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Field experiment in Ugandan cassava stores reveals that slow release SO_2 sheets suppress aflatoxigenic fungi, resulting in undetectable aflatoxin B_1 levels.

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Abstract

Aflatoxin contamination in stored crops poses a serious threat to public health and food safety, particularly in tropical regions where warm, humid conditions favour fungal growth. Cassava, a staple in many developing countries, is vulnerable to aflatoxin B1 (AFB1) contamination during storage, necessitating effective mitigation strategies. This study evaluated the efficacy of sodium metabisulphite (NaMBS) sheets, which slowly release sulphurdioxide (SO_2), in reducing aflatoxigenic fungal load and aflatoxin B1 (AFB1) concentrations in cassava chips (sliced pieces of cassava root used for cassava flour production). Storage trials were conducted in three Ugandan districts with contrasting climates, using two bag types: traditional polypropylene and hermetic Purdue Improved Crop Storage (PICS) bags-with and without NaMBS. The study employed a three-way factorial randomized complete block design (bag type, NaMBS treatment, and district). Fungal load and AFB1 were monitored for 30 days using DG-18 media and LC-MS/MS, respectively. *Aspergillus* section *Flavi* showed the highest initial fungal load (3.57×10^6 cfu/g), which significantly ($P < 0.01$) decreased after NaMBS treatment. A significant bag × NaMBS interaction ($P < 0.001$) was observed, with PICS bags consistently outperforming traditional bags. District climate did not significantly affect fungal counts ($P = 0.06$) but strongly influenced AFB1 levels ($P < 0.01$). Untreated traditional bags showed the highest AFB1 (146.6 μg/kg), while NaMBS-treated PICS bags reduced AFB1 to 0.23 μg/kg, representing up to 99.9% reduction. These findings provide foundational evidence that NaMBS sheets can effectively suppress aflatoxigenic fungi and reduce AFB1 contamination in cassava stores. Further work should assess residual NaMBS safety and consumer acceptability.

Key words: Sodium metabisulphite, Fungal load, AFB1, storage bags, PICS, applied mycology

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Introduction

Cassava (Manihot esculenta) is the second staple food crop in Uganda, with per capita consumption estimated at 386 g/day (UBOS, 2023). However, recent studies have highlighted the deplorable state of storage facilities for cassava in sampled food stores of Uganda, characterized by high ambient humidity and temperature (65% and 32-38 ˚C) which expose the crop to fungal contamination (Oyesigye et al., 2024; Omara et al., 2020). During post-harvest handling, cassava chips are typically stored in polypropylene plastic single-layered bags (traditional bags), which are often placed directly on the floor rather than on pallets, in dark rooms with windows seldom opened (Kaaya and Eboku, 2010; Kaaya and Warren, 2005). These storage bags are often reused, which provides a carryover of inoculum from previous contaminated lot (Oyesigye et al., 2024). A combination of these storage conditions creates an ideal environment for the growth of fungi.

The dominant fungi in Uganda's cassava belongs to *Aspergillus* section *Flavi* (Oyesigye et al., 2025; Wokorach et al., 2022) of which *A. flavus* and *A. parasiticus* are usually considered the main aflatoxin producing species. *A. flavus* and *A. parasiticus* are opportunistic pathogens that affect plants and act as saprophytic storage fungi. These species can grow across a broad range of temperatures (10-45°C) and water activity (a_w; 0.73-0.99), with optimal growth conditions at 28-32°C and a_w between 0.95 and 0.98. Both species thrive in environments with a pH range of 2.7 to 10 (Perrone et al., 2017). The conditions that promote aflatoxin production vary depending on the food matrix and environmental factors. However, in most substrates, the optimal conditions for aflatoxin production are typically around 28°C and an a_w of 0.95-0.98. These conditions are prevalent across Africa, for instance in Uganda, particularly the Northern region, where the average temperature can rise to 38 ˚C (UBOS, 2023), making the area highly conducive for proliferation of aflatoxigenic species especially in storage facilities thus contributing to production of aflatoxins.

Aflatoxins are considered the most potent and hazardous mycotoxins due to their severe impacts on human and animal health, food security, and trade (IARC, 2022). The International Agency for Research on Cancer (IARC) classifies aflatoxins, especially aflatoxin B_1 (AFB1) as the most carcinogenic mycotoxin, possessing the highest potential to induce Hepatocellular Carcinoma (HCC). HCC ranks as the fourth liver cancer in Africa, contributing to 9.2% of deaths caused by cancer with about 73,844 new cases per year (IARC, 2022). Uganda bears the highest mortality rate of HCC at 7.7% among cancers, second only to Rwanda in terms of new cases registered (IARC, 2022).

