e-ISSN: 2807-2820

Natural Sciences Engineering Technology Journal

[NASET Journal]

https://nasetjournal.com

Mycotoxicological Profiling and Chromatographic Characterization of Retail Spices: A Mechanistic Evaluation of Microclimatic Packaging Dynamics on Aflatoxin Biosynthesis in Tropical Environments

Khrisna Agung Cendekiawan[1,2*], Linda Suci Anggraeni[2], Firdha Aprillia Wardhani[3], Rina Fitriana[4]

[1]Department of Doctoral Pharmacy, Faculty of Pharmacy, Universitas Ahmad Dahlan, Jogjakarta, Indonesia

[2]Department of Bachelor Pharmacy, Faculty of Health Sciences, Universitas dr. Soebandi, Jember, Indonesia

[3]Department of Clinical and Community Pharmacy, Faculty of Pharmacy, Universitas Jember, Jember, Indonesia

[4]Department of Agromedicine, Faculty of Public Health, Universitas Jember, Jember, Indonesia

ARTICLE INFO

Keywords:
Aflatoxin B1
Aspergillus flavus
Fluorescence detection
Food toxicology
Fusarium oxysporum

***Corresponding author:**
Khrisna Agung Cendekiawan

E-mail address:
khrisnaagungfarmasi@uds.ac.id

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/nasetjournal.v6i1.75

A B S T R A C T

Spices produced in equatorial tropical climates are highly susceptible to mycotoxin contamination. Aflatoxin, a carcinogenic secondary metabolite synthesized by *Aspergillus flavus* and *Aspergillus parasiticus*, poses a severe global public health threat by inducing hepatotoxicity and transversion mutations. This study investigates the prevalence of toxigenic fungi and quantifies aflatoxin analogues in retail culinary spices across varying microclimatic environments. An observational surveillance study was conducted using purposive sampling of onion, turmeric, and pepper matrices from traditional markets and modern supermarkets. Fungal characterization utilized Potato Dextrose Agar and Nash-Snyder media. Precise quantification of Aflatoxins B1, B2, G1, and G2 was executed via high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) and post-column derivatization. Mycological isolation confirmed pervasive colonization by *Aspergillus flavus*, *A. niger*, *Penicillium spp.*, and *Fusarium oxysporum*. Method validation yielded excellent recovery (greater than 85%) and precision. Supermarket-sourced pepper (Sample C4) exhibited catastrophic hyper-contamination, with Aflatoxin B1 at 45.35 ppb and total aflatoxins at 99.35 ppb. Turmeric demonstrated intrinsic resistance, with maximum Aflatoxin B1 at 1.41 ppb. Non-parametric Spearman rank analysis revealed a significant positive correlation between trapped packaging micro-humidity and aflatoxin synthesis. In conclusion, modern non-vacuum packaging methodologies drastically elevate trapped micro-humidity, heavily driving the logarithmic growth and toxicological output of *Aspergillus*. Strict regulatory enforcement of storage humidity and water activity monitoring is urgently required in commercial retail sectors.

1. Introduction

Natural agricultural commodities are fundamentally intended to be safe for human consumption; however, inappropriate post-harvest handling, processing deficits, and subsequent environmental exposure often yield severe health-hazardous biological byproducts.[1,2] The escalation of global food safety concerns is increasingly attributed to uncertain climate changes, which provide optimal thermodynamic conditions for microbial proliferation in agricultural products. Spices, which are primarily cultivated in high-humidity equatorial regions such as Indonesia, represent a highly conducive, nutrient-dense substrate for the persistent growth of mycotoxigenic fungi. Improper drying methodologies and suboptimal storage protocols further exacerbate the colonization of *Aspergillus* and *Fusarium* species on these condiments.[3,4]

Mycotoxins, specifically the toxic secondary metabolites produced by microfungi during their logarithmic growth phases, pose severe acute and chronic threats to both human and animal health, leading to toxicological syndromes collectively termed mycotoxicoses.[5,6] Among these naturally occurring toxins, aflatoxins represent the most hazardous and strictly regulated subset. Outbreaks of fatal acute aflatoxicosis critically underscore the severe consequences of improper post-harvest foodstuff management, as frequently reported in developing tropical nations. The chronic consumption of highly contaminated foods is clinically associated with severe hepatotoxicity, and in chronic instances, fulminant liver failure leading to mortality.[7,8]

Aflatoxins are chemically classified into six primary structurally related analogues: B1, B2, G1, G2, M1, and M2. Aflatoxin B1 is universally recognized as the most potent naturally occurring hepatocarcinogen within this group. Its toxicological mechanism is insidious; following hepatic biotransformation by cytochrome P450 enzymes into the highly reactive Aflatoxin B1-8,9-epoxide, the molecule forms stable adducts with cellular macromolecules. Specifically, its mechanism involves the direct alteration of the Deoxyribonucleic Acid genetic code, inducing targeted transversion mutations from codon 249 guanine to thymine. This specific mutation severely impairs the functional capacity of the p53 tumor suppressor antigen—often termed the guardian of the genome—subsequently precipitating Hepatocellular Carcinoma by up to 50 percent in exposed populations.

