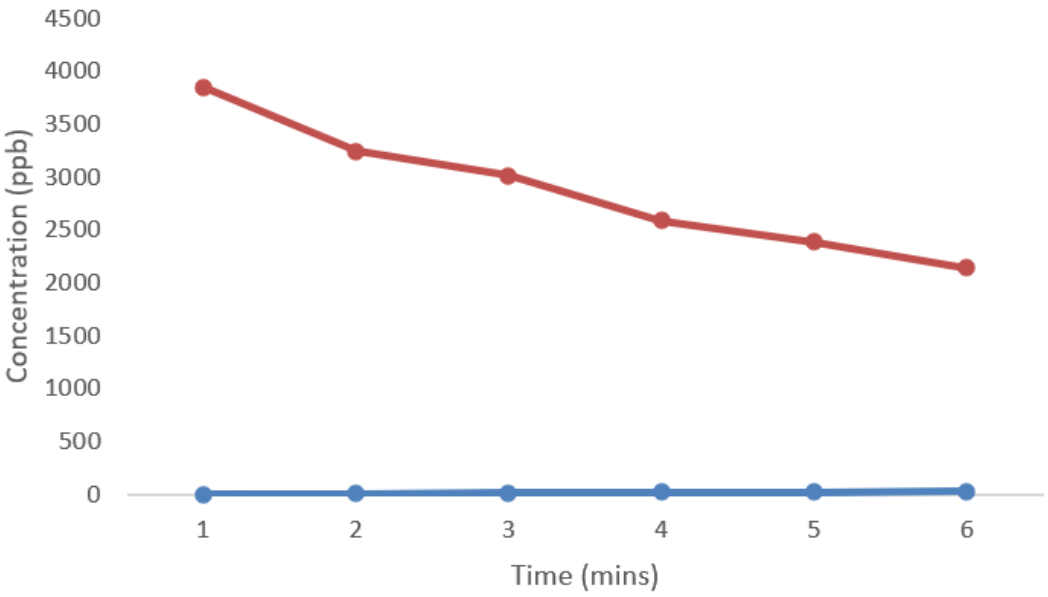

Study 1
y = 0.0177x - 0.0539
R² = 0.9835
ln A0- ln At
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
0
10
20
30
40
Time (mins)
lnA-lnT
Linear (lnA-lnT)


Concentration (ppb)
3500
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
Time (mins)

Study 2:

Temperature	Time of Exposure (mins)	Weight of peanuts (g)	Concentration of AFB1 (ppm)
40℃	0 (untreated & control)	5.928	3.847
	5	6.630	3.242
	15	5.543	3.014
	20	5.783	2.587
	25	5.974	2.386
	30	5.754	2.143





First-order rate constant (k) was calculated from the intercepts of the ln A0 – ln At vs Time graph and was found to be in the range of 0.05 min $^{-}$

3.2 Shelf-Life study of Peanuts by Exposure to UV radiation and degradation of AFB1

Study with fresh packaged peanut samples

Date	**Type of Treatment**	**Weight of peanuts (g)**	**Level of Aflatoxin (ppm)**
28.11.2023	Untreated (Fresh packaged sample taken)	5.011	3.698
	UV treatment given for 30 minutes		
04.12.2023	Untreated	4.727	4.027
	Treated	4.828	3.768
05.12.2023	UV treatment given for 30 minutes		
08.12.2023	Untreated	4.810	4.596
	Treated	4.676	3.710
	Untreated	4.780	4.844

11.12.2023	Treated	4.723	3.787
12.12.2023	UV treatment given for 30 minutes		
15.12.2023	Untreated	4.236	4.781
	Treated	4.970	3.674
19.12.2023	Untreated	4.815	4.988
	Treated	5.6	3.714
	UV treatment given for 30 minutes		
23.12.2023	Untreated	4.2	4.947
	Treated	4.15	3.871
02.01.2024	Untreated	4.4	5.106
	Treated	4.730	3.906
03.01.2024	UV treatment given for 30 minutes		
	Untreated	5.05	5.140

08.01.2024	Treated	4.89	4.118
10.01.2024	UV treatment given for 30 minutes		
16.01.2024	Untreated	5.09	5.147
	Treated	5.13	4.194
19.01.2024	Untreated	5.32	5.244
	Treated	5.377	4.210
	UV treatment given for 30 minutes		
24.01.2024	Untreated	5.25	5.274
	Treated	5.52	4.295