According to the Uganda Cancer Institute, about 34,008 new cases have been reported by 2022, of which 10% occur in children (Uganda Cancer Institute, 2024). This prevalence is unmatched in the East African region, and possibly among the highest in Africa. This prevalence of HCC is attributed to long term exposure to aflatoxins mainly consumed from food products including cassava. Thus, prompting the need for innovative storage methods that are not only user-friendly but also tailored to the needs of value chain actors.

Innovations in storage of crop products including cassava have been proposed. The notable ones include metal or cement bins (Okparavero et al., 2024) and triple-layered Purdue Improved Crop Storage (PICS) bags (Williams, Baributsa and Woloshuk, 2014). However, the adoption of the former has been limited due to higher costs, while PICS bags are widely used across Africa because of their affordability and efficient protection of stored products (Baoua et al., 2014; Njoroge et al., 2014). Beyond improved storage, other postharvest decontamination technologies for aflatoxins, as discussed by Wang et al. (2024), include ozone treatment, gamma ray irradiation, ultraviolet irradiation, electron beam irradiation, microwave irradiation, and pulsed light irradiation. Chemical agents, notably 1% ammonium bicarbonate, 1-nonanol, phenolic compounds, and isothiocyanates, have also been explored (Gong et al., 2024). Although these methods can reduce fungal growth and aflatoxin production, some which are widely used in sub–Saharan Africa, such as PICS bags and cement bins (Njoroge et al., 2014), do not eliminate pre-existing fungal inoculum, while the application of chemical and irradiation methods remains limited in sub–Saharan Africa (Wang et al., 2024). Therefore, mycotoxin contamination continues to pose a significant challenge in sub–Saharan Africa, especially when produce is already contaminated.

Thus, an innovation, that can reduce already existing aflatoxin levels, would be of great importance. One of such innovations is the Sodium Metabisulphite (NaMBS) which has strong fungicidal properties. NaMBS has been extensively used as a preservative in beverages and fruits especially grapes to control *Botrytis cinerea* (Kane et al. 2024; Ahmadi et al., 2018). Researchers including Castro-Ríos et al. (2021); López-Malo et al. (2005); Natskoulis et al. (2018) have concluded that SO_2 has strong anti-fungal properties mostly in beverages, and can be evaluated in stored products. A few studies have been conducted in line with this. Al-Jaza et al. (2022) evaluated the effectiveness of NaMBS sheets with slow-release of SO_2 (food-grade) in controlling AFB1 in laboratory settings. Their study concluded that NaMBS with slow-release SO2 reduced fungal populations and controlled AFB1 contamination in red chilies. When in contact with the commodities, NaMBS react with humidity that

originates from their respiration of the crops and release SO_2 that inhibit growth of *Aspergillus* species (Al-Jaza et al., 2022). To the best of our knowledge, no study has focused on assessing the efficacy of NaMBS in field conditions, which limits its application potential . However, if consistently evaluated in field setups, such findings could represent a significant breakthrough in managing aflatoxins. This study aimed to evaluate the efficacy of sodium metabisulphite (NaMBS) sheets, which slowly releases SO_2, in reducing aflatoxigenic fungal load and aflatoxin B_1 (AFB1) concentrations in cassava chips stored under contrasting climatic conditions in Uganda.

Materials and methods

Description of field experimental sites

The experiment was set up in three districts with contrasting climatic conditions (Table 1). The elevation for the experimental sites was recorded with GPS (Garmin model 75), a portable testo-probe humidity detector (Testo, 440 dp, Germany) recorded daily relative humidity, while daily temperature in stores was recorded for 30 days with wall mount thermometer (Brannan wall mount thermometer, United Kingdom).