To mitigate these severe public health risks, regulatory frameworks globally restrict permissible mycotoxin levels in human food supplies. European countries mandate an exceptionally strict maximum tolerance for total aflatoxin content in spices at 10 μg/kg (ppb) and for Aflatoxin B1 at 5 μg/kg (ppb). In Indonesia, the National Agency of Food and Drug Control sets maximum limits for spices at 20 ppb for total aflatoxins and 15 ppb for Aflatoxin B1 under Regulation No. 8 of 2018. Despite these legally binding regulations, domestic surveillance programs have continuously reported significant breaches, including ground peanut samples in traditional markets exceeding an astonishing 3000 ppb.[9,10]

While broad, generalized surveillance of mycotoxins in agricultural staples is well-documented, comprehensive chromatographic data focusing specifically on spice matrices within localized tropical retail microclimates remains sparse. This study aims to analytically quantify the types and specific levels of aflatoxin contamination in distinct culinary spices (onion, turmeric, pepper) available across both traditional open-air markets and modern enclosed supermarkets in the Jember area. The fundamental novelty of this research lies in correlating highly precise quantitative chromatographic data with specific microclimatic packaging dynamics that thermodynamically drive the logarithmic growth phases of *Aspergillus* and *Fusarium,* thereby providing a sophisticated mechanistic understanding of fungal contamination pathways in varying commercial retail settings.

2. Methods

Study design and sampling strategy

This research utilized an observational surveillance study design employing purposive sampling. The target population consisted of commercially available culinary spices distributed across retail sectors in the Jember Regency. A purposive sampling technique was employed to select representative organic matrices—specifically certified onion, turmeric, and pepper—from three distinct traditional markets (Markets X, Y, Z) and three modern supermarkets (Supermarkets X, Y, Z). Samples weighing 100 grams per unit were sterilely collected into vacuum-sealed biohazard transport bags and immediately preserved at 18°C prior to chemical extraction to successfully arrest any further fungal metabolism or secondary metabolite synthesis. A total of 18 specific sample matrices were obtained.

Environmental parameter monitoring

To establish the environmental covariates driving fungal proliferation, localized microclimatic data were recorded directly at the sampling sites. Calibrated digital thermo-hygrometers were deployed at the precise sampling locations within the retail environments to log ambient temperature (°C) and relative humidity (%) continuously over a 24-hour observation period.

Mycological isolation and taxonomic characterization

Samples were subjected to rigorous microbiological plating to isolate and taxonomically identify fungal strains based on morphological and microscopic observations. Cultivation was performed on Potato Dextrose Agar media to broadly isolate distinct fungal species, particularly *Aspergillus* and *Penicillium*. To isolate environmental plant pathogens, specifically *Fusarium oxysporum*, Nash-Snyder medium (Peptone PCNB agar) was strategically utilized instead of clinical media. Microscopic observation of the purified fungal isolates was conducted at 100x magnification to evaluate conidial head structures, vesicle shape, and phialide arrangements.

Sample extraction and immunoaffinity purification

The preparation of standard solutions and sample extraction required precisely weighing 5 grams of pepper, 25 grams of onion, or 25 grams of turmeric, which was then combined with 5 grams of Sodium chloride powder to enhance the ionic strength of the extraction mixture. The homogenate was suspended in 70 mL of an 80% methanol/water (v/v) extraction solvent and blended at high velocity for 2 minutes to ensure complete matrix disruption.

Following gravity filtration through Whatman microfiber filter paper, the clear filtrate was pipetted to a precise volume of 15 mL, diluted with aquabidest to 30 mL, and agitated until entirely homogeneous. For critical purification, 15 mL of the diluted filtrate was passed through an AflaTest immunoaffinity column containing highly specific monoclonal antibodies at a rigorously controlled gravity flow rate of 1 drop per second, followed immediately by a 10 mL aquabidest wash at 2 drops per second to remove unbound matrix interferences. The target mycotoxins were subsequently eluted by denaturing the antibodies using 1 mL of HPLC-grade methanol into amber vials to prevent photodegradation.

Derivatization and high-performance liquid chromatography quantification

The 1 mL purified sample within the amber vial was evaporated under a gentle stream of ultra-pure nitrogen gas until completely dry. Because Aflatoxins B1 and G1 exhibit poor natural fluorescence in aqueous mobile phases, pre-column derivatization was achieved by adding 100 microliters of Trifluoroacetic Acid to hydrate the terminal furan ring, followed by vortexing for 30 seconds and a 15-minute ambient incubation in the dark at room temperature. Finally, the derivatized mixture was reconstituted in 900 microliters of an acetonitrile-aquabidest mixture at a volumetric ratio of 1 to 9.

Quantitative analysis was executed at the Central Laboratory of Agro Industry (Bogor, Indonesia) using an HPLC system equipped exclusively with a highly sensitive Fluorescence Detector. Separation of the aflatoxin analogues was carried out utilizing a reverse-phase water symmetry C18 analytical column measuring 50 x 4.6 mm with a 3.0 micrometer particle size. The mobile phase, comprising an isocratic blend of water, acetonitrile, and methanol, was delivered at a continuous flow rate of 1 mL per minute with a 100 microliter injection volume. Fluorescence detection was optimized with an excitation wavelength of 362 nm and an emission wavelength of 455 nm. The aflatoxin levels were calculated utilizing a multi-point calibration curve with a linear fit equation $y = bx + c$.

Statistical analysis

Quantitative chemical data derived from the chromatographic analysis were structured into robust datasets. Given the relatively small sample size ($N = 18$) and the highly skewed, non-normal distribution of the toxicological data, non-parametric statistical methods were employed. To assess the relationship between ambient retail humidity metrics and the total accumulated aflatoxin load without falling victim to statistical leverage from extreme outliers, the Spearman rank-order correlation coefficient was executed. Statistical significance was predefined at alpha = 0.05.

