



Novel decontamination approaches and their potential application for post-harvest aflatoxin control

Helina Marshall^{a,*}, Julie P. Meneely^a, Brian Quinn^a, Yueju Zhao^b, Paula Bourke^{a,c},
Brendan F. Gilmore^a, Guangtao Zhang^b, Christopher T. Elliott^a

^a Institute for Global Food Security, School of Biological Sciences, Queens University Belfast, Belfast, UK

^b Mars Global Food Safety Center, Yanqi Economic Development Zone, Beijing, China

^c School of Biosystems and Food Engineering, University College Dublin, Belfield, Dublin 4, Ireland

ARTICLE INFO

Keywords:

Aflatoxins
Decontamination
Control
Detoxification

ABSTRACT

Background: Aflatoxin is considered to be the most important mycotoxin in the world for human food and animal feed. Current strategies for the reduction of mycotoxins in food and feed includes both prevention and removal. It is clear that the development and implementation of novel decontamination methods is critical for the protection of human and animal health.

Scope and approach: This review focuses on post-harvest- biological, chemical and physical processes that could potentially be applied to aflatoxin decontamination. The application of novel technologies are reviewed in detail, as well as the advantages, disadvantages and limitations of these methods. This review investigates the potential for novel approaches to achieve aflatoxin decontamination.

Key findings and conclusion: The limitations that are associated with conventional methods of mycotoxin removal have led to ongoing research into alternative decontamination methods using novel technologies. The combination of fluorescence-based sorting to remove highly contaminated produce, paired with a secondary decontamination process is believed to have great potential to deliver effective reduction in aflatoxin contamination, whilst retaining the organoleptic and nutritional profile, and preventing significant food waste. Novel decontamination approaches when applied to aflatoxin decontamination are of huge interest and a growing need for global food security.

1. Introduction

The quality, safety and availability of food are growing worldwide concerns especially as the global population continues to increase against a backdrop of massive changes to the global climate. Mycotoxins are secondary metabolites produced by filamentous fungi that contaminate a number of food and feed crops. Contamination of food and feed poses a very serious health risk for both humans and animals, but also has the potential to impact the health of the environment and negatively influence the global economy (Bueno et al., 2015). The Food and Agriculture Organization of the United Nations (FAO) estimated that approximately 25% of the world's crop may be contaminated with mycotoxins, with contamination occurring both pre- and post-harvest, but recently this figure has been shown to range between 60 and 80% (Eskola et al., 2019). Better detection methods and climate change are the major factors that have likely elevated these figures. The effects that

some food-borne mycotoxins have on health ("mycotoxicosis") can vary quite markedly from a very acute and severe sickness to a more chronic disease, with many studies showing them to have the ability to be carcinogenic, mutagenic and immunosuppressive. Of the many known fungal toxins, there are approximately 400 with recognized toxigenic effects (Jard et al., 2011), and those of greatest concern are produced by fungal species belonging to the *Aspergillus*, *Fusarium*, and *Penicillium* genera (Reddy et al., 2010). Many fungal species can produce more than one mycotoxin. The production of mycotoxins is dependent on a number of physical, biological and environmental factors that can be conducive to fungal contamination and growth. The most important are temperature and relative humidity, however insect infestation, physical damage, carbon dioxide levels, availability of nutrients, inoculum concentrations and microbial interactions all influence infection and contamination (Wielogórska et al., 2016). The use of pesticides, fungicides and fertilizers also help to promote mycotoxin production (D' mello & Macdonald,

* Corresponding author.

E-mail address: h.marshall@qub.ac.uk (H. Marshall).

<https://doi.org/10.1016/j.tifs.2020.11.001>

Received 17 September 2020; Received in revised form 26 October 2020; Accepted 2 November 2020

Available online 6 November 2020

0924-2244/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1997).

There are six major groups of mycotoxins that are most toxic to humans and animals and highly regulated globally: fumonisins, ochratoxin A, zearalenone, deoxynivalenol, T-2 toxin and the compounds that this review will primarily focus on, aflatoxins (Karlovsky et al., 2016).

Aflatoxin contamination is an important issue globally for both human food and animal feed (Williams et al., 2004). They are potent carcinogens and are the most toxic of all mycotoxins. Aflatoxigenic fungi, most commonly *Aspergillus flavus* and *Aspergillus parasiticus*, are ubiquitous worldwide and often found in soil used for crop cultivation in both temperate and tropical areas. There are more than 18 different aflatoxins, however, Aflatoxins B₁, B₂, G₁ and G₂ are considered the most toxic, and are listed as Class 1 carcinogens by the International Agency for Research on Cancer (IARC, 2002), meaning that they are known to cause cancer in humans. In the DALY estimates in the 2015 FAO/WHO report, four chemicals which already have a substantial impact on the foodborne burden of disease, including aflatoxins, were evaluated and the chemical with the least uncertainty and the greatest number of DALYs was aflatoxin (WHO, 2015). Aflatoxin is not only of concern to human health but also to that of livestock. In poultry, consumption of high levels of aflatoxin B₁ (AFB₁) can result in liver damage which can ultimately lead to death. Furthermore, aflatoxins impair productivity and reproductive efficiency, resulting in decreased egg production, inferior shell and carcass quality and a greater susceptibility to disease. Chronic exposure in pigs presents as liver damage and in cattle, symptoms include reduced weight gain, damage to the liver and kidney and reduced milk production (Monson et al., 2015). Aflatoxin B₁ contamination in dairy animals is highly detrimental as the toxin can be carried into the milk in the form of aflatoxin M₁ (AFM₁), the hydroxylated metabolite of AFB₁ (Creppy, 2002). AFM₁ in both milk and other dairy products could be a risk to human health. As well as being found as a contaminant in cow's milk, AFM₁ has been detected in human breast milk (Fakhri et al., 2019).

