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Article

Mycotoxin Assessment of Leaf-Wrapped Foods: Characterization of Endophytic Fungal Diversity and Multi-Mycotoxin Profiles in Traditional Eko and Iru

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ABSTRACT: The use of wrapping leaves serves to protect foods and enhance their flavor. However, these leaves naturally harbor endophytic microorganisms, which may include species that are beneficial or belong to genera with known toxigenic or pathogenic potential. This study characterizes fungal communities and mycotoxin profiles in West African foods wrapped in *Megaphrynium macrostachyum* leaves, identifying how beneficial endophytes transition into pathogenic risks. Using ITS gene amplicon sequencing and LC-MS/MS analysis, this study revealed a significant restructuring of the endophytic mycobiome during wrapping of Iru and Eko. The process created a niche that favored toxigenic fungi, specifically replacing protective *Paecilomyces* spp. with mycotoxin producers and identifying a potential transfer of *Fusarium* spp. from leaves to food. The detection of regulated mycotoxins (aflatoxin B1, fumonisins, zearalenone) and emerging mycotoxins (brevianamide F, bikaverin) in wrapped foods underscores major public health risks. Eko samples exhibited higher concentrations of mycotoxins produced by *Aspergillus* species, while Iru control samples contained an aflatoxin B1 concentration (5.52 μg/kg), raising public health concerns. These findings emphasize quality control and toxin monitoring across the production chain to ensure the safety of traditional leaf-wrapped foods.

KEYWORDS: *Toxigenic, Megaphrynium macrostachyum, Fungi, ITS, Wrapping leaves, Endophytes*

1. INTRODUCTION

The use of wrapping leaves in the traditional processing and preservation of fermented foods is an age-old practice crucial to food security and the cultural identity of West African populations.[1] Wrapping leaves serve multiple functions, including flavor enhancement and food packaging, as well as protecting against environmental contaminants.[2] Common examples of wrapping leaves utilized in West Africa include *Thaumatococcus daniellii*, *Megaphrynium macrostachyum*, and *Musa paradisiaca*. These leaves are used in the processing of traditional West African foods, such as Eko (a solidified form of cooked, fermented maize gruel) and Iru (fermented *Parkia biglobosa* seeds).[1,3−5] Despite the significant benefits offered by these leaves, they can harbor microorganisms, known as endophytes, which may affect public and human health either positively or negatively. Endophytes are bacteria, fungi, and archaea that reside within plant tissues, either transiently or persistently, throughout the plant's life cycle without causing harm to their host.[6] These endophytes form reciprocal interactions with their host plants, contributing to plant growth promotion, pathogen inhibition, elimination of soil pollutants, and improved resistance to abiotic stresses such as salinity, drought, and extreme temperatures, all common threats to agricultural food production.[7−9] Endophytic fungi that colonize healthy plant tissues can transition from a mutualistic or commensal state to a pathogenic state when the host plants experience abiotic and/or biotic stress without changing their genome.[10] Stressors can cause disruption in the regulatory mechanisms that maintain asymptomatic colonization, thus leading to an imbalance in the metabolic gene clusters for the activation of toxigenic genes in the endophytic fungi.[11,12]

Filamentous fungi, or molds, synthesize a structurally diverse group of secondary metabolites, which are often tiny molecular-weight chemicals known as mycotoxins. Mycotoxins are fungal secondary metabolites that are toxic to humans, animals, and plants.[13,14] Based on their effects, these toxins are categorized as teratogens, carcinogens, mutagens, and allergens, impacting critical organ systems such as the liver, kidney, nervous system, and immune system.[13,15−17]

The mycobiota found in leaf-wrapped fermented foods such as Eko and Iru includes endophytic fungal genera that may be either beneficial (e.g., *Saccharomyces*) or harmful (e.g., *Aspergillus*, *Fusarium*, and *Penicillium*).[18−20] Contamination of fermented foods by fungal species has been reported, with *Aspergillus* species often dominating cereal-based foods (e.g., Ogi, Eko) and alkaline fermented condiments (e.g., Iru).[21] Specific to Eko, diverse toxigenic and spoilage fungal species have been documented, including *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium herbarum*, *Geotrichum candidum*, *Mucor*