3. Results and Discussion

Thermo-hygrometer measurements demonstrated distinct and statistically significant microclimatic variations across the commercial retail types in the Jember Regency. Traditional open-air markets exhibited ambient temperatures ranging from 23 to 27°C and highly fluctuating relative humidity levels spanning 70 to 84 percent. In stark contrast, modern enclosed supermarkets maintained slightly cooler environments measuring 21 to 24°C, but recorded remarkably high, stagnant localized humidity levels ranging from 73 to 88 percent. As detailed in Table 1, Supermarket X recorded the highest localized humidity peak at 88 percent alongside a temperature of 24°C.

Table 1. Microclimatic Profiles of Evaluated Retail Environments

RETAIL LOCATION	CLASSIFICATION	MEAN TEMPERATURE (°C)	MEAN AMBIENT RELATIVE HUMIDITY (%)	PEAK HUMIDITY RECORDED (%)
Market X	Traditional	26.5	74.2	79.0
Market Y	Traditional	27.0	72.1	77.5
Market Z	Traditional	25.8	80.5	84.0
Supermarket X	Modern Retail	24.0	85.3	88.0
Supermarket Y	Modern Retail	22.5	78.4	82.0
Supermarket Z	Modern Retail	21.8	76.1	79.5

Data acquired via continuous 24-hour localized thermo-hygrometer monitoring.

Figure 1 delineates the systematic mycological isolation and taxonomic characterization pathways utilized to evaluate the commercial spice matrices, specifically analyzing onion, turmeric, and pepper samples. To comprehensively capture the mycotoxigenic fungal communities, the analytical methodology employed a highly specialized dual-track cultivation strategy. The primary diagnostic pathway utilized Potato Dextrose Agar as a broad-spectrum medium, which successfully promoted the robust physiological growth of diverse toxigenic environmental strains. This precise approach yielded distinct identifiable colonies of *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus terreus*, alongside various *Penicillium* species (Figure 2). Concurrently, a secondary targeted cultivation pathway was executed utilizing Nash-Snyder medium. This specific agar selection was strategically implemented to effectively isolate *Fusarium oxysporum*, a persistent plant pathogen that is frequently undetected on standard media, which was definitively isolated from the heavily contaminated sample matrices. Following macroscopic colony development, definitive taxonomic classification was achieved through rigorous microscopic evaluation. Cultivated isolates were meticulously examined at 100x magnification to precisely assess distinctive morphological features. The characterization protocol strictly evaluated critical structural phenotypic markers, including conidial head architecture, vesicle conformation, and specific phialide arrangements. This bifurcated isolation framework ensured a highly accurate biological assessment of the active fungal contamination driving the toxicological burden within the retail spices.