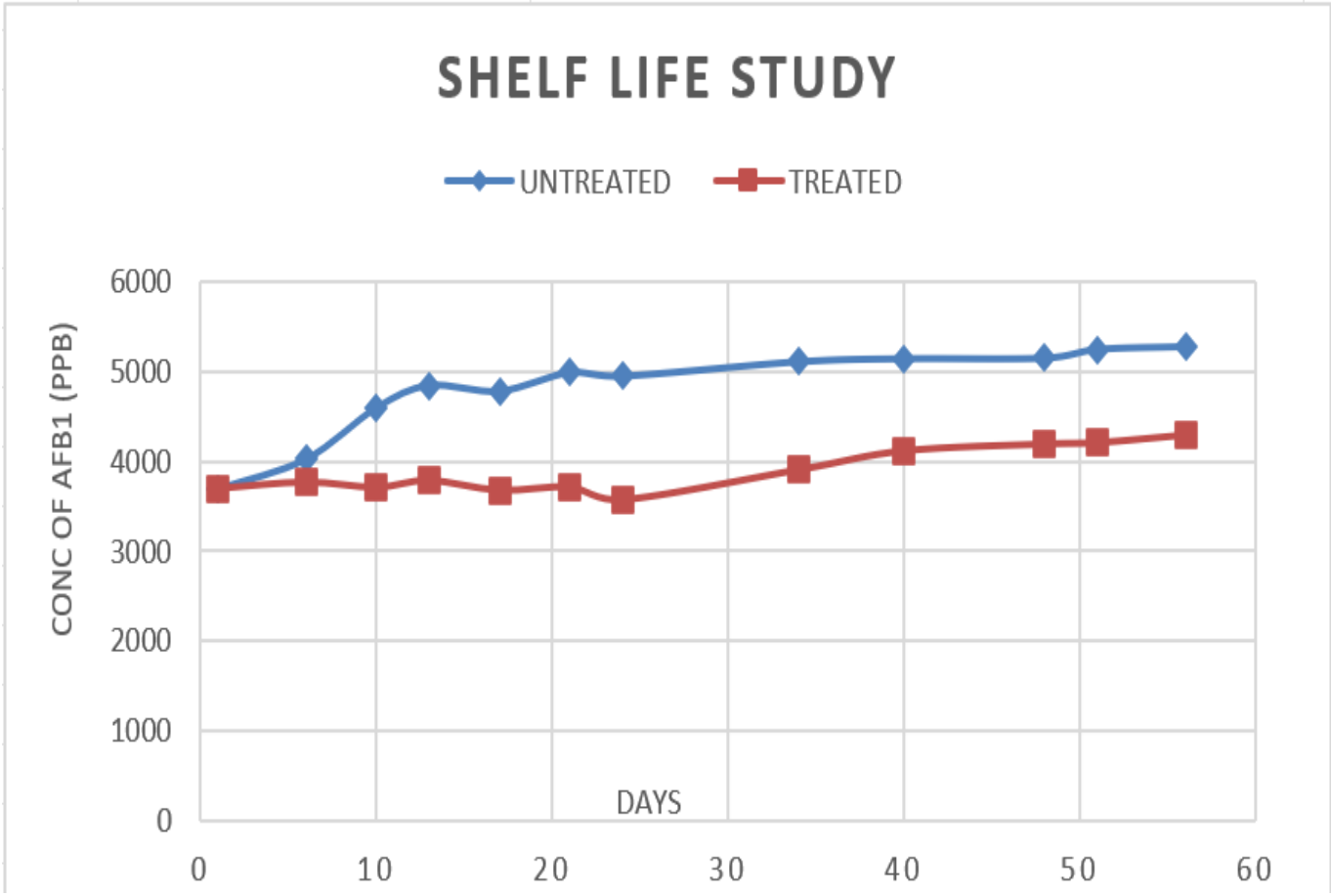
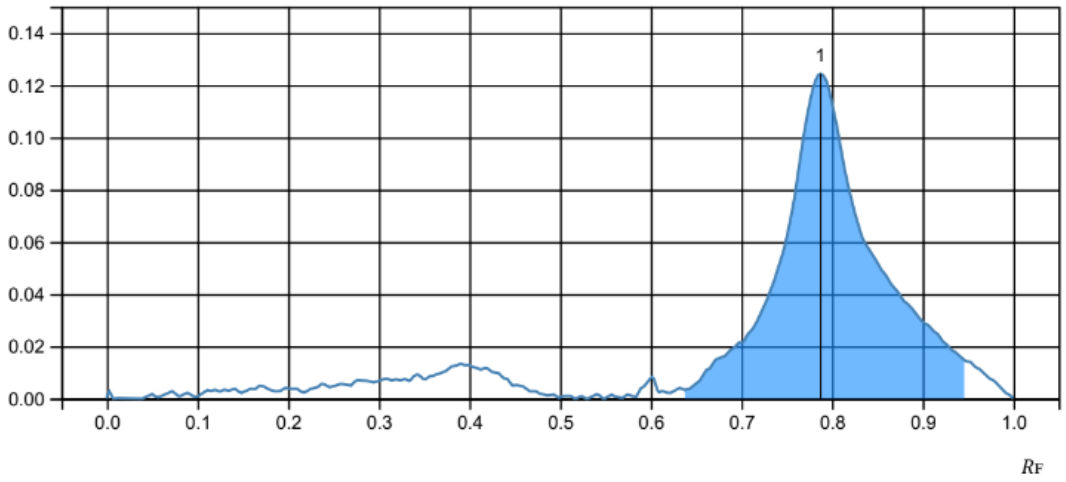

