MATHEMATICAL MODELLING OF AFLATOXIN B1 DECONTAMINATION BY INACTIVATED CELLS OF *Lactobacillus rhamnosus* GG IN DRY-CURED MEAT PRODUCTS - A CASE STUDY OF SAMARELLA

A THESIS SUBMITTED TO THE GRADUATE INSTITUTE OF HEALTH SCIENCES OF NEAR EAST UNIVERSITY

By
HAFIZU IBRAHIM KADEMI

In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Hygiene and Technology

NICOSIA, 2019

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DEDICATION

To the entire ummah

APPROVAL

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DECLARATION

I hereby declare that, all the information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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Date:

ABSTRACT

Dry-cured meat products are consumed in various regions of the world and consumers are increasingly demanding better quality and safety of these products. Despite stringent and tight legislations, many products including dry-cured meat products that exceed maximum permissible limits (MPLs) of aflatoxins (AFs) reach the markets, as complete removal of these contaminants is somehow not feasible or rather complex. Application of probiotics to decontaminate AFs from food systems is well known and widely studied. The decontamination process is attributed to either metabolic degradation or physical absorption by probiotics cell wall components. Several factors such as concentration of probiotics and AFs in food**,** food matrix, probiotics number of binding sites, stability of probiotics/AFs complex and environmental conditions are claimed to affect the decontamination efficacy of probiotics. This study presents a mathematical model for the decontamination process of AFB_1 by the inactivated cells of probiotic strain *Lactobacillus rhamnosus GG* in the matrix of Samarella. The objective of the model is to show the mitigation effect of *L. rhamnosus GG* on the preformed toxins and thus serve as prediction tool for AFB_1 quantitative risk assessment in Cypriot traditional dry-cured meat product tsamarella (samarella). According to the developed model, the rate of formation of AFB_1/*L. rhamnosus GG* complex (α) is the major indicator for AFB_1decontamination. Consequently, the predictivity of the model indicates that the rates of formation of AFB_1/*L. rhamnosus GG* complexes determine how fast, rigorous, and effective the mitigation of aflatoxins B_1 in the matrix of samarella can be achieved. Potential *L. rhamnosus GG* with high number of binding sites should thus be applied in dry-cured meats to prevent or reduce the rate of AFB_1 contamination. The developed model can be used to support investigations involving removal of mycotoxins in various food matrices, and to compare and contrast the effectiveness of various control strategies especially designed for decontaminating aflatoxins from food systems.

***Keywords*:** Dry-cured meat; Samarella; *Lactobacillus rhamnosus* GG; AFB_1 decontamination; Mathematical modelling; Michaelis-Menten kinetics; Aflatoxins; Probiotics

ÖZET

Kuru-kürlenmiş et ürünleri dünyanın çeşitli bölgelerinde tüketilmektedir ve bu ürünler için kalite ve güvenlik talebi gün geçtikçe artmaktadır. Sıkı yasal düzenlemelere rağmen, aflatoksinlerin (AF) izin verilen maksimum limitlerini (MPL) aşan birçok ürün ve kuru kürlenmiş et ürünleri piyasalara ulaşmaktadır. Bu kontaminantların gıda matriksinden tamamen uzaklaştırılması mümkün olmamaktadır. Probiyotiklerin AF'leri gıda sistemlerinden uzaklaştırmak için kullanılması bir süredir üzerinde çalışılan bir konudur. Dekontaminasyon işlemi, AF'lerin metabolik bozulmaları veya probiyotik hücre duvarı bileşenleri tarafından emilimi gibi fiziksel mekanizmalara dayanmaktadır. Gıdalardaki probiyotiklerin ve AF'lerin konsantrasyonu, gıda matrisi, probiyotiklerin / AF'lerin stabilitesi ve çevresel koşullar gibi çeşitli faktörlerin probiyotiklerin dekontaminasyon etkinliğini etkilediği belirtilmektedir. Bu çalışmada, Tsamarella'da (samarella) matrisindeki probiyotik suş *Lactobacillus rhamnosus* GG'nin inaktive edilmiş hücreleri tarafından AFB_1'in dekontaminasyon süreci için matematiksel bir model oluşturulmuştur. Modelin amacı, elde edilen sayısal veriler ile *L. rhamnosus* GG'nin oluşmuş toksinler üzerindeki azaltıcı etkisini göstermek ve böylece Kıbrıs'ın geleneksel kuru-kürlenmiş et ürünü olan samarella için AFB_1'in kantitatif risk değerlendirmesinde kullanmaktır. Geliştirilen modele göre AFB_1/*L. rhamnosus* GG kompleksi oranını temsil eden (α) AFB_1 dekontaminasyonunun ana göstergesidir. Modelleme neticesinde elde edilen sonuçlara göre bu komplekslerin oluşum oranlarının, samarella matrisinde aflatoksin B1'in ne kadar hızlı ve etkili bir şekilde azaltılabileceğini göstermektedir. Geliştirilen model, çeşitli gıda matrislerinde mikotoksinlerin dekontaminasyonunu içeren araştırmaları desteklemek ve özellikle gıda sistemlerinden aflatoksinleri dekontamine etmek için tasarlanmış çeşitli kontrol stratejilerinin etkinliğini karşılaştırmak için kullanılabilecektir.

***Anahtar Kelimeler*:** Kuru-kürlenmiş et; samarella; *Lactobacillus rhamnosus* GG; AFB_1 dekontaminasyonu; Matematiksel modelleme; Michaelis-Menten kinetiği; Aflatoksin; Probiyotikler.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

FAO: Food and Agriculture Organization

MPLs: Maximum permissible limits

AFs: Aflatoxins

APFBP: Atmospheric Pressure Fluidized Bed Plasma

OTA: Ochratoxin A

AFB_1: Aflatoxin B_1

IARC: International Agency for Research on Cancer

LAB: Lactic Acid Bacteria

EU: European Union

ALARA: As low as reasonably achievable

EFSA: European Food Safety Authority,

OTA: Ochratoxin

ZEN: Zearalenone

DON: Deoxynivalenol

EC: European Commission

FDA: Food and Drug Administration

FB_1: Fumonisin B_1

ELISA: Enzyme-linked immunosorbent assay

HPLC-FLD: High performance liquid chromatography coupled with fluorescence detector **DLLME:** Dispersive liquid-liquid microextraction

IAC: Immunoaffinity column

kGy: Kilogray

CFU: Colony forming unit

SEM: Scanning electron microscopy

DON: Deoxynivalenol

DAPACP: Double atmospheric pressure plasma

ENNs: Enniatins

EOs: Essential oils

GRAS: Generally recognized as safe

MIC: Minimum Inhibitory Concentration

MFC: Minimal fungicidal concentration

aw: Water activity

RH: Relative Humidity

TLC: Thin layer chromatography

IITA: The International Institute of Tropical Agriculture

uHPLC-MS: Ultrahigh performance liquid chromatography mass spectrometry

ng/kg: nanogram/kilogram

µg/kg: macrogram/kilogram

CHAPTER ONE

1.1 Introduction

Meat and meat products are highly nutritious and valuable food resources; they are rich in proteins, lipids, vitamins, and minerals necessary for the sustenance of life (Kademi et al., 2019a). Consequently, they are the most widely consumed food products of animal origin (Siekmann et al., 2003; Sofos and Geornaras, 2010) Food and Agriculture Organization (FAO) reported that global meat production and consumption have more than trebled during the last decade and it will continue to increase by 233–300 million tons between 2000 and 2020 (Murphy et al., 2003).

Depending on the purpose and demand, different kind of meat products can be prepared or manufactured. Some of the products required numerous and complex production processes such as fermented meat products while in others, few processing steps are needed to make a complete product of desired characteristics (Castro-Giraldez et al., 2010; Bingol et al., 2014; Kademi et al., 2019a). Drying and salting are some of the common operations used in high quality meat production; for which the main goals are to conserve the product by depressing the water activity and to transform the sensorial properties of fresh meat to the final meat products (Doğruer et al., 2013; Amiryousefi et al., 2012). Fresh meat is a very suitable matrix that can be contaminated by bacteria, and fungi and the action of certain enzymes, through different microbiological, physicochemical, and biochemical processes on the main components of meat (Kademi et al., 2019). Traditionally known as “Cypriot pastrami”, Samarella is one of the kinds of sun dried and salted meat made by de-boned sheep and mainly goats’ meat (Ulusoy et al., 2018).

Dietary exposure to mycotoxins is a matter of great concern for regulatory bodies all over the world. Despite stringent and tight legislations, many food products that exceed maximum permissible limits (MPLs) of aflatoxins (AFs) reach the markets, as complete removal of these contaminants is somehow not feasible or rather complex (Chiocchetti et al., 2019). AFs and Ochratoxin A (OTA) are the most important mycotoxins in dry-meat products in terms of prevalence and associated toxicity (Montanha et al., 2018). Contamination of meat products with aflatoxigenic fungi and production of AFs can occur at different points of the production and manufacturing steps, from the farm (animal feed contamination) to the commercial or artisanal production or storage of the final product (Kademi et al., 2017).

Once the product is contaminated, decontamination may be a tedious process or even impossible to achieve. This is due to the resistance of mycotoxins to extreme environmental conditions as well as to physical, chemical and biological treatments specifically designed for their inactivation/decontamination (Kabak, 2009). Mycotoxins in meat and meat products are therefore required to be intensively controlled from farm to fork (Asefa et al., 2011).

Pitt et al. (2000) mentioned that the single most effective and beneficial measure that could be made in human diets would be the elimination of mycotoxins. Application of microbial cells aiming to decontaminate AFs in food products has provided an opportunity to reduce the occurrence and deleterious effects of AFs in food for human consumption. Thus, incorporation of microbial cells as far as they might not leave undesirable compounds in food matrices is being searched and tried to be integrated in the food industry (Chiocchetti et al., 2019).

1.2 Research Questions

i. Why we are building up the model?
ii. Why we select to study on Aflatoxin B_1 (AFB_1)?
iii. What is the prevalence of AFB_1 in dry meat products?
iv. Why samarella?
v. Why we select *Lactobacillus rhamnosus* GG?
vi. Why inactivated cells of *Lactobacillus rhamnosus* GG?

1.3 Answers

i. Mathematical modeling along with simulation and optimization offer several advantages especially in the development of processes and control strategies which significantly reduce the overall time for dealing with food safety issues. Models can be applied to check significant kinetic differences, investigate mechanisms and correlations, quantitatively describe or predict phenomena to enable optimal control. On the other hand, in recent years mathematical modelling is regarded as a first step (primary) and rapid tool for predicting phenomena, therefore took significant part of laboratory analysis since it reduces the cost of the analysis. The main goal of this study is to apply a mathematical model for the decontamination process of AFB_1 by the inactivated cells of probiotic *L. rhamnosus* GG in the matrix of Samarella. The objective of the model is to

show the mitigation effect of *L. rhamnosus* GG on the preformed toxins and thus serve as prediction tool for AFB_1 quantitative risk assessment in this traditionally produced sundried-cured meat product. Samarella is a sun-dried meat product; thus application of inactivated cells of *L. rhamnosus* GG could be used to decontaminate AFB_1 and prevent possible fermentation in the final product.

ii. This study was planned because AFB_1 is the most common with wide occurrence in raw and processed food products, and has a higher toxigenic potential compared to other AFs In 1987, the International Agency for Research on Cancer (IARC) classified AFB_1 in group 1 as a human carcinogen (potential cancer-causing compound). Thus, a sound detoxification method is needed for controlling AFB_1, as one of the most harmful mycotoxins in animal production and food industry.

iii. Dry-cured meat products are consumed in various regions of the world and, consumers are increasingly demanding better quality and safety of these products. Some fungal species can produce mycotoxins in dry-cured meats, such as AFs and ochratoxins, which, when ingested, can produce carcinogenic and mutagenic effects in humans. Contamination of these products can occur at different points of the production chain, from the field (animal contaminated with feed) to the production or storage of the final product. Although the presence of mycotoxins in dry-cured meats has been reported in several regions of the world, the presence of these contaminants are not legislated in most countries. Therefore, it is important to put in place methods to identify and reduce the contamination of dry-cured meats, minimizing the consumption and deleterious effects caused by mycotoxins.

iv. Nutritionally, samarella is commonly consumed traditional meat product in the Island and there is no research or survey study on this product in terms of food safety and public health in particular risk of aflatoxicosis, therefore it must be studied to assess the safety of the product in terms of prevalence of AFs. Mathematical modelling approach used here is also not applied in any food product(s) for the prediction of aflatoxicosis risks. Thus it is aimed to put spotlight on one of the prevention methods against AFB_1 risk.

v. Several species of probiotic lactic acid bacteria (LAB), bifidobacteria and yeasts (Vinderola et al., 2000) are widely applied in food fermentation and as starter cultures as as well as for mycotoxin decontamination (Shetty & Jespersen, 2007; Rahaie et al., 2012) in the food and beverage industry. *L. rhamnosus* has emerged from the 1980s as the most researched probiotic species. Furthermore, they are microbial species with a history of safe use, which again is due to their inclusion in a variety of foods. This might explain, together with their suitability for large scale cultivation, why the most studied Lactobacillus species for human application is *L. rhamnosus*. This species can be recovered from some fermented foods and the intestinal and vaginal tracts, and strains appear to possess a number of interesting characteristics suitable for use in humans. *L. rhamnosus* GG, isolated in 1983 in Boston, it was quickly commercialized with the idea that it could provide health benefits, with the first paper suggesting this published in 1993. Since then, of course, it has become the most researched probiotic strain, primarily for gut health, with over 900 publications on PubMed database. *L. rhamnosus* is widely applied mycotoxin binder, possibly because of its better binding capacity.

vi. Because many investigations have proven that inactivated (non-viable) cells of *L. rhamnosus* GG have equal or better AFs binding capacities compared to viable ones. Moreover, it is of advantage to food groups such as sun-dried meats that do not require the presence of viable cells as they may cause fermentation in matrix. Thus, we presumed that when inactivated cells are added into the matrix of samarella, it might quencher the preformed AFB_1 and hence prevent the risks of aflatoxicosis.

CHAPTER TWO

2.1 Dry-cured meat products

2.1.1 Definition, Types and the Technology of Dry-cured Meat Products

Drying method can be accepted as one of the oldest preservation techniques which used to prolong shelf-life of foods. Dried-cured meat products are very common and well known worldwide with a long history of safe used. The meat preservation includes preventing or delaying microbial spoilage, autolysis, avoidance of weight loss and any changes in taste or texture (Macrae et al., 1997; Ayanwale et al., 2007). Preservation methods include use of low or high temperatures, reduction of aw or water contents or use of chemical preservatives. Drying meat under natural conditions with the presence humidity and circulation of the air, including direct influence of sun rays is the oldest way of drying in the past. It consists of a gradual dehydration of pieces of meat cut to a specific uniform shape that permits the equal and simultaneous drying of whole batches of meat (Heinz et al., 1990). The most important aim is to reduce the drying time and improve the quality of different dehydrated food products. Many alternative techniques have been developed in time and still being developed. These techniques include, vacuum drying, freeze-drying, and the use of dielectric heating sources, such as microwaves (Bampi et al., 2019; Manafzadeh et al., 2013). Dry-cured meats are consumed in various regions of the world and many types of traditionally produced meat products were reported in literature with different names as represented Table 2.1.

Consumers are increasingly demanding better quality and safety of these products. However, under unfavorable conditions, toxigenic molds in dry-cured meat products synthesize mycotoxins which are amongst the most important contaminants of meat and meat products (Tabuc et al., 2004).

2.1.2 The Mycobiota of Dry-cured Meat Products

The predominant mycobiota of dry-cured meat, especially sun-dried and salted meat products comprises of *Aspergillus*, *Fusarium* and *Penicillium* species (Adeyeye, 2016). Although there are certain molds desirable to be present in dry-cured meat products, as they actively participate to

acquisition and improvement of organoleptic qualities of these products, but the undeniable fact is most, if not all of the above mentioned fungal genera can contaminate dry-cured meats by producing certain mycotoxins such as AFs and Ochratoxin (OTA), which, when ingested, can produce toxicological and deleterious effects in humans (Montanha et al., 2018).

2.1.3 Traditional Cypriot Dried Meat Product Samarella (Tsamarella)

Samarella (tsamarella-τσαμαρέλλα in Greek) which is called Cypriot pastrami is a type of sun-dried meat product. It is very popular in both south and north part of the Island and even can be consumed in breakfast traditionally. This traditional dry-cured meat product of Cyprus is made by de-boned sheep and mainly goats' meat, salted and dried in the sun for preservation (Hakeri 2003; Kabataş 2007). Samarella was popular to be produced in the past in the foothills of the Trodos Mountain, especially in the areas of Paphos and Dillirga for the purpose of meat preservation, generally from the meat of mouflon (Yorgancıoğlu 2000). Today it is produced from the meat of goat or sheep of mature age and is still dried in the sun by traditional methods. After drying, it is washed and sprinkled with dry oregano which gives unique flavor. Samarella, considered as an appetizer in Cypriot food culture today, used to be consumed with tomatoes for breakfast and also added into some dishes such as dry beans, cracked wheat, etc.

Samarella is also a very important Cypriot meat product that is under protection of The Slow Food Presidia project. The Presidium aims to promote samarella in local markets and to bring it to international attention. According to the accepted rules of samarella manufacturing of Presidium, only flesh from the thigh of the animal should be used because it is the leanest, most highly valued cut and best provides the earthy, rustic flavor and smoothness they are looking for. The leg is butterflied and cut into strips, immersed in salt and oregano and left to dry in the sun (Anon 2020). Traditional production of samarella on the north side of Cyprus was described in flow chart in Figure 2.1. Unfortunately, the information contained in the scientific sources about samarella is quite limited. We have come across a single scientific study (Ulusoy et al., 2018) for this product that has been specifically addressed in terms of technology and laboratory analysis. Additionally, microbiological and mycological studies on this product have not been reported, and the risk of aflatoxin has not been studied.