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mucedo, *Neurospora sitophilia*, and *Penicillium* spp.[22] Toxigenic fungal species, particularly *Aspergillus* spp., have also been observed in Iru.[21] Physical manipulation of wrapping leaves during harvesting, cutting, and wrapping can be portrayed as stress signals to plant cells and endophytic fungi, potentially promoting the activity or proliferation of such toxigenic species. Hydrothermal treatment or a combination of moisture, heat, and prolonged incubation during fermentation of wrapping leaves creates a favorable moist-warm environment that supports mycotoxin biosynthesis in *Aspergillus* and *Fusarium* species.[23−25] The leaves are also a possible source of substrate for fungal biofilms, which could enhance the diffusion of mycotoxins into the food.[23] However, studies portraying wrapping leaves as the primary source of mycotoxins in Eko and Iru are still limited.[26] In a recent study, Adeleke et al.[27] evaluated the diversity and predicted functions of endophytic bacteria and endophytic fungi associated with different food wrapping leaves, including *M. macrostachyum*. However, a key novelty of this study lies in the application of a validated LC-MS/MS multimycotoxin panel to quantify cocontamination, an approach that offers a fresh analytical insight, thus moving beyond single-toxin analyses. One of the set objectives of this study is to characterize endophytic fungal diversity in food products (Eko and Iru) as well as *M. macrostachyum* and to ascertain it as a possible primary vector for contamination.This study also links the endophytic mycobiota of the wrapping leaves to the mycotoxin profiles of the wrapped foods. This research provides essential data for mycological risk assessment of artisanally processed fermented foods. The findings provide evidence-based recommendations for artisanal food processors and contribute to the development of food safety guidelines applicable across sub-Saharan Africa, where comparable leaf-wrapping practices are widespread.

2. METHODS

2.1. Sampling

Two professional vendors, one for Iru and the other for Eko, were recruited from Ibadan, Nigeria to prepare packaged samples of Iru and Eko, which were wrapped in *M. macrostachyum* leaves. The samples were collected in November 2023 at a farm in Ibadan (coordinates: 7°08′14.6″N, 3°54′40.7″E), with three samples gathered per test. The surface-sterilized leaf samples[28] were handed over to the vendors, who wrapped the Iru and Eko food items in triplicate. Control samples for the leaves and food items were also in triplicate. The vendors followed the normal traditional procedure for wrapping the food items. After 5 days, food samples were collected from the wrapped items. The collected material specifically consisted of samples taken from the contact point between the leaves and the food. Both the food and leaf samples were transported on ice to the microbiology laboratory at the North-West University in Potchefstroom, South Africa, for further analyses.

2.2. Analysis of Fungal Diversity in Wrapping Leaves and Wrapped Food

2.2.1. ITS Gene Amplicon Sequencing and Library Preparation. DNA extraction was performed using the DNeasy PowerFood Microbial Kit for food samples and the DNeasy Plant Pro Kit (Qiagen, Hilden, Germany) for leaf samples, respectively. Extracted DNA quality was assessed with a NanoDrop spectrophotometer (Thermo Scientific). The fungal ITS2 gene region was amplified using primers fITS7 (5′-GTGARTCATCGAATCTTTG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′).[29,30] Each Polymerase Chain Reaction (PCR) mixture comprised 15 μL of Phusion High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of each primer, and 10 ng of template DNA. The thermal cycling program included an initial denaturation at 98 °C for 1 min, followed by 30 cycles consisting of 98 °C for 10 s, 50 °C for 30 s, and 72 °C for 30 s, concluding with a final extension step at 72 °C for 5 min. Sequencing libraries were prepared utilizing the NEBNext Ultra II DNA Library Prep Kit. Library quality control was performed using a Qubit 2.0 Fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100. Sequencing was subsequently executed on an Illumina NovaSeq platform, yielding 250 bp reads.

2.2.2. Bioinformatics. Paired-end reads were demultiplexed according to their unique bar codes. Barcode and adapter sequences were trimmed using Trimmomatic v0.40.[31] The filtered reads underwent quality checking with FastQC v0.12 and were processed using the QIIME2 pipeline.[32] Amplicon sequence variants (ASVs) were inferred through DADA2.[33] Taxonomic assignment of representative sequences was carried out using the Scikit-learn classifier in conjunction with the UNITE database. The endophytic fungal community composition was analyzed based on relative abundance, alpha diversity (observed species and Chao1 indices), and beta diversity (PCoA). Data analysis for high-throughput microbiome profiling and visualization, including biomarker discovery, was performed using Phyloseq and MicrobiotaProcess.[34]

2.3. Multi-Mycotoxin Analysis of Wrapped Food Samples

Three samples were prepared per test for mycotoxin profiling of the wrapped Eko and Iru samples using the dilute and shoot method.[3,35] This method is capable of analyzing approximately 800 metabolites in food samples. The chemicals used included liquid chromatography (LC) grade methanol (Merck, Darmstadt, Germany), glacial acetic acid (Merck, Darmstadt, Germany), LC gradient grade acetonitrile (VWR, Leuven, Belgium), and mass spectrometry (MS) grade ammonium acetate. Mycotoxin standards sourced from Biopure Referenzsubstanzen GmbH (Tulln, Austria), with certified purities of ≥ 95%, consistent with the validated reference library described by Sulyok et al.[35] LC-MS grade water was purified using the reverse osmosis method via the Elga Purelab ultra-analytic system (Veolia Water, Bucks, UK).