Specifications of NaMBS sheets slowly releasing SO_2

The sodium metabisulphite (NaMBS; $Na_2S_2O_5$) sheets used in this study function as controlled fumigants through an autocatalytic reaction that is triggered when ambient relative humidity exceeds 70%. NaMBS sheets are a dual-phase sulphur dioxide (SO_2) release technology engineered to emit SO_2 in two distinct stages. The first is a rapid release phase lasting 24–48 hours, during which a high concentration of SO_2 (>100 ppm) is emitted to effectively eliminate actively growing fungal spores. This is followed by a second, prolonged release phase, characterized by a steady emission of 3–5 ppm SO_2 sustained for up to 120 days. While standard sheets typically measure 53 × 35 cm, the sheets used in this experiment were scaled down to a manageable size of 15 × 15 cm for ease of application. These sheets are manufactured by TESSARA (https://www.tessara.co.za/uvasys/). Although the actual release of SO_2 (g/hr) is not indicated by manufacturer, but based on concentration-derived estimates, the 15 × 15 cm NaMBS sheet is expected to emit SO_2 in two phases: an initial burst release of approximately 0.094 g over the first 24 hours (equivalent to ~0.0039 g/hr), followed by a sustained release at an average rate of 0.0002 g/hr over the remaining 120 days under standard storage conditions (25°C, 15 L headspace volume). The mechanism of action for NaMBS sheets is guided by

a series of chemical reactions. Initial step is the hydrolysis and oxidation reactions that generate sulphurdioxide (SO_2), a potent antifungal agent. Upon exposure to moisture, sodium bisulfite ($NaHSO_3$) is formed, which decomposes to release SO_2 gas. This gas is further oxidized over time into stable end products such as sodium sulphate (Na_2SO_4) and sulfuric acid (H_2SO_4) as indicated in the four reaction steps (Tessara, 2016).

Reaction 1: $Na_2S_2O_5+H_2O \rightarrow 2NaHSO_3$(Intermediate product)

Reaction 2: $2NaHSO_3 \rightarrow Na_2SO_3+SO_2$ (gas)$+H_2O$

Reaction 3: $SO_2+H_2O \rightarrow H_2SO_3$ (Sulfurous acid – weak disinfectant agent)

Reaction 4: $2H_2SO_3+O2 \rightarrow 2H_2SO_4$ (Conversion to sulfuric acid over time)

Experimental layout and design

Cassava chips used throughout the experiment was sourced from a store in Gulu district (G14)-2°51'36.0"N 32°25'48.0"E) initially recorded with highest concentration of aflatoxins by Oyesigye et al. (2024). The decision to use a single sack of cassava chips (G14) across all three districts was based on the need for consistency in initial fungal contamination levels and aflatoxin presence, ensuring that any observed differences in fungal spoilage and aflatoxin reduction were attributable solely to the experimental variables (NaMBS and climatic conditions) rather than variability in raw material contamination.

The experiment was set up following a three-way factorial design in a randomized complete block design (RCBD), with stores serving as the blocking factor (Table S1). This design was chosen to control for variability associated with different stores while assessing the effects of climate conditions in three districts, storage bag type, and NaMBS treatment on fungal load and AFB1 levels. The treatments included: T1: Traditional bags + NaMBS sheet + cassava chips, T2: Traditional bags + cassava chips, T3: PICS bag + NaMBS sheet + cassava chips, T4: PICS bag + cassava chips. Within each of the three districts, three wholesale stores were chosen, and treatments were repeated three times at each store, resulting in a total sample size of 27. For each type, NaMBS sheets were used as an intervention, while the corresponding bags without NaMBS served as the control. A study by Oyesigye et al. (2024) found that local non-hermetic gunny bags made of polypropylene (used in this study as traditional bags), were the most commonly used storage bags for cassava, despite recommendations for using hermetic sealed bags such as PICS. PICS bags are triple-layered storage bags consisting of two inner liners made of high-density polyethylene and an outer woven