Table 2.1: Different Types of Dried Meat and their Countries/Regions

Product Name	**Technology(ies) Applied**	**Country/Region**
Aliya	Sun-drying	Kenya
Bakkwa or rougan	Curing and Drying	China
Biltong	Curing and Drying	Southern Africa
Bògoǫ	Drying and Smoking	Northern Canada
Borts	Air-Drying	Mongolia
Bresaola	Air-Drying and Curing	Northern Italy
Bündnerfleisch	Air-Drying	Kanton Graubünden, Switzerland
Carnedesol	Sun-Drying and Curing	Brazil
Carne seca	Air-Drying	Mexico
Cecina	Smoking, Drying and Salting	Northwestern Spain and Mexico
Chipped beef	Drying	Southern Africa
Charque	Drying and Salting	Brazil and other South American countries
Droëwors	Drying	Southern Africa
Fenalår	Curing and Drying	Norway
Corned beef	Marinating and Baking	Pakistan
Jerked beef	Drying, Curing and Salting	Brazil and other South American countries
Kawaab	Air-Drying	Hyderabad, India.
Kilishi	Drying	Nigeria
Kuivaliha	Air-Drying	Northern Finland
Laap mei	Air-Drying	Southern China
Lahndi	Air-Drying and Curing	Pakistan
Mipku	Air-Drying	Northern Canada
Odka	Sun-Drying	Somalia and other East African countries
Pânsâwân	Smoking and Drying	Western Canada and United States
Pastirma	Air-Drying and Curing	Armenia, Greece, Turkey and the Balkans
Pindang	Drying	the Philippines
Po	Drying	Korea
Qwanta	Air-Drying and Curing	Ethiopia and other East African countries
Suho meso	Smoking	Bosnia
Sukuti	Air-Drying	Nepal
Walliser Rohschinken	Air-Drying	Switzerland
Yukpo	Drying	Korea

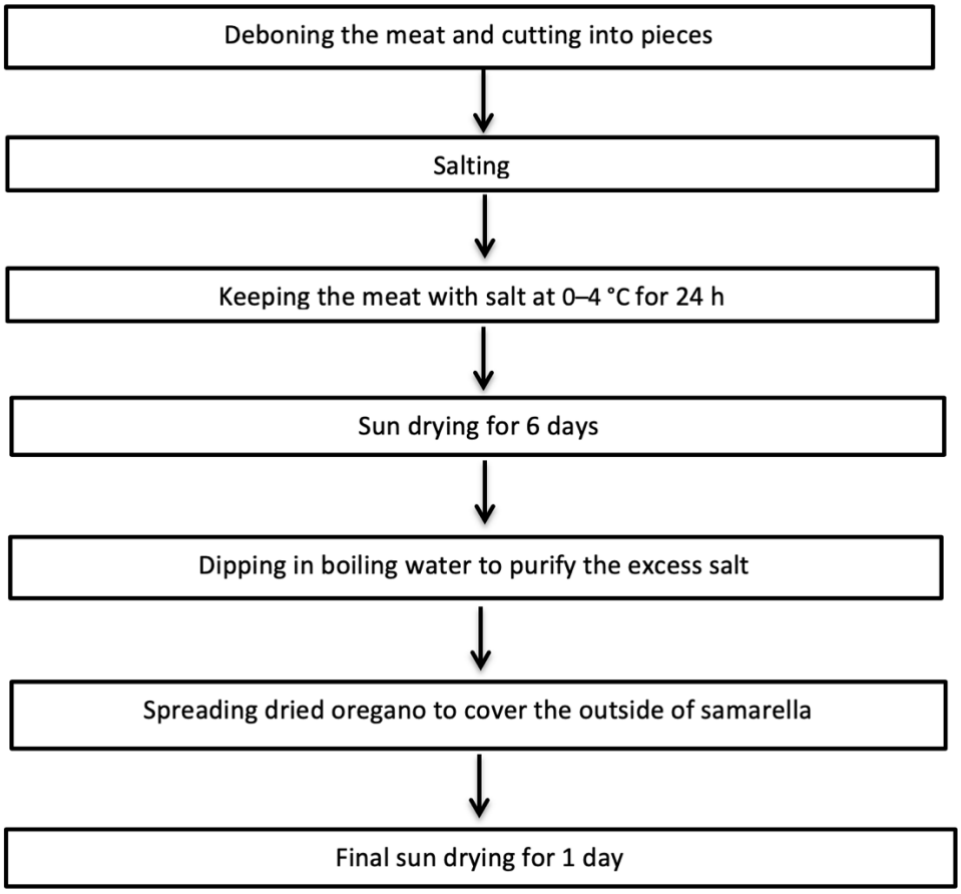


Figure 2.1: Production Flow Chart of Samarella (Tsamarella)

2.2 Mycotoxin Dynamics in the Food and Animal Feed Chains

It is evident that humans suffer from the vast number of contaminants in foods, which could be natural (such as mycotoxins, allergens, chemical factors, and plant toxins), or artificial (pesticide and veterinary drug residues, and food additives) (CAC, 2010; Nasreddine & Parent-Massin, 2002; Munro, 1976). Mycotoxins are among the natural contaminants of particular importance as far as public health is concern. This is due to their widespread distribution in foods and feeds, and the resulting array of severe clinical conditions they posed to humans and animals. Hence, when they accumulate in the body of humans or animals they could produce toxicological effects (Marroquín-Cardona et al., 2014).

The resulting diseases from mycotoxins are referred to as mycotoxicoses, characterized by carcinogenic, genotoxic, teratogenic, nephrotoxic, hepatotoxic, immunotoxic, amongst other debilitating clinical conditions (Bhat et al., 2010; Benkerroum, 2016) and even possible death in times of high exposure (Sherif et al., 2009; Paterson & Lima, 2010).

At the moment, more than 400 mycotoxins have been identified and presented in the literature (Kabak, 2009). However, aflatoxins (produced by the genus *Aspergillus*), fumonisin, trichothecenes, zearalenone, and deoxynivalenol (produced by the genus *Fusarium*), patulin (produced by the genera *Aspergillus*, *Byssochlamys* and *Penicillium*), ochratoxin (produced by the genera *Aspergillus* and *Penicillium*) and ergotamine (produced by the genera *Aspergillus*, *Claviceps*, *Penicillium* and *Rhizopus*) are the most significant in terms of the severity of the health consequences they posed to humans and animals (Bhat et al., 2010).

Although, epidemiological studies of human populations can provide direct evidence of adverse health effects, yet the issue of combating the concentrations of AFs in foods could be a difficult task considering the cloud of uncertainties which might arise with respect to levels of exposure, constrains in procuring representative samples of food from subsistence farmers, multiple vulnerable crops and other relevant confounding risk factors which may mask or otherwise obscure any effects of the putative causative agent within food supply chain (Krewski et al., 1984; Strosnider etal., 2006). Consequently, this situation requires numerous and sound approaches to set up the possible limits which may prevent or reduce toxicological effects to humans, taking into account the natural occurrence and effects of handling and food processing methods to the concentration of aflatoxins from the initial stages of contamination (Figure 1) to postharvest stages (from farm to fork), since respective limits are under debate for other mycotoxins (Zöllner & Mayer-Helm, 2006; Kademi et al., 2017).

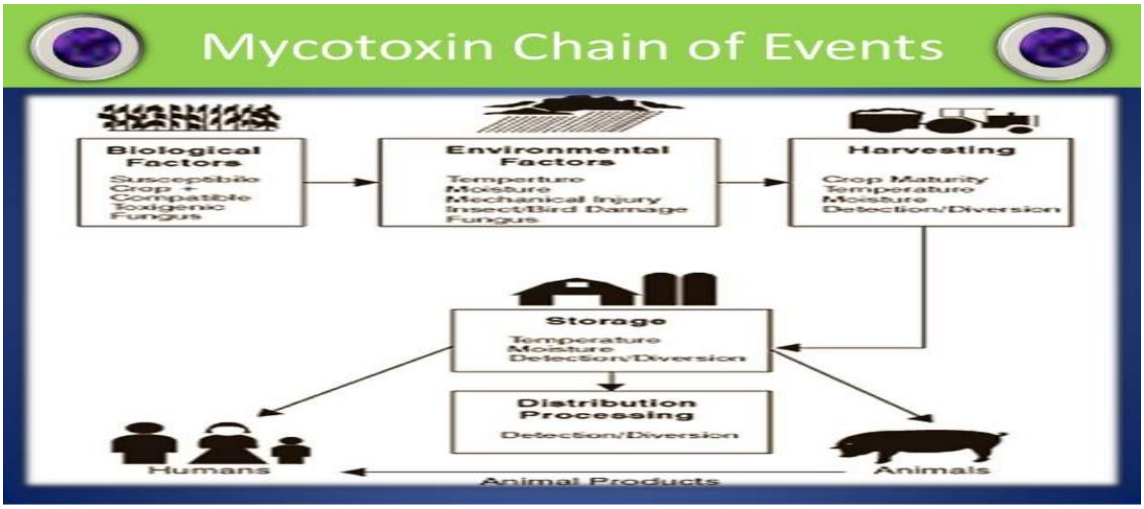


Figure 2.1: Mycotoxins dynamics in the food and feed chain (Retrieved from: https://www.slideshare.net/babasahebkumbhar/mycotoxins-26124352)

2.3 Aflatoxins in Food and Feed: Occurrence and Toxicity

Amongst the different kind of mycotoxins, AFs are the principal and most challenging in foods and animal feeds due to high prevalence, associated toxicity (in particular mutagenicity, carcinogenicity and teratogenicity) and high temperature and heat resistance during food processing (Kademi et al., 2017). The carcinogenic effect of AFs has been demonstrated both in humans and in animals by feed contamination and by human consumption of contaminated meat and milk (Eckhardt et al., 2014). AFs are fungal metabolites highly toxic, teratogenic, mutagenic and carcinogenic (Moss, 1998, IARC, 2002, Jager et al., 2013, Atherstone et al., 2014). Aflatoxin is a designation from "a", "fla" and "toxin" for *Aspergillus*, *flavus* and toxin (resulting poison) respectively (Ellis et al., 1991). These toxins were discovered in the past six decades in an outbreak involving poultry (turkeys) and farm animals in the United Kingdom. The suspected cause of the outbreak was contaminated peanuts imported from Brazil, resulting in the death of hundreds of thousands of poultry and farm animals. The incidence is correlated with *Aspergillus flavus* contamination levels. *Aspergillus* species (*A. flavus* and *A. parasiticus*) are the dominant producers of AFs (Bennett and Klich 2003).

The occurrence and level of AFs contamination from farm to table (Figure 2.1) is influenced by the kind of *Aspergillus* species present, farming system, handling and storage practices, and several other factors (Paterson & Lima, 2010) that may contribute to the occurrence and severity of these toxins to humans for example, genetic make-up, drying or evapo-transpiration, soil

nature, moisture deficit, and insect infestations (Wagacha & Muthomi, 2008). In addition, AFs contamination in foods and feeds affects crop and animal production thereby causing significant economic losses. Accordingly, high AFs exposure is attributed to high liver cancer incidence (Pitt, 2000; Liu & Wu, 2010). Other debilitating clinical conditions associated with AFs include alteration and impairment of child growth, enhancement of edema and kwashiorkor in malnourished adults and children respectively (Turner et al., 2003; Wu & Khlangwiset, 2010; Coulter et al., 1986; Hendrickse, 1982).

Presently, there are 18 various types of aflatoxins, the most important ones are Blue ($B_1$and B_2), Green (G_1 and G_2), B_1 Metabolite (M_1), B_2 Metabolite (M_2), B_2A and G_2A (Stroka and Anklam 2000; Creppy, 2002; Bennett and Klich 2003; Zinedine & Mañes, 2009). Blue and Green are referred to their characteristic fluorescence lights emitted during the course of separation with thin-layer chromatography. For M-types, these compounds are normally not found on crops, but their metabolites are found in meat, eggs and dairy and their products of animals fed with contaminated feedstuffs (De Ruyck et al., 2015, Paterson & Lima, 2010).

In dry-cured meats, four forms of AFs namely; B_1, B_2, G_1 and G_2, are considered as the most important (Doruk et al., 2018; Montanha et al., 2018). AFB_1 ($C_{17}H_{12}O_6$), structural configuration in (Figure 2.2) is the most common and has a higher toxigenic potential compared to other AFs (Coulombe, 1991). In 1987, IARC classified AFB_1 in group 1 as a human carcinogen (IARC, 2002, Jager et al., 2013, Bernáldez et al., 2013, Iqbal et al., 2014).

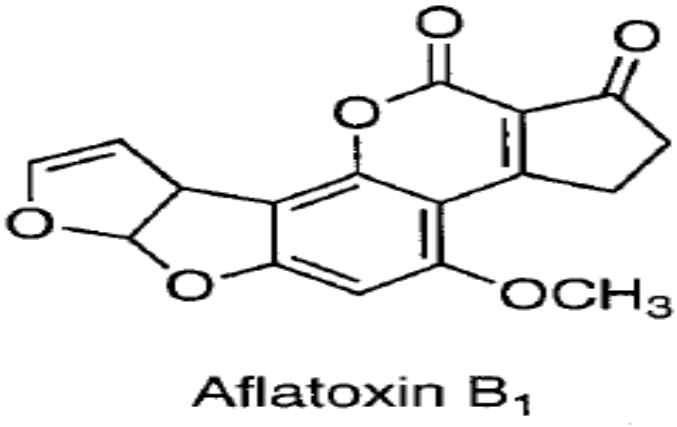


Figure 2.2: Chemical structure of AFB_1

2.3.1 Legislative Limits of Aflatoxins in Food and Feed

Scientific evidence and legislation for AFs limits which are toxicologically acceptable are needed to estimate the exposure to these important mycotoxins; these actions are usually carried out in the agricultural practice, storage of products and control of the products intended for human or animal consumption (Berg, 2003; van Egmond et al., 2007; Kan & Meijer, 2007). For almost two decades, the European Union (EU) is committed setting up standards based on toxicological examination. The allowable limit of contamination in foods is governed by the principle of as low as reasonably achievable (ALARA), normally derived from the frequency distribution of the respective food classes (at the 90–95th percentile), taking into account the outcome of the risk assessment and the analytical capabilities (European Food Safety Authority, EFSA, 2012). However, during that time, similar approaches have been recommended for the establishment of safe limits of certain mycotoxins (de Koe, 1999).

Currently, maximum tolerable levels and guideline levels have been established for aflatoxins (AFs), ochratoxin (OTA), zearalenone (ZEN) and deoxynivalenol (DON) ranging from ppb to ppt for various food and feed products (Price et al., 1999; Anonymous, 2000; EC, 2001; Hussein & Brasel, 2001). The standardized safe limit worldwide for total AFs in foods intended for human consumption ranges from 4-30μg/kg (Udomkun et al., 2017). Hence, when strictest limits will be adopted, foods and feeds from tropical and subtropical countries will face both economic losses and additional costs related to meeting those standards. Likewise, when the allowable limits are not so strong, there might be high exposure to these toxins (Strosnider et al., 2006; Wagacha & Muthomi, 2008; Wu & Khlangwiset, 2010).

Measures have been set up (Figure 2.3) by the relevant authorities in many countries and some international organizations to contain AFs levels (Juan et al., 2012; Udomkun et al., 2017), especially for agricultural products from countries with hot climates to satisfactorily harmonize foods and feeds trade. The recommended maximum level of AFB1 and total aflatoxins in human foods is 2 μg/kg and 4 μg/kg according to the European Community, Codex Alimentarius and Cyprus regulations (European Commission, EC, 2007 and 2010), and Iranian maximum tolerated level is 15 ng/g (Taheri et al., 2012). According to Food and Drug Administration (FDA) of the United States, the maximum levels of AFs in all foods intended for human consumption is 20μg/kg, 0.5μg/kg is set for milk and dairy products and 20 to 300μg/kg for animal feed (FAO/WHO, 1999). The maximum permissible level of AFB_1 in Turkey and

Nigeria is 2 µg/kg and 20 µg/kg, respectively (Official Journal of Turkish Republic, 2002; Farombi, 2006).

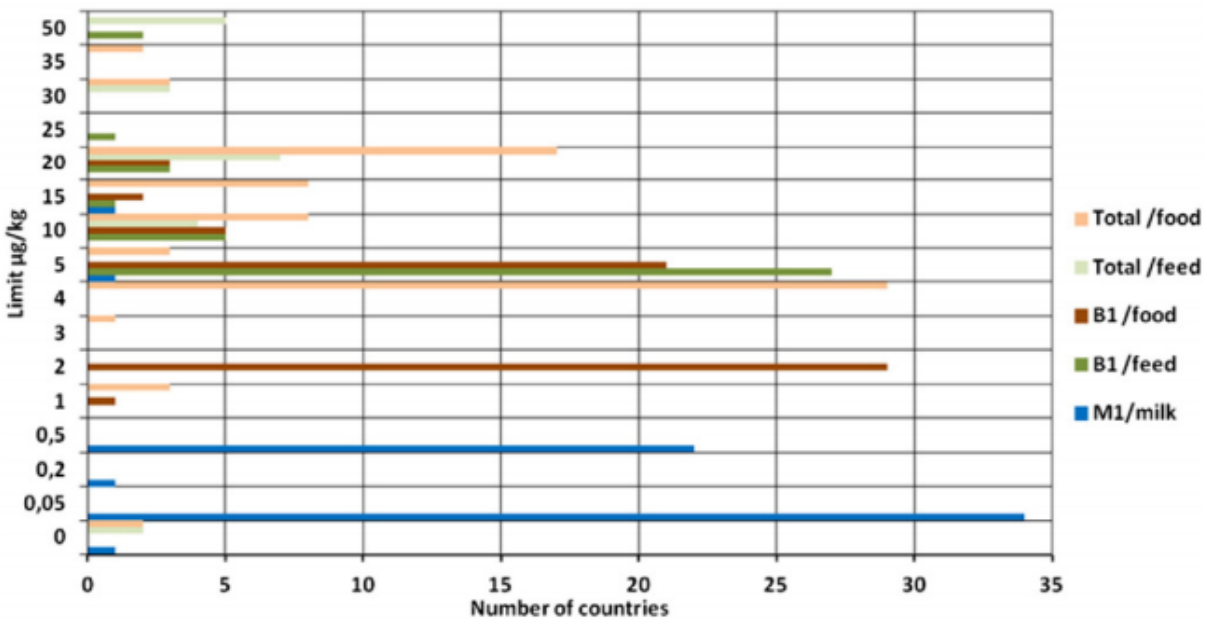


Figure 2.3: Worldwide Distribution Limits of Aflatoxins in Food and Feed (Ahlberg et al., 2015)

2.3.2 Aflatoxigenic Fungi in Meat and Meat Products

The prevalence of molds capable of producing AFs in fresh or processed meat show a serious concern to consumers since the concentrations of AFs were found to be high from the outcomes of the experimental studies (Aziz & Youssef, 1991, Adeyeye, 2016). Some fungal genera such as *Aspergillus*, *Fusarium* and *Penicillium* can be grown in meat medium (Adeyeye, 2016). As in most food products and foodstuffs, the environmental conditions (for example temperature, relative humidity singly or their combination) usage of meat ingredients affect the production of AFs by toxigenic molds in meat and meat products. As a result, atoxigenic and toxigenic molds often grow on dried and cured meats during storage (Rodrigues et al., 2019).