2.3.1. Metabolite Extraction and Recovery Estimation. A 5 g portion of the food sample was weighed into a 50 mL polypropylene tube (Sarstedt, Nümbrecht, Germany) and homogenized using 20 mL of the extraction solvent (acetonitrile/water/acetic acid, 79:20:1 v/v/v). Samples were extracted for 90 min using a GFL 3017 rotary shaker (GFL, Burgwedel, Germany). The resulting extract was then diluted with an additional 20 mL of the extraction solvent. Direct injection of the diluted extract into the LC-MS/MS instrument was employed, leveraging sedimentation due to the gravity of the diluted extracts. To determine the apparent recoveries of the analytes, five samples (0.25 g each) were spiked. The solvents in the spiked samples were allowed to evaporate overnight at ambient temperature to establish equilibrium between the analytes and the sample matrix. These spiked samples were subsequently extracted (in 1 mL of solvent), diluted, and analyzed according to the procedure previously described by Sulyok et al.[35]

2.3.2. LC-MS/MS Parameters. Mycotoxin screening was conducted using a QTrap 5500 LC-MS/MS System (Applied Biosystem, Foster City, CA, USA) coupled with a TurboIonSpray electrospray ionization (ESI) source and a 1290 series HPLC system (Agilent, Waldbronn, Germany). Chromatographic separation was achieved at 25 °C using a Gemini C18-column (150 mm × 4.6 mm i.d., 5 μm particle size), which was fitted with a C18 security guard cartridge (4 × 3 mm i.d.) (Phenomenex, Torrance, CA, USA). The specific chromatographic method, along with the detailed chromatographic and mass spectrometric parameters used in this study, is described by Sulyok et al.[35] This validated method enables simultaneous acquisition of multiple reaction monitoring (MRM) transitions for >500 mycotoxins and secondary metabolites, including regulated mycotoxins (aflatoxin B1, fumonisins B1−B4, zearalenone) and emerging/nonregulated fungal and bacterial metabolites. Quantification was performed using external calibration curves constructed with certified reference standards across the expected concentration range, applying apparent recovery correction where appropriate. Method performance was validated by Sulyok et al.,[35] with key parameters including limit of detection (LOD) and limit of quantification (LOQ) in the low μg/kg

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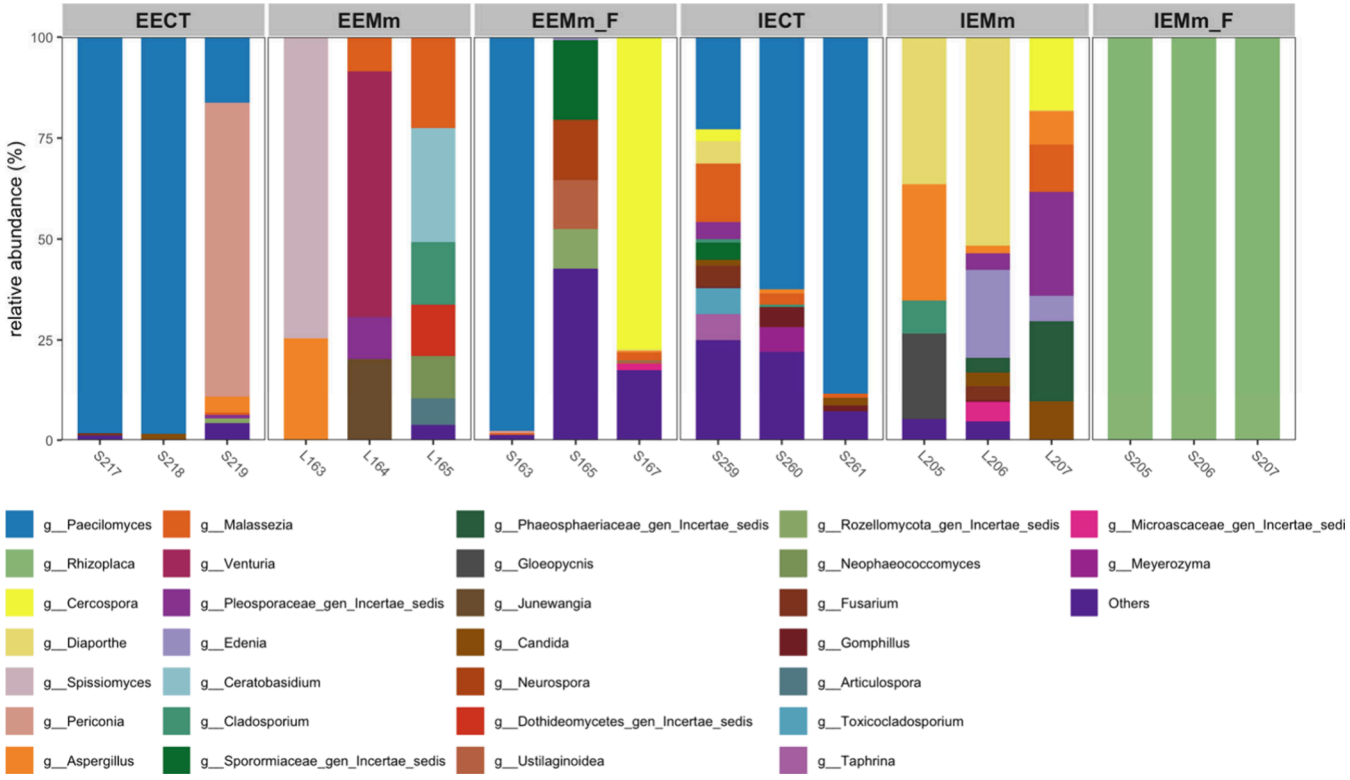