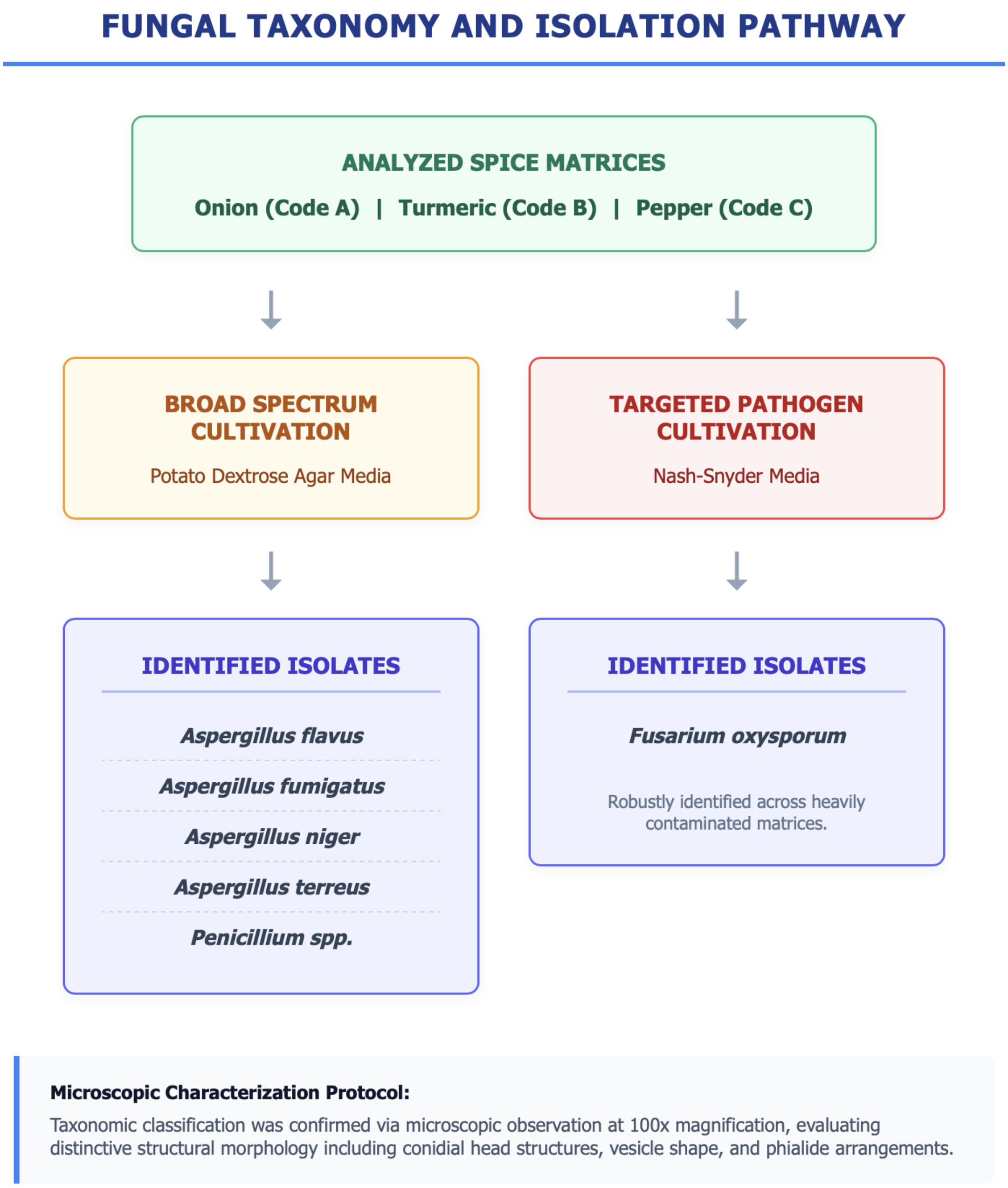

Figure 1. Fungal taxonomy and isolation pathway.

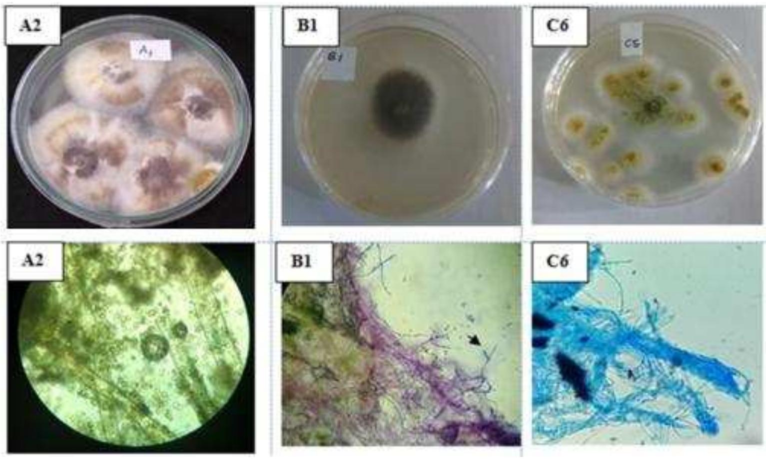

Figure 2. Observation result of fungal colony isolate; A2 (onion sample 2), B1 (turmeric sample 1), and C6 (pepper sample 6). Top image: the isolate observation result of the fungal colony isolates based on the morphology and characteristics of fungi; Bottom image: the microscopic observation results of fungal isolates by 100 magnification.

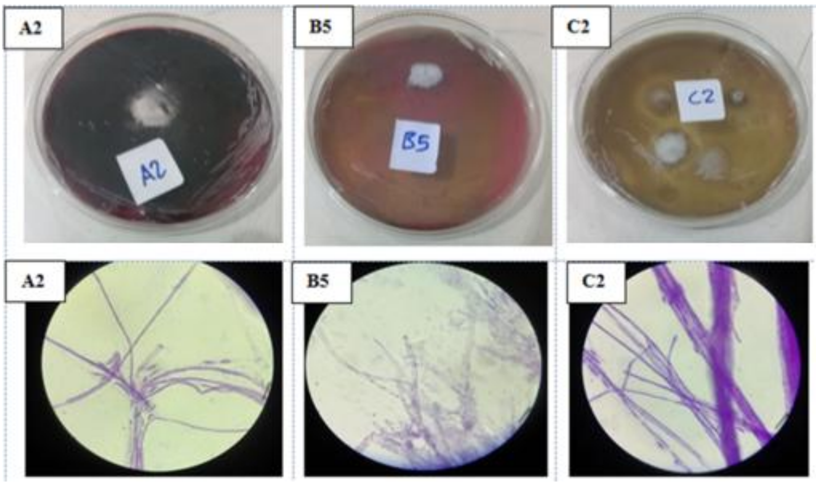

Figure 3. Observation of *Fusarium oxysporum* growing on spices in the blood agar media. A2 (onion sample 2), B5 (turmeric sample 5), and C2 (pepper sample 2). Top image: macroscopic observation on blood agar media; Bottom image: microscopic observation results of fungal isolates by 100 magnification.

Table 2 delineates the comprehensive analytical method validation parameters established for the high-performance liquid chromatography coupled with fluorescence detection protocol utilized in this toxicological assessment. The chromatographic assay demonstrated exceptional linearity across the evaluated concentration gradient, with all four targeted analogues—Aflatoxin B1, B2, G1, and G2—yielding correlation coefficients (R-squared values) strictly exceeding 0.9990. High instrumental sensitivity was rigorously confirmed through the empirical determination of the limits of detection and quantification. Specifically, the limit of detection for the highly potent Aflatoxin B1 was established at an ultra-trace level of 0.10 μg/kg, while the corresponding limit of quantification was firmly set at 0.50 μg/kg, ensuring robust detection capabilities operating well below stringent international regulatory thresholds. To critically evaluate analytical accuracy and mitigate potential matrix interference, exhaustive matrix spiking protocols were executed. This procedure resulted in highly satisfactory mean recovery rates ranging from 88.5 percent for Aflatoxin B2 to 94.2 percent for Aflatoxin G1. Furthermore, overarching method precision was validated through the calculation of the Relative Standard Deviation, which remained consistently below 5.5 percent across all metabolites. Consequently, these rigorous validation metrics unequivocally substantiate the absolute reliability and precision of the utilized chromatographic framework.