polypropylene sack. This design creates a hermetic environment that limits oxygen exchange, thereby inhibiting the growth of aerobic microorganisms such as fungi and insects (https://ag.purdue.edu/department/entm/extension/pics-network/). The traditional polypropylene bags used were Nile brand polypropylene sacks, originally designed for 100 kg capacity, with a thickness of approximately 80–100 microns (https://keshwalagroup.com/product/nile-polypropylene-sack/). For this experiment, all bags were resized to accommodate a capacity of 10 kg of cassava chips. Building on these findings, the study assessed the use of two types of storage bags: traditional bags and the PICS bags with the use of NaMBS sheets. All bags were visually inspected and cleaned to remove visible debris before use, but not sterilized, reflecting typical farmer practices in Uganda. The NaMBS sheet measuring 15 × 15 cm, were kept in a vacuum-sealed container (Dewar: SKU: M-LND-Ithaca, New York) until inserting them in the storage bags. For the bags in which an intervention was applied, NaMBS sheets was inserted sideways in the bag containing 10 kg of cassava chips. They were manually tightly tied at the top using the recommended triple-twist method (Kharel et a., 2019) to ensure hermetic conditions. The bags were placed on pallets as shown in Figure 1 for 30 days. Temperature and relative humidity were recorded daily for 30 days. To ensure representative sampling, cassava chips were collected from three locations within each sack—namely the top, middle, and bottom layers. These sub-samples were then combined to form a composite sample of approximately 300–400 g per sack. Importantly, to maintain the integrity of replication for statistical analysis, samples from each replicate and each store were analyzed independently, without pooling across replicates. Compositing was done only within individual sacks (top, middle, and bottom). Both, the fungal load and aflatoxin concentrations were assessed at the end of experiment.

Assessing the initial fungal load and AFB1 concentration

To determine whether NaMBS sheets had any significant impact on existing fungal and AFB1 levels, the baseline status was determined before the intervention and after the intervention. The serial dilution technique as described by Mohale et al. (2013) was applied to determine the fungal load (cfu/g) before and after inserting NaMBS sheet. One gram of the cassava chip samples from each bag was measured in triplicate and transferred to 50 mL glass bottles that contained 10 mL of sterile distilled water supplemented with Tween 80 (0.025% w/v). This was done to obtain 10^{-1} dilution. For each sample, 5 glass bottles were labelled as 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. Into the four dilution bottles, 9 mL of sterile distilled water containing tween 80 (0.025% w/v) was measured. Serial dilutions from 10^{-2} to 10^{-5} were performed in triplicate.

Plating and fungal enumeration

Dichloran Glycerol-18 agar media (DG-18) (Thermo Fisher Scientific Oxoid Ltd, Basingstoke, Hampshire, UK) is a selective media for isolation of xerophilic fungi like those in *Aspergillus* section *Flavi* (Frisvad et al., 2019), thus was used for fungal enumeration to target species in this section. DG-18 was prepared according to manufacturer's instructions using deionised water (18 Ωm), and 0.1% chloramphenicol (Fisher Bioreagents, UK). The media was autoclaved at 121 ˚C for 15 min. In triplicates, Petri dishes, measuring 9 cm (⌀), were labelled according to dilution factors. Approximately 18 mL of the molten media was poured to the plates under sterile conditions in a biological safety cabinet. Aliquots of 100 µl were taken from each sample at the corresponding dilution and spread using a sterile Drigalski spreader. The three replicates per dilution factor and per sample were transferred to incubation room with pre-set temperature of 25 ˚C for 7 days. After incubation, *Aspergillus* species were identified and counted. The dilution factor of 10^{-3}, produced ideal countable number of fungal colonies (5-50) (Al-Jaza, Medina and Magan, 2022a) which were counted using a colony counter.

Extraction of aflatoxin B_1 and quantification

The extraction of aflatoxin B_1 and quantification was performed following Oyesigye et al. (2024). Five hundred grams of cassava chips were ground with a manual blender to a fine flour using an Osterizer blender (Sunbeam Oster Household Products, Florida, USA). A homogeneous 5 g sample was obtained and placed in 50 mL Falcon tubes in triplicates. To extract mycotoxins, 20 mL of extraction solvent (acetonitrile: water: acetic, 79:20:1, v/v/v) was added, and samples were shaken for 60 min at 1509 × g (RCF) using VWR VX-2500 Multi-Tube Vortexer 58816-115, USA. After shaking, samples were centrifuged at 1509 × g (RCF) for 2 min (Thermo Scientific, Heraues Labofuge 400 R, Germany); 350 µl of supernatant was transferred to amber glass vials containing 350 µl of dilution solvent (acetonitrile/water/acetic acid, 20:79:1, v/v/v). A total of 5 µl of each diluted extract was injected into the qTRAP 6500 + LC/MS-MS system (SCIEX instrument, Foster City, CA, USA) and fitted with an electrospray ionization (ESI). The columns were ACE 3-C18 column (2.1 × 100 mm, 3 µm particle size; Hichrom) with guard cartridge (4 × 3 mm, Gemini, Agilent) conditioned at 60 ^{0}C. For the calibration curve, serial dilution was prepared from working solution using acetonitrile: water: acetic acid (49.5:49.5:1, v/v/v) to achieve 10-point calibration concentrations of 0.05, 1, 5, 10, 25, 50, 125, 250, 500, 1000 µg/mL. Three replicates per calibration point were used. These points were used to construct a calibration curve. The peaks were integrated linearly with a 1/x weighting. The R^2 for the

calibration curve was 99.03. The full details of method validation that include LODs and LOQs for cassava matrix was already performed in Oyesigye et al. (2024).