Figure 1. Relative abundance of the fungal community in the wrapped Eko (EEMm_F), unwrapped Eko (EECT), wrapped Iru (IEMm_F), unwrapped Iru (IECT), and wrapping leaves (EEMm and IEMm).

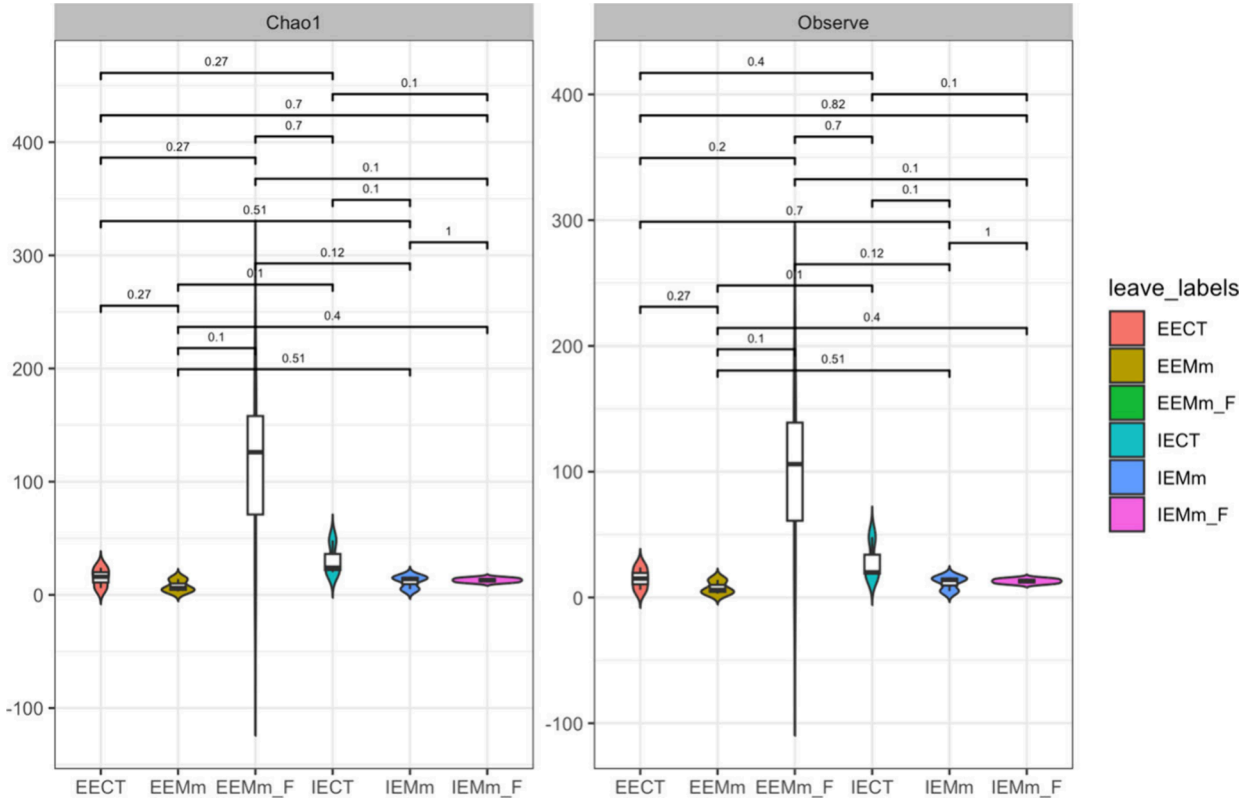

Figure 2. Alpha diversity indices for the fungal endophytes in the wrapped Eko (EEMm_F), unwrapped Eko (EECT), wrapped Iru (IEMm_F), unwrapped Iru (IECT), and wrapping leaves (EEMm and IEMm).

range, apparent recoveries of 70−120%, and within-day precision of RSD ≤ 20%.