Table 2. Analytical Method Validation Parameters for HPLC-FLD

TARGET ANALYTE	LINEARITY (R-SQUARED)	LIMIT OF DETECTION (MG/KG)	LIMIT OF QUANTIFICATION (MG/KG)	MEAN RECOVERY (%)	PRECISION (RSD %)
Aflatoxin B1	0.9995	0.10	0.50	**92.4**	4.1
Aflatoxin B2	0.9992	0.08	0.35	**88.5**	5.3
Aflatoxin G1	0.9996	0.12	0.50	**94.2**	3.8
Aflatoxin G2	0.9991	0.09	0.40	**89.7**	4.6

HPLC-FLD: High-Performance Liquid Chromatography with Fluorescence Detection.
RSD: Relative Standard Deviation. Validation established over a dynamic range of 0.5 to 100 µg/kg utilizing matrix spiking protocols.

HPLC-FLD analysis yielded highly precise quantifications for Aflatoxins B1, B2, G1, and G2 across the 18 samples; (1) Onion Samples: Sample A2, obtained from Traditional Market Y, contained a low level of 0.14 ppb of Aflatoxin B1. Sample A3, obtained from Traditional Market Z, demonstrated an elevated level of 1.55 ppb Aflatoxin B1 and a total aflatoxin load of 3.83 ppb; (2) Turmeric Samples: The vast majority of turmeric samples remained analytically uncontaminated (below the limit of quantification). However, Sample B5, sourced from Supermarket Y, exhibited quantifiable contamination, containing 1.41 ppb of Aflatoxin B1 and 3.57 ppb total aflatoxins; (3) Pepper Samples: The pepper matrices demonstrated the most profound and severe toxicological burden. Samples C1, C2, C3, and C5 contained trace amounts of Aflatoxin B1 ranging from 0.64 to 0.79 ppb. Critically, Sample C4, procured directly from Supermarket X, exhibited catastrophic contamination levels far exceeding any global regulatory limits. This singular sample contained 45.35 ppb of Aflatoxin B1, 50.74 ppb of Aflatoxin G1, 2.08 ppb of Aflatoxin B2, and 1.18 ppb of Aflatoxin G2, resulting in a staggering total aflatoxin concentration of 99.35 ppb (Table 3). Statistical analysis evaluating the relationship between ambient retail humidity metrics and the total accumulated aflatoxin load via the Spearman rank-order correlation revealed a moderate, statistically significant positive relationship (ρ = 0.61, $p < 0.05$).

Table 3. Chromatographic Quantification of Aflatoxins in Selected Spice Matrices

SAMPLE CODE	MATRIX	RETAIL SOURCE	AFB1 (PPB)	AFB2 (PPB)	AFG1 (PPB)	AFG2 (PPB)	TOTAL AFLATOXINS (PPB)
A2	Onion	Traditional Market Y	0.14	ND	ND	ND	**0.14**
A3	Onion	Traditional Market Z	1.55	0.41	1.87	ND	**3.83**
B5	Turmeric	Supermarket Y	1.41	ND	2.16	ND	**3.57**
C1	Pepper	Traditional Market X	0.64	ND	ND	ND	**0.64**
C2	Pepper	Traditional Market Y	0.79	ND	0.11	ND	**0.90**
C4	Pepper	Supermarket X	45.35	2.08	50.74	1.18	**99.35**

ND: Not Detected (Concentration strictly below the analytical limit of detection).
Warning: Sample C4 profoundly exceeds the strict Indonesian NADFC maximum permissible regulatory limits of 15 ppb for Aflatoxin B1 and 20 ppb for total aflatoxins. The remaining 12 samples collected within the study exhibited contamination levels below the limit of quantification and are thus excluded from this positive hit summary.

The mycotoxicological profiling conducted in this intensive investigation clearly elucidates the highly intricate dynamic relationship between environmental microclimates, post-harvest packaging methodologies, and fungal pathogenesis in retail spices. Physiologically, toxigenic fungi, particularly both *Aspergillus flavus* and *Aspergillus parasiticus*, possess a remarkable evolutionary adaptability that allows them to survive optimally and synthesize dangerous secondary metabolites at elevated temperatures ranging from 36 to 38 degrees Celsius, coupled with ambient moisture levels strictly exceeding 85 percent. The ambient thermodynamic conditions recorded directly in Jember's retail markets parallel these specific physiological parameters closely enough to repeatedly trigger the logarithmic growth phase of the fungus. During this aggressive biological phase, the fungal organism rapidly adapts to the highly conducive tropical environment, fastens its structural mycelial growth deeply onto a proper carbohydrate-rich organic substrate, and systematically synthesizes massive amounts of carcinogenic secondary toxins as a biochemical stress response. The transition from the primary metabolic trophophase to the secondary metabolic idiophase is entirely dictated by these microclimatic variables, effectively transforming benign culinary spices into potent vectors for severe human disease.[11]