To safeguard the health of consumers, many countries have set maximum permitted limits for aflatoxin and other mycotoxins, in human food and animal feed. For example, within the European Union (EU), the permitted levels range from 0.1 to 12 ppb for AFB₁, from 4 to 15 ppb for total aflatoxins and 0.025–0.05 ppb for AFM₁ in particular foodstuffs (Commission Regulation EU No 165/2010) (Commission, 2010). Whereas, in the United States of America (USA), the Food and Drug

Administration (FDA) have set a maximum concentration of 20 ppb for AFB₁ in foods. Likewise, different guidelines exist between the EU and USA for animal feed. The FDA regulatory levels for animal feed products intended for finishing cattle, swine and poultry are set at a maximum of 300 ppb for cottonseed meal, corn and peanut products, 100 ppb for those used in breeding and 20 ppb for all other animals (FDA, 2019). In the EU, the legislative limits for AFB₁ range from 5 to 20 ppb for animal feed materials (EU, 2003).

Aflatoxins are highly stable chemical compounds with decomposition temperatures that fall within the range of 237–306 °C (Rustom, 1997) and are therefore not destroyed by regular thermal processing or cooking. Unfortunately, there is no single step that can be taken that can prevent or eradicate mycotoxin contamination but there are a range of control strategies that can be implemented to help tackle the issue.

Aflatoxin control can be targeted at two stages along the supply chain: pre-harvest and post-harvest (Fig. 1.). Pre-harvest primarily focuses on the prevention of toxin formation where possible, by preventing or eliminating fungal growth. Pre-harvest mitigation focuses on good agricultural practice which includes pest control and the correct use of fungicides. A novel pre-harvest approach is based on biocontrol measures. Nontoxigenic strains of the *Aspergillus* fungi that are unable to produce aflatoxin are applied to the crop field, establish themselves, compete and displace toxigenic strains, resulting in the reduction of aflatoxins (Senghor et al., 2020). Reducing the risk of aflatoxin contamination post-harvest depends largely on correct crop storage, ensuring it remains dry, as *Aspergillus* like many fungi, thrive in humid environments. Although prevention of fungal contamination may be the key to reduce the impact of mycotoxins on both human and animal health, current practices do not fully address this issue, especially in lesser economically developed countries (Wielogorska, Mooney, et al., 2019). The combination of good agricultural practices and the use of proper controlled storage conditions are used to minimize the potential for mycotoxin contamination, however these strategies have been shown to be unable to assure elimination of mycotoxin producing organisms. Decontamination techniques are further needed to control aflatoxin risk.

Physical, chemical and biological decontaminations are three major ways. Many of the traditional methods employed to tackle aflatoxin contamination involve basic physical processes such as sorting and sieving, washing, milling and thermal treatment (Özer et al., 2018,

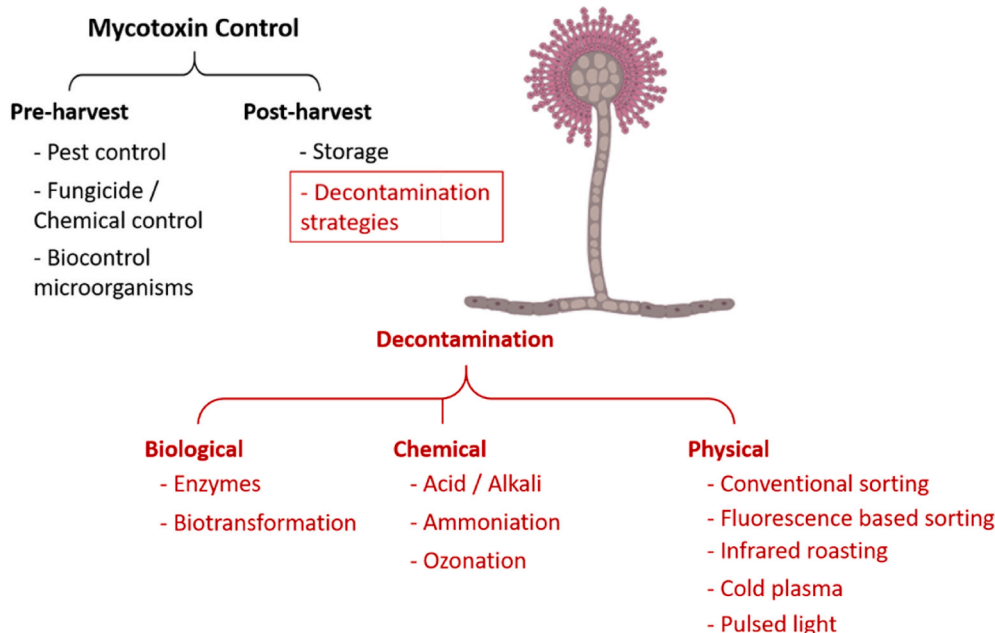


Fig. 1. Pre- and post-harvest prevention and decontaminations processes for mycotoxin control.

Yilmaz et al., 2018). Aflatoxins can be broken down not only by chemicals, such as certain acids, alkalis and oxidising agents, but also by microorganisms/enzymes (Boudergue et al., 2009). It is clear that the various preventative measures that are in place are not sufficient to eliminate all the potential risks of aflatoxin contamination in feed and food commodities. The development and implementation of highly efficient novel decontamination strategies will become increasingly critical for the protection of both human and animal health. This review aims to investigate innovative approaches with great potential to achieve aflatoxin decontamination at postharvest stage.

2. Novel methods for Aflatoxin decontamination

How to effectively minimize the loss of crops due to aflatoxin contamination is an urgent but complex issue, when aflatoxins are observed in food/feed at the postharvest stage. A two-step aflatoxin decontamination strategy is discussed below (Fig. 2). When considering the potential combination of technologies, it is important to examine the advantages and disadvantages of each method (Table 3) beyond solely being effective in detoxifying aflatoxins. While this is the most important goal, if during the processing the product is damaged or the nutritional profile negatively affected, that technique cannot be considered as fit for purpose for uptake within the human or animal food chains.

2.1. Sorting

Unprocessed cereals and grains may contain dust and admixtures, with damaged kernels usually containing most of the mycotoxin contamination (Johansson et al., 2006). Generally, these agricultural products will initially go through the process of sorting. This practise is supported by the fact that mycotoxin contamination tends to have a skewed distribution, with the majority of toxin found in a small number of grains or kernels (Kabak et al., 2006).