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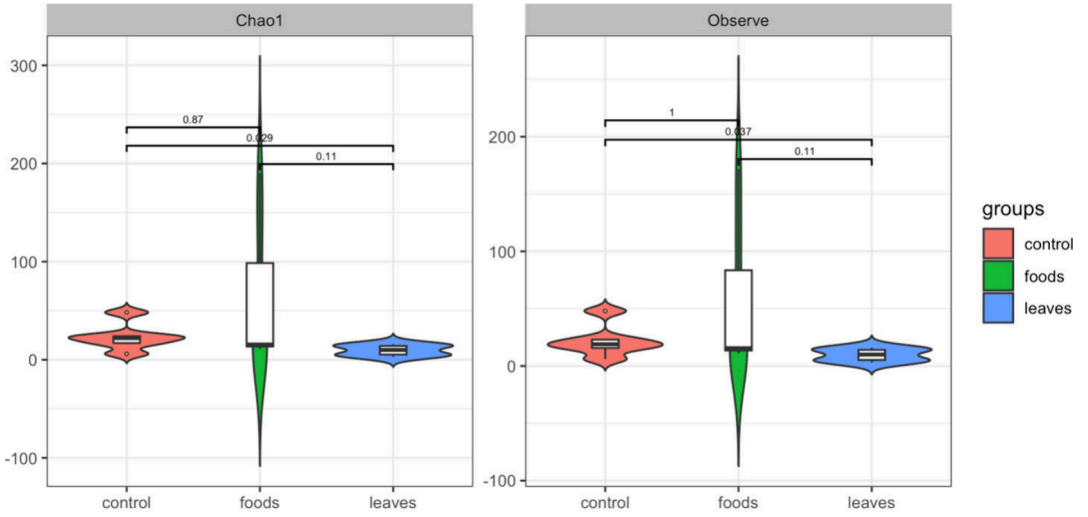

Figure 3. Alpha diversity indices for the distribution of fungal endophytes in the combined wrapped food, unwrapped food (control), and wrapping leaves.

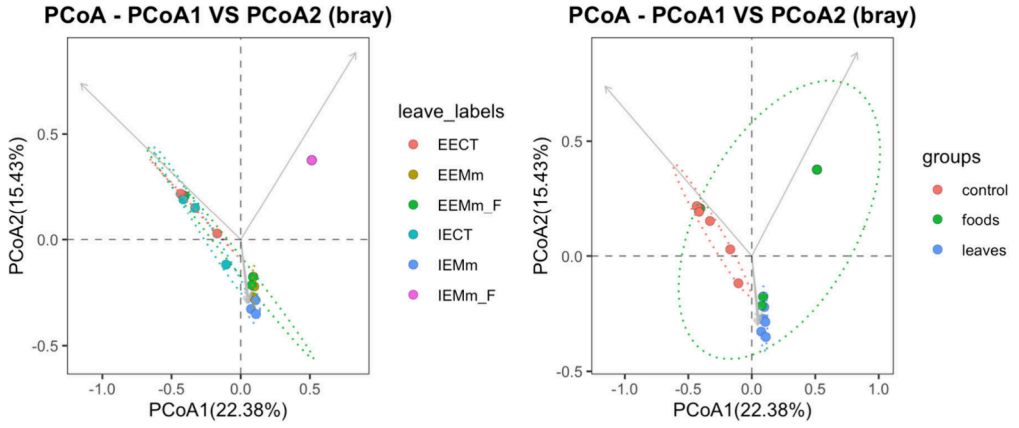

Figure 4. Beta diversity index for the distribution of fungal endophytes in the wrapped Eko (EEMm_F), unwrapped Eko (EECT), wrapped Iru (IEMm_F), unwrapped Iru (IECT), and wrapping leaves (EEMm and IEMm).

2.4. Statistical Analysis

Alpha diversity comparisons (Chao1, Observed species) were assessed using the Kruskal–Wallis nonparametric test (Shapiro–Wilk confirmed non-normality; $p < 0.05$ in most groups). Beta diversity was assessed by PERMANOVA using the vegan and phyloseq packages in R. Mycotoxin concentration differences between paired wrapped and control samples were assessed using Wilcoxon signed-rank tests. Statistical significance threshold: $\alpha = 0.05$.

3. RESULTS

3.1. Fungal Diversity in Wrapping Leaves and Wrapped Food

The mycobiome within the endophytic community of the wrapping leaves and wrapped food samples was assessed through the direct PCR amplification of the ITS region. The genus *Paecilomyces* was the most abundant and was consistently detected in the control samples of both Eko and Iru; however, it was absent among the 30 most abundant genera in all leaf samples (Figure 1). Notably, the wrapped Iru sample was almost entirely dominated by the genus *Rhizoplaca*, a genus that was not significantly detected in either the associated leaf sample or the control Iru sample. Overall, a marked shift in the abundance of fungal genera was observed when comparing wrapped and unwrapped foods. For example, after the wrapping of Eko, the dominant genera were *Saccharomyces*, *Malassezia*, and *Aspergillus*.

