

Original Article

Prevention of the Growth of Fungi and Aflatoxins in Bread Leftovers Used as Animal Feed

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ABSTRACT

Background: Aflatoxin contamination poses a significant threat to food safety, particularly in bread products used as animal feed. This review explores various strategies for preventing fungal growth and aflatoxin production.

Objective: To assess the efficacy of traditional and emerging decontamination methods for managing aflatoxin contamination in food commodities, with a focus on bread products.

Methods: A systematic review of literature from databases such as PubMed and ScienceDirect was conducted, focusing on studies published in the last two decades. The review included chemical, physical, and biological decontamination methods, as well as natural antifungal agents derived from medicinal plants.

Results: Chemical methods such as sodium hypochlorite and sodium bisulfite showed varying degrees of efficacy in reducing aflatoxin levels. Natural antifungal agents like garlic, neem leaves, and eucalyptus leaves demonstrated significant antifungal properties. The prevalence of aflatoxins in bread samples varied across different countries, highlighting the global challenge of aflatoxin contamination.

Conclusion: Emerging decontamination methods offer promising alternatives to traditional techniques for managing aflatoxin contamination. Further research is needed to optimize these methods and ensure their safety for human consumption.

Keywords: Aflatoxin, Fungal Contamination, Food Safety, Decontamination Methods, Natural Antifungal Agents, Bread Products

INTRODUCTION

Food availability and safety are critical in today's society, particularly with the growing global population. Ensuring an adequate, easily accessible, and nutritionally complete food supply is essential for maintaining public health and socioeconomic welfare (1). However, food can contain toxic substances that are harmful to human health. Each year, contaminated food leads to millions of cases of illness and death due to foodborne infections (2). This review focuses on the essential connection between food security and safety, highlighting the importance of strategies for providing safe access to food for all people and investigating the challenges that developing countries face in maintaining food quality standards. The literature also discusses the unique threat of fungal contamination and its consequences for food security and safety (3).

Mycotoxins, derived from the Latin word "toxicum," meaning "poison," and the Greek word "mukos," meaning "fungus," are toxic compounds produced by filamentous fungi such as *Aspergillus*, *Penicillium*, and *Fusarium*. These toxic compounds contaminate food at various stages, such as pre-harvest and during storage, posing a risk to the health of both humans and animals. Environmental factors, including temperature, humidity, and agricultural practices, promote the growth of mycotoxins, specifically aflatoxins. Fungal

growth thrives in warm and humid environments, leading to increased levels of mycotoxin contamination. Aflatoxins, primarily produced by *Aspergillus flavus* and *parasiticus*, are of major concern due to their significant toxicity (3). Mycotoxins can lead to adverse effects such as cancer, genetic mutation, and congenital abnormalities (4). Aflatoxin B1 (AFB1) is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC), indicating a significant risk for cancer. Aside from immunosuppression, some mycotoxins can also cause neurotoxicity and gastrointestinal problems. The occurrence of "Turkey X disease" during the 1960s, linked to peanut meal contaminated with aflatoxin, highlights the harmful effects of these poisons on human health (5).

Aflatoxins predominantly affect the liver, causing hemorrhagic necrosis, bile duct growth, and fatty infiltration, which potentially result in hepatocellular carcinoma (HCC). The development of HCC caused by aflatoxin is influenced by genetic vulnerability (5). Aflatoxins can harm DNA and change gene expression, promoting cancer development. Additionally, aflatoxins have immunotoxic properties, potentially diminishing immune responses and affecting vaccine efficacy (6). Aflatoxin exposure is also associated with hindered child development, characterized by stunted growth, fatigue, and being below the expected weight (7). This vulnerability is caused by lower physical size, less robust immune systems, and exposure to harmful substances through the placenta or breast milk. Multiple studies have demonstrated a robust association between aflatoxin consumption and negative birth outcomes, such as stunted growth and kwashiorkor (8). Aflatoxins can also interfere with the neurological system by impacting neurodevelopment, possibly by forming DNA/protein adducts, causing oxidative stress, and disrupting mitochondrial function. Autopsy investigations have shown the presence of aflatoxin in the brains of children with kwashiorkor, indicating possible neurotoxicity (9).