Hand sorting is still a primary method to remove aflatoxin contamination, especially in lesser economically developed countries

(Matumba et al., 2015). Various versions of sorting machines have been in use since the late 1800s (Karlovsy et al., 2016) that separated samples based on weight and size, however technology has significantly advanced since then. Studies have shown that machine sorting using pre-defined parameters of physical characteristics (colour, size, density) to be effective (De Mello & Scussel, 2009), whereby, if any sample differs from the pre-set limits, it is rejected and removed (Fraenkel, 1962). Fluorescence based sorting (based on the Bühler Lumovision™) is a new technology which facilitates analysis at a scale which minimises the risk of aflatoxin contamination whilst reducing the amount of food waste. The ability to detect aflatoxin using ultraviolet (UV) light is well known. The Bright Greenish Yellow Fluorescence (BGYF) test is used as a presumptive test to identify aflatoxin contamination. The BGY-Fluorescence is produced by the reaction of kojic acid formed by *A. flavus* or *A. parasiticus*, aflatoxin producing fungi, or the mycotoxin itself and peroxidase enzyme present in the plant tissues. Technological advances have resulted in the development of a novel platform for the significant reduction of aflatoxin contamination in maize. This approach exploits the fluorescent properties associated with kojic acid and combines a camera built and optimised using hyperspectral fluorescence data with an LED-based UV lighting system to detect and sort out contaminated kernels at the speed of 15 tonnes per hour, with a reduction in aflatoxin contamination averaging at 85–90% in tests to date. Furthermore, losses of non-contaminated produce have been reported to be less than 5% (Bühler, 2018). Reducing food waste is critical in terms of feeding the world's growing population and to avoid the economic losses associated with aflatoxin contamination. The implementation of such technologies is hugely important, as testing for aflatoxin can be unreliable due to the heterogeneous distribution of the toxin in dried food products. Considering the accuracy, speed and low losses, the authors of this review are of the opinion that this approach presents itself as a promising technology that could make a significant impact in preventing aflatoxin contaminated feed and food reaching the markets. Limitations are that it has not yet been extensively tested across the full range of important cereal crops and a broader evidence base will

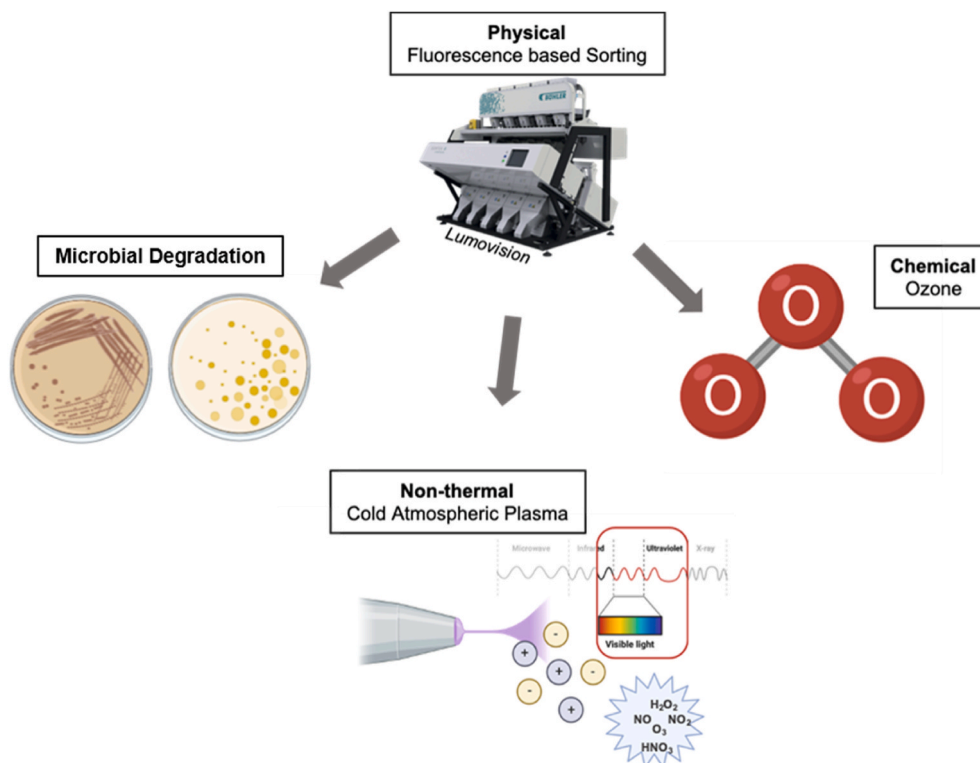


Fig. 2. Graphical representation of novel potential aflatoxin decontamination approaches (Lumovision image reproduced with permission from Bühler Group).

be necessary prior to industry implementation.

2.2. Repurposing contaminated grains for feed

Sorting effectively reduces the aflatoxin level of grains, whereby contaminated kernels are sorted out. In order to avoid aflatoxin contaminated food waste from re-entering the food chain, various decontamination methods have been reported. Under European legislation (Commission Regulation No 2015/786) (Commission, 2015), a detoxification process can only be carried out on products intended for animal feed. Decontamination processes must not result in the endangerment of animal, public or environmental health, or adversely alter the characteristics of the feed. Application of detoxification methods may only be carried out following a scientific assessment by the European Food Safety Authority (EFSA), and determination that the process complies with the predefined acceptability criteria (Table 1). According to this criteria, novelty and potential for application, one method in each category was selected and reviewed.

2.2.1. Microbial degradation of aflatoxins

There are studies that demonstrate the capability of a number of different microbial species to degrade aflatoxins, likely as a survival strategy. Microbial degradation primarily detoxifies aflatoxins using catabolic pathways that target the furofuran, lactone and difuran rings, leading to less toxic molecules (Cao et al., 2011; Mishra & Das, 2003). *Bacillus subtilis* ANSB060 isolated from fish intestines were shown to effectively detoxify aflatoxin, alongside possessing antimicrobial activity and resistance to the harsh intestinal environment. The protective effects of supplementation of *B. subtilis* ANSB060 in the aflatoxin-contaminated diets on layers, broilers, ducks, dairy cows have been verified *in vivo* (Fan et al., 2013, 2015; Guo et al., 2019; Zhang

Table 1
Acceptability criteria for mycotoxin detoxification as defined by EFSA.