Bullerman et al. (1969a) evaluated the effects of temperatures on AFs production by *Aspergillus* species in fresh and cured meat. The studied meats were beef, smoked ham and

smoked bacon. Meat type, mold strain, storage temperatures, length of storage period and mold spores produced were considered as the factors responsible for the concentration and type of AFs produced. Three temperatures (15, 20 and 30°C) were used. Meats stored at 20°C formed high levels of AFs. As much as 630μg/g have been detected in one sample of meat in which AFG_1 was dominant. High levels of AFB_1 and AFG_1 could be produced in temperatures below 30°C. Compared to *Aspergillus flavus*, *Aspergillus parasiticus* produced more AFB_1 and AFG_1 at 30°C.

Bullerman et al. (1969b) investigated the effects of environmental conditions and curing ingredients on the growth of toxigenic *Aspergillus* molds and AFs production in cured and aged European-type salamis. The result show that, could the meat be smoked extensively under low temperatures and humidity of less than 15°C and 75%, the growth of *Aspergillus flavus* and *Aspergillus parasiticus* can be depressed and AFs production prevented. Considerable AFs reduction could be achieved due to the presence of curing ingredients such as pepper and sodium nitrite during the aging period of 2 months.

In Egypt, Aziz & Youssef (1991) evaluated the incidence of AFs and AFs-producing molds in fresh and processed meat products. 215 samples of fresh and processed meat products and 130 samples of meat ingredients used in the meat industry were evaluated. The ingredients examined include black pepper, coriander, cumin, curry, dried garlic, dried onion, rosemary and white pepper. All the analysed ingredients were contaminated with molds. Coriander and turmeric were found to be contaminated with AF (B_1 and G_1). *Aspergillus* and *Penicillium* species were the predominant molds isolated and detected from both processed meats and meat ingredients. Processed meat products had the highest mold count compared to fresh and canned ones. The processed meat products analysed include luncheon meat, beef burger, sausage, hot-dog and kubeka. AF (B_1 and B_2) were detected in the all processed meats analysed. None of the samples of fresh, canned, salami, beefsteak and minced were contaminated with AFs. The contamination of the processed meat products was attributed to the addition of meat ingredients probably contaminated before or during meat production.

In Croatia, (Cvetnić & Pepeljnjak, 1995) investigated the prevalence of aflatoxigenic molds in various smoke-dried meat products. 420 samples were collected from individual households in different region of Croatia and analysed for the presence of aflatoxigenic molds. *A. flavus* and *A. parasiticus* were present in 17.8% of the samples. Ability of AF-producing molds was tested in

75 isolates. *A. flavus* isolates produced mainly aflatoxin B_1, at various concentrations from 1.4–3.12 mg/kg. Some isolates of A. parasiticus produced all four aflatoxins B_1 B_2 G_1 G_2, while the other ones produced AF B_1 + G_1 only, with concentrations of AFs from 0.1 to 450 mg/kg.

Prevalence of aflatoxigenic molds and AFs in luncheon meat was studied in Egypt by Ismail and Zaky (1999). Fifty (50) samples (25 each) were collected from two different companies and analysed out of which seven (7) samples were positive for AFs. AFB_1 was detected in 4 and 3 samples from the two different companies at concentrations between 0.5 μg/kg - 11.1 μg/kg. The most prevalent aflatoxigenic species isolated was *A. flavus*.

Prevalence of *Aspergillus*, *Penicillium*, *Cladosporium*, *Mucor*, *Scopulariopsis*, *Candida* and *Rhodotorula* molds were reported in 15 randomly selected samples of fresh ground beef. *Aspergillus* species were the most prevalent. Further analysis showed that *Aspergillus flavus* produce 25 to 45μg/kg amount of AFB_1 in ground beef (Youssef et al., 1999).

Refai et al. (2003) investigated the incidence of molds and AF residues in Basterma, a traditionally cured Egyptian meat. According to these authors *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Fusarium* and *Cladosporium* were the most prevalent fungal genera in Basterma and its ingredients. Basterma samples were contaminated with total AF (B_1, B_2, G_1 and G_2) in range of 2.8 to 47μg/kg during the summer and 7.2 to 29μg/kg in winter.

Incidence of aflatoxigenic fungi was also reported from different meat and meat products including game sausages, semi-dry sausages and fermented dry-meats in Croatia. The most prevalent fungi in the analysed meat products were found to be *Aspergillus* and *Penicillium* species (Markov et al., 2013).

In Iran, a study was conducted to assess the presence of aflatoxigenic molds and AFB_1 in sausages and burgers marketed in various locations. 53 and 45 samples of burgers and sausages respectively were randomly collected during a period of six months and analyzed by enzyme-linked immunosorbent assay (ELISA) method. Prevalence of A. flavus was reported in 8.9% of burger samples. Moreover, the authors also reported that 6.3% and 4.9% of burger and sausage samples were contaminated with > 1 ng/g of AFB_1 (Maktabi et al., 2016).

Poultry meat products were investigated by Morshdy et al. (2016) for the presence molds and AF residues. Hundred samples (20 of each) burger, coated fillet, fillet, luncheon and liver were randomly collected from markets in Zagazig City, Egypt. *A. flavus* and *A. parasiticus* were identified in 23% and 3% of the samples respectively. The mean values of AFB_1 in coated fillet,

liver, burger, luncheon and fillet were 0.80±0.2 µg/kg, 0.48±0.1 µg/kg, 0.43±0.14 µg/kg, 0.36±0.05 µg/kg and 0.09±0.02 µg/kg, respectively.

Prevalence of aflatoxigenic molds were reported in fresh meat and meat products sold in Port Harcourt, Nigeria (Omorodion & Odu, 2014). The analyzed meat samples include beef, chicken and pork. 30.77% of the samples were contaminated with *Aspergillus* spp. In another study, Zohri et al. (2014) reported that *A. flavus* and *A. parasiticus* were detected in beef burger and sausage samples. AFB_1 was found in 10 % of sausage samples; however, none of the samples of beef burger contain AFB_1.

Prevalence of surface molds in some Croatian traditional dry-cured meats and correlation with AFB1accumulation were studied by Zadravec et al. (2020). Effects of environmental conditions and production technologies on the growth of mycotoxigenic molds in sampled Croatian prosciuttos and fermented sausages were evaluated. The authors reported that the contaminating molds were *Penicillium* (79%), *Aspergillus* (11%), *Eurotium* (7%) and *Mucor* (4%). The maximal value of AFB_1 was 1.92µg/kg detected in 8% of the samples.

2.3.3 Incidence of Aflatoxins in Meat and Meat Products

Most survey studies on the prevalence of AFs in the foods of animal origin are predominantly in milk and dairy products due to the fact that most of the ingested AFB_1 and B_2 are metabolized (carry-over effects) into less toxic group of AFs M_1 and M_2 found in milk of ruminant animals, commonly referred to as “milk aflatoxins”. However, when meat producing animals are fed with highly contaminated feeds and/or meat production ingredients are heavily contaminated, the most toxic group AF (B_1, B_2, G_1 and G_2) residues (Figure 2.4) can be present in meat and meat products (Herzallah, 2009; Markov et al., 2013; Iqbal et al., 2014; Amirkhizi et al., 2015; Pleadin et al., 2015 Montanha et al., 2018).

Consequently, many researchers from different parts of the world investigated the occurrence of AFs in different kind of livestock (cattle and birds) meats. AFs were detected in both fresh and processed muscles such as minced meat, burgers, luncheon, sausages and cured and aged meat products and offal meats like gizzard, liver, and kidney (Aziz & Youssef, 1991; Refai et al., 2003; El-Desouky et al., 2014; Amirkhizi et al., 2015). The following paragraphs summarize the

natural incidence of AFs in meats and as a result of contamination of ingredients used in the preparation or manufacture of meat and meat products.

A survey for the incidence of AFs in different foods of animal origin; milk, eggs and beef were conducted by Herzallah (2009) in Jordan. Two hundred and twenty (220) meat samples (poultry, lamb, goat and beef) were collected from different locations and markets during the period of 5 months, in 2007. Two categories (fresh and imported) were analyzed. Sample of 1kg from the selected meat types were taken on weekly basis for the period of 5 months in winter and spring. The incidence levels of total AFs in the samples were respectively, 13.3% and 6.7% for winter and spring. AF (B_1, B_2, G_1 and G_2) were reported with AFB_1 having the highest average concentration between 2.53 to 3.25 and 2.85 to 3.46µg/kg for fresh and imported meats respectively. The imported meat showed high degree of contamination compared with fresh ones which could be due to different types of feed intake.

Markov et al. (2013) carried out a study to determine the possible presence of AFB_1, ochratoxin A and citrinin in different types of meat products; game sausages, semi-dry sausages and fermented dry-meat products. Ninety (90) samples were collected from different producers across Croatian markets. The incidence of AFB_1 and OTA were quantified by a newly developed competitive ELISA method whereas citrinin was quantified (CIT) with high performance liquid chromatography coupled with fluorescent detector. 68.88% of the examined samples were positive for both mycotoxins. OTA has the highest percentage up to 64.4% followed by AFB_1 10% and CIT 4.44%.

A survey study was carried out to evaluate the incidence of aflatoxigenic molds and AFs in fresh and sundried meat products in Nigeria. Eighty samples of fresh and sun-dried cuts of cow beef and offal (liver, kidney and heart) were collected randomly from some markets in Ibadan, Nigeria. *Aspergillus* species including *A. flavus* were detected in dried meats. AF (B_1, B_2, G_1 and G_2) were also detected in varying concentrations. The mean concentrations of AFB_1 in fresh beef, heart, kidney and liver were 0.01 µg/kg, 0.03 µg/kg, 0.04 µg/kg and 0.07 µg/kg respectively. The mean concentrations of AFB_1 in dried beef, liver, heart and kidney were 0.001 µg/kg, 0.002 µg/kg, 0.014 µg/kg and 0.035 µg/kg respectively (Oyero & Oyefolu, 2010).

Shaltout et al. (2014) reported the prevalence of AFs in some meat products collected from Kaliobia governorates, Egypt. Hundred samples of basterma, kofta, luncheon and sausage were collected randomly and examined by HPLC. The average concentration of AFB_1 in kofta,

sausage, luncheon and basterma were 13.38± 1.52 µg/kg, 9.03± 1.17 µg/kg, 8.8±0.95 µg/kg and 4.53±0. 61 µg/kg, respectively.

In Pakistan, the incidence of mycotoxins in chicken meats and eggs were investigated by Iqbal et al. (2014). One hundred and fifteen (115) and eighty (80) samples of chicken meat and eggs respectively were analyzed. Domestic chickens, broilers and layers chickens are the representative samples of meat products while farm and domestic eggs used as representative samples. AFs, OTA and Zearalenone (ZEN) were present in all the analyzed samples. Briefly, 35% of chicken meat and 28% of eggs were contaminated with AFs. Chicken offal carries the highest percentage in both mycotoxins analyzed. Maximum levels of total AFs (8.01µg/kg), and AFB_1 (7.86µg/kg) were found in chicken livers. OTA were found in 41% of chicken meat and 35% of eggs; with maximum concentration of 4.70µg/kg in the liver. For ZEN, 52% of chicken meat and 32% of eggs were contaminated with ZEN. Maximum level of ZEN was also detected in the chicken livers as 5.10µg/kg.

It can be observed that chicken livers are more prone to contamination by mycotoxins. Liver is the main organ where mycotoxins accumulate. For example, AFB_1 and B_2 are metabolized into AFM_1 and M_2 respectively in the liver. Consequently, El-Desouky et al. (2014) carried out an investigation for the occurrence of AFB_1 and AFM_1 in some chicken organs collected from different retail markets in Egypt. Samples of gizzard, liver and heart were randomly collected from March to June of 2014. According to these authors, 45% of chicken liver, 32% of gizzard and 25% of heart samples were contaminated with AFB_1 as revealed by high performance liquid chromatography coupled with fluorescence detector (HPLC-FLD). Similarly, 33, 25 and 22% of chicken liver, gizzard and heart samples respectively were contaminated with AFM_1.

In Iran, prevalence of AFB_1 was also investigated in chicken livers and eggs available in Tabriz market, Iran. AFB_1 was extracted with newly developed dispersive liquid-liquid microextraction (DLLME) and quantified by high performance liquid chromatography coupled with UV detector. DLLME is a fast, economical and more sensitive method of extraction compared with commonly used immunoaffinity column (IAC) for AFs determination in foods. According to these authors, 72% and 58% of the liver and eggs samples respectively were contaminated with AFB_1 ranging from 0.30 to 16.36 µg/kg (Amirkhizi et al., 2015).

A four-year (2011-2014) cross-sectional study was conducted to investigate the prevalence of AFB_1 and OTA in different traditional meat products from different regions and markets in Croatia (Pleadin et al., 2015). Mycotoxins levels were analyzed by ELISA and HPLC-FLD methods. In total, 410 samples of pork meats including different types of hams, dry fermented sausages, bacon and cooked sausages were collected and analyzed. All the meat products excluding bacon were contaminated with AFB1 ranging from 0.89 to 1.06 for hams, 0.96 to 1.29 dry fermented sausages and 1.18 to 1.69 μg/kg for cooked sausages. The maximum OTA detected in the dry fermented sausages and hams were closely 5 to 10 times (5.10 to 9.95μg/kg) higher than 1μg/kg which is the MPLs recommended for pork meat products in some European countries.

Occurrence of AFs and OTA in meat products marketed in Mansoura, Egypt was evaluated by Abd-Elghany and Sallam (2015). A total of 50 samples (25 each of beef luncheon and beef burger) were purchased and analyzed by VICAM AflaTest and OchraTest immunoaffinity fluorometric method. All the samples were contaminated with both AFT and OTA. The mean values of AFs and OTA for beef luncheon were 1.1 μg/kg and 5.23 μg/kg whereas, for beef burger the values were 3.22 μg/kg and 4.55 μg/kg, respectively.

Darwish et al. (2016) studied the prevalence of mold and AFs in frozen meat cuts and giblets. Eighty (80) samples (twenty of each) frozen chicken breast, thigh, gizzards and livers were randomly collected from different regions of Zagazig city, Egypt. Gizzards and livers had the highest incidence of *Aspergillus* (90% & 80%). All the samples were contaminated with *A. flavus* and *A. parasiticus*. AFs contaminations ranged from 0.1 μg/kg, 0.3 μg/kg, 1.5 μg/kg and 3.3 μg/kg for chicken thigh, breasts, gizzards and livers, respectively.

Incidence of mold and AFs in bovine offal was studied by Atia and Darwish (2017). Fifty (50) Samples (10 of each) intestine, kidney, liver, muscle and rumen were randomly collected and analyzed. In total, 35.29% of the samples were contaminated with *Aspergillus* spp., *A. flavus* were identified in 6.82%. AFs were detected in 40%, 30% and 30% of liver, intestine and rumen samples respectively. None of the kidney and muscles samples were contaminated with AFs.

Sineque et al. (2017) investigated the incidence of AFB_1 in chicken meat products sold in Maputo, Mozambique. Samples of industrial and local chicken livers and gizzards were collected and analyzed by ELISA method. AFB_1 was detected in 39% and 13.8% of liver samples and

gizzards, respectively. The mean concentrations of AFB_1 were 1.73µg/kg and 1.07µg/kg in chicken livers and gizzards, respectively.

A survey study was conducted in Turkey by Cavus et al. (2018) aimed at determining mold and AFs contamination in fresh meat products and ingredients used for the preparation of meat products. The meat products include Turkish sucuk, sausage, and pastirma while meat ingredients were black pepper, coriander, fenugreek powder, red paprika and spice mix. *A. flavus* was detected in most of the investigated samples. HPLC analysis showed that 50% and 65% of the meat products and ingredients respectively were contaminated with AFB_1.

In Bosnia and Herzegovina, Smajlović et al. (2018) evaluated the prevalence of *Aspergillus* molds in traditional Bosnian sausages (*sudžuk)*. A total of 145 samples (105 samples artisanally and 40 industrially produced) were randomly collected from retails in the Sarajevo region. All in all, four and seven *A. flavus* and *A. parasiticus* were identified, respectively.