Global regulations for aflatoxin control in food have become increasingly comprehensive and stringent. These regulations consider both toxicological risks and practical limitations to ensure consumer safety. The European Union, for instance, has established maximum permissible limits (MPLs) for various food commodities to mitigate the health risks associated with aflatoxin exposure (10). Implementing strict EU aflatoxin restrictions in low- and middle-income countries (LMICs) in Asia and Sub-Saharan Africa would likely be unfeasible due to economic hardships and limited resources for compliance. Therefore, it is crucial to employ alternative methods before and after harvesting to lower aflatoxin levels and lessen contamination in commercially traded food and animal feed (11).

Bread, a staple in many diets, is susceptible to fungal contamination, particularly aflatoxins. Various studies have reported the prevalence of aflatoxins in bread across different countries, highlighting the global nature of this issue (12). For instance, aflatoxin contamination in bread has been documented in countries such as Nigeria, Iran, Spain, and Pakistan, with varying levels of contamination (13). Addressing this contamination is vital for food safety and public health.

Efforts to prevent fungal growth and aflatoxin production involve both pre- and post-harvest strategies. Pre-harvest approaches focus on farm management practices to minimize fungal establishment and infection. Post-harvest decontamination and detoxification procedures aim to eradicate or reduce mycotoxin levels to acceptable thresholds. These methods include physical, biological, and chemical decontamination techniques (14). Chemical methods, such as using sodium silicate, calcium chloride, calcium carbonate, and silica gel, have shown promise in preventing fungal growth and aflatoxin production in food items (15). Additionally, natural antifungal agents derived from medicinal plants like garlic, neem leaves, and eucalyptus leaves offer potential sustainable alternatives for controlling fungal contamination (16).

This study aims to fill the research gap by thoroughly assessing the effectiveness and outlook for various new decontamination methods. By exploring unconventional techniques, such as the use of chemicals and medicinal plants with antifungal properties, this research contributes significantly to the field of food safety and aflatoxin contamination mitigation.

MATERIAL AND METHODS

This study was conducted following the ethical guidelines outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board (IRB) of the respective institutions involved in the research. The review article design incorporated a comprehensive assessment of existing literature, including a systematic review of scientific articles, reports, and relevant publications on the prevention of fungal growth and aflatoxin production in food items.

The review included studies that examined various decontamination methods for aflatoxins, focusing on chemical, physical, and biological approaches. Specific criteria for inclusion were established, such as studies published in peer-reviewed journals, those conducted within the last two decades, and research involving aflatoxin contamination in food commodities. The search strategy employed databases such as PubMed, ScienceDirect, and Google Scholar, using keywords like "aflatoxin prevention," "fungal contamination," "food safety," "chemical decontamination," and "medicinal plants."

Data extraction involved collecting information on study design, methods, decontamination techniques, and outcomes. The review aimed to evaluate the efficacy of various methods in reducing aflatoxin levels and preventing fungal growth. Statistical analysis was

performed where applicable, and findings were synthesized to provide a comprehensive overview of current and emerging decontamination strategies.

RESULTS

The analysis included a variety of studies addressing aflatoxin contamination in food commodities. The results are summarized in the following tables, highlighting the efficacy of different decontamination methods and the prevalence of aflatoxins in various food items.

Table 1. Efficacy of Chemical Decontamination Methods

Food Additive	Concentration	Exposure Duration	Temperature	Reduction in Aflatoxin Level
Sodium Bisulfite (NaHSO ₃)	0.5%	48 hours	-	20% in maize samples
Sodium Hypochlorite (NaClO)	0.25%	48 hours	60°C	Complete breakdown of Aflatoxin B
Potassium Metabisulfite (K ₂ S ₂ O ₅)	-	-	-	Remain unaffected
Sodium Hydrosulfite (Na ₂ S ₂ O ₄)	-	-	-	Remain unaffected
Sulfuric Acid (H ₂ SO ₄)	-	-	-	Remain unaffected
Ammonium Peroxodisulfate (NH ₄) ₂ S ₂ O ₈)	-	-	-	Remain unaffected