(Micro)biological	Chemical	Physical
The process is effective and irreversible	The process is effective and irreversible	The process is effective
The process is performed with a fully characterized and acceptable (micro) biological agent	The process is performed with a fully characterized and acceptable chemical substance	The process does not adversely affect the characteristics and the nature of the feed
The process does not result in harmful residues of the (micro) biological agent used in the detoxified feed	The process does not result in harmful residues of the chemical substance used in the detoxified feed	A safe disposal of the removed part of the feed is guaranteed
The process does not result in metabolites of the contaminant that endanger animal, public and environmental health	The process does not result in reaction products of the contaminant that may endanger animal, public and environmental health	
The process does not adversely affect the characteristics and nature of the feed	The process does not adversely affect the characteristics and nature of the feed	

et al., 2017).

With a greater number of aflatoxin degrading strains now well studied, solid state fermentation (SSF) would be a potential method for aflatoxin decontamination in feed. A study by Zhou et al. (2017) screened traditional fermented Chinese foods for their ability to detoxify aflatoxin from peanut meal, and found that aerobic SSF by *Zygosaccharomyces rouxii* was effective in reducing residual aflatoxin. However, SSF in combination with heating for 15 min at 100 °C reduced the residual AFB₁ rate to 2.48% (Zhou et al., 2017). Heat treatment (10 min at 100 °C) combined with anaerobic SSF using *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies *bulgaricus* also demonstrated biotransformation of aflatoxin in peanut meal up to 100% (Chen et al., 2015). SSF of cottonseed meal using *Cellulosimicrobium funkei* not only demonstrated significant aflatoxin degradation *in vitro* but was also shown *in vivo* to improve the growth performance of ducklings and alleviate AFB₁-mediated aflatoxicosis (Liu et al., 2017).

2.2.2. Ozone

Ozone (O₃) is a strong oxidant that has many uses in the food industry, such as water remediation, pesticide degradation, and decontamination of fresh produce. Ozone has WHO, FDA and FAO approval for use as an antimicrobial agent for the treatment, storage and processing of foods in gas and aqueous phases (FDA, 2001). During the decontamination process, ozone can be applied in three different forms – dry, watery and moist (Mallakian et al., 2017). Ozone-mediated aflatoxin degradation occurs due to an electrophilic attack on the C8–C9 double bond of the furan ring in its molecular structure, leading to the formation of primary ozonides, which are followed by rearrangement into aldehydes, ketones and organic acids (Jalali & Avagyan, 2016). The application of ozone for aflatoxin decontamination is limited to certain

Table 2
A summary of Ozone (O₃) treatment parameters for reduction of aflatoxin.

Food product	Ozone parameters	Outcome
Peanut ^{a, b}	O ₃ concentrations: 13–21 mg/L Times: 24–96 h	25% reduction in AFB ₁ and 30% reduction in total aflatoxin
	O ₃ concentrations: 3–7.5 mg/L Times: 10–120 min	65.9% reduction in AFB ₁ and 65.8% reduction in total aflatoxin
	O ₃ concentrations: 50 mg/L Times: 60 h	89.4% reduction in AFB ₁
Corn ^{c, d}	O ₃ concentrations: 40–90 mg/L Times: 5–40 min	Up to 88% reduction in AFB ₁
	O ₃ concentrations: 20–40 mg/L Times: 120–480 min	Up to 57% reduction in aflatoxin levels
Wheat ^{e, f}	O ₃ concentrations: 20–40 mg/L Times: 5–40 min	84–97% reduction in AFB ₁
	O ₃ concentrations: 40–60 mg/L Times: 30–180 min	81–95% reduction in total aflatoxin
Pistachio ^g	O ₃ concentrations: 5–9 mg/L Times: 140 and 420 min	23% reduction in AFB ₁ and 24% reduction in total aflatoxin
Red Pepper ^h	O ₃ concentrations: 40 & 80 mg/L Times: 20–40 min	6.1–74.1% reduction in AFB ₁

^a de Alencar et al. (2012).

^b Chen et al. (2014).

^c Luo et al. (2014).

^d Porto et al. (2019).

^e El-Desouky et al. (2012).

^f Savi et al. (2015).

^g Akbas and Ozdemir (2006).

^h Kamber et al. (2017).

food products, with detoxification efficiency increasing with time and ozone concentration (Akbas & Ozdemir, 2006; Inan et al., 2007). However when applying ozone to certain food products, the time and concentration must be taken into account (Table 2) to mitigate damage to the product's nutritional properties, for example, a low ozone concentration and short treatment time is thought optimal to reduce the impact on the nutritional/micro nutritional value of peanuts (Chen et al., 2014).

There are already numerous areas within the food industry that ozone is applied to, such as treating fruits and vegetables to extend shelf-life, sterilization of food plant equipment and waste water, as well as the inhibition of microbial growth. It is a rapid and highly efficient method for aflatoxin decontamination that could be upscaled with ease. In certain food matrices, such as cereals (Zhu, 2018), ozone has been shown not to significantly alter the nutritional components, however in other food products, non-optimised usage can lead to negative effects on physiology and quality, resulting in a change of colour and in some cases the production of an undesirable odour (Khadre et al., 2001). Ozone may also inhibit growth, germination and sporulation of mycotoxin producing fungi, though this effect is dependent on a number of factors, such as fungal species, O₃ concentration, as well as exposure time. The use of ozone has a low energy consumption but still may not be a cost-effective option in developing countries. Though the FDA has already approved ozone as a direct additive to food for other applications, further safety studies of degradation products and residues, both *in vitro* and *in vivo* are needed.

2.2.3. Cold plasma

Cold plasma is a unique form of the fourth state of matter, and an emerging technology that shows great potential in various applications within the food industry. Plasma is a quasi-neutral ionized gas which is primarily composed of free electrons, photons and ions (Pankaj et al., 2014). Plasma can be generated using different combinations of temperature and pressure and sorted into two types of plasma – thermal and

non-thermal. Thermal plasma is generated under high pressure and high power, resulting in plasmas with high temperature and uniform distribution between electrons and neutral species (Eliasson & Kogelschatz, 1991; Scholtz et al., 2015). Non-thermal plasma is generated under low pressure and low power conditions, and differs from thermal plasma in that it is only partially ionized, having a greater number of neutral species, the temperature of which can be closer to ambient, and thereby leading to it also being known as cold plasma. The mechanisms of cold plasma depend on the system employed, but may comprise a combined effect of electric field, UV and reactive gas species. These may comprise ions (H⁺, H₃O⁺, O⁺, H⁻, O⁻, OH⁻, N₂⁺), molecular species (N₂, O₂, O₃, H₂O₂) and reactive radicals (O•, H•, OH•, NO•).