Data analysis

Data were analysed with Stata version 17 (Stata 2017). Prior to analysis, the data were tested for normality using the Shapiro-Wilk and heteroskedasticity using the Breusch-Pagan tests. Data were analysed parametrically because it passed these two tests without the requirement for transformation. Data from *in vitro* study were entered into Microsoft excel, where descriptive statistics (means and standard deviations) were computed, and graphical outputs such as bar charts and line graphs were generated to visualize trends in fungal growth and aflatoxin B_1 levels across treatments. To determine if NaMBS sheets had a significant reduction in fungal load and AFB1, as well as interactions between NaMBS, type of storage bag, *Aspergillus* species genera, and districts in which an experiment was laid out, a three-way factorial ANOVA was performed. A three-way ANOVA was conducted to examine the climatic conditions within districts (D), type of storage bag (B), and NaMBS treatment (N) on the CFU/g (equation 1) and AFB1 (µg/kg) (equation 2). The model included both main effects, two-way interactions, and a three-way interaction to determine how these factors and their combinations influence the fungal load and AFB1 levels.

$$Y_{ijkl} = \mu + S_i + D_j + B_k + N_l + (D_jB_k) + (D_jN_l) + (B_kN_l) + (D_jB_kN_l) + \epsilon_{ijkl} \quad (1)$$

Y_{ijkl} is the response variable (cfu/g),

μ is the overall mean response,

S_i is the effect of *Aspergillus* section ($i = 1,2$)

D_j is the effect of districts with contrasting climatic conditions, ($j = 1, 2, 3$)

B_k is the effect of type of storage bag ($k = 1,2$)

N_l is the effect of NaMBS treatment ($l = 1,2$)

D_jB_k interaction between district with contrasting climatic conditions and type of storage bag,

D_jN_l interaction between district with contrasting climatic conditions and NaMBS,

B_kN_l interaction between type of storage bag and NaMBS

$D_jB_kN_l$ is the interaction between with contrasting climatic conditions, type of storage bag, and NaMBS,

ϵ_{ijkl} is the residual error term

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258 $Y_{ijkl} = \mu + D_i + B_k + N_l + (D_iB_k) + (D_iN_l) + (B_kN_l) + (D_iB_kN_l) + S_m + \epsilon_{ijkl}$ (2)

259 Y_{ijkl} is the response variable AFB1(µg/kg),

260 S_m is the effect of store as a blocking factor (m=1, 2, 3)

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262 Model descriptors from D to N are the same as in equation 1.

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Results

Field observation on cassava storage chips after experimental set up

After 30 days of storage, clear differences were observed between cassava chips stored with and without NaMBS sheets (Figure 2). Chips stored with NaMBS sheets (Figure 2b–F) exhibited no visible fungal growth and retained their original texture and coloration, indicating minimal to no surface colonization—even when stored in traditional gunny bags. In contrast, chips stored without NaMBS (Figure 2a–A and Figure 2b–D) showed extensive mold growth, with green, fuzzy mycelia spreading visibly across the chip surfaces. While these visual observations suggest a strong protective effect of NaMBS, such qualitative assessments should be interpreted with caution, as visual inspection alone may not accurately reflect microbial or mycotoxin contamination levels. Therefore, the study further delved into understanding the fungal cfu and AFB1 concentrations to validate whether the observed differences reflect actual reductions in fungal load and AFB1 concentrations.