Karmi (2019) study the prevalence of AFs and OTA in meat products marketed in Aswan, Egypt. 25 samples (each of) basterma, burger, luncheon, minced meat and kofta totaling 125 were aseptically collected and brought to laboratory for analysis. Competitive direct enzyme linked immunosorbent assay was applied for the detection of mycotoxins. The highest rate of contamination of AFs and OTA was 92% in both luncheon and basterma samples respectively.

In Portugal, Rodrigues et al. (2019) carried out an investigation on fresh and dry-cured meat products aimed at determining the contamination rate of OTA and AFB_1, and the potential molds responsible for the contamination. A total of 128 samples of pork fresh legs and pork, goat and sheep dry-cured legs and shoulders were analyzed. *Penicillium* spp. (66%) were the dominant mold isolates. Aflatoxigenic molds representing 31% of the mold isolates including *A. flavus, A. parasiticus* and *A. nomius* were also identified from the meat products analyzed. *A. flavus* was reported in all the meat products analyzed. 40% of the sampled pork fresh legs were found to be contaminated with OTA at concentrations below 1 µg/kg. Although aflatoxigenic molds were identified, AFB_1 could not be detected in any of the samples analyzed.

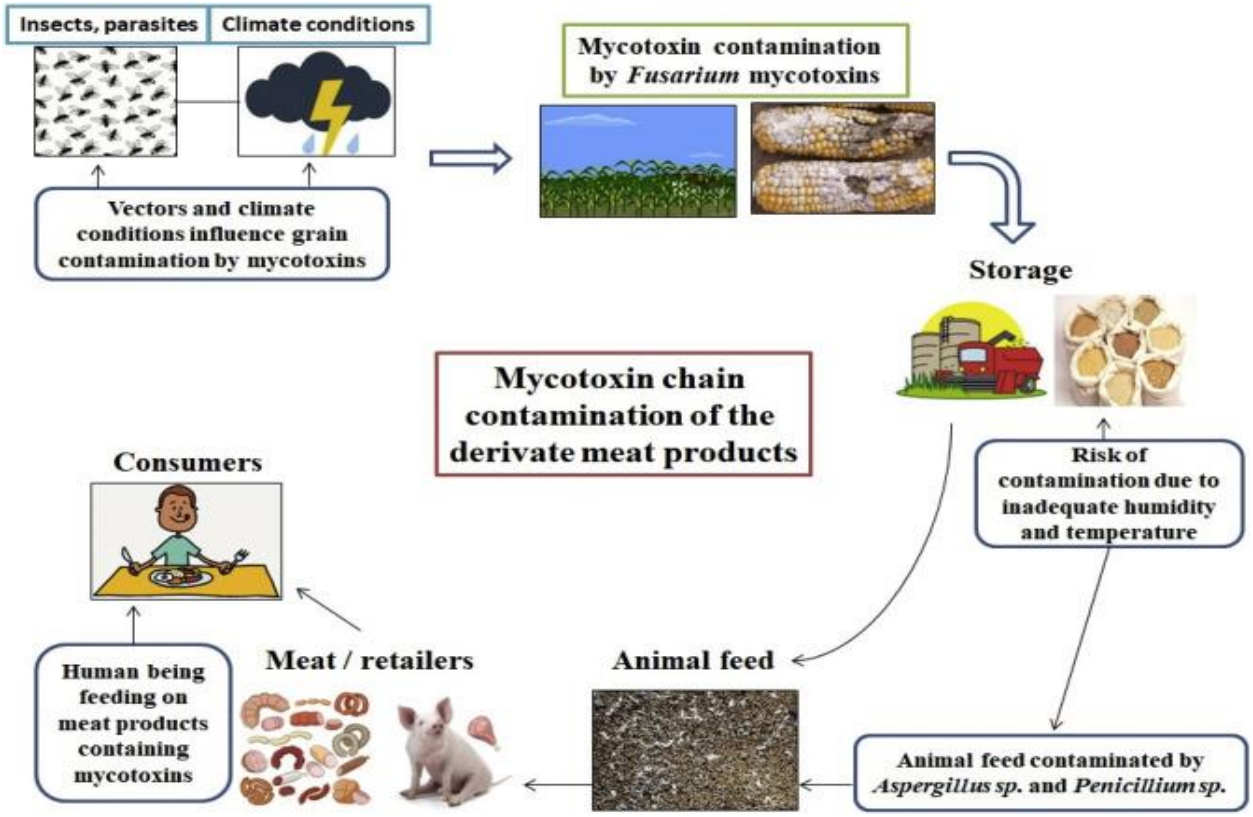


Figure 2.4: Mycotoxins dynamics in the meat production chain (Montanha et al., 2018).

2.4 Decontamination Methods of Aflatoxins in Meat and Meat Products

Despite the considerable risk posed by AFs and its prevalence, these contaminants are not legislated in meat products in many countries (Montanha et al., 2018). For example, only OTA has MPL for pork products in some European countries. Therefore, there are urgent need for methods to identify, reduce or eliminate AFs from dry-cured meats in an effort to minimize the consumption and deleterious effects caused by these toxins. Physical, chemical and biological control strategies have been proposed (Galvano et al., 2001; Kabak et al., 2006). Methods for aflatoxins decontamination in food products and foodstuffs (Figure 5) was reviewed by Ismail et al. (2018).

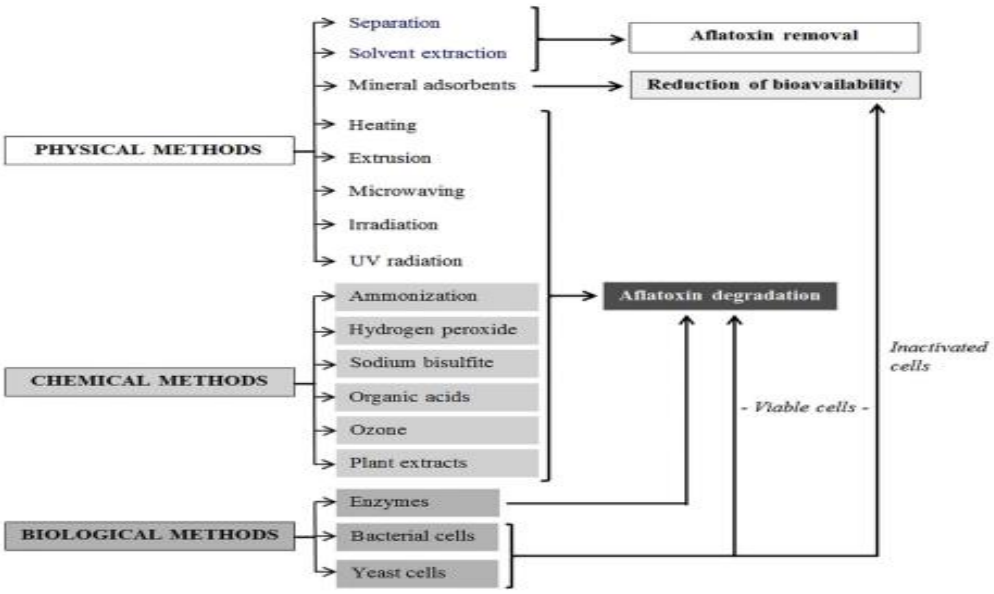


Figure 2.5: Methods of Aflatoxins Decontamination in Foods (Ismail et al., 2018)

2.4.1 Physical Method for AFs Decontamination in Meat and Meat Products

As can be seen in Figure 2.5, methods such as separation, solvent extraction, mineral adsorbents, heating, microwaving, irradiation and UV radiation are the most widely applied physical methods for AFs decontamination in food. The incidence of AFs and AF-producing molds in meat and meat products may occur at any point along the food supply chain. Effects of some physical method on Aflatoxigenic mold growth and AFs production in meat and meat products have been studied by several researchers.

Application of irradiation for food quality and safety had been in existence for several decades. Food irradiation involves subjecting the material to either gamma, X-rays or electron beams (Youssef et al., 1999). Gamma-irradiation is a physical treatment technique based on the emission of high energy photons by radioactive elements (mostly Cobalt-60) aimed at destroying the DNA of the target microbial entities. Gamma irradiation can interact with water and other substrate constituents thereby creating free radicals and ions to destroy the DNA of microbial entities (Calado et al., 2014). Gamma-irradiation is measured in Kilogray (kGy). Concerning the relationship between the doses of irradiation and mycotoxin decontamination, it was observed that AFs are extremely resistant to irradiation in dried surface and more sensitive in solution. Therefore, it can be understood that penetration and decontamination effects of irradiation

technology on mycotoxins in meat products will be more practicable in semi-dried meat products. It was reported that at 4 kGy irradiation doses, viable molds could be completely decontaminated from food matrices. In solute, irradiation dose of 1kGy is enough to decontaminate AFB_1. In addition, several factors (Figures 2.6 and 2.7) may influence the decontamination efficacy of molds and mycotoxins from food matrices by irradiation technology (Calado et al., 2014).

Youssef et al. (1999) evaluated the impact of γ-irradiation on the growth of *Aspergillus flavus* and AFB1 accumulation in sampled ground beef. Briefly 300g of sampled ground beef were exposed to 10KGy doses of gamma radiation and an inoculum of 10^6 CFU/g *A. flavus* was added and stored at 5°C following 15 days of incubation. HPLC analysis showed that AFB_1 could be detected in 20% of the samples and 1.5 kGy doses could be applied to completely inhibit *A. flavus* growth and AFB_1 production. Effects of γ-irradiation on *Aspergillus* growth and AFB_1 accumulation in spices used for dried cured meat (Egyptian basterma) production and meat products was evaluated by Refai et al. (2003) and found that 5 kGy doses of γ-irradiation will be adequate to decontaminate fungal spores and AFs accumulation from basterma samples.

Impact of gamma irradiation on aflatoxigenic molds growth and AFB_1 reduction was evaluated by Markov et al. (2015). The authors showed that 5 kGy doses of irradiation could be used to depressed *Aspergillus parasiticus*, *Aspergillus flavus*, and *Aspergillus niger* both in pure and mixed culture form. Decontamination of AFB_1 is concentration dependent, the higher the concentration, the lower the effect of irradiation. 69.8% AFB_1 could be reduced with 5 kGy, while with 10 kGy, 94.5% of AFB_1 reduction could be achieved.

In the study of Hassan (2017), UV-radiation was applied to decontaminate AFB_1 in various meat products including beef and chicken meats. The results revealed that at distance of 60 cm the contaminated beef containing 975 μg/kg and chicken meat containing 217ug/kg AFB_1 was reduced to 111 μg/kg and 30 μg/kg respectively during 30 minute of exposure.

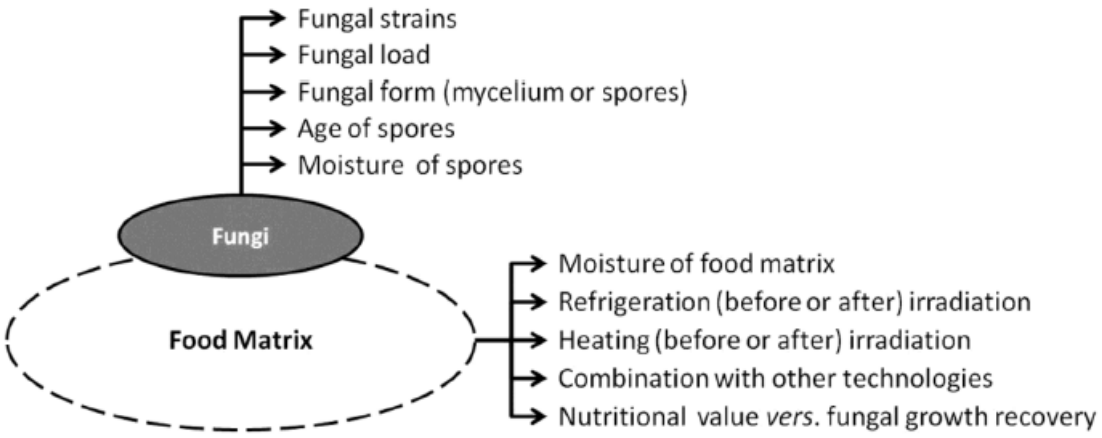


Figure 2.6: Factors that may Influence Decontamination of Fungal Spores by Irradiation Method (Calado et al., 2014)

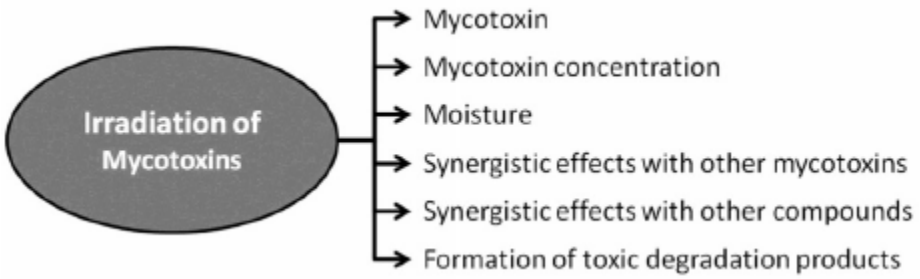


Figure 2.7: Factors that may Influence Decontamination of Mycotoxins by Irradiation Method (Calado et al., 2014)

In addition to physical methods for aflatoxigenic molds decontamination and AFs degradation presented by Ismail and others (2018), plasma and near infrared spectroscopic methods can be used to produce aflatoxigenic molds and AF-free food products (Gavahian & Cullen, 2019).

Plasma technology (Figure 2.8) refers to the application of ionized reactive species (electrons and free radical ions) and UV radiation generated by several means including glow discharge, dielectric barrier discharge and radio frequency (Ekezie et al., 2017; Gavahian et al., 2018). Plasma can be generated at different pressure and temperature scales; categorized as thermal and non-thermal (cold) plasma. The fungi inactivation effects of cold plasma can be correlated with the plasma composition for example, atomic oxygen, and plasma generated reactive species, and treatment conditions (Avramidis et al., 2010; Hayashi et al., 2014; Gavahian & Cullen, 2019).

As mentioned earlier, *Aspergillus* and *Fusarium* species are among the inherent mycotoxigenic molds in dry-cured meats. Suhem et al. (2013) reported that *A. flavus* growth could be inhibited by plasma jet at a power of 20-40 watts within 5-25 minutes. According to multiple regression analysis, the optimum process condition to inhibit *A. flavus* in culture medium is 40W in 25 minutes (Suhem et al., 2013).

Dasan et al. (2016) studied the effects of atmospheric pressure fluidized bed plasma to inactivate the spores of *A. parasiticus* and *A. flavus*. The results revealed that 5 min of plasma treatment at a power of 0.66 Kw and frequency of 25 KHz caused a reduction in the number of *A. parasiticus* and *A. flavus* to 4.50 CFU/g and 4.19 CFU/g, respectively. The authors observed that the decontamination efficiency of *Aspergillus* depend on the rate of input voltage and frequency of the method. Further analysis by scanning electron microscopy indicated that the plasma disrupts the integrity of the *Aspergillus* cellular structure and disperse the cell contents (Dasan et al., 2016).

Dasan et al. (2016) evaluated the correlation between atmospheric pressure fluidized bed plasma (APFBP) process parameters and aflatoxigenic spores viability of *A. flavus* and *A. parasiticus* treated with dry air or nitrogen plasma for up to 5 min in two different fluidizing bed reactors of APFBP system at various plasma parameters. The decontamination effect of APFBP on *Aspergillus* spp. spores increased with the applied reference voltage and the frequency. The killing effect of plasma on the spores decreased as the diameter of the fluidized bed reactor increased. The fungicidal effects on *A. flavus* (4.17 log) and *A. parasiticus* (4.09 log) were found for air plasma treatment after 5 min. Due to the formation of active plasma species in the presence of oxygen, the air plasma generated at APFBP system was more effective than nitrogen plasma on decontamination of *Aspergillus* spp. spores, according to the scanning electron microscopy (SEM).

In another study, the application of cold plasma technology for the inactivation of *A. parasiticus* and *A. flavus* was carried out by Devi et al. (2017). SEM results showed that cell structures of the fungal spores were completely disintegrated by reactive species of plasma through electroporation and etching. According to the authors, increasing the time and plasma power enhanced the inactivation effects on both *Aspergillus* species. Plasma power of 60W in 24 min reduced the growth of *A. parasiticus* and *A. flavus* by 98% and 99%, respectively (Devi et al., 2017).

In addition to the risks of Aflatoxicosis due to production of AFs by *Aspergillus* species, the presence of *Fusarium* species such as *F. oxysporum* and *F. sacchari* in dry-cured meats may contribute to additional mycotoxicoses such as nausea and vomiting. Abbasian et al. (2017) explore the ability of cold plasma technology for Deoxynivalenol (DON) and T2 reduction produced by *Fusarium* species grown on malt and yeast extract agar. Argon cold double atmospheric pressure plasma (DAPACP) with high voltage of approximately 25KHZ is applied to a high voltage electrode during 30, 60 and 180 seconds. Plasma jet treatments cause a relative reduction in concentration of DON and T2 toxins in time-dependent manner (Abbasian et al., 2017).

In the study of Ten Bosch et al. (2017), the decontamination effect of cold plasma technology on mycotoxins produced by *Aspergillus* and *Fusarium* species was explored. Fumonisin B_1 (FB_1), DON, T2, Enniatins (ENNs) and Sterigmatocystin could be completely eliminated in one minute of atmospheric plasma treatment. The authors also observed that food matrix and mycotoxin chemical structure can affects the decontamination efficiency of plasma technology (Ten Bosch et al., 2017).