Although cold plasma is still in its technological infancy, it has already demonstrated considerable potential in a number of areas within the food industry, such as pesticide degradation (Gavahian & Khane-gah, 2019; Misra et al., 2014) and antimicrobial activity (Han et al., 2020; Kim & Min, 2018; Misra et al., 2014), with a growing number of studies showing the potential for cold plasma treated food products, for example, flour (Menkovska et al., 2014), peanut (Ji et al., 2018) and wheat (Los et al., 2018) to name a few, and has been demonstrated as a potential alternative approach to aflatoxin decontamination (Wielogorska, Ahmed, et al., 2019).

It is thought that the mechanism for degradation of aflatoxin is dependent on the gas used to generate the plasma, thus defining the reactive species produced that go on to interact with the mycotoxin structure (Shi et al., 2017). Microwave argon plasma at atmospheric pressure for 5 s was demonstrated to be sufficient for complete AFB₁ degradation on a glass substrate (Park et al., 2007), with 15 min of nitrogen gas plasma generated using a static induction thyristor as a power supply leading to a 90% reduction in AFB₁ also on a glass substrate (Sakudo et al., 2017). Radio frequency plasma at 300 W demonstrated an 88% reduction in AFB₁ after 10 min (Wang et al., 2015). By separating degradation products of AFB₁ following 5 min of high-voltage atmospheric cold plasma treatment, and determining the identity of

Table 3
The advantages and limitations of decontamination technologies.

	Physical		Microbial	Chemical
	Lumo-vision™	Cold Plasma	Microbial degradation	Ozone
Decontamination mechanism	n/a	targets the furofuran ring*	catabolic pathways that target the furofuran, lactone and difuran rings	electrophilic attack on the C8–C9 double bond of the furan ring
Decontamination target				
•Mycotoxin	x**	✓	✓	✓
•Whole fungus	x**	✓	✓	✓
•Spores	x**	✓	✓	✓
For the application to mycotoxins:				
•Laboratory-based	n/a	✓	✓	✓
•Prototype	✓	x	✓	x
•Commercially available	x	x	x	x
Technique widely used	✓	✓	✓	✓
•On mycotoxins	✓	x	✓	x
Advantages and Limitations				
Industrial speed	✓	undetermined	✓	✓***
Energy efficient	✓	✓	✓	✓
Can handle bulk material	✓	undetermined	✓	✓
Regulatory approval	✓	x	x	✓****
Diversity of mechanisms	x	✓	x	x
Decontamination Effects				
Negative effects on organoleptic properties	x	undetermined	x	✓***
Negative impact on food quality and nutrition	x	undetermined	x	✓***
Non-toxic degradation products	n/a	undetermined	undetermined	undetermined

* Mechanism for degradation of aflatoxin is dependent on the gas used to generate the plasma.

**Lumovision™ is regulatory approved as a sorting machine.

*** Condition dependent.

**** For other applications within the food industry.

these compounds, Shi et al. (2017) showed that AFB₁ degraded into six main products and as with many of the aforementioned detoxification mechanisms, cold plasma targets aflatoxin B₁ at its furan ring, involving hydrogenation, hydration and oxidation.

Cold plasma is a technology with scalability and the capacity to be optimised to suit varying food matrices and mycotoxins, it has the potential to be a cost effective, sustainable method, requiring a reduced energy input in comparison to many other methods. Studies to date have shown cold plasma to degrade mycotoxins on the surface of foods such as peanuts (Ji et al., 2018), without resulting in significant changes to the nutritional composition or organoleptic properties, whilst generating degradation products that have been demonstrated to be less toxic (Wang et al., 2015). The actions of cold plasma are non-thermal and do not require the use of any applied chemicals and therefore do not result in heat damage or formation of chemical residues, thus aligning with many ecological and environmental regulations. However, some knowledge gaps remain concerning tailoring the technology to the product and specific toxin risk, as well as proving safety of the treated commodities, functional and nutritional characteristics which differ for each product. The effectiveness of this technology depends on a number of parameters, such as the surface characteristics of the food material and the structure and concentration of the target mycotoxin. As cold plasma will principally treat only the surface, poor penetration of the reactive species and irregularly shaped produce may potentially present a challenge, which may be addressed through process engineering to fluidise or rotate feed to enhance surface exposure. Many studies to date employ noble gases as inducer gases, which, while effective, is an uneconomic path for large scale food processing. Engineering and design allowing air to be employed as a working gas for large scale grains processing is required. Furthermore, for any new technology to be considered for application to food processing, it is important to consider what degradation products are generated through treatment and assess the toxicity of these. Although a small number of studies have demonstrated some *in vitro* cytotoxicity (Boehm & Bourke, 2018; Patange et al., 2018), these are often associated with liquid mediated treatments and further development of this technique will warrant additional confirmative investigation *in vitro* and *in vivo*.

3. Summary and future perspectives

With an estimated 60–80% (above the detectable levels) of crops globally contaminated with mycotoxins, there is an urgent need for new, post-harvest measures to cope with the challenge (Eskola et al., 2019). Mycotoxin decontamination approaches when applied to food products, must be multidimensional as there is no single technique that can be applied universally to deal with the growing problem. These approaches must be proven not to cause changes to the nutrient profiles and organoleptic properties of crops, it is also critical that the appropriate toxicity/cytotoxicity testing is performed to ensure that any secondary degradation products formed are not harmful to animal or human health.

This review has set out to understand the state of the art in terms of detoxification of cereal crops from aflatoxins and the innovations that are emerging. Aflatoxin contamination is not a new problem, however it continues to increase in severity and causes massive food safety issues as well as substantial economic losses and increasing levels of food waste. It must be considered as one of the major challenges to global food security and innovative solutions are required. The combination of two novel technologies has the potential to have a substantial impact on addressing this challenge.