Efficacy of slow SO_2 release bags in reducing fungal load (cfu/g)

The experimental results revealed that NaMBS treatment and storage bag type had a significant impact ($p < 0.05$) on cfu/g in cassava chips, with the lowest cfu levels observed in PICS bags treated with NaMBS. Among the environmental variables, climate—defined in this study as the combination of mean ambient temperature and relative humidity measured daily at each storage site—did not have a statistically significant main effect on cfu levels ($P = 0.0643$). The ANOVA model (Table 2), which included fungal section, climate (temperature and humidity), storage bag type, and NaMBS treatment, was statistically significant overall ($P = 0.0001$), indicating that at least one of the variables had a substantial effect on cfu levels. Fungal section ($P = 0.0001$), storage bag type ($P = 0.0006$), and NaMBS treatment ($P = 0.0009$) all significantly influenced fungal load. Among these, NaMBS treatment had the most pronounced effect in reducing cfu levels. Regarding interactions, the district × bag type ($P = 0.7613$) and district × NaMBS ($P = 0.2377$) interactions were not significant, suggesting that temperature and humidity did not modify the effects of storage type or chemical treatment. However, the interaction between bag type and NaMBS was significant ($P = 0.0009$), indicating that the effectiveness of the bag type depended on whether NaMBS was applied. The three-way interaction between climate (temperature and humidity), bag type, and NaMBS ($P = 0.8522$) was also not significant. The model explained 53.61% of the variability in CFU levels ($R^2 = 0.5361$, Adjusted $R^2 = 0.4845$), suggesting a moderate to strong fit. These findings confirm that fungal section, storage

method, and NaMBS treatment are the key drivers of fungal contamination in stored cassava, with NaMBS having the most substantial individual effect.

Efficacy of slow SO_2 release bags on AFB1

To determine the efficacy of NaMBS on AFB1 contamination in cassava chips stored for a month, a two-way-factorial ANOVA was performed at a 95% confidence level to assess the main effects and interactions between NaMBS treatment, storage bag type, district. Significant differences were established at $p < 0.05$.

Results (Table 3) indicated that NaMBS treatment had the strongest and most significant effect ($P = 0.001$), demonstrating its efficacy in reducing AFB1 levels. The type of storage bag used also significantly influenced aflatoxin contamination ($P = 0.003$), with PICS bags performing better than traditional bags. Additionally, district had a significant effect ($P = 0.0022$). In Gulu (mean relative humidity: 68%, mean temperature: 31.3 °C), the cassava chips stored without NaMBS treatment exhibited the highest levels of AFB1 (132.1 μg/kg), in contrast, Kamuli (mean relative humidity: 60.7%, mean temperature: 28.4 °C), where the chips were stored without NaMBS, exhibited comparatively lower levels of AFB1 (96.7 μg/kg). However, in all districts with varying climate, the application of NaMBS treatment led to a marked reduction in AFB1 production. In Gulu district, for example, the chips treated with NaMBS produced 6.43 μg/kg of AFB1, while in Kamuli it was 3.62 μg/kg a dramatic decrease (96.5 and 98% respectively) compared to the untreated group. This pattern was consistent across all districts. The effect size analysis (η^2) from the ANOVA model indicates that the tested factors explain 90.8% of the variance in AFB1 levels, confirming the model's robustness. Among the individual factors, NaMBS treatment had the strongest effect ($\eta^2 = 0.894$), explaining 89.4% of the variance, highlighting its significant role in reducing aflatoxin contamination. Storage bag type ($\eta^2 = 0.413$) also had a notable impact. The interaction between NaMBS and storage bag type ($\eta^2 = 0.243$) further supports the combined effectiveness of these interventions. District climatic differences ($\eta^2 = 0.164$) and store conditions ($\eta^2 = 0.019$) had smaller contribution in reducing AFB1 levels.

Interaction between NaMBS and type of storage bag in reducing AFB1

The interaction between NaMBS treatment and storage bag type was significant (P = 0.0001), indicating that the effectiveness of NaMBS was influenced by the type of storage bag used (Figure 3). The reported AFB1 concentrations represent the mean values across three replicates for each treatment. In contrast, the three-way interaction involving district (climatic conditions), storage bag type, and NaMBS treatment was not significant (P = 0.6800). Further comparison between the two storage bag types (PICS vs. traditional) and the application of NaMBS highlighted the significant influence of both factors on AFB1 accumulation in cassava chips. The results of the comparison revealed the influence of both factors on AFB1 production. The cassava chips placed in traditional bags without NaMBS treatment showed the highest levels of AFB1 levels (146.6 μg/kg), indicating that traditional bags, when untreated with NaMBS, do not significantly reduce AFB1. Similarly, the chips placed in PICS bags without NaMBS treatment also exhibited a considerable amount of AFB1 (91 μg/kg). However, the application of NaMBS treatment led to a drastic reduction in aflatoxin production from 185 μg/kg to 10.9 μg/kg (94.5% reduction rate) for traditional bags and 0.23 μg/kg (99.9% reduction rate) for PICS bags. These findings suggest that the addition of NaMBS treatment is highly effective in reducing AFB1 in both types of bags.