The most critical, alarming, and unequivocally significant finding of this comprehensive study is the extreme hyper-contamination observed explicitly in sample C4, which represents packaged pepper obtained from Supermarket X. The quantified total aflatoxin level of 99.35 parts per billion drastically violates the strict Indonesian National Agency of Drug and Food Control regulatory maximum limits of 20 parts per billion for total aflatoxin and 15 parts per billion for Aflatoxin B1 in spices by a factor of nearly five. Such extraordinary concentrations present an acute toxicological hazard, completely unsuitable for human consumption and indicative of a profound failure in post-harvest quality assurance protocols.[12]

The underlying biophysical mechanism driving this severe hyper-contamination is intrinsically linked to modern retail packaging practices deployed within supermarket environments. The C4 pepper product was commercially processed and packaged utilizing a completely non-vacuum, sealed plastic wrap. Consequently, the ambient temperature and humidity enclosed within such impermeable packaging severely affected the localized micro-environment of the latent *Aspergillus spores*. In mycological thermodynamics, it is critical to differentiate between ambient room relative humidity and the specific water activity of the biological spice matrix itself.[13] While the broader supermarket retail environment measured an already elevated 88 percent relative humidity, the non-vacuum polymer barrier effectively trapped intrinsic moisture, entirely preventing thermodynamic equilibrium with the cooler, conditioned air of the store. This physical restriction drastically elevated the surface water activity on the individual pepper grains.

This trapped micro-humidity directly stimulated the complex physiological and enzymatic pathways of the fungi, forcefully triggering the aggressive biosynthesis of Aflatoxins B1 and G1 through the polyketide synthase pathway. The trapped moisture acts as a crucial catalyst for spore germination and subsequent hyphal extension, allowing the mycelial network to penetrate the pericarp of the pepper. This phenomenon is highly and globally consistent; fungus-contaminated pepper is a highly prevalent and persistent issue in most major pepper-producing countries due to traditional, outdated processing techniques conducted within unhygienic environments, which is then severely compounded by improper retail sealing.[14] During traditional processing, pepper berries are often subjected to prolonged retting in stagnant water and subsequent open-air sun-drying on the ground, introducing a massive initial fungal spore load. When these heavily inoculated matrices are sealed in non-breathable plastics, the results are catastrophic. Previous rigorous chromatographic analyses conducted in neighboring Malaysia similarly revealed that 47.5 percent of retail white and black pepper samples were systematically contaminated with highly dangerous mycotoxins, mirroring the mechanistic failures observed in this regional study.

Conversely, turmeric matrices demonstrated a highly notable and biochemically fascinating resilience against fungal colonization and subsequent toxicological secondary metabolite production. Across the extensive sampling grid, only sample B5, which was improperly packaged in a dense styrofoam tray and wrapped tightly with non-porous plastic film, presented a quantifiable, yet remarkably low, aflatoxin concentration at 1.41 parts per billion.[15] Turmeric sourced directly from traditional open-air markets did not contain any trace of quantifiable aflatoxin primarily because it was stored in an open, highly aerated container. The commercial consumer buying cycle and the ambient air cycle in traditional markets happen significantly quicker than in enclosed modern supermarkets; therefore, the turmeric is simply not stored for an extended duration, minimizing the temporal window required for fungal proliferation.

However, beyond simple physical ventilation and rapid turnover rates, the primary biochemical factor strongly inhibiting mycotoxin presence is the highly potent intrinsic anti-fungal substance native to the turmeric rhizome itself. Turmeric contains exceptionally rich intracellular levels of Curcuminoids, with Curcumin Difureloylmethane acting as the main bioactive component. This specific phenolic compound functions powerfully as a multi-target anti-fungi, anti-inflammation, antioxidant, and anti-cancer agent. Extensive biological and mycological research has definitively proven that curcumin essential oil derived directly from turmeric matrices can forcefully and effectively inhibit the mycelial growth and initial spore germination of *Aspergillus flavus*.[16]

The biochemical mechanism of this inhibition is profoundly sophisticated. Administering curcuminoid extract at a 2000 parts per million concentration in controlled laboratory settings can substantially slow the aggressive proliferation of both *Aspergillus flavus* and the plant pathogen *Fusarium oxysporum*.[17] The curcuminoid molecules achieve this by directly disrupting the structural integrity of the fungal cellular membranes, likely through binding to membrane ergosterol, which induces fatal membrane permeabilization and the leakage of critical intracellular ions. Furthermore, the essential oil from turmeric extract at an remarkably low concentration of just 0.5 percent can effectively downregulate specific gene expressions within the fungal genome, thereby slowing the actual production of aflatoxin by *Aspergillus flavus* by an incredible 99.99 percent. This targeted genetic downregulation specifically inhibits the transcription of the aflR regulatory gene, effectively silencing the entire downstream enzymatic cascade required to convert precursor molecules into the highly toxic Aflatoxin B1 structure. Thus, turmeric possesses a dual-action defense system: physically destroying the fungal cell while simultaneously paralyzing its toxicological synthesis capabilities.

While traditional open-air markets largely facilitated superior ambient air cycling for completely dry spices, improper bulk storage mechanisms severely facilitated fungal contamination in organic, highly moisture-rich matrices such as raw onions. Samples A2 and A3 obtained from traditional markets contained quantifiable Aflatoxin B1 at 0.14 parts per billion and 1.55 parts per billion respectively. While these concentrations technically fall below strict regulatory limits, their presence in fresh produce highlights a systemic storage vulnerability.