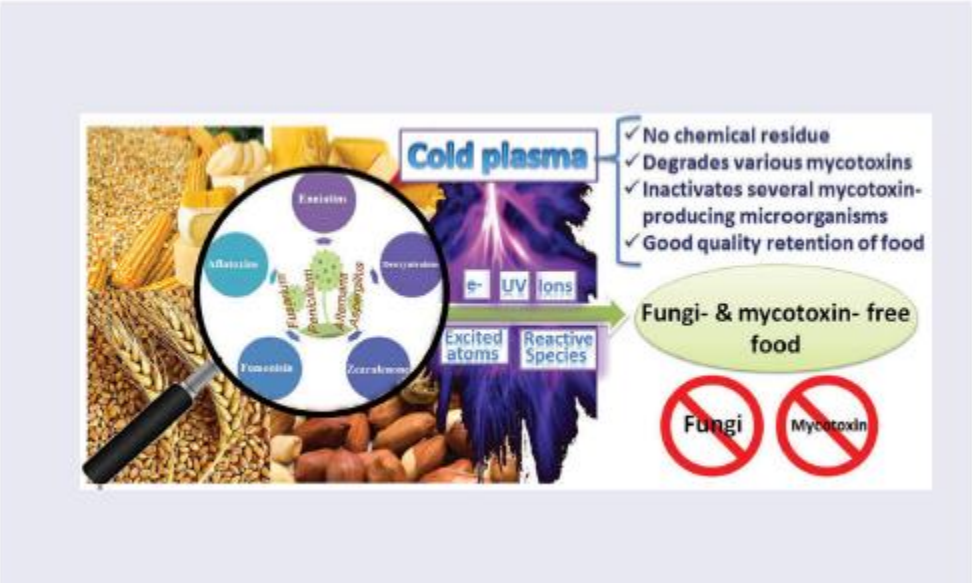


Figure 2.8: Cold Plasma Technique for Mycotoxin-free Food (Gavahian & Cullen, 2019)

Similarly, decontamination of mycotoxins by plasma technology is governed by the composition and characteristics of the plasma used (for example oxygen concentration and hydroxyl radicals) as well as the presence of photons and UV-radiation (Gavahian & Cullen,

2019). Devi et al. (2017) studied the effects of cold plasma treatment on *A. flavus* and *A. parasiticus* growth and AFs reduction. Combined effects of atmospheric cold plasma power-time of 40 and 60W at 0, 12 and 15 min was used to determine the effectiveness of the process against the AFs produced by *A. parasiticus* and *A. flavus*. According to the authors plasma treatment reduced AFB_1 production of *A. flavus* and *A. parasiticus* by up to 97 and 95%, respectively.

Ren et al. (2017) investigated the effects of food composition including moisture and alpha-tocopherol on the cold plasma-induced decontamination of AFB_1. The authors observed that addition of moisture to matrix improved the decontamination effects of cold plasma and reduced the AFB_1 concentration by 98% as compared with treatment without water of 62%. The presence of alpha-tocopherol also reduces the decontamination effect of plasma technology.

Decontamination effects of cold plasma on AFB_1 were investigated by Sakudo et al. (2017). Plasma treatment reduced the concentration of AFB_1 from 200μg/L to approximately 20μg/L during 15 min according to ELISA method. Further analysis by HPLC showed that AFB_1 molecules were converted into smaller fragments. The authors noted that AFB_1 decontamination is due to the presence of reactive species and proposed that adjustment of reactive species can increase the decontamination effects of cold plasma technology (Sakudo et al., 2017).

Shi et al. 2017 studied the effects of process parameters, including plasma exposure time (1, 2, 5, 10, 20, and 30 min), carrier gas type (air and a modified atmosphere gas mixture containing 65% O_2, 30% CO_2, and 5% N_2), and relative humidity, (RH of 5, 40, 80%) on plasma-induced degradation of AFs. The results showed that the relative humidity and carrier gas composition affected the formation of reactive species and that the modified atmosphere gas mixture yielded a greater concentration of ozone than using atmospheric air as the carrier gas according to optical emission spectroscopy. These conditions (i.e., modified atmosphere gas mixture and high RH) improved the detoxification effects of cold plasma against AFS. In addition, the authors observed that AFs degradation is time dependent as 62% and 82% of AFs was degraded after 1 and 10 min plasma treatment at the RH of 40%, respectively.

Siciliano et al. (2016) evaluated the effects of plasma process parameters, including exposure time (1–12 min), input power (400–-1150 W), and working gas composition, on AF degradation. The sensitivity of different types of AFs (i.e., B_1, B_2, G_1, and G_2) to the plasma treatment was also studied. According to the results, AFB_2 and AFG_2 were more resistant to cold plasma

treatment than AFB_1 and AFG_1. In addition, AFG_1 was found to be more resistant to plasma treatment than AFB_1. Up to 70% of AFB_1 could be reduced (at the input power of 1 kW and exposure time of 12 min) by plasma technology without negatively affecting the organoleptic properties of the products. Similar results regarding the effectiveness of cold plasma in reducing the total AF were reported by Spadaro et al. (2015) wherein 70% of AFs degraded upon 12 min exposure to a 1000 W plasma treatment.

2.4.2 Chemical Method for AFs Decontamination in Meat and Meat Products

The application of ozone to control aflatoxigenic molds and AFs production is a well-known chemical method. In the last few years, there have being increased in the application of ozone to inhibit aflatoxigenic fungal growth and AFs production in food products (Ismail et al., 2018). Ozonation refers to a process by which triatomic form of oxygen (O_3) is decomposed by attacking the radical hydroxyl (OH) of AFs thereby making them less toxic. Ozone has been shown to control AF-producing molds and to degrade the preformed AFs in some food products such as peanuts in which a reduction of approximately 25% of AFB_1 was reported (de Alencar et al., 2012). However, there are scarce literature on application of ozone for AFs decontamination in meat and meat products. El-Desouky et al. (2014) reported the application of ozonated water for AFB_1 and AFM_1 decontamination in samples of chicken gizzard, hearts and liver. The results showed the effectiveness of this method to eliminate AFB_1 and AFM_1 from chicken organs.

Another important chemical method for inhibition of aflatoxigenic molds and AFs production is use of essential oils (EOs). Potential of EOs from plant extracts against aflatoxigenic molds and AFs production in various food matrices have been investigated by several researchers (Ponzilacqua et al., 2018). Plant extracts and essential oils (EOs) have been studied as fungal growth inhibitors and regarded as safe alternatives in the prevention of mycotoxins occurrence (Sandosskumar et al., 2017; Patil et al., 2010; El-Habib et al., 2012; Iram et al., 2015; Yooussef et al., 2016). Some aqueous plant extracts have chemically active compounds that inhibit the biosynthesis of AFs (Reddy et al., 2009) which have increased the scientific attention on these issues. A number of recent publications have shown the efficacy of plant extracts against *Aspergillus* and/or AFs production. The mechanism of action is by denaturing enzymes responsible for spore germination and interfering with amino acids involved in fungal germination. EOs damage the enzymatic system of fungal cells by reducing the synthesis of

proteins and structural compounds (Iram et al., 2015). Some compounds, such as monoterpenes and limonene, have been identified as potential inhibitors of pectin methylsterase, which is responsible for building the main components of the cell wall in fungi (Marei et al., 2012). According to Cardile et al. (2009) application of EOs in food products have been approved by the FDA, being classified as GRAS. EOs antimicrobial actions involve several chemical compounds found in plants, and this activity cannot be attributed to a single cell mechanism, but a set of them (Kitic et al., 2005).

Karapinar (1985) studied the antifungal effect of various EOs from different herbs and plant extracts including aniseed, bay leaves, citrus peel, ground mint, red pepper, sage and thyme on growth of *A. parasiticus* and AFs production. Thyme extracts has the highest antifungal effect among all the EOs tested. However, orange and lemon EOs have the highest MIC (1.6%) effects on growth *A. parasiticus* and AFs production during 10 days incubation.

Vilela et al. (2009) tested *Eucalyptus globulus* (common eucalyptus) EO and its main component alone (1,8-cineol) against *A. flavus* and *A. parasiticus.* They observed that 1,8-cineol has lower antifungal activity than the EO. These findings suggest that other components found in lower levels in the oil may be critical for promoting synergism and enhancing the effects (Burt, 2004).

Xing et al. (2010) reported the antifungal activity of cinnamon EO against *A. flavus* proliferation. According to the results, *A. flavus* growth could be depressed by the action cinnamaldehyde which is the main constituent of cinnamon EO.

Medeiros et al. (2011) evaluated the potential of EOs from *Pittosporum undulatum* (rich in hydrocarbon, monoterpenes and sesquiterpenes) against *A. flavus* growth. The authors used EOs at three different concentrations 0.1 µL/mL, 0.2 µL/mL and 0.3 µL/mL. 0.3 µL/mL could be used to achieve optimum growth inhibition of *A. flavus* and AFB_1 production.

In another study, El-Habib (2012) investigated antifungal effects of basil, dill coriander, marjoram, mint rosemary and thyme EOs against *A. flavus* and AFB_1 production. Dill EOs have the highest inhibitory effects against AFB1 formation, whereas basil and thyme EOs are the most efficient in terms of *A. flavus* growth inhibition.

Becerril et al. (2013) studied the antifungal activity of EOs from cinnamon on *A. flavus* growth inhibition. The MIC and minimal fungicidal concentration (MFC) were determined directly by

macrodilution. The authors observed that a strong antifungal activity will be achieved with MIC and MFC of 0.05 - 0.1 mg/ml and 0.05 – 0.2 mg/ml, respectively.

Ferreira et al. (2013) ascertained the inhibitory effects of *Curcuma longa* plant extracts on AFB_1 and AFB_2 production. Addition of 0.5% (v/v) of the *C. longa* oil on yeasts extract sucrose (YES) medium showed a reduction of AFB1 and AFB2 by 96% and 98.6%, respectively.

Younos et al. (2018) investigated the antifungal activities of some plant extracts including *Euphorbia cotinifolia L*., *E. tirucalli L*. and *Rhus coriaria L.* against aflatoxigenic molds and AFs in processed meat and meat products sold in Egypt. 48 samples of uncooked processed meat products, including basterma, beef burger; luncheon meat and sausage (12 of each type) were collected and analyzed. AFs were more prevalent in Basterma samples according to thin layer chromatography (TLC) method. The highest AFB_1 contamination was also in Basterma, at concentration of 0.340 ng/kg. It was also found that, all tested plant extracts were found to significantly decrease the growth of *A. flavus* and *A. parasiticus* at all different concentrations. The most effective plant extract against tested molds was *R. coriaria L.* extract.

Effect of environmental conditions and smoked paprika on aflatoxigenic molds growth and AFs production during ripening of dry-cured sausages was studied by (Sánchez-Montero et al., 2019). Water activity (aw) values of 0.98, 0.94 and 0.87 and temperature of 20–25 °C was used. Smoked paprika at 1-3% was applied. The authors claimed that there was almost complete reduction of *A. flavus* growth and AFB_1 formation at 2% concentration.

2.4.3 Biological Method for AFs Decontamination in Meat and Meat Products

Although, physical and chemical methods can be used to inhibit growth of AF-producing mold and AFs accumulation in meat products, some shortcomings such as loss of nutritional value, change in organoleptic properties, equipment's demands and other related practical difficulties might render their potent applications in the meat industry unviable (Montanha et al., 2018). Therefore, a promising alternative is the adoption of biological method using microorganisms or microbial by-products such enzymes and bacteriocins as mycotoxins sequestering agents (Ismail et al., 2018; Rahaie et al., 2012).

Mycotoxin-producing molds and mycotoxins can be decontaminated from food systems by introducing atoxigenic microorganisms in foodstuffs or microbial cells and/or their by-products

such as enzymes, bacteriocins peptides, organic acids etc. in food products to compete for resources necessary for their survival or accumulation (Ismail et al., 2018).

Decontamination of AF-producing molds by introducing Atoxigenic specie offers an attractive alternative as it has being regarded as chief, safe and more natural for food industry and consumers in their continuous demand for chemical free foods (Ismail et al., 2018). Several biocontrol systems are available in the market. They served as pre and postharvest strategies for mycotoxins decontamination in and animal feeds foodstuffs. The most recent ones are Afla-guard™ (Figure 2.9) and Aflasafe™ (Figure 2.10) from Syngenta and The International Institute of Tropical Agriculture (IITA) productions respectively. Both of them operate on the principle of inclusion of atoxigenic AF-producing molds in the matrices of the products intended for humans and animal consumption. They are reliable AF-decontamination tools as > 90% inhibition of AF producing mold can be achieved (Verheecke et al., 2016).

Figure 2.9: Afla-Guard® biocontrol product using barley as the career matrix (Retrieved from http://progressivecrop.com/2019/11/biocontrol-of-aflatoxin-contamination-in-nut-crops-is-working/)

Figure 2.10: Application of Aflasafe in crops (Retrieved from: https://hiveminer.com/Tags/aflasafe%2Carachishypogaea)

2.5 Application of Microbial Cells and Microbial Cell By-Products for the Inhibition of Aflatoxigenic Fungal Growth and AFs Formation

Besides biocontrol systems, another approach is the inclusion of microbial cells and microbial cell capable of inhibiting mold growth. Several researchers documented the application of microbial cells against aflatoxigenic mold growth and AFs formation; and most of the reports showed the potential of LAB and autochthonous (natural inhabitants) microorganisms (Dalié et al., 2010; Crowley et al. 2013; Bianchini, 2015).

In vitro ability of *L. rhamnosus* GG and *L. rhamnosus* LC705 to inhibit *A. flavus* growth and AFs production was evaluated by Nada et al. (2010). The inhibitory effect of *L. rhamnosus* GG and *L. rhamnosus* LC705 against AFs production were 98.8% and 85.2%, respectively.

Other specialist studied the effect of *L. bulgaricus* and *L. acidophilus* on *A. parasiticus* growth and AFs production (Karunaratne et al., 1990). The authors reported 100% inhibition of AFs production.

Since early 1990s, the decontamination ability of AFs by microbial enzymes has been evaluated, although the decontamination mechanisms are not fully understood. Different types of enzymes from different sources have been studied for AFs reduction and thus served as potential decontamination agents of AFs in foods (Shcherbakova et al., 2015; Ismail et al., 2018). AFB_1 decontamination ability of laccase in citrate buffer was tested by (Hontanaya et al., 2015). The

enzyme was able to reduce up to 67% of AFB_1 in the medium. However, number of AFs decontaminating enzymes in literature is scarce, probably due to certain complexities in the enzyme-mycotoxins decontamination process (Zhu et al., 2016).

LAB species produced certain metabolites such as cyclic peptides, reutin, organic acids, and bacteriocins which can affect fungal growth and AFs formation. In this respect, Guimarães et al. (2018) tested antifungal effects of organic acids on *A. flavus* and AFs production. The results revealed that AFs formation could be reduced by 91% whereas 32% inhibition of *A. flavus* growth was reported.

Decontaminating potential of antifungal compounds produced by *L. brevis* and *L. paracasei* against *A. flavus* and *A. parasiticus* growth and AFB_1 production has been evaluated by Gomaa et al. (2018). L. brevis showed the highest reduction of AFB_1 production by *A. flavus* and *A. parasiticus*, 96.31 and 90.43%, respectively.

Sezer et al. (2013) investigated the effectiveness of LAB and their bacteriocins in decontaminating AFB_1 from solution. According to the results, bacteriocins could remove 90% AFB1 from the medium.

2.5.1 Application of Probiotics as Bio-protective Agents in Meat and Meat Products

Since time immemorial, LAB have been used traditionally as preservative in the food and agro-allied industries for many reasons including prevention of food spoilage and shelf lives extension. In recent decades, applications of LAB and yeasts in food production and preservation and as nutritional supplements have being increasing (Bianchini & Bullerman, 2009). Application of microbial cells aiming to decontaminate AFs in food products has provided yet another opportunity to reduce occurrence and deleterious effects of AFs in food for human consumption. Thus, incorporation of microbial cells as far as they might not leave undesirable compounds in food matrices can be an appropriate choice (Chiocchetti et al., 2019). Several species of probiotic LAB, bifidobacteria and yeasts (Vinderola et al., 2000) are widely applied in food fermentation and as starter cultures as well as for mycotoxin decontamination (Shetty & Jespersen, 2007; Rahaie et al., 2012) in the food and beverage industry.

LAB are group of gram-positive, acid tolerant, and non-sporulating and non-respiring microorganisms that share common metabolic and physiological characteristics. Morphologically, they appear either rod-shaped or spherical known as bacilli or cocci

respectively. LAB can be categorized into four genera viz; *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Pediococcus*. They can be distinguished by the production of lactic acid as major end product during fermentation of the substrates (basically aldehydes and ketones). In addition to lactic acid, LAB produce several hurdles such as bacteriocins and other antimicrobial peptides against several spoilage and pathogenic microorganisms. Due to their high nutritional requirements, they are generally grown in enriched media and are found abundantly in food products such as cereals and cereal-based products, dairy products and meat and meat products (Dalié et al., 2010). Probiotics are microorganisms which upon ingestion in adequate amount confer health benefits by enhancing and modulating the gastrointestinal functioning of the host (FAO, 2001). Probiotics are known to decontaminate AFs in different matrices and even inhibit the growth of mycotoxigenic fungi to achieve the so-called food bioprotection (Ahlberg et al., 2015; Oliveira et al., 2014; Shetty et al., 2007).

In general, the GRAS status of probiotic LAB enhances the potential of these group of microorganisms to be exploited in large scale commercial applications as biological control agents in processed foods to prevent mold growth, improve shelf life and reduce health hazards associated with mycotoxins (Shetty and Jespersen, 2006; Bianchini and Bullerman, 2009; Taheur et al., 2017).

Probiotics and some food autochthonous microorganisms have shown promising results against aflatoxigenic fungal growth and AFs production in meat and meat products (Simoncini et al., 2014; Ibrahim et al., 2018). However, careful selection of any microorganisms to be used as bioprotection agents in dry cured meats must be done as matrix status and environmental factors such as pH, water activity and temperature might influence the antagonistic effects and AFs reduction in the meat products (Peromingo et al., 2019).