Table 2. Prevalence of Aflatoxins in Bread Samples

Country	Type of Sample	Year	Aflatoxin Type	Total Samples/Positive Samples	Range (mg/kg)	Detection Method	References
Colombia	Bread	2019	AFB1	246/95	0.41	HPLC-FD	(12)
Nigeria	Brown Bread	2004	AFB1, AFB2, AFG1	150/11	0.00072, 0.00041, 0.00017	TLC	(13)
Iran	Bread	2019	AFG1	Moldy bread sample	0.0056	HPLC-FLD	(14)
Iran	Bread	2016	AFB1	180/29	0.064	HPLC	(15)
Spain	Bread	1983	AFB1, AFG1	100/50	0.016, 0.012	TLC, HPLC	(16)

The results demonstrated varying degrees of success in aflatoxin decontamination using different chemical methods. Sodium hypochlorite showed complete breakdown of aflatoxin B, whereas other chemicals like potassium metabisulfite and sulfuric acid remained unaffected. The prevalence of aflatoxins in bread samples across different countries highlighted the global challenge of managing aflatoxin contamination in food items.

DISCUSSION

The findings from this review underscore the significant threat posed by aflatoxin contamination to global agricultural security and public health. Traditional decontamination methods, while effective to some extent, have limitations in fully addressing the persistence and stability of aflatoxins in food commodities. Emerging decontamination techniques, such as the use of chemical agents like sodium silicate, calcium chloride, and silica gel, offer promising alternatives. These methods have demonstrated varying levels of efficacy in reducing aflatoxin contamination, but further research is needed to optimize their application and ensure their safety for human consumption (17).

The use of medicinal plants with inherent antifungal properties, such as garlic, neem leaves, and eucalyptus leaves, presents a sustainable and environmentally friendly approach to controlling fungal contamination. These natural antifungal agents have shown effectiveness against a wide range of fungal pathogens, with mechanisms that include disrupting fungal cell membranes, inhibiting enzyme activity, and generating oxidative stress within fungal cells (18). However, the variability in bioactive compound concentrations and the need for standardization in their application remain challenges that require further investigation.

The strengths of this review lie in its comprehensive assessment of both traditional and emerging decontamination methods, providing a broad perspective on current strategies for managing aflatoxin contamination. The incorporation of various studies from different geographical regions enhances the generalizability of the findings. However, the review also highlights several limitations,

including the scarcity of recent studies focusing on novel decontamination technologies and the need for more rigorous clinical trials to validate the efficacy and safety of these methods.

Future research should focus on optimizing the concentration and application methods of chemical and natural antifungal agents, as well as exploring the potential synergistic effects of combining different decontamination strategies. Additionally, developing cost-effective and scalable solutions for low- and middle-income countries is crucial to ensuring food safety and reducing the public health burden associated with aflatoxin contamination (19, 20).

CONCLUSION

Emerging decontamination methods offer promising alternatives to traditional techniques for managing aflatoxin contamination. Further research is needed to optimize these methods and ensure their safety for human consumption.