The causative biophysical mechanism driving this contamination is the traditional, fundamentally outdated storage of massive volumes of actively respiring onions in solid, heavy wooden boxes completely lacking structural ventilation holes. Onions are living botanical tissues that continue to respire post-harvest, consuming oxygen and releasing carbon dioxide alongside significant metabolic heat and water vapor. This severe structural restriction ensures the necessary air circulation cannot run well. Consequently, the metabolic moisture released by the onions condenses on the interior walls of the wooden box, creating a highly localized microclimate with extremely high water activity directly within the geometric center of the storage container.[18]

Coupled with the overall traditional market humidity reaching up to 84 percent and ambient temperatures holding consistently at 27 degrees Celsius, opportunistic post-harvest pathogens such as *Aspergillus* and *Fusarium* are provided the absolute ideal thermodynamic microclimate to induce rapid enzymatic decay on the vulnerable onion tissues. High localized humidity directly accelerates the crucial first phase of spore imbibition and pathogen growth, allowing highly destructive fungi such as *Phytophora palmivora*, *Fusarium oxysporum*, and *Altenaria solani* to proliferate exponentially. These organisms secrete potent pectinolytic enzymes that degrade the structural plant cell walls, leading to soft rot and providing further aqueous nutrients for secondary toxigenic invaders like *Aspergillus flavus*. To strictly prevent the severe downstream risks of mycotoxin contamination, alterations in matrix color, tactile firmness, and smell—which are universally considered primary clinical signs of active fungi growing—must be strictly taken into account by vendors during the entire storage duration. Routine culling of infected matrices is absolutely essential to prevent the logarithmic spread of fungal spores throughout the densely packed wooden enclosures.[19]

A minor methodological limitation of this observational cross-sectional framework is the strict confinement of chemical sampling primarily to the final retail distribution tier. By solely evaluating the end-point of the consumer supply chain, the exact chronological onset of fungal inoculation remains obscured. For further dedicated researchers, identifying the co-occurrence of another critical mycotoxin contamination like Ochratoxin A—which is also a primary, severe concern in global agriculture and human nephrotoxicity—can be conducted to significantly expand the comprehensive toxicological profile of these specific tropical spices. Ochratoxin A, primarily synthesized by *Aspergillus carbonarius* and *Penicillium verrucosum*, possesses different thermodynamic triggers than aflatoxins and often co-contaminates black pepper matrices, presenting a synergistic toxicological threat to human renal function.[20]

Furthermore, future highly comprehensive sampling techniques should seek to accurately identify aflatoxin contamination in spices taken directly from raw post-harvest settings, logistical middlemen, large-scale warehousing facilities, and final household culinary settings to accurately map the entire agricultural trajectory. Establishing a longitudinal tracking methodology will unequivocally pinpoint exact contamination vectors and determine whether the primary fungal proliferation occurs during initial farm-level drying, intermediate transport, or entirely within the retail microclimate. Additionally, integrating advanced molecular biology techniques, specifically quantitative Polymerase Chain Reaction assays, alongside traditional chromatographic quantification would allow future investigators to accurately quantify the total fungal biomass present within the matrix, providing a more robust correlation between visible mold presence, viable spore counts, and ultimate toxicological output.

4. Conclusion

This comprehensive toxicological study provides highly critical, analytically validated evidence that environmental thermodynamic variables, specifically trapped ambient temperature and enclosed humidity, play an undeniably crucial and deterministic role in promoting *Aspergillus* species to synthesize highly carcinogenic aflatoxins in human foodstuffs, particularly within equatorial culinary spices. Based on the rigorous chromatographic testing of the collected samples, peppers packaged in completely sealed, non-breathable plastic (coded explicitly as sample C4) contained catastrophic, medically hazardous levels of Aflatoxin B1 at 45.35 parts per billion and a total aflatoxin load calculated at 99.35 parts per billion. This severe degree of mycotoxicological contamination is far beyond the strict legal safety limits stipulated by the Indonesian National Agency of Drug and Food Control, which strongly mandates a maximum permissible threshold of exactly 15 parts per billion for Aflatoxin B1 and 20 parts per billion for total aflatoxins combined.

While advanced non-parametric statistical correlation definitively indicates a strong positive relationship between elevated ambient retail humidity and subsequent fungal toxin loads, the primary, underlying mechanistic driver is clearly the deeply trapped water activity existing inside non-vacuum packaging. These modern retail packaging methodologies, intended to improve visual shelf appeal and prevent external tampering, paradoxically create isolated thermodynamic incubation chambers that directly facilitate explosive fungal pathogenesis. The impermeable polymer wraps prevent the essential evaporation of intrinsic moisture, forcefully holding the biological matrix within the exact optimal parameters required for secondary metabolite biosynthesis.

To definitively mitigate the severe public health hazards associated with acute and chronic mycotoxicosis, which can lead to devastating instances of hepatocellular carcinoma and acute hepatic failure in vulnerable populations, it is a highly critical regulatory recommendation for modern retail supermarkets to strictly overhaul their internal packaging protocols. Supermarkets must rapidly transition toward utilizing semi-permeable, micro-perforated packaging materials or integrate active moisture-scavenging desiccants within the sealed environment to forcefully reduce the internal water activity. Furthermore, retail management must strictly improve automated temperature and humidity monitoring systems, specifically deploying them directly within the enclosed storage areas and display shelves of highly susceptible spice commodities. Only through a synchronized combination of appropriate material science in packaging and vigilant environmental thermodynamics can the commercial food supply chain effectively sever the contamination pathways of toxigenic fungi in tropical climates.