Simoncini et al. (2014) evaluated the efficacy of two autochthonous yeast strains to inhibit *Penicillium nordicum* growth and OTA accumulation in dry-cured hams. Effects of a_w and meat surface disinfection on growth and toxin accumulation were taken into account. Briefly strains of *Debaryomyces hansenii* and *Hyphopichia burtonii* (10^6 cells/cm^2 meat surface) were co-cultured with *P. nordicum* in dry-cured meat model at 0.88 and 0.92 a_w. Compared to the control sample, the growth of *P. nordicum* and OTA accumulation could be reduced between 1 and 3 log, and 0.10 to 1.06µg/kg; irrespective of the environmental conditions influence.

Ibrahim et al. (2018) evaluated the AFs mitigation capacities of two probiotic strains in some meat and meat products sold in different supermarkets in Egypt. The meat samples analysed include Burger, minced meat, luncheon and sausage. *L. acidophilus* and *Bifidobacterium lactis* were used for decontaminating AFs from the meat samples. The results showed that 98.3 and 88% of AFs could be decontaminated by *Bifidobacterium lactis* and *L. acidophilus* respectively.

In the study of Karmi (2019), 97.2% and 61% of AFs and OTA respectively spiked in burger were decontaminated by *L. acidophilus*.

Peromingo et al. (2019) investigated the antagonistic effects of autochthonous yeasts isolated from dry-cured meats on *A. parasiticus* growth and AF (B_1 and G_1) production in dry-cured ham and dry-fermented sausages. *D. hansenii* strains were co-cultured with *A. parasiticus* in the above mentioned dry cured meat models with the aim to prevent *A. parasiticus* growth and decontaminate the preformed AFs. Co-cultured of *A. parasiticus* and *D. hansenii* strains 125G and 253H were grown in meat-based media at 25°C for 14 days whereas the preformed AFs were detected by ultrahigh performance liquid chromatography mass spectrometry (uHPLC-MS). *A. parasiticus* growth and AFs production in the presence of *D. hansenii* could not be depressed at 0.99 aw. However, the authors reported that at 0.92 aw, *D. hansenii* can significantly reduce the concentration of AFs in all the meat-model systems. The concentration of AFB_1 could be reduced in dry-fermented sausages from 69.88-45.10 ng/g and 55.81-29.13ng/g for strain 125G and 253H respectively. AFB_1 concentration in dry-cured ham was found to be less than limit of detection by uHPLC-MS (Peromingo et al., 2019).

2.5.2 Nature and Kinetics of AFs Decontamination by Probiotics

Numerous investigations and reports were available in the literature to explain the AFs decontamination mechanisms by probiotic microorganisms (Bueno et al., 2007; El-Nezami et al., 1998; Karunaratne et al., 1990; Lee et al., 2003; Oatley et al., 2000; Peltonen et al., 2000; Peltonen et al., 2001; Pierides et al., 2000; Pizzolito et al., 2011). Thus far, the decontamination mechanisms have been largely attributed to either metabolic degradation or physical absorption by probiotics cell wall components (Haskard et al., 2000; Lahtinen et al., 2004; Lili et al., 2017) the latter case was favored because there were no significant differences in toxins removal between the viable and inactivated cells. To extensively understand the mitigation process of AFs by probiotics, a chemo-biological kinetics of AFs and probiotics (Figure 2.11) with

subsequent AFs removal has been established (Bueno et al., 2007; Haskard et al., 2000; Lee et al., 2003). Physical adsorption of AFB_1 by *L. rhamnosus* GG is attributed to cell wall peptidoglycans, with no role of cell wall proteins, lipids, exopolysaccharides, and minerals (Lahtinen et al., 2004).

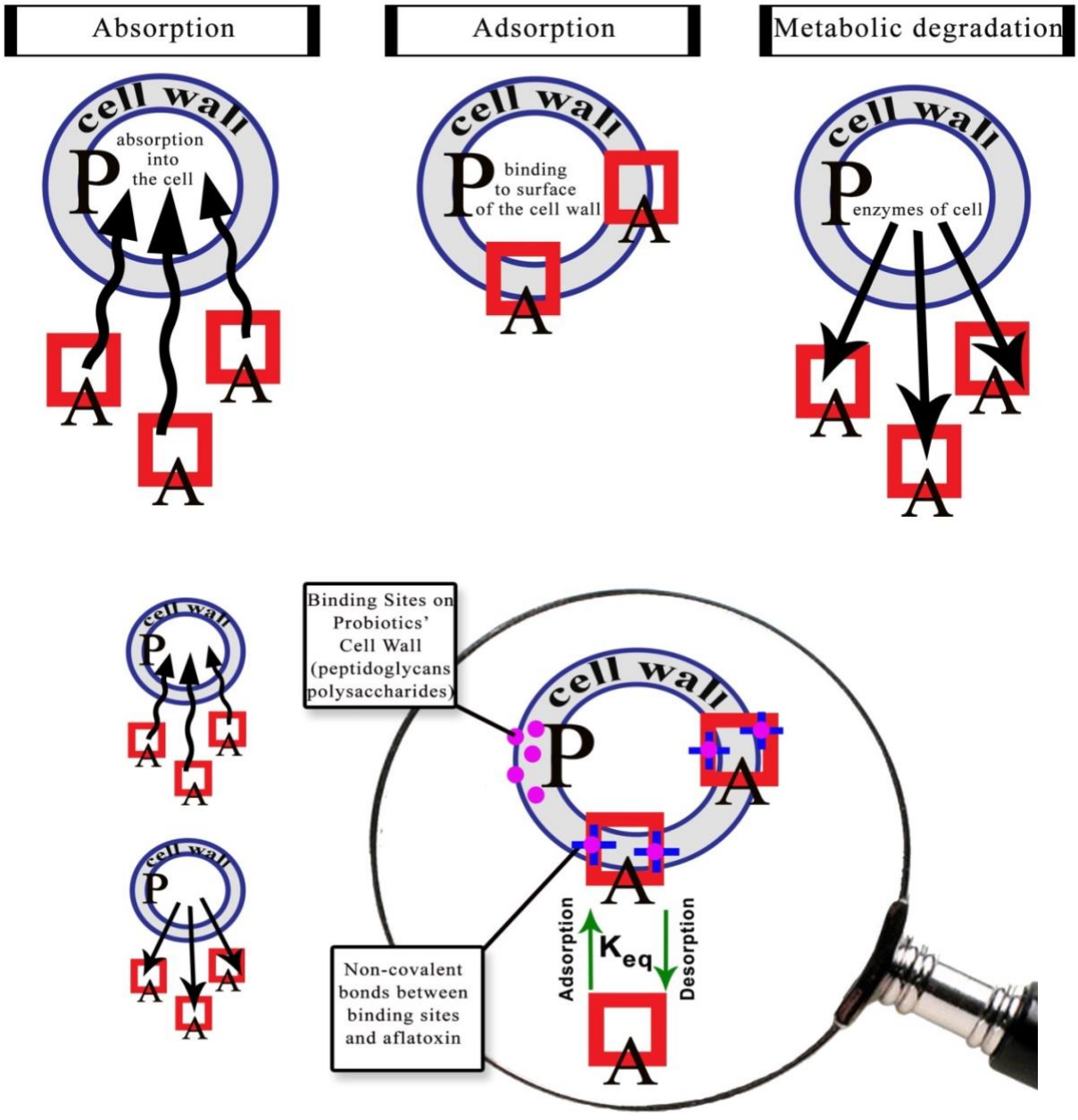


Figure 2.11: Mechanisms of AFs Decontamination by Probiotics with Focus on Adsorption/Desorption Kinetics

2.5.3 Factors Affecting the *In vitro* Binding Efficiency of AFB1 by *L. rhamnosus*

Several factors such as initial concentration of probiotics and AFs in food**,** type of probiotic strain, probiotics state (viable or inactivated), food matrix, stability of probiotics/AFs complex and environmental conditions are claimed to affect the decontamination efficacy of probiotics. There is no consensus on the precise role of these factors in determining the binding of AF to the bacterial cell wall. The following examples will illustrate the current understanding of the role of these factors in bacterial ability to bind AFB_1 (Sadiq et al., 2019). Figure 2.12

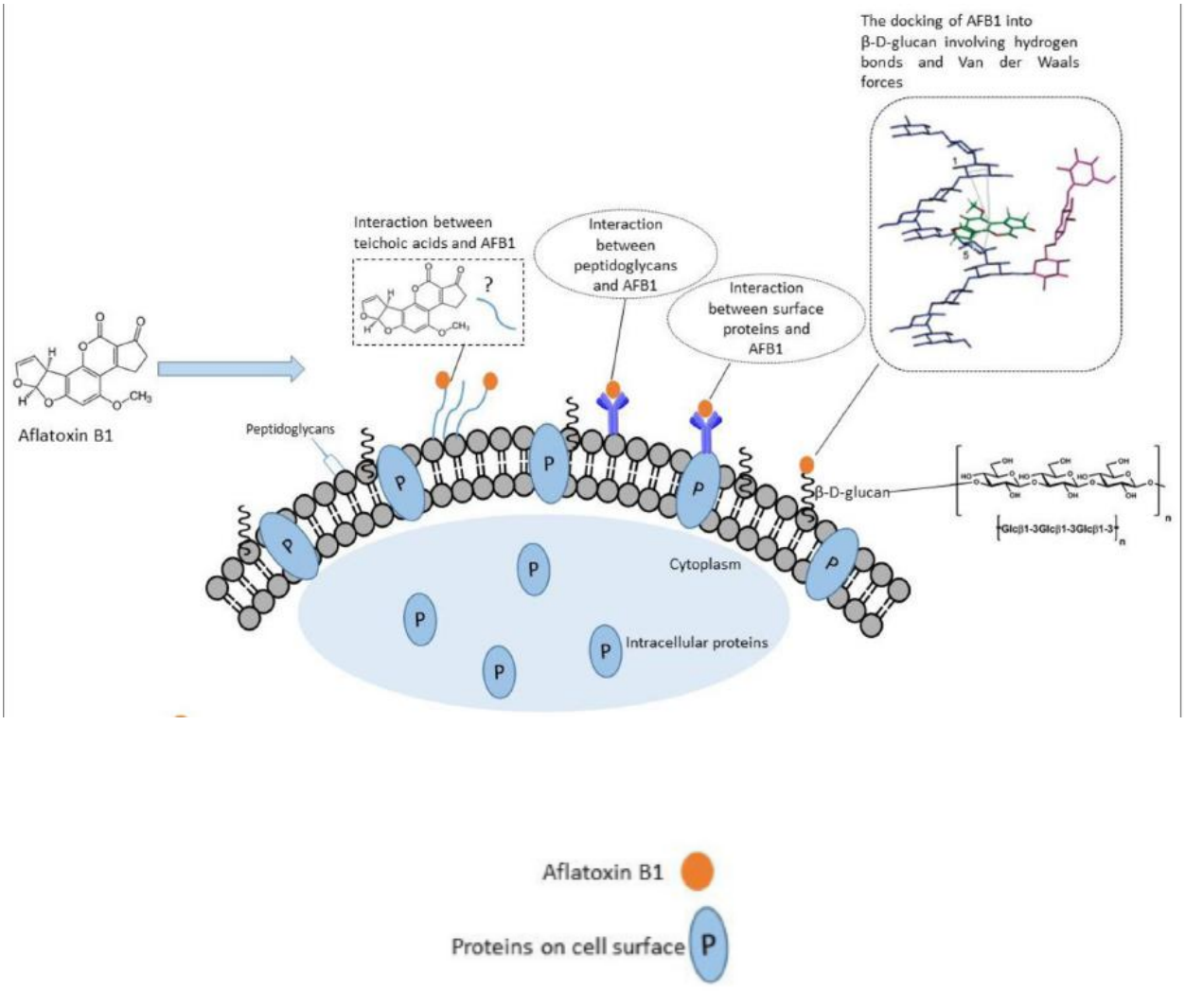


Figure 2.12: Interactions of AFB_1 with Probiotic Cell Main Components (Sadiq et al., 2019)

2.5.4 Efficacy of Viable and Inactivated *L. rhamnosus* GG for AFB_1 Decontamination

Cell viability is one of the good characteristics of probiotics; long as the health benefits is needed. Thus, tremendous efforts were attempted to discover whether decontamination of AFs depends on cell viability. As a consequence, several heat-killed, freeze-dried and viable LAB, bifidobacteria and yeasts were examined for their efficiencies to decontaminate AFs in solution and food matrices (Rahaie et al., 2012; Kabak & Ozbey, 2012; Bovo et al., 2013; Corassin et al., 2013; Elsanhoty et al., 2014).

In the study of El-Nezami et al. (1998a), the potential of several dairy strains of LAB including *L. rhamnosus* subsp. *L. rhamnosus* GG and *L. rhamnosus* LC-705 were tested for their ability to decontaminate AFB_1 during four incubation periods (0, 24, 48 and 72 hr). Different LAB concentrations from $5x10^9$ to $2x10^{10}$ CFU/ml were used. Different temperature conditions of 4, 25 and 37°C were used to evaluate the freeze-dried cells. As much as 82% of AFB_1 (concentrations 5, 10 and 50 mg/ml) could be removed from the solution. From this study, the authors concluded that AFB_1 binding efficiency depends on the temperature and bacterial concentration. Thus, the most efficient strains were further screened and tested again at varying bacterial concentrations. It was observed that $2x10^{10}$ CFU/ml of *L. rhamnosus* GG and *L. rhamnosus* LC-705 could remove almost all the AFB_1 (0.1% and 13% remained unbound), respectively. An average of $2x10^9$ CFU/ml is sufficient enough to maximally decontaminate AFB_1. Heat-killed bacteria have the highest binding capacity. The percentage of binding is in the order of 81 and 82% for heat-treated *L. rhamnosus* LC-705 and *L. rhamnosus* GG. Freeze-drying has a negative effect for both strains.

From the results of the above study (El-Nezami et al., 1998a) it can be understood that heat treatments have no effects on bacterial-toxin binding. Thus, El-Nezami et al. (1998b) further study the effects of physical and chemical treatments. AFB_1 decontamination ability of *L. rhamnosus* GG and *L. rhamnosus* LC-705 were examined following adjustment of different chemical and physical conditions by HPLC method. An appreciable amount of AFB_1 (50%) could be decontaminated by both strains without any treatment(s). Temperature and pH were found to significantly affect the AFB_1 decontamination abilities of both *L.* strains. Decontamination abilities are in the order of 99.9 and 96.6% for *L. rhamnosus* GG and *Lb. rhamnosus* LC-705 at 37°C and pH 2. It was reported that increased in alkalinity reduced the decontamination efficiencies for both strains.

In continuous search for better decontaminating abilities of *L. rhamnosus* strains, Haskard et al. (2000) examined decontaminating capacity of physico-chemically treated *L. rhamnosus* GG as lyophilized pellets by HPLC-FLD. Addition of NaCl and $CaCl_2$ during the late exponential to early stationary phase were found to increase AFB_1decontaminating capacity of *L. rhamnosus* strain GG as 80% approximately.

In another study, viable and physico-chemically altered strains of *L. rhamnosus* GG and *L. rhamnosus* LC-705 were assessed for their decontaminating abilities against AFB_1 by Haskard et al. (2001) through repetitive aqueous washes by an indirect competitive inhibition enzyme-linked immunosorbent assay (ELISA). After several acid treatments, 71% of AFB_1 remain attached to bacterial cells. Moreover, approximately 90% of retained AFB_1 could be recovered by solvent extraction method. However, variations in pH (from 2 to 10) and temperatures (4 to 37°C) did not show any effect on the retained AFB_1.

In the study of Turbic et al. (2002) viable and inactivated cells of *L. rhamnosus* GG and *L. rhamnosus* LC-705 were evaluated for their ability to bind several mutagens including AFB_1. The authors showed that both bacteria could bind AFB_1 effectively, as much as 77% and 92% AFB_1 was absorbed by *L. rhamnosus* LC-705 and *L. rhamnosus* GG respectively.

Zinedine et al. (2005) tested the AFB_1 decontaminating capacity of five physico-chemically treated *L. rhamnosus* strains sourced from sourdough. At varying pH (3, 4.5 and 5.5) and temperature (15, 25 and 37°C), the bacteria could decontaminate 23% to 45% of free AFB_1.

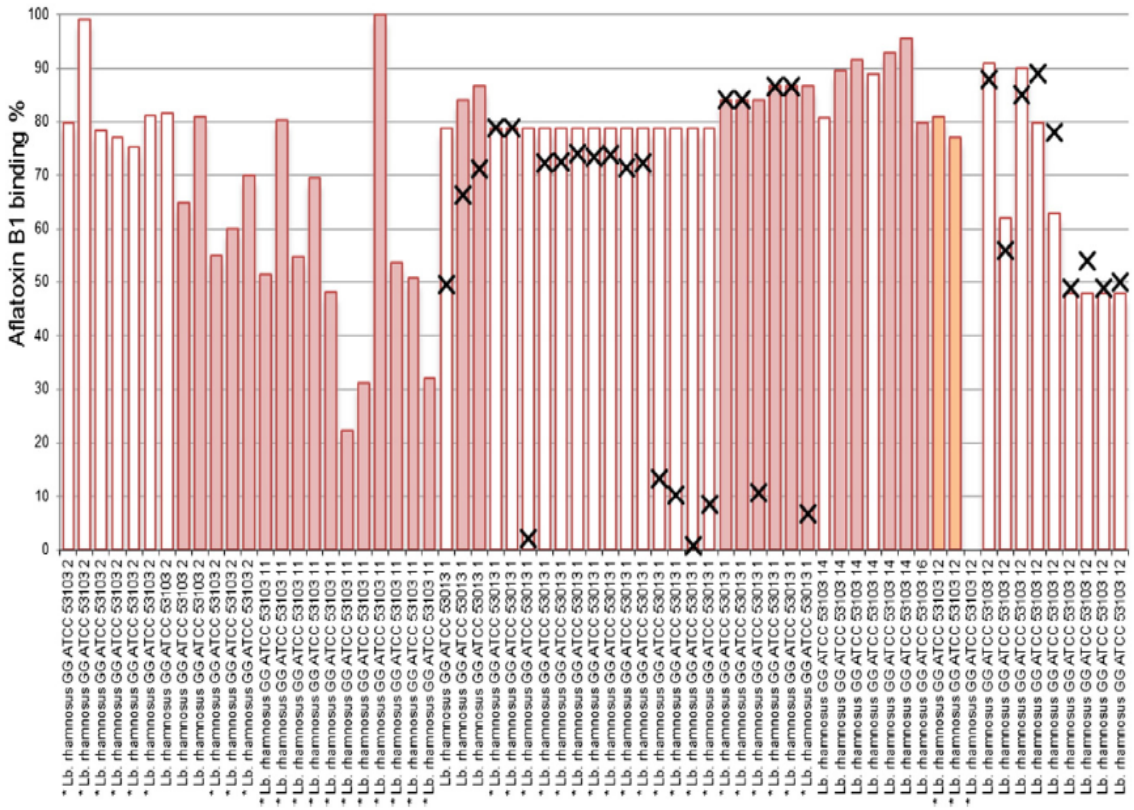


Figure 2.13: Application of *L. rhamnosus* GG for AFB_1 Decontamination (Ahlberg et al., 2015) Red= killed bacteria. * = variation in conditions (e.g. pH, temperature, concentration, e.t.c). X = binding stability after distinct treatments. Orange = cell free.