SHELF LIFE STUDY
UNTREATED
TREATED
CONC OF AFB1 (PPB)
6000
5000
4000
3000
2000
1000
0
DAYS
0
10
20
30
40
50
60

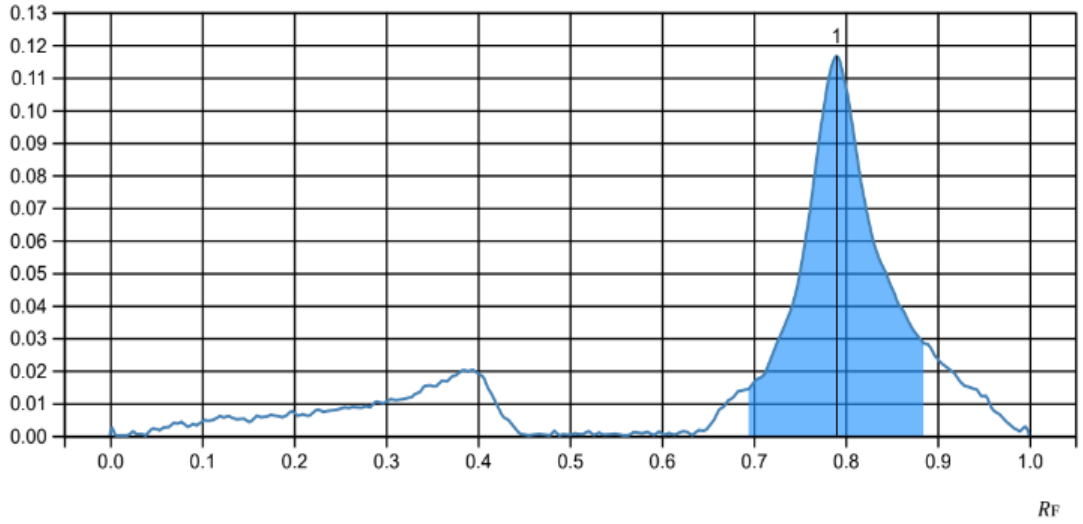
FIG 23: Untreated Peanuts after 56 days of storage

FIG 24: Treated peanuts after 56 days of storage and intermittent UV treatments



Peak	Start		Max			End		Area	
#	R_F	H	R_F	H	%	R_F	H	A	%
1	0.637	0.0033	0.787	0.1244	100.00	0.949	0.0143	0.01466	100.00