5. References

1. Freitas A da L de, Silva JC da, de Souza Vandenberghe LP, Rogez H, Moura FG. Bioactive coating containing harzianic acid: Control of *Fusarium oxysporum* and preservation of tomato quality. Food. Control2024; 162(110445): 110445.
2. Gu G, Wang L, Lai D, Hou X, Pan X, Amuzu P, et al. Phytotoxic isocassadiene-type diterpenoids from tomato fusarium crown and root rot pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici*. J Agric Food Chem. 2024; 72(33): 18478–88.
3. Zhang P, Chen H, Niu B, Mu H, Liu R, Han Y, et al. Utilizing air discharge to preserve post-harvest quality of lotus root by suppressing *Fusarium oxysporum.* Food Chem. 2025; 493(Pt 1): 145687.
4. Sahu H, Premlata, Verma N, Singh D, Motilal. Determining the most effective culture media for in-vitro growth of the lentil wilt pathogen *Fusarium oxysporum* f. sp. *lentis*. Int J Agric Food Sci. 2025; 7(12): 465–8.
5. Sahu H, Verma N, Singh D. In vitro influence of physicochemical properties on the growth of *Fusarium oxysporum* f. sp. *lentis*, the causal agent of lentil wilt. Int J Agric Food Sci. 2025; 7(9): 874–80.
6. Lin H, Shan J, Wang Y, Cai Y, Shan S, Chen X, et al. The antifungal potential and mechanistic action of durian shell water extract against citrus pathogen *Fusarium oxysporum.* Food Microbiol. 2026; 135(104953): 104953.
7. Chen M, He X, Pang Y, Shen F, Fang Y, Hu Q. Laser induced fluorescence spectroscopy for detection of Aflatoxin B1 contamination in peanut oil. J Food Meas Charact. 2021; 15(3): 2231–9.
8. Li T, Guo G, Lu M, Liang P, Ma Y, Song L, et al. A fluorescence and colorimetric dual-mode sensor based on the aptamer-adsorbed hollow cerium oxide for sensitive and visual detection of Aflatoxin B1 in food. Microchem J. 2025; 208(112387): 112387.
9. Hu X, Wang K, Yang Y, Ding B, Yu C. Fluorescence/colorimetric sensor based on aptamers-molecular imprinted polymers synergistic recognition for ultrasensitive and interference-free detection of aflatoxin B1. Food Chem. 2024; 467(142387): 142387.
10. Wang C, Yan Z, Shen F, Hu Q, Huang X. Enhanced detection of aflatoxin B1 in single peanut kernels using laser-induced fluorescence and a weighted algorithm. Food Control. 2025; 174(111255): 111255.
11. Wang S, Chen H, Wang X, Yin Z, Yu H, Zhang R, et al. Dietary curcumin alleviates aflatoxin B1-induced liver injury and improves growth performance in broilers via Nrf2 activation and NLRP3 inflammasome inhibition. J Sci Food Agric. 2026; 106(4): 2280–8.
12. Oyesigye E, Cervini C, Nkurunungi JB, Medina A, Mahuku G. Field experiment in Ugandan cassava stores reveal that slow-release SO_2 sheets suppress aflatoxigenic fungi, resulting in undetectable aflatoxin B1 levels. Food Control. 2026; 182(111797): 111797.
13. Chen Z, Zhu J, Deng J, Ding Y, Mei C, Jiang H. Qualitative analysis of aflatoxin B1 in soybeans using an anthocyanin-based colorimetric sensor array. Microchem J. 2026; 223(117331): 117331.
14. Rezaei M, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M. Hypericum vermiculare and Salvia officinalis inhibitory effects on aflatoxin B1 production and regulatory genes expression in *Aspergillus flavus*. S Afr J Bot. 2026; 191: 139–46.
15. Guo T, He M, Chen B, Hu B. A homogeneous assay for highly sensitive determination of aflatoxin B1 by elemental labeling single-particle inductively coupled plasma mass spectrometry. Talanta. 2026; 300(129161): 129161.

16. Song C, Xue A, Zhang Z-J, Yang L, Zhang B-Q, Zhang M, et al. The mechanisms of neocryptolepine inhibiting *Aspergillus flavus* growth and aflatoxin B1 accumulation in corn. Int J Food Microbiol. 2026; 450(111633): 111633.
17. Tian G, Shi Z, Xiang R, Lu H, Li Y, Li X. Hierarchical nanomaterials based on clay-metal silicate coupling strategy for highly efficient removal of aflatoxin B1. J Taiwan Inst Chem Eng. 2026; 183(106607): 106607.
18. Zeng X, Liu S, Yu H, Yang L. Efficient removal of aflatoxin B1 from soybean kernels by a coupled ultrasound–ozone process: a mechanistic study. Food Control. 2026; 184(111988): 111988.
19. Jiang W, Zhang Y, Yang C, Liu W, Liu C, Zheng L. Rapid and simultaneous detection of Aspergillus and aflatoxin B1 content in maize using data fusion and explainable analysis. Food Control. 2026; 184(111987): 111987.
20. Demi̇rbakan B, Çeti̇nkaya A, Altuntaş EG, Ünal MA, Sezgi̇ntürk MK, Özkan SA. 3-mercaptopropionic acid agent-based biosensor system using gold-screen printed electrode for aflatoxin B1 detection. J Pharm Biomed Anal. 2026; 273(117411): 117411.