2.6 Application of *L. rhamnosus* for AFB_1 decontamination in Food

Ability of *L. rhamnosus* to decontaminate AFs from various food matrices such as cereal and cereal products, nuts and spices, milk and dairy products and meat and meat products has been extensively explored (Pierides et al., 2000; Zinedine et al., 2005; Rahaie et al., 2012; Kabak & Ozbey, 2012; Bovo et al., 2013; Corassin et al., 2013; Elsanhoty et al., 2014; Taheur et al., 2017; Ibrahim et al., 2018; Panwar et a., 2019; Wochner et al., 2019). From the reports compiled by Ahlberg et al. (2015) *L. rhamnosus* GG is the most research and successful probiotic for the inhibition of mycotoxigenic fungal growth and control of mycotoxins in food systems. Table 2.2 presents the summary of studies involving decontamination of AFB_1 by *L. rhamnosus* in various food matrices.

AFB_1 mitigation efficacy of viable and inactivated (heat and acid treated) *Saccharomyces cerevisiae* and *L. rhamnosus* GG cells in pistachio nuts was evaluated by Rahaie et al. (2012). Initial concentration of AFB_1, temperature and pH are some of the factors considered to evaluate

AFB_1 the adsorption potential of probiotic microorganisms tested. Thus, to fully evaluate the mitigation capacities of the tested probiotic strains, two different initial concentrations of AFB_1 were used. It was shown that 40% and 35% of AFB_1 was adsorbed by *Saccharomyces cerevisiae* and *L. rhamnosus* GG, respectively with 10 μg/kg initial concentrations. Moreover, the authors also noted that adsorption potential increase with increase in the initial concentrations of AFB_1. *S. cerevisiae* and *L. rhamnosus* GG decontaminate 70% and 60% respectively, with initial concentration of 20 μg/kg. Heat treatment increases the decontamination efficacy, *S. cerevisiae* adsorbed 55% and 75% while *L. rhamnosus* GG could adsorb 85% and 90% for the first and second concentrations. Acid treatment also increases the decontamination efficacy for yeast and bacterium to 60% and 85% in first concentration, and 73% and 90% for second concentration, respectively.

Elsanhoty et al. (2013) assessed the *in vitro* AFs decontaminating potential of viable and inactivated (heat treated) probiotics pool including *L. rhamnosus* in wheat flour used for Baladi bread making. The probiotics species tested were *L. acidophilus* ATCC 20552, *L. rhamnosus* TISTR 541, *L. sanfranciscensis* DSM20451 and *Bifidobacterium angulatum* DSMZ 20098. The stability of probiotics/AFs complexes formed was also assessed. Three different wheat flour formulations contaminated with AFs were studied including formulation (A) as control fermented by *Saccharomyces cerevisiae* (bakery yeast), formulation (B) fermented by *L. rhamnosus* TISTR 541 and formulation (C) fermented by the mixture of *Saccharomyces cerevisiae* and *L. rhamnosus*. According to the authors, *L. rhamnosus* has the highest AFB_1 decontaminating potential (35.8% and 72.7% for viable and inactivated cells, respectively) and formulation C was the best AFs decontaminating strategy.

In the study of Bovo et al. (2014), AFB_1decontaminating potential of inactivated *L. rhamnosus* in milk medium was tested. The authors reported that 43.7% and 25.8% of AFB_1 was removed from the medium by *L. rhamnosus* at pH 3.0 and 6.0, respectively.

The AFB_1decontaminating efficiency of *L. rhamnosus* in whole milk at both the optimum temperature (37°C) and chilled (4°C) for one week was examined by Marrez et al. (2018).
L. rhamnosus showed rapid removal of AFB_1 (10%) at 0 time and higher removal (77.6%) was achieved at optimum temperature within one day. However, the binding capacity increased to 79.4% during seven days at chilled temperature. The AFB_1 adsorption/desorption kinetics of *L. rhamnosus* after 3 days and 7 days was 44.4% and 29.7%, respectively.

Table 2.1: AFB_1 Decontamination Efficacies of *L. rhamnosus* Strains in Various Food Matrices

***L. rhamnosus* Strain**	**Initial Concentration of AFB1 (µg/kg/mL)**	**% of AFB1 Removal**	**Food Matrix**	**Reference**
L. rhamnosus GG	20	90%	Pistachio nut	Rahaie et al. (2012)
L. rhamnosus TISTR 541	29.7	72.7%	Bread	Elsanhoty et al. (2013)
L. rhamnosus	10	43.7%	Milk whey	Bovo et al. (2014)
L. rhamnosus	$49.1x10^{-3}$	79.4%	Whole milk	Marrez et al. (2018)

2.7 Concept of Mathematical Modelling in Food Mycology and Mycotoxicology

Models for microbial inactivation have been developed in the 1920s and, in 1980s rapid progress in predictive microbiology (Zwietering & den Besten, 2011). Some people will consider that a computational model is a computer tool that can make predictions of growth and inactivation kinetics, or predict dynamics over a food chain. One can also argue that a computer tool is not the model, but the mathematical equations that are implemented in the program. Others can view that the model is not the mathematical equations, but the set of assumptions that are made, that result in mathematical equations (Prandini et al., 2009).

A further conceptualization is that a model is a simplified representation of reality. This then is defined as a set of assumptions that will result in mathematical equations, which can be programmed in a computer tool. Model can be linear or nonlinear if all the operators in mathematical model exhibit linearity; the resulting mathematical model is defined as linear. A model is considered to be nonlinear otherwise (Prandini et al., 2009).

Depending on the goal of the model, probabilistic and mechanistic models can be developed. Probabilistic (also known as kinetic models) are used to predict fungal growth and toxin formation under controlled conditions in a given time (Garcia et al., 2009; Molina and Giannuzzi, 2002). Mechanistic or semi mechanistic models are based on parametric differential equations from a set of well-established phenomena, for example mechanism of mycotoxins degradation (Gibson and Hocking, 1997; Pitt, 1993).

Mathematical modeling has long been used to predict the extent of fungal growth and colonization in foodstuffs as a function of environmental conditions (Molina and Giannuzzi,

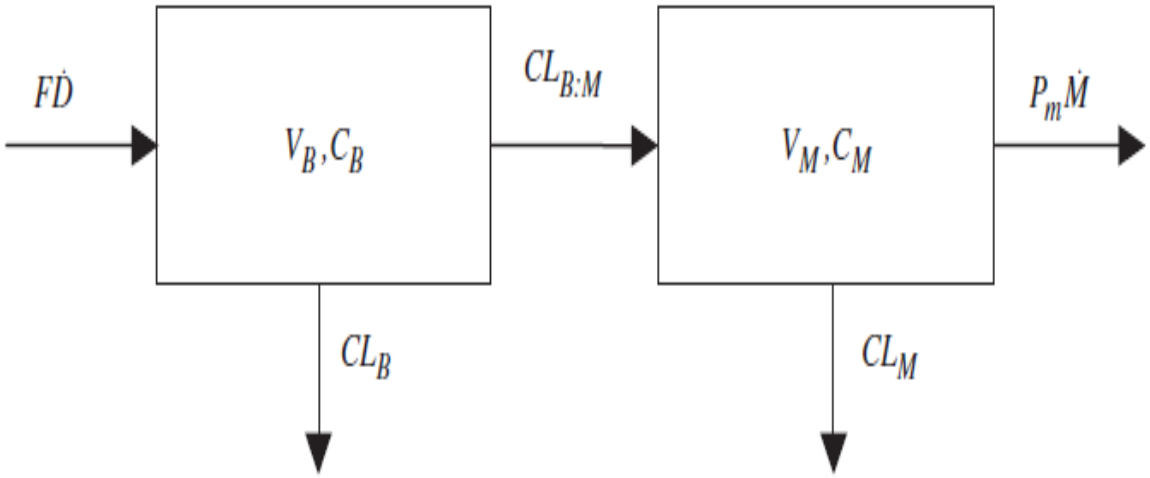
2002). Pitt (1993) developed some mathematical functions to deal with mold growth and AFs degradation in food under the influence of environmental factors. Kinetic Modeling of AFs in the food supply chain have been previously studied by several researchers such as Van Eijkeren et al. (2006) predicting the conversion of ingested AFB_1 in cows to AFM_1, Zhang et al. (2011), Martins et al (2017) and Wang et al. (2018) predicting AFs degradation in food after thermal treatments and Kademi et al. (2019b) prediction of AFs risk reduction in food products. These kinds of models could provide an insight into whether the maximum levels of these toxins will be exceeded. Summary of these models will be given:

2.7.1 Kinetic Modelling of AFB_1 from Feed to Cow's Milk

The first line in Figure 2.14 shows the rate of change of the body burden of AFB_1 dA_B/dt after fractional systemic uptake F of the daily ingested amount D through contaminated feed and the concurrent clearance from plasma by elimination (CL_B) and AFM_1 formation $CL_{B:M}$.

The second line shows the rate of change of the body burden of AFM_1 i.e dA_M/dt, and its concurrent clearance from plasma by elimination (CLM) and excretion through milk, PmM.

Briefly, Van Eijkeren model explains similar experimental outcomes from different investigations into carry-over of AFs from feed to milk. When cows are fed with contaminated rations that do not exceed 5 μg/kg (threshold quantity of AFB_1 in animal feed) set by the EU, the model predicts that raw cow's milk would not likely exceed the MPLs of 0.05 μg/kg AFM_1.



$$\frac{dA_B}{dt} = F\dot{D} - (CL_B + CL_{B:M}) \cdot C_B$$

$$\frac{dA_M}{dt} = CL_{B:M} \cdot C_B - (CL_M + P_m\dot{M}) \cdot C_M$$

Figure 2.14: Two kinetic one-compartment models for the conversion of AFB_1 to AFM_1 in the lactating cow milk

2.7.2 Kinetic Modelling of AFs Decontamination in Food Matrix

As at the present time, kinetics modelling of AFs in food products after thermal treatments has been studied by the following researchers:

In the study of Zhang et al (2011), the kinetic models of the AFB_1 conversion were constructed based on the differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). To be clear, the kinetic models based on the DSC and TGA were named as Model A and Model B, respectively.

CHAPTER THREE

Methodology

This chapter presents a mathematical model describing the mitigation process of AFB_1 by *Lactobacillus rhamnosus* strain GG in the matrix of samarella, a traditionally produced sun-dried and salted meat product in Cyprus. The objective of the model is to show the mitigation effect of *L. rhamnosus* GG on the preformed toxins and thus serve as prediction tool for AFB_1 quantitative risk assessment in this traditionally made dry-cured meat model. Stability analyses of the model as well as numerical simulations were carried out to support the analytic result. To describe and support how the propose model works, numerical simulations were carried out using MATLAB R2017a.

3.1 Model Construction

The model was constructed according to the procedure described by Kademi et al. (2019b), as follows:

Let A and L be the concentration of AFB_1 and *L. rhamnosus* GG in the matrix of samarella respectively. The description of the model parameters was given in Table 1. The degradation mechanism is given by

$$\frac{dA}{dt} = r_1 A(1 - kA) - \frac{\mu AL}{\beta + L}$$
$$\frac{dL}{dt} = r_2 L - \alpha AL \tag{1}$$
$$A(0) = A_0 > 0 \; and \; L(0) = L_0 > 0$$

In the first line of the model (1), the first term A (in μg/kg) ; represents the occurrence of AFB_1 which is considered to be logistic with concentration of AFB_1 to be formed as $\frac{1}{k}$ and intrinsic occurrence rate r_1. The second term β, describes the interaction of AFB_1 with *L. rhamnosus* GG which usually, depends on the binding ability of *L. rhamnosus* GG and the number of binding sites in the cell of *L. rhamnosus* GG. This term is of Michaelis-Menten form to show the saturated effects in the action of *L. rhamnosus* GG on AFB_1, with μ being the decontamination ability of *L. rhamnosus* GG. Whereas in the second line of the model system (1), the first term

L $(in\ CFU/mg)$, implies the natural occurrence/technological addition of *L. rhamnosus* GG in the matrix of samarella at the rate r_2 and the second term AL represents AFB_1/*L. rhamnosus* GG complex; with α being the reaction rate.

Table 3.1: Description of Model Parameters

Parameter	Description
r_1	Intrinsic production rate of AFB_1
$\frac{1}{k}$	Concentration of AFB_1 that can be formed within the matrix of samarella
μ	Decontamination ability of *L. rhamnosus* GG
β	Half-saturation for the association term
r_2	Rate of occurrence/application of *L. rhamnosus* GG
α	Rate of formation of AFB_1- *L. rhamnosus* GG complex

3.2 Positivity of Solution

Assume that $A_0 > 0\ and\ L_0 > 0$, then from system (1) we have

$$\frac{dL}{dt} = (r_2 - \alpha A)L, \qquad then$$

$$L(t) = L_0 e^{(r_2 - \alpha A)T} > 0\ \ for\ all\ t \in [0, T].$$

Similarly,

$$\frac{dA}{dt} = r_1 A(1 - kA) - \frac{\mu AL}{\beta + L}, implies\ that$$

$$\frac{dA}{dt} \geq -r_1 kA^2 - \frac{\mu AL}{\beta + L}.$$

Hence, solving the ODE as a Bernoulli equation we have $A(t) \geq 0$ for all t between 0 and T. Hence, the solutions are positive.

3.3 Boundedness of Solution

We determine solutions that are upper bounds (super solutions) of A and P in system (1). From the first line of model system (1), assuming that A_{max} is an upper bound solution associated with A and given that $(t) \geq 0\ and\ L(t) \geq 0 \forall t \in [0,T]$ (by positivity of solution), where T is the estimated shelf life of Samarella, then

$\frac{dA_{max}}{dt} = r_1 A$, which implies $A_{max}(t) = A_0 e^{r_1 T}$.

Similarly, $L_{max} = L_0 e^{r_2 T}$.

By using A_{max} and L_{max}, we can form a set of upper bound solutions for system (1).
Denoting the upper solutions by $\bar{A}$ and $\bar{L}$ we have

$$\frac{d\bar{A}}{dt} = r_1 \bar{A}$$

$$\frac{d\bar{L}}{dt} = r_2 \bar{L}$$

that is bounded on a finite time interval, which can also be written as

$$\begin{pmatrix} \bar{A} \\ \bar{L} \end{pmatrix}' = \begin{pmatrix} r_1 & 0 \\ 0 & r_2 \end{pmatrix} \begin{pmatrix} \bar{A} \\ \bar{L} \end{pmatrix}$$

Where$' = \frac{d}{dt}$.

We now have a linear system in finite time with bounded coefficients; hence the super solutions $\bar{A}\ and\ \bar{L}$ are uniformly bounded. Therefore, using a comparison result, system (1) is also bounded.

3.4 Equilibrium Analysis

Equating the right-hand side of system (1) to zero and solving simultaneously for A and L we have the following equilibrium points:

$$E_0 = \{0,0\},\quad E_1 = \left\{\frac{1}{k}, 0\right\}, and\ E_2 = \left\{\frac{r_2}{\alpha}, \frac{r_1\beta(\alpha - kr_2)}{r_1 kr_2 + \mu\alpha - r_1\alpha}\right\}.$$

$E_0$is referred to as the AFB1- *L. rhamnosus* GG free equilibrium.

$E_1$is considered to be "dangerous equilibrium", where we have *L. rhamnosus* GG -free and the AFB_1 reaches their maximum allowable limits.

$E_2$is the coexistence of AFB_1 and *L. rhamnosus* GG.

3.5 Existence of Equilibria

The equilibrium E_0 and E_1 always exists, while E_2 exists only if the following conditions are satisfied:

i. $\alpha > kr_2$ and $r_1 kr_2 + \mu\alpha > r_1\alpha$

OR

ii. $\alpha < kr_2$ and $r_1 kr_2 + \mu\alpha < r_1\alpha$

iii. From (i), we have $\frac{r_2}{\alpha} < \frac{1}{k}$ which means that the equilibrium exist when the ratio of the occurrence of *L. rhamnosus GG* to the rate of formation of aflatoxin- *L. rhamnosus GG* complex is greater than their maximum allowable limits; which is not likely the case. Therefore, we choose (ii).

iv. The equilibrium point E_2 exist if $\alpha < kr_2$ and $r_1 kr_2 + \mu\alpha < r_1\alpha$.

3.5 Stability Analysis

In this section we check the stability of the equilibrium points.