FIG 24: HPTLC spectrum curve for untreated peanuts

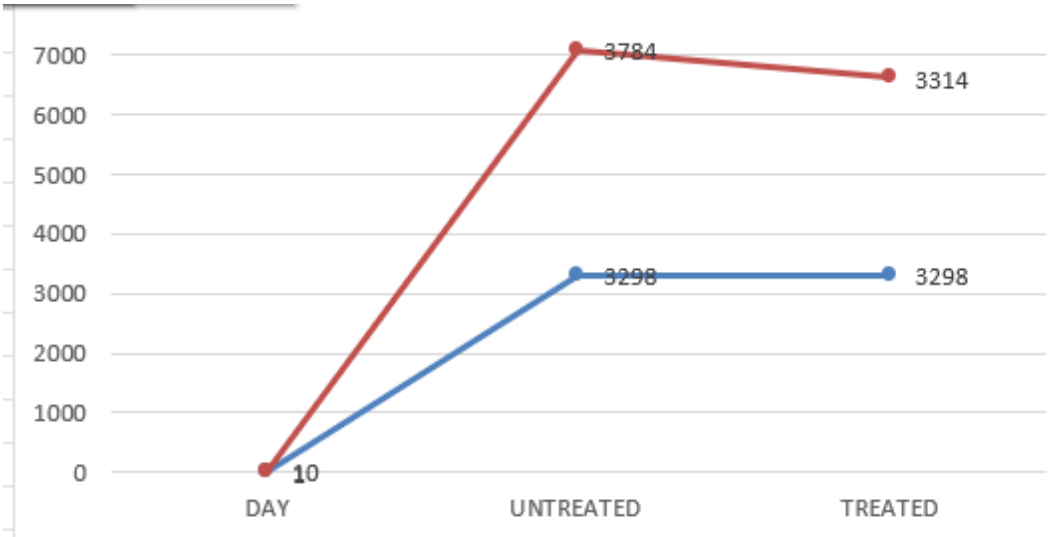


Peak	Start		Max			End		Area	
#	R_F	H	R_F	H	%	R_F	H	A	%
1	0.686	0.0138	0.790	0.1165	100.00	0.887	0.0281	0.01108	100.00

FIG 25: HPTLC spectrum curve for treated peanuts

Study with random peanut samples from local market

Date	Type of Treatment	Weight of peanuts (g)	Level of Aflatoxin (ppm)
16.03.2024	Untreated (Random sample from local market)	6.603	3.298
	UV treatment given for 30 minutes		
01.04.2024	Untreated	5.751	3.784
	Treated	6.745	3.314




Untreated.