Analysis of the richness and evenness of the fungal endophytes indicated that the species diversity was generally low across individual samples, encompassing wrapped foods, unwrapped foods, and wrapping leaves. The wrapped Eko sample was the only exception, exhibiting a slightly higher diversity. Conversely, the wrapped Iru sample recorded the lowest diversity. When diversity was compared across all sample types, no significant differences were observed based on Chao1 and observed species indices ($p > 0.05$) (Figure 2). However, a significant difference (Chao1, $p = 0.029$; observed, $p = 0.037$) was detected when comparing pooled unwrapped food samples with wrapping leaves, a difference that was not evident when comparing wrapped foods (Figure 3).

Additionally, β diversity analysis, performed using principal coordinate analysis (PCoA), revealed distinct variations among individual wrapped and unwrapped food samples (PCoA1 = 22.38%, PCoA2 = 15.43%). This variation was particularly pronounced in the wrapped Iru sample (Figure 4).

3.2. Multi-Mycotoxin Profile of Wrapped Food Samples

A total of 31 metabolites (comprising 29 fungal and two bacterial metabolites) were detected in the wrapped food

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samples. Twenty-nine of the 31 metabolites were quantified (Supplementary Table 1). Eko samples contained a greater diversity of metabolites compared with Iru samples (Table 1). Furthermore, regulated mycotoxins were found in Eko samples, but not consistently in Iru samples.

Table 1. Mycotoxins and Bacterial Metabolites Identified in Food Samples (Concentration in μg/kg)

mycotoxin	Eko control	Eko sample	Iru control	Iru sample
Regulated Mycotoxins				
aflatoxin B1	0.34	0.4	5.52	<LOD
fumonisin B1	23	33.3	<LOD	<LOD
fumonisin B2	19.9	32.9	<LOD	<LOD
fumonisin B3	9.4	9.7	<LOD	<LOD
fumonisin B4	<LOD	15.1	<LOD	<LOD
hydrolyzed fumonisin B1	<LOD	0.84	<LOD	<LOD
total fumonisin	52.3	91.8	<LOD	<LOD
zearalenone	0.95	1.1	<LOD	<LOD
Emerging Toxins				
asperglaucide	52.98	72.36	<LOD	5.46
3-nitropropionic acid	22.1	42.6	<LOD	<LOD
asperphenamate	31.48	45.03	<LOD	<LOD
beauvericin	<LOD	0.13	<LOD	<LOD
bikaverin	2.19	4.72	2850	<LOD
brevianamid F	<LOD	3.64	20832	2858
epiequisetin	<LOD	0.25	<LOD	<LOD
equisetin	0.74	0.97	20.9	<LOD
fellutanine A	<LOD	<LOD	<LOD	21.7
monocerin	<LOD	3.64	280.93	<LOD
quinolactacin A	1.91	2.72	<LOD	<LOD
quinolactacin B	0.1	0.15	199	<LOD
rugulusovin	<LOD	4	199	223
tenuazonic acid	<LOD	<LOD	40.92	36.57
tryptophol	<LOD	<LOD	<LOD	27.14
Bacterial Metabolites				
surfactin A[a]	376400	220600	7328000	24680000
surfactin B[a]	88720	79480	0.00	7789000

[a]No quantitative standard was available for surfactin A and B.

In wrapped Eko samples, the concentration of both regulated and emerging mycotoxins increased relative to the control Eko sample. In the wrapped Iru samples, only asperglaucide (5.46 μg/kg), fellutanine A (21.7 μg/kg), and tryptophol (27.14 μg/kg) showed increased metabolite concentrations compared to the control (<LOD). While quantitative standards were not available for bacterial metabolites in Eko samples, concentrations of both surfactin A and B decreased in the wrapped Eko compared to its control. However, in the wrapped Iru samples, the concentrations of surfactin A and B decreased compared to the Iru control. Toxins produced by *Aspergillus* species were detected at higher concentrations in the wrapped Eko sample than in its control. Conversely, in Iru samples, toxins produced by *Fusarium* species were detected at higher concentrations in the control sample than in the wrapped sample (Figure 5).