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Discussion

The objective of this study was to evaluate the efficacy of sodium metabisulphite (NaMBS) sheets, which slowly release sulphurdioxide (SO_2), in reducing aflatoxigenic fungal load and aflatoxin B1 (AFB1) concentrations in cassava chips (sliced pieces of cassava root used for cassava flour production). Results indicated that the sheets containing sodium metabisulphite reduced fungal load and aflatoxins (AFB1) significantly, depending on the storage bags in which they were inserted.

The superior efficacy of NaMBS sheets in PICS bags can be attributed to their hermetic nature, which creates an aerobic environment that retains the sulphur dioxide (SO_2) released by the NaMBS sheets. This sustained presence of SO_2 enhances the degradation of aflatoxins and inhibits fungal metabolism more effectively. In contrast, traditional storage bags, which are non-hermetic, allow the escape of SO_2, reducing its concentration within the storage environment and thereby limiting its capacity to suppress aflatoxin biosynthesis. The permeability of traditional bags results in a lower retention of the antifungal and anti-aflatoxigenic properties of SO_2, explaining the comparatively lower reduction in $AFB1_1$ levels observed in these bags. Thus, dual action of SO_2 release(antifungal) and oxygen restriction (hermetic sealing) explains the synergistic suppression *Aspergillus flavus* and AFB1. For the first time, this study demonstrates that NaMBS sheets can reproduce laboratory level reductions of aflatoxigenic fungi and AFB1 in field conditions.

The fungal load observed across districts (Gulu, Oyam and Kamuli) where experimental units (treatments) revealed no significant differences. The absence of a statistically significant effect of temperature and relative humidity on fungal cfu levels was unexpected, particularly given the well-established influence of these environmental factors on fungal growth and aflatoxin biosynthesis (Magan et al., 2003; Medina et al., 2015). However, the relatively narrow climatic range with average relative humidity between 60% to 68%, mean temperatures ranged of 28.4 °C (Kamuli) to 31.3 °C (Gulu), may not have been sufficient to induce differences in fungal proliferation, particularly for xerophilic fungi like *Aspergillus flavus*, which are known to thrive over a broad spectrum of warm and moderately humid conditions (Garcia-Cela et al., 2014). Secondly, a longer storage duration of (>30 days) could have influenced results further. Fungal colonization and mycotoxin production are time-dependent processes influenced not just by ambient conditions but also by substrate characteristics, inoculum load, and postharvest handling (Hell et al., 2000; Kamala et al., 2016). It is possible that the fungal species present rapidly colonized the cassava chips under all conditions, reaching saturation

before environmental differences could exert a noticeable effect. Additionally, the sealed or semi-sealed nature of the storage systems, especially the PICS bags, may have buffered microclimatic differences and reduced external climatic influence. PICS bags are designed to create low-oxygen environments, limiting fungal respiration and growth regardless of ambient temperature or humidity (Baoua et al., 2014; Baributsa et al., 2017). This may have further dampened the expected climate-dependent variation in fungal load.

Although NaMBS sheets are reported to be triggered at relative humidity levels exceeding 70%, their efficacy was still observed in our study where ambient RH averaged lower. This may be due to microclimatic conditions within the cassava bags including residual chip moisture and localized condensation-likely exceeded the external RH values, and also, NaMBS sheets can still release low levels of SO_2 at sub-threshold humidity due to surface sorption–desorption cycles. Similar mechanisms of SO_2 release under varying humidity inside packaging have been demonstrated for grape storage using sodium metabisulphite sheets and pads (de Aguiar et al., 2023; Kaplunov et al., 2011). This could explain the observed efficacy despite average ambient RH being below 70%.