The use of fluorescence-based sorting prior to any further decontamination procedure has the primary benefit of preventing significant loss of produce as a result of aflatoxin presence. Fluorescence-based sorting also has the potential to mitigate the need for further decontamination due to the ability of the technology to minimise rejection. The use of cold plasma or ozone, as secondary decontamination steps,

would be minimally invasive and leave behind no chemical residue or bacterial product. In testing to date, cold plasma treatment has not resulted in negative effects on nutritional and organoleptic properties of the food matrices studied. The application of ozone in a non-optimised manner can however lead to nutritional degradation and undesirable changes to the sensory profile. All three techniques have however demonstrated a level of efficacy in reducing a number of mycotoxins present, though it has not been established how these technologies may handle large quantities of materials.

Originally developed by NASA, Technology Readiness Level (TRL) is a term used to describe a measurement system that assesses the maturity of a specific technology. There are nine technology readiness levels, TRL 1 being the lowest, representing a technology that is in its infancy and defined only by its basic principles, and TRL 9 being the highest and assigned to systems that are proven in their operational environment. When assessing TRL levels for the technologies discussed in this review, it is important not to just consider how advanced the technique may be in principal, but how operational that system would be in terms of industrial uptake. Fluorescence-based sorting, specifically Lumovision™ technology (Bühler) has been explicitly designed for use against aflatoxin and is in pilot scale testing currently, so would therefore, in the authors opinion, have a designated TRL of between 6 and 8. Microbial degradation, cold plasma and ozone are commercially available technologies that are continually being improved. However, in this capacity, though a higher quantity of research has been carried out regarding mycotoxins and both ozone and cold plasma, knowledge gaps remain for scalability, efficacy and safety, with limited combinatorial evaluations performed. Whilst these are under intensive investigation currently, employing cold plasma or microbial degradation alone or in combination sits at a lower TRL (TRL 3–5), however the authors believe ozone should be assigned a higher TRL of 5–7.

Naturally occurring toxins pose a unique challenge to food safety. They are unavoidable and their occurrence is ubiquitous and unpredictable. The destruction of contaminated products or their diversion to use in feed is not always practical, particularly in countries where there are high levels of food insecurity. A range of strategies to reduce mycotoxin formation in the field as well as during storage have been developed but despite these efforts, substantial contamination of feed and food continues to occur. Novel approaches will be required in helping to address the needs of global food safety and security, thus further research should be focused to better understand the safety, efficacy and economic benefits of these novel approaches, when applied to aflatoxin decontamination.

Acknowledgements

This study was supported by The Mars Global Food Safety Center, Beijing, China.

References

- Akbas, M. Y., & Ozdemir, M. (2006). Effect of different ozone treatments on aflatoxin degradation and physicochemical properties of pistachios. *Journal of the Science of Food and Agriculture*, 86, 2099–2104.
- Boehm, D., & Bourke, P. (2018). Safety implications of plasma-induced effects in living cells—a review of *in vitro* and *in vivo* findings. *Biological Chemistry*, 400, 3–17.
- Boudergue, C., Burel, C., Dragacci, S., Favrot, M. C., Fremy, J. M., Massimi, C., Prigent, P., Debongnie, P., Pussemier, L., & Boudra, H. (2009). Review of mycotoxin-detoxifying agents used as feed additives: Mode of action, efficacy and feed/food safety. *Efsa Supporting Publications*, 6, 22e.
- Bueno, D., Istambouli, G., Muñoz, R., & Marty, J. L. (2015). Determination of mycotoxins in food: A review of bioanalytical to analytical methods. *Applied Spectroscopy Reviews*, 50, 728–774.
- Bühler. (2018). *Lumovision™: Bühler's contribution to solving the aflatoxin challenge*.
- Cao, H., Liu, D., Mo, X., Xie, C., & Yao, D. (2011). A fungal enzyme with the ability of aflatoxin B₁ conversion: Purification and ESI-MS/MS identification. *Microbiological Research*, 166, 475–483.
- Chen, Y., Kong, Q., Chi, C., Shan, S., & Guan, B. (2015). Biotransformation of aflatoxin B₁ and aflatoxin G₁ in peanut meal by anaerobic solid fermentation of *Streptococcus*

- thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus*. *International Journal of Food Microbiology*, 211, 1–5.
- Chen, R., Ma, F., Li, P.-W., Zhang, W., Ding, X.-X., Zhang, Q., Li, M., Wang, Y.-R., & Xu, B.-C. (2014). Effect of ozone on aflatoxins detoxification and nutritional quality of peanuts. *Food Chemistry*, 146, 284–288.
- Commission, E. (2010). Commission Regulation (Eu) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Official Journal of the European Union*, L 50/8.
- Commission, E. (2015). *Commission Regulation (Ec) No 2015/786 of 19 May 2015 defining acceptability criteria for detoxification processes applied to products intended for animal feed as provided for in Directive 2002/32/EC of the European Parliament and of the Council Official Journal of the European Union*, L 125/10.
- Creppy, E. E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology letters*, 127, 19–28.
- D'mello, J. P. F., & Macdonald, A. M. C. (1997). Mycotoxins. *Animal Feed Science and Technology*, 69, 155–166.
- De Alencar, E. R., Faroni, L. R. D. A., Soares, N. D. F. F., Da Silva, W. A., & Da Silva Carvalho, M. C. (2012). Efficacy of ozone as a fungicidal and detoxifying agent of aflatoxins in peanuts. *Journal of the Science of Food and Agriculture*, 92, 899–905.
- De Mello, F. R., & Scussel, V. M. (2009). Development of physical and optical methods for in-shell Brazil nuts sorting and aflatoxin reduction. *Journal of Agricultural Science*, 1, 3.
- El-Desouky, T., Sharoba, A., El-Desouky, A., El-Mansy, H., & Naguib, K. (2012). Evaluation of ozone gas as an anti-aflatoxin B1 in wheat grains during storage. *Journal of Agroalimentary Processes and Technologies*, 18, 13–19.
- Eliasson, B., & Kogelschatz, U. (1991). Nonequilibrium volume plasma chemical processing. *IEEE Transactions on Plasma Science*, 19, 1063–1077.
- Eskola, M., Kos, G., Elliott, C. T., Hajslová, J., Mayar, S., & Krska, R. (2019). Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'Fao estimate' of 25%. *Critical Reviews in Food Science and Nutrition*, 1–17.
- EU. (2003). Commission directive 2003/100/EC of 31 October 2003 amending annex I of directive 2002/32/EC of the European parliament and of the council on undesirable substances in animal feed. *Off. J. Eur. Union*, 285, 33–37.
- Fakhri, Y., Rahmani, J., Oliveira, C. A. F., Franco, L. T., Corassin, C. H., Saba, S., Rafique, J., & Khaneghah, A. M. (2019). Aflatoxin M1 in human breast milk: A global systematic review, meta-analysis, and risk assessment study (Monte Carlo simulation). *Trends in Food Science & Technology*, 88, 333–342.
- Fan, Y., Zhao, L., Ji, C., Li, X., Jia, R., Xi, L., Zhang, J., & Ma, Q. (2015). Protective effects of *Bacillus subtilis* ANSB060 on serum biochemistry, histopathological changes and antioxidant enzyme activities of broilers fed moldy peanut meal naturally contaminated with aflatoxins. *Toxins*, 7, 3330–3343.
- Fan, Y., Zhao, L., Ma, Q., Li, X., Shi, H., Zhou, T., Zhang, J., & Ji, C. (2013). Effects of *Bacillus subtilis* ANSB060 on growth performance, meat quality and aflatoxin residues in broilers fed moldy peanut meal naturally contaminated with aflatoxins. *Food and Chemical Toxicology*, 59, 748–753.
- FDA. (2001). Secondary direct food additives permitted in food for human consumption. *Federal Register*, 66, 33829–33830.
- FDA. (2019). **United States-food and Drug administration regulation section sec. 683.100: Action levels for aflatoxins in animal food [online]** [Accessed June 08 2020] <https://www.fda.gov/media/121202/download>.
- Fraenkel, H. (1962). *Colour sorting machine*. Us Patent.
- Gavahian, M., & Khaneghah, A. M. (2019). Cold plasma as a tool for the elimination of food contaminants: Recent advances and future trends. *Critical Reviews in Food Science and Nutrition*, 1–12.
- Guo, Y., Zhang, Y., Wei, C., Ma, Q., Ji, C., Zhang, J., & Zhao, L. (2019). Efficacy of *Bacillus subtilis* ANSB060 biodegradation product for the reduction of the milk aflatoxin M1 content of dairy cows exposed to aflatoxin B1. *Toxins*, 11, 161.
- Han, J.-Y., Song, W.-J., Eom, S., Kim, S. B., & Kang, D.-H. (2020). Antimicrobial efficacy of cold plasma treatment against food-borne pathogens on various foods. *Journal of Physics D: Applied Physics*, 53, 204003.
- IARC. (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, 82, 1–556.
- Inan, F., Pala, M., & Doymaz, I. (2007). Use of ozone in detoxification of aflatoxin B1 in red pepper. *Journal of Stored Products Research*, 43, 425–429.
- Jalali, L., & Avagyan, G. (2016). *Evaluation of contamination of Aspergillus flavus and aflatoxin production in pistachio cultivars and investigation of a chemical controlling method*.
- Jard, G., Liboz, T., Mathieu, F., Guyonvarc'h, A., & Lebrihi, A. (2011). Review of mycotoxin reduction in food and feed: From prevention in the field to detoxification by adsorption or transformation. *Food Additives & Contaminants: Part A*, 28, 1590–1609.
- Ji, H., Dong, S., Han, F., Li, Y., Chen, G., Li, L., & Chen, Y. (2018). Effects of dielectric barrier discharge (DBD) cold plasma treatment on physicochemical and functional properties of peanut protein. *Food and Bioprocess Technology*, 11, 344–354.
- Johansson, A. S., Whitaker, T. B., Hagler, W. M., Bowman, D. T., Slate, A. B., & Payne, G. (2006). Predicting aflatoxin and fumonisin in shelled corn lots using poor-quality grade components. *Journal of AOAC International*, 89, 433–440.
- Kabak, B., Dobson, A. D., & Var, I. L. (2006). Strategies to prevent mycotoxin contamination of food and animal feed: A review. *Critical Reviews in Food Science and Nutrition*, 46, 593–619.
- Kamber, U., Gülbaz, G., Aksu, P., & Doğan, A. (2017). Detoxification of aflatoxin B1 in red pepper (*Capsicum annuum* L.) by ozone treatment and its effect on microbiological and sensory quality. *Journal of Food Processing and Preservation*, 41, Article e13102.
- Karlovsky, P., Suman, M., Berthiller, F., De Meester, J., Eisenbrand, G., Perrin, I., Oswald, I. P., Speijers, G., Chiodini, A., Recker, T., & Dussort, P. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research*, 32, 179–205.
- Khadre, M., Yousef, A., & Kim, J. G. (2001). Microbiological aspects of ozone applications in food: A review. *Journal of Food Science*, 66, 1242–1252.
- Kim, J. H., & Min, S. C. (2018). Moisture vaporization-combined helium dielectric barrier discharge-cold plasma treatment for microbial decontamination of onion flakes. *Food Control*, 84, 321–329.