4. DISCUSSION

This study demonstrates significant restructuring of the endophytic mycobiome associated with the traditional food-wrapping process, along with an assessment of its mycotoxin-producing potential. Overall, a substantial shift in the microbial community structure was evident, following the wrapping process. The pronounced dominance of *Rhizoplaca* sp., which is typically described as an epiphyte,[36] in the mycobiota of wrapped Iru samples is remarkable, especially since it was absent from both the control Iru and the wrapping leaves. This suggests that the synergistic interaction between Iru and the wrapping leaves established a favorable habitat that promoted the proliferation of this genus, leading to an unusual ecological shift. Extracts from *Rhizoplaca* facilitate the green synthesis of nanoparticles, which have shown promise as a biocontrol agent against bacterial and fungal pathogens.[37] This biocontrol trait could explain their dominance following the wrapping process as lichenized fungi such as *Rhizoplaca* have been naturally associated with the possession of antimicrobial properties (biocontrol); however, studies specifically regarding the genus *Rhizoplaca* remain limited.[38,39]

Initially, *Paecilomyces*, an endophytic genus recognized for its biocontrol capabilities, predominated in the unwrapped food samples (EECT and IECT). According to the literature, *Paecilomyces* species can produce antimicrobial metabolites and may suppress competing microbes.[40] The observed decrease in the relative abundance of *Paecilomyces* following

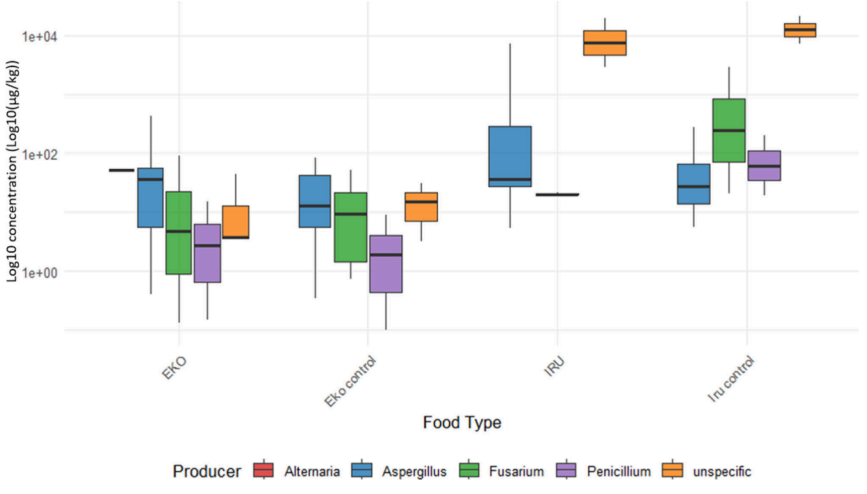

Figure 5. Variation in mycotoxin concentrations based on food source and fungi producer.

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wrapping, coupled with the proliferation of potential toxigenic fungi, challenges the assumption of safety often associated with the traditional practice of using wrapped leaves for food products. However, it has been reported that some *Paecilomyces* species may produce leucinostatins, peptide antibiotics with cytotoxic properties that are usually overlooked during mycotoxin screenings.[41,42] Along these lines, the ecological and food safety implications of fungal community shifts during wrapping warrant further investigation.

The identification and predominance of *Aspergillus* and *Cercospora* species suggest a potential food safety hazard owing to their capacity for mycotoxin production.[43,44] Although certain *Aspergillus* species can exist as endophytes, their presence in this ready-to-eat product primarily signals a potential contamination risk.[45] In contrast to the other dominant genera, *Malassezia*, a lipid-dependent fungus typically part of the natural skin flora, has not been associated with mycotoxin production.[46]

The detection of the mycotoxin-producing fungus, *Fusarium*, on the wrapping leaves (IEMm) and subsequently in the wrapped Eko (EEMm_F) raises the possibility that the leaf may be a source of contamination that compromises food quality. *Fusarium* species demonstrate ecological versatility, capable of being beneficial or pathogenic to their host.[47] The co-occurrence of *Fusarium* with other potentially toxigenic genera such as *Aspergillus* and *Penicillium* warrants further attention.[48,49]

The low Chao1 index signifies a reduced fungal diversity within the wrapping leaves. This reduction may be attributed to a stressed ecosystem, potentially arising from postharvest processing of the leaves (such as drying and storage), which likely depleted the endophytic fungal population. The low endophytic fungal diversity and population observed here might increase the risk of mycotoxin contamination by promoting the proliferation of resilient mycotoxin-producing fungi due to reduced microbial competition.[50,51] Nevertheless, the effect of wrapping on the overall fungal diversity was not statistically significant on the sampled scale.