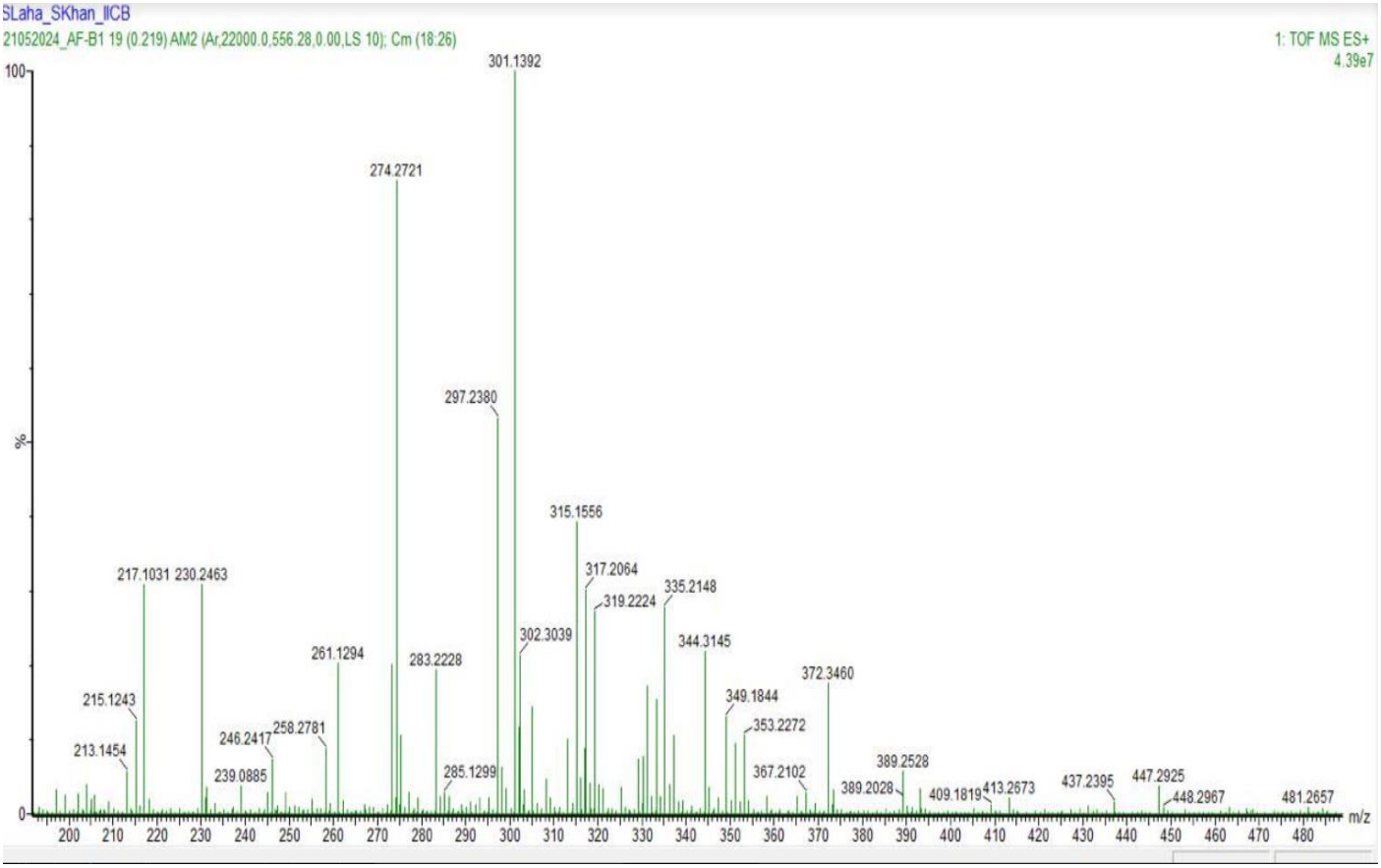

Treated

3.3 Microbiological Study of Peanuts after exposure to UV radiation

Fig 27: Change in optical density of media after inoculating with untreated and treated peanuts

Wavelength	Absorbance of media with untreated sample	Absorbance of media with treated sample
600 nm	2.176 A	1.683 A
620 nm	2.139 A	1.651 A

3.4 Analysis of Degraded By-products Post treatment using LC-MS



m/z ratio	Chemical formula
261.1296	$C_{14}H_{13}O_5$
274.2723	$C_{14}H_9O_6$
275.2763	$C_{14}H_{11}O_6$

305.1563	$C_{16}H_{17}O_6$
313.2107	$C_{17}H_{13}O_6$ (Aflatoxin B1)
315.1559	$C_{17}H_{15}O_7$
319.2226	$C_{16}H_{17}O_7$
330.3360	C17H13O7
335.2152	C16H17O8
372.3464	C19H17O8

By – products after degradation of Aflatoxin B1 by UV-plasma method:

- C16H17O6
- C17H15O7
- C14H13O5
- C14H11O6
- C17H13O7
- C19H19O8

These chemical compounds which were proposed by researchers through different mechanisms were found in peanuts which were UV treated.

3.5 Free Fatty Acid Analysis of Peanut oil

Acid Value = $\frac{56.1*V*N}{W}$

Free Fatty Acid as oleic acid % by weight = 28.2*N*V/W

Sample	Weight of oil (g)	Normality (N)	Titre Value	FFA%
Treated Peanut Oil	3.461	0.1	1.4	1.140
Untreated Peanut Oil	3.07	0.1	1.2	1.102

Thus, there is no such significant difference in free fatty quantity between UV treated and untreated peanut oil. So we can conclude that UV treatment does not cause any change to chemical quality of peanut oil.

Conclusion:

The degradation kinetics of AFBI 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 Bl degradation in a fluidized bed, but several key conclusions, future research directions, and implications can be drawn from the findings:

1. The study has shown that UV-Plasma assisted fluidized bed drying can effectively degrade aflatoxin Bl in peanuts with a degradation rate constant 0.05 min- (at 40 °C), making it a viable technique for mycotoxin reduction.
2. The shelf life study (56 days and 15 days simultaneously) clearly indicated that the peanuts which were given UV treatment at intermediate intervals were more fresh and less prone to microbiological deterioration. The aflatoxin level of UV treated peanuts were significantly lower than the untreated peanuts.
3. The optical density value of media contaminated with untreated peanuts were more than that contaminated with treated peanuts which clearly indicated that treated peanuts have less microbial load.
4. By – products after degradation of Aflatoxin B1 by UV-plasma method: $C_{16}H_{17}O_6$, $C_{17}H_{15}O$, $C_{14}H_{13}O_5$, $C_{14}H_{11}O_6$, $C_{17}H_{13}O_7$, $C_{19}H_{19}O_8$. These chemical compounds which were proposed by researchers through different mechanisms were found in peanuts which were UV treated which is a indication that aflatoxin B1 was degraded
5. There is no such significant difference in free fatty quantity between UV treated and untreated peanut oil. So we can conclude that UV treatment does not cause any change to chemical quality of peanut oil

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