- Liu, J., Song, W.-J., Zhang, N.-Y., Tan, J., Krumm, C., Sun, L.-H., & Qi, D.-S. (2017). Biotransformation of aflatoxin B1 in cottonseed meal by fermentation of *Cellulosimicrobium funkei* in duckling diet. *Poultry Science*, 96, 923–930.
- Los, A., Ziuzina, D., Akkermans, S., Boehm, D., Cullen, P. J., Van Impe, J., & Bourke, P. (2018). Improving microbiological safety and quality characteristics of wheat and barley by high voltage atmospheric cold plasma closed processing. *Food Research International*, 106, 509–521.
- Luo, X., Wang, R., Wang, L., Li, Y., Bian, Y., & Chen, Z. (2014). Effect of ozone treatment on aflatoxin B1 and safety evaluation of ozonized corn. *Food Control*, 37, 171–176.
- Mallakian, S., Rezaeezhad, R., Jalali, M., & Ghobadi, F. (2017). The effect of ozone gas on destruction and detoxification of aflatoxin. *Bulletin de la Société Royale des Sciences de Liège*, 86, 1–6.
- Matumba, L., Van Poucke, C., Njumbe Ediage, E., Jacobs, B., & De Saeger, S. (2015). Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. *Food Additives & Contaminants: Part A*, 32, 960–969.
- Menkovska, M., Mangova, M., & Dimitrov, K. (2014). Effect of cold plasma on wheat flour and bread making quality. *Macedonian Journal of Animal Science*, 4, 27–30.
- Mishra, H., & Das, C. (2003). *A review on biological control and metabolism of aflatoxin*.
- Misra, N., Moiseev, T., Patil, S., Pankaj, S., Bourke, P., Mosnier, J., Keener, K., & Cullen, P. (2014). Cold plasma in modified atmospheres for post-harvest treatment of strawberries. *Food and Bioprocess Technology*, 7, 3045–3054.
- Monson, M. S., Coulombe, R. A., & Reed, K. M. (2015). Aflatoxicosis: Lessons from toxicity and responses to aflatoxin B1 in poultry. *Agriculture*, 5, 742–777.
- Özer, M., Yilmaz Tuncel, N., & Tuncel, N. B. (2018). The effects of infrared stabilized immature rice grain flour in gluten-free bread preparation. *Cereal Chemistry*, 95, 527–535.
- Pankaj, S. K., Bueno-Ferrer, C., Misra, N., Milosavljević, V., O'donnell, C., Bourke, P., Keener, K., & Cullen, P. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science & Technology*, 35, 5–17.
- Park, B. J., Takatori, K., Sugita-Konishi, Y., Kim, I.-H., Lee, M.-H., Han, D.-W., Chung, K.-H., Hyun, S. O., & Park, J.-C. (2007). Degradation of mycotoxins using microwave-induced argon plasma at atmospheric pressure. *Surface and Coatings Technology*, 201, 5733–5737.
- Patange, A., Boehm, D., Giltrap, M., Lu, P., Cullen, P., & Bourke, P. (2018). Assessment of the disinfection capacity and eco-toxicological impact of atmospheric cold plasma for treatment of food industry effluents. *The Science of the Total Environment*, 631, 298–307.
- Porto, Y. D., Trombete, F. M., Freitas-Silva, O., De Castro, I. M., Direito, G. M., & Ascheri, J. L. R. (2019). Gaseous Ozonation to reduce aflatoxins levels and microbial contamination in corn grits. *Microorganisms*, 7, 220.
- Reddy, K. R. N., Salleh, B., Saad, B., Abbas, H. K., Abel, C. A., & Shier, W. T. (2010). An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Reviews*, 29, 3–26.
- Rustom, I. Y. (1997). Aflatoxin in food and feed: Occurrence, legislation and inactivation by physical methods. *Food Chemistry*, 59, 57–67.
- Sakudo, A., Toyokawa, Y., Misawa, T., & Imanishi, Y. (2017). Degradation and detoxification of aflatoxin B1 using nitrogen gas plasma generated by a static induction thyristor as a pulsed power supply. *Food Control*, 73, 619–626.
- Savi, G. D., Piacentini, K. C., & Scussel, V. M. (2015). Ozone treatment efficiency in *Aspergillus* and *Penicillium* growth inhibition and mycotoxin degradation of stored wheat grains (*Triticum aestivum* L.). *Journal of Food Processing and Preservation*, 39, 940–948.
- Scholtz, V., Pazlarova, J., Souskova, H., Khun, J., & Julak, J. (2015). Nonthermal plasma—a tool for decontamination and disinfection. *Biotechnology Advances*, 33, 1108–1119.
- Senghor, L., Ortega-Beltran, A., Atehnkeng, J., Callicott, K., Cotty, P., & Bandyopadhyay, R. (2020). The atoxigenic biocontrol product Atlasafe Sn01 is a valuable tool to mitigate aflatoxin contamination of both maize and groundnut cultivated in Senegal. *Plant Disease*, 104, 510–520.
- Shi, H., Cooper, B., Strohshine, R. L., Ileleji, K. E., & Keener, K. M. (2017). Structures of degradation products and degradation pathways of aflatoxin B1 by high-voltage atmospheric cold plasma (HVACP) treatment. *Journal of Agricultural and Food Chemistry*, 65, 6222–6230.
- Wang, S.-Q., Huang, G.-Q., Li, Y.-P., Xiao, J.-X., Zhang, Y., & Jiang, W.-L. (2015). Degradation of aflatoxin B1 by low-temperature radio frequency plasma and degradation product elucidation. *European Food Research and Technology*, 241, 103–113.
- WHO. (2015). *WHO estimates of the global burden of foodborne diseases: Foodborne disease burden epidemiology reference group 2007-2015*. World Health Organization.
- Wielogorska, E., Ahmed, Y., Meneely, J., Graham, W. G., Elliott, C. T., & Gilmore, B. F. (2019). A holistic study to understand the detoxification of mycotoxins in maize and impact on its molecular integrity using cold atmospheric plasma treatment. *Food Chemistry*, 301, 125281.
- Wielogorska, E., Macdonald, S., & Elliott, C. (2016). A review of the efficacy of mycotoxin detoxifying agents used in feed in light of changing global environment and legislation. *World Mycotoxin Journal*, 9, 419–433.

- Wielogorska, E., Mooney, M., Eskola, M., Ezekiel, C. N., Stranska, M., Krska, R., & Elliott, C. (2019). Occurrence and human-health impacts of mycotoxins in Somalia. *Journal of Agricultural and Food Chemistry*, *67*, 2052–2060.
- Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D. (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition*, *80*, 1106–1122.
- Yilmaz, F., Tuncel, N. Y., & Tuncel, N. B. (2018). Stabilization of immature rice grain using infrared radiation. *Food Chemistry*, *253*, 269–276.
- Zhang, L., Ma, Q., Ma, S., Zhang, J., Jia, R., Ji, C., & Zhao, L. (2017). Ameliorating effects of *Bacillus subtilis* Ansb060 on growth performance, antioxidant functions, and aflatoxin residues in ducks fed diets contaminated with aflatoxins. *Toxins*, *9*, 1.
- Zhou, G., Chen, Y., Kong, Q., Ma, Y., & Liu, Y. (2017). Detoxification of aflatoxin B1 by *Zygosaccharomyces rouxii* with solid state fermentation in peanut meal. *Toxins*, *9*, 42.
- Zhu, F. (2018). Effect of ozone treatment on the quality of grain products. *Food Chemistry*, *264*, 358–366.