Although the fungal community exhibited low species richness and evenness (which were not significantly different across most samples), this aligns with reports that above-ground plant parts, such as leaves, harbor fewer endophytic species than roots due to exposure to environmental stress.[52,53] Despite this inherently low diversity, the findings confirm that the wrapping leaves contributed to the observed fungal compositional shift. Estimates of endophytic fungal richness and observed species counts indicated high diversity in the control samples, contrasting with the low diversity found in the food and wrap leaves. This disparity suggests an adverse effect of the food and wrapping leaves on the endophytic fungal community, with the wrapping leaves showing a particularly high reduction in fungal population.[54]

The significant difference observed in the beta diversity analysis (PCoA) emphasizes a major shift in the fungal community structure of the wrapped food. There is significant compositional variability between wrapped and unwrapped foods and among different samples. The distinct separation of the wrapped Iru sample in multivariate space suggests that it harbors a unique fungal community compared to the other foods and wrapping leaves. This separation potentially indicates that toxigenic fungi that were less prevalent in other foods (Eko) or in unwrapped controls might form part of the distinct fungal community, dominating the Iru samples. Consequently, wrapping practices and food types could influence the endophytic fungal community structure, thereby modifying the mycotoxin contamination risk profile for different traditional foods.

As previously suggested, the wrapping of Iru may have encouraged the proliferation of specific fungal groups, particularly those associated with mycotoxin production. This hypothesis is corroborated by the multimycotoxin results (Table 1). This observation resulted from the dominance of *Fusarium* and "unspecific fungi". The chemical analysis results correlate with the increased concentrations of various mycotoxins associated with these fungi. The prevalence of regulated mycotoxins such as aflatoxin B1 and fumonisins in the Eko samples and controls is consistent with previous findings indicating that maize-based fermented food products are highly susceptible to *Fusarium* contamination, in contrast to legume-based fermented food products.[21]

Although aflatoxin B1 was detected in both Eko and Iru, its concentration in Eko remained within regulatory limits, whereas the contamination level in Iru exceeded regulatory limits. This highlights that fermentation cannot completely eradicate aflatoxins, especially when raw materials are highly contaminated.[55] The lower mycotoxin concentrations observed in Eko samples may be due to lower levels of toxigenic fungi, suggesting that Iru is particularly susceptible to certain fungi when wrapped. These findings are highly significant for food safety and public health, especially considering the known link between these toxins and severe health implications, such as increased cancer risk and compromised immunity.[13]

The increased concentrations of fumonisins and the presence of zearalenone further support the observed dominance of *Fusarium* species in the Eko samples. The detection of high concentrations of emerging mycotoxins (brevianamide F and bikaverin), linked to *Aspergillus* and *Penicillium* species, specifically in the Iru control samples, emphasizes the potential for the accumulation of nonregulated toxins in wrapped Iru. This observation is consistent with studies by Adebayo et al.,[22] which indicate a primary fungal community shift resulting from processing conditions such as wrapping. Furthermore, the emergence of other metabolites (tenuazonic acid, rugulusovin, and fellutanine A) suggests the dominance of a potentially more resistant fungal consortium.

Considering the regulatory limit for aflatoxin B1 in food products set by the US FDA is typically 20 μg/kg, the European Union (EU) has adopted a stricter approach, setting the limit for aflatoxin B1 in food products for humans at just 2 μg/kg.[56] The detected concentration of 5.52 μg/kg in the Iru control sample raises public health concerns about aflatoxin exposure. The absence of mycotoxins in certain samples despite the presence of toxigenic fungi suggests that contamination likely occurred during the handling, storage, and processing of the food products, rather than exclusively from the wrapping leaves.

Identification of bacterial metabolites, specifically surfactin A and surfactin B, in food products is crucial for evaluating the overall safety and quality profile. Surfactin metabolites are typically produced by *Bacillus* species and are known for their surfactant and antimicrobial (biopreservation) properties. Elevated surfactin concentrations are indicators of potential bacterial fermentation and suggest the potential for biocontrol of toxigenic fungi given that some bacteria can bind, degrade, or partially detoxify mycotoxins. However, the full effects of these metabolites on food quality and safety are not yet fully understood; high concentrations of surfactin in food products

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could impact food sensory quality and pose unknown health risks.[57−59]

This study demonstrates that wrapping food in *M. macrostachyum* leaves significantly modifies the food's mycobiota, creating a niche that promotes the growth of endophytic, unusual, and toxigenic fungi. Our findings highlight a remarkable shift in the fungal community that is possibly driven by the wrapping process. Specifically, the synergy between the food products and *M. macrostachyum* leaves promoted the proliferation of *Rhizoplaca* spp., while simultaneously facilitating the transfer of toxigenic fungi (*Fusarium* spp.) from the leaves into the food. The low fungal diversity associated with the wrapping leaves, likely due to postharvest processing/handling, facilitated the proliferation of resilient toxigenic fungi. The detection of both regulated and emerging mycotoxins underscores the associated health risks and further suggests that significant contamination arises during the handling and wrapping stages of food products. These findings stress the critical need for proactive quality control and vigilant monitoring of toxins (both fungal and bacterial metabolites) throughout the entire production chain to ensure the safety of traditional leaf-wrapped foods.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.6c00243.

Supplementary Table 1 (PDF)

Background on the study (PDF)

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Notes

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