From system (1), we derive the Jacobian matrix

$$J(A,L) = \begin{pmatrix} r_1 - 2r_1 kA - \dfrac{\mu L}{L+\beta} & -\dfrac{(L+\beta)\mu A - \mu AL}{(L+\beta)^2} \\ -\alpha L & r_2 - \alpha A \end{pmatrix}$$

3.5.1 Aflatoxin-probiotics free equilibrium: $\boldsymbol{E_0 = \{0,0\}}$

From J, we have

$$J(\boldsymbol{E_0}) = \begin{pmatrix} r_1 & 0 \\ 0 & r_2 \end{pmatrix}$$

The eigenvalues of $J(\boldsymbol{E_0})$ are given by $\lambda_1 = r_1$ and $\lambda_2 = r_2$. Since all the eigenvalues are positive, hence, E_0 is an unstable equilibrium point.

3.5.2 Dangerous equilibrium: $\boldsymbol{E_1} = \left\{\frac{\mathbf{1}}{\boldsymbol{k}}, \mathbf{0}\right\}$

Similarly, the eigenvalues of $J(\boldsymbol{E_1})$ are $\lambda_1 = -r_1$ and $\lambda_2 = r_2 - \frac{\alpha}{k}$. Then, $\lambda_1 < 0$ and $\lambda_2 < 0$ if and only if $\frac{r_2}{\alpha} < \frac{1}{k}$. Hence, E_1 is stable if and only if $\frac{r_2}{\alpha} < \frac{1}{k}$.

3.5.3 Coexistence equilibrium: $\boldsymbol{E_2} = \left\{\frac{\boldsymbol{r_2}}{\boldsymbol{\alpha}}, \frac{\boldsymbol{r_1\beta(\alpha - kr_2)}}{\boldsymbol{r_1 kr_2 + \mu\alpha - r_1\alpha}}\right\}$

Assume the equilibrium point exist, then $\boldsymbol{E_2}$ is stable if the following conditions are satisfied:

i. $\boldsymbol{r_1(\alpha - kr_2) - \mu\alpha > 0}$ **and** $\boldsymbol{\alpha > kr_2}$

OR

ii. $\boldsymbol{r_1(\alpha - kr_2) - \mu\alpha < 0}$ **and** $\boldsymbol{\alpha < kr_2}$

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Results

According to a theoretical model proposed by Bueno et al. (2007), the number of probiotics binding sites (called M-value) and reaction equilibrium constant (called K-value) are the primary factors for mitigation efficiencies (MxK) among probiotic microorganisms. The model follows the Machaelis-Menten protocols of adsorption-desorption phenomena by probiotics to AFB_1. Similar phenomenon was followed by mathematical model constructed in this study. However, in our model one probiotics is selected and simulated results show that the reaction equilibrium constant (defined in our model as α-value) which is the rate of formation of AFB_1/*L. rhamnosus* GG complex is the important factor responsible for decontamination of AFB_1 by *L. rhamnosus* GG. Hence, from Fig. 4.1, for example, we can see that to some extent *L. rhamnosus* GG adequately remove/decontaminate AFB_1 at a given time and for a given rate of formation of AFB_1/*L. rhamnosus* GG complex. Increasing this rate shows that the occurrence and rate of formation of AFB_1 is reduced faster as can be observed from Figures 4.2, 4.3 and 4.4. This shows that the rate of formation of AFB_1/*L. rhamnosus* GG complex is vital and sensitive in the whole process, as it will determine how fast, rigorous, and effective the mitigation of AFB_1 in a matrix of Samarella can be achieved. Therefore, the model presented here established that the decontamination capacity of *L. rhamnosus* GG depends on the rate of formation of AFB_1/*L. rhamnosus* GG complex as previously reported by theoretical model and experimental results (Bueno et al., 2007; Lee et al., 2003).

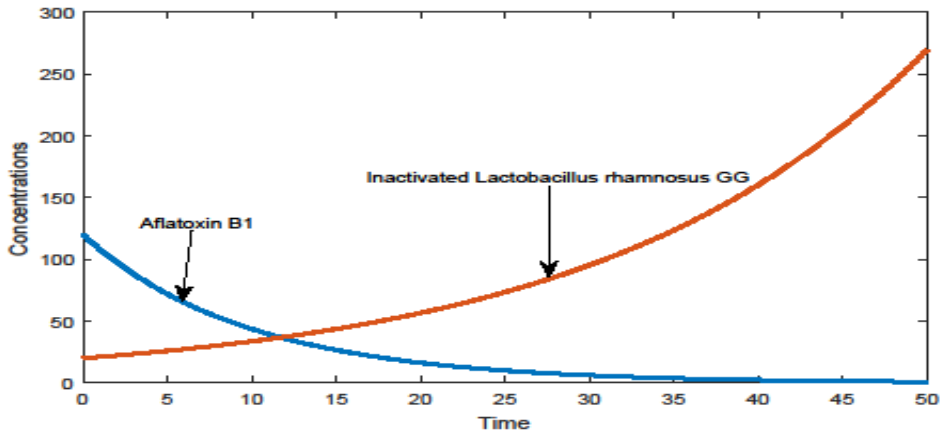


Figure 4.1: Decontamination Mechanism of AFB_1 by Inactivated *L. rhamnosus* GG in Samarella. The AFB_1/*L. rhamnosus* GG complex $(\alpha) = 0.10$. Precisely, (α) is the point of intersection between Inactivated *L. rhamnosus* GG and AFB_1 concentration

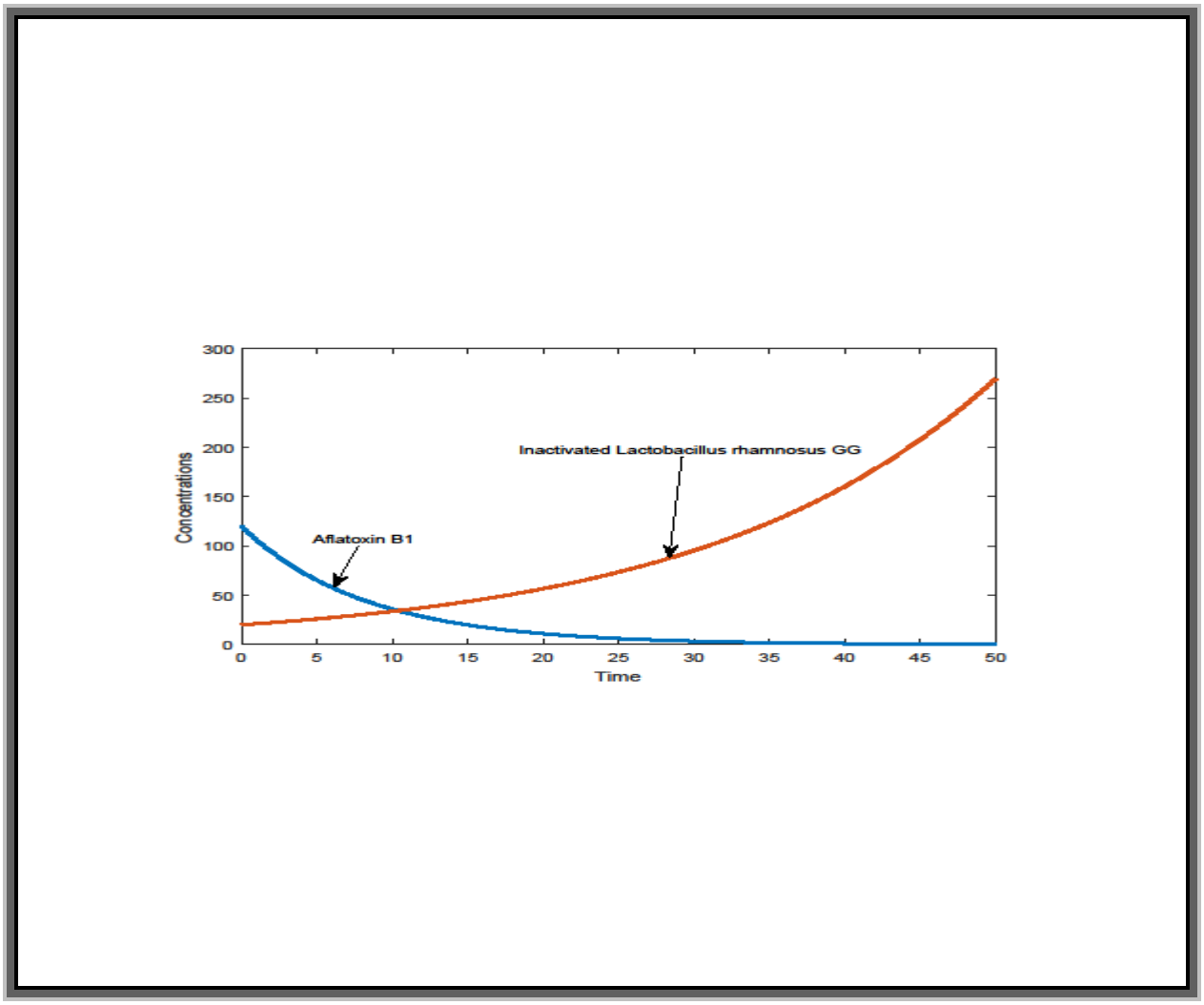


Figure 4.2: Decontamination Mechanism of AFB_1 by Inactivated *L. rhamnosus* GG in Samarella. The AFB_1/*L. rhamnosus* GG complex $(\alpha) = 0.12$

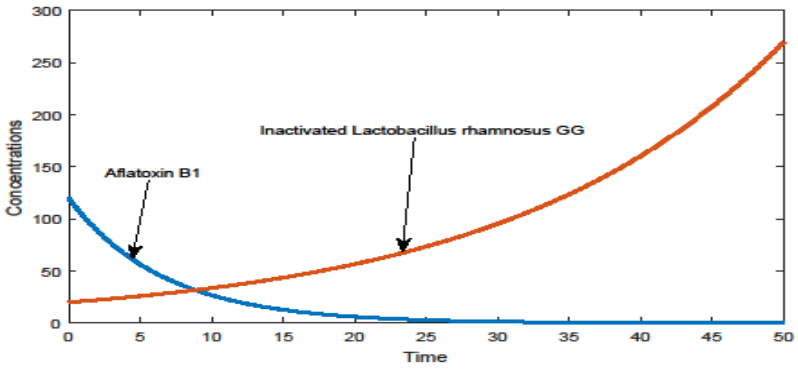


Figure 4.3: Decontamination Mechanism of AFB_1 by Inactivated *L. rhamnosus* GG in Samarella. The AFB_1/*L. rhamnosus* GG complex $(\alpha) = 0.15$

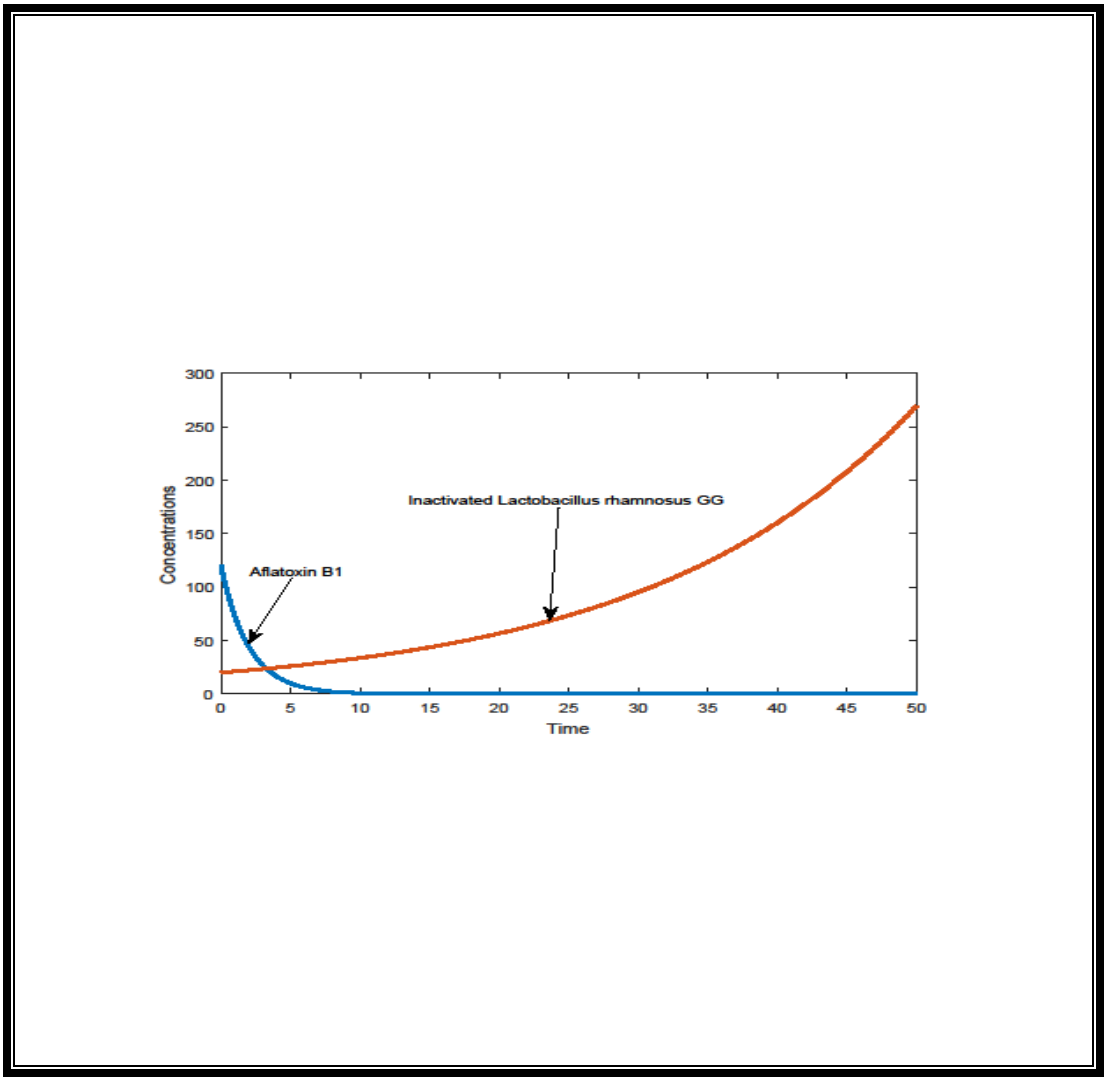


Figure 4.4: Decontamination Mechanism of AFB_1 by Inactivated *L. rhamnosus* GG in Samarella. The AFB_1/*L. rhamnosus* GG complex $(\alpha) = 0.50$

4.2 Adsorption-Desorption Kinetics of AFB_1-Probiotics

Connors (1990) defined kinetics as the study of rates of chemical reactions and/or processes, depending on the relationship of time, temperature, and conversion rate of a reaction and/or process. To the best of our knowledge there is no literature that modeled the mitigation process of AFB_1 by probiotic *L. rhamnosus* GG in meat and meat products per se dry-cured meats. Ibrahim et al. (2018) evaluated the AFs mitigation capacities of two probiotic strains in some

meat and meat products sold in different supermarkets in Egypt. *L. acidophilus* and *Bifidobacterium lactis* were used for decontaminating AFs from the meat samples. The results showed that 98.3 and 88% of AFs could be removed by *Bifidobacterium lactis* and *L. acidophilus* respectively. According to a theoretical model proposed by Bueno et al. (2007), the number of probiotics binding sites (called M-value) and reaction equilibrium constant (called K-value) are the primary factors for mitigation efficiencies (MxK) among probiotic microorganisms. The model follows the Machaelis-Menten protocols of adsorption-desorption phenomena by probiotics to AFB_1. Similar phenomenon was followed by mathematical model constructed in this study. Zhang et al. (2011) constructed an Arrhenius equation based model for decontaminating AFB_1 after thermal treatments of corn, peanuts and rice. Temperature, time and AFB_1 degradation rate were considered as factors which might affect the final AFB_1 content in the processed samples. The model could be able to predict accurately the AFB_1 content.

CHAPTER FIVE

5.1 Conclusion

In conclusion, application of probiotics to decontaminate AFs from food systems is well known and widely studied. The present study applied a mathematical model for the decontamination process of AFB_1 by the inactivated cells of probiotic strain *L. rhamnosus* GG in the matrix of samarella.

The decontamination process is attributed to either metabolic degradation or physical absorption by probiotics cell wall components. Several factors such as concentration of probiotics and AFs in food**,** food matrix, stability of probiotics/AFs complex and environmental conditions are claimed to affect the decontamination efficacy of probiotics. The number of probiotics binding sites (called M-value) and reaction equilibrium constant (called K-value) are the primary factors for mitigation efficiencies (MxK) among probiotic microorganisms. However, in our model one probiotics is selected and simulated results show that the reaction equilibrium constant (defined in our model as α-value) which is the rate of formation of AFB_1/*L. rhamnosus* GG complex is the important factor responsible for decontamination of AFB_1 by *L. rhamnosus* GG. The predictivity of the model indicates that the rates of formation of AFB_1- *L. rhamnosus* GG complexes determine how fast, rigorous, and effective the mitigation of AFB_1 in the matrix of Samarella can be achieved. This shows that the rate of formation of AFB_1/*L. rhamnosus* GG complex is vital and sensitive in the whole process, as it will determine how fast, rigorous, and effective the mitigation of AFB_1 in a matrix of samarella can be achieved.

5.2 Recommendations

To this end, we proposed to include the inactivated cells of *L. rhamnosus* GG in samarella to ameliorate or mitigate toxic effects of the preformed AFB_1 since samarella is sun-dried and cured, not fermented meat products, addition of inactivated cells may prevent the possible fermentation in the final products.

The developed model can be used to support investigations involving removal of mycotoxins in various food matrices, and to compare and contrast the effectiveness of various control strategies especially designed for decontaminating AFs from food systems.

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