REFERENCES

1. Mahato DK, Lee KE, Kamle M, Devi S, Dewangan KN, Kumar P, Kang SG. Aflatoxins in Food and Feed: An Overview on Prevalence, Detection and Control Strategies. *Front Microbiol.* 2019;10:2266.
2. O’Riordan MJ, Wilkinson MG. A Survey of the Incidence and Level of Aflatoxin Contamination in a Range of Imported Spice Preparations on the Irish Retail Market. *Food Chem.* 2008;107(4):1429-1435.
3. Stoev SD. Food Safety and Increasing Hazard of Mycotoxin Occurrence in Foods and Feeds. *Crit Rev Food Sci Nutr.* 2013;53(9):887-901.
4. Pleadin J, Frece J, Markov K. Mycotoxins in Food and Feed. *Adv Food Nutr Res.* 2019;89:297-345.
5. Chauhan R, Singh J, Sachdev T, Basu T, Malhotra BD. Recent Advances in Mycotoxins Detection. *Biosens Bioelectron.* 2016;81:532-545.
6. Alshannaq A, Yu JH. Occurrence, Toxicity, and Analysis of Major Mycotoxins in Food. *Int J Environ Res Public Health.* 2017;14(6):632.
7. Abrar M, Anjum FM, Butt MS, Pasha I, Randhawa MA, Saeed F, Waqas K. Aflatoxins: Biosynthesis, Occurrence, Toxicity, and Remedies. *Crit Rev Food Sci Nutr.* 2013;53(8):862-874.
8. European Food Safety Authority (EFSA). Opinion of the Scientific Panel on Contaminants in the Food Chain [CONTAM] Related to the Potential Increase of Consumer Health Risk by a Possible Increase of the Existing Maximum Levels for Aflatoxins in Almonds, Hazelnuts and Pistachios and Derived Products. *EFSA J.* 2007;5(3):446.
9. Vidal A, Marín S, Ramos AJ, Cano-Sancho G, Sanchis V. Determination of Aflatoxins, Deoxynivalenol, Ochratoxin A and Zearalenone in Wheat and Oat Based Bran Supplements Sold in the Spanish Market. *Food Chem Toxicol.* 2013;53:133-138.
10. Pizzolitto RP, Armando MR, Combina M, Cavaglieri LR, Dalcero AM, Salvano MA. Evaluation of *Saccharomyces cerevisiae* Strains as Probiotic Agent with Aflatoxin B1 Adsorption Ability for Use in Poultry Feedstuffs. *J Environ Sci Health B.* 2012;47(10):933-941.
11. Pierron A, Alassane-Kpembé I, Oswald IP. Impact of Mycotoxin on Immune Response and Consequences for Pig Health. *Anim Nutr.* 2016;2(2):63-68.
12. Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP. Modification of Immune Function through Exposure to Dietary Aflatoxin in Gambian Children. *Environ Health Perspect.* 2003;111(2):217-220.
13. Smith LE, Mbuya MN, Prendergast AJ, Turner PC, Ruboko S, Humphrey JH, Stoltzfus RJ. Determinants of Recent Aflatoxin Exposure among Pregnant Women in Rural Zimbabwe. *Mol Nutr Food Res.* 2017;61(9):1601049.
14. Hatem NL, Hassab HM, Al-Rahman EMA, El-Deeb SA, Ahmed RLES. Prevalence of Aflatoxins in Blood and Urine of Egyptian Infants with Protein–Energy Malnutrition. *Food Nutr Bull.* 2005;26(1):49-56.
15. Ahlberg S, Grace D, Kiarie G, Kirino Y, Lindahl J. A Risk Assessment of Aflatoxin M1 Exposure in Low and Mid-Income Dairy Consumers in Kenya. *Toxins.* 2018;10(9):348.
16. Alamu EO, Gondwe T, Akello J, Maziya-Dixon B, Mukanga M. Relationship between Serum Aflatoxin Concentrations and the Nutritional Status of Children Aged 6–24 Months from Zambia. *Int J Food Sci Nutr.* 2020;71(5):593-603.
17. Kamala A, Kimanya M, De Meulenaer B, Kolsteren P, Jacxsens L, Haesaert G, Lachat C. Post-harvest Interventions Decrease Aflatoxin and Fumonisin Contamination in Maize and Subsequent Dietary Exposure in Tanzanian Infants: A Cluster Randomised-Controlled Trial. *World Mycotoxin J.* 2018;11(3):447-458.
18. Van Egmond HP. Natural Toxins: Risks, Regulations and the Analytical Situation in Europe. *Anal Bioanal Chem.* 2004;378:1152-1160.

19. Ismail A, Gonçalves BL, de Neeff DV, Ponzilacqua B, Coppa CF, Hintzsche H, Oliveira CA. Aflatoxin in Foodstuffs: Occurrence and Recent Advances in Decontamination. *Food Res Int.* 2018;113:74-85.