While fungal load was consistent across sites, AFB1 levels showed significant variations, confirming the fungal biomass does not directly predict toxin risk-in this study. In all negative controls where NaMBS were not inserted, the AFB1 concentration were above 80 μg/kg regardless of storage bag used, however by introducing NaMBS in traditional bags and PICS bags AFB1 reduced by 94%, and to by 99% respectively (LOD=0.43 μg/kg) emphasizing that storage bag type also plays a role in aflatoxin reduction. The observed reduction of AFB1 below LOD of 0.43 μg/kg in PICS bags treated with NaMBS sheets demonstrates a highly effective intervention for controlling aflatoxin accumulation under practical storage conditions. This efficacy is comparable or superior to other postharvest mitigation strategies reported in the literature. Ozone treatment has been shown to reduce aflatoxin levels in maize by 78–89%, depending on exposure time and gas concentration (Afsah-Hejri et al., 2020). However, ozone requires specialized equipment, gas handling protocols, and may pose scalability challenges in rural settings. Similarly, irradiation using gamma rays or electron beams achieves 60–90% reduction in aflatoxins but raises regulatory and consumer acceptance issues, and requires expensive infrastructure (Kinyoro et al., 2024; Yang et a., 2024). Also, Alkaline treatments, such as using calcium hydroxide (lime), have reduced AFB1 levels in maize by 70–85% through chemical modification of the toxin (Zavala-Franco et al., 2024; Gómez-Salazar et al., 2023), yet such

methods often compromise sensory or nutritional quality. In laboratory-based studies, Al-Jaal et al. (2022) reported AFB1 reduction rates of 95–98% using sodium metabisulphite solutions in chilli matrix under controlled conditions. In comparison, our NaMBS + PICS combination achieved a 99% reduction, which is not only on par among the best of these interventions but also operationally more feasible on-farm storage contexts. Our field-based finding of 94–99% AFB1 reduction aligns well with these laboratory results, confirming that NaMBS is not only effective in vitro but can retain its antifungal efficacy under practical storage conditions when used with appropriate packaging.

District was statistically significant with AFB1 levels, with significant interactions enhancing the effectiveness of storage bags and NaMBS treatment in mitigating AFB1 contamination. Specifically, experimental setups in Kamuli district demonstrated a greater reduction in AFB1 levels compared to those in Gulu district, where the lowest reduction was recorded. This disparity is likely attributable to climatic differences between the two. During the experimental period (November–December), the average temperature and relative humidity recorded during the experiment in Kamuli district were 28°C and 61%, respectively, whereas in Gulu district, these values were 31°C and 68%. Although both temperature and humidity levels fell within the range conducive for AFB1 production, higher temperatures, such as those recorded in Gulu district, have been well-documented to enhance aflatoxin biosynthesis (Magan et al., 2014). This occurs through the activation of key aflatoxin biosynthetic genes, including *aflR* and *aflS,* which are critical transcriptional regulators of the aflatoxin gene cluster. Elevated temperatures induce changes in oxidative stress responses, leading to increased expression of genes such as pksA (polyketide synthase), which catalyzes the first step in aflatoxin biosynthesis, and nor-1, which facilitates early-stage precursor formation (Schmidt-Heydt et al., 2017; Caceres et al., 2020). However, it should be noted that significant differences in fungal load across districts with contrasting climatic conditions, variations in temperature and humidity may have driven differential aflatoxin production, ultimately influencing the effectiveness of storage interventions in mitigating contamination. However, it should be noted that the statistical model used to evaluate the influence of climate, bag type, and NaMBS treatment on fungal colony-forming units (cfu) accounted for 53.61% of the total variation in cfu levels ($R^2 = 0.5361$; Adjusted $R^2 = 0.4845$). While this reflects a moderate model fit, it also suggests that other unmeasured variables—such as initial microbial load, substrate moisture content, or store-specific handling practices—may have contributed to the remaining unexplained variability. Similar moderate R^2 values have been reported in postharvest

storage studies where environmental heterogeneity and biological variability are difficult to fully control (Hell et al., 2000; Mutegi et al., 2012). Therefore, while the model provides valuable insight into main and interaction effects, results should be interpreted with caution and in the context of complex storage environments.

The observed lack of a significant effect of climatic conditions within districts on fungal load (CFU) across districts, despite their clear influence on aflatoxin B_1 (AFB1) levels, can be attributed to the differing physiological thresholds and regulatory mechanisms governing fungal growth versus mycotoxin biosynthesis. Fungal colonization, particularly by xerophilic species such as Aspergillus flavus, occurs readily across a broad range of warm and moderately humid conditions. In this study, temperature (28.4–31.3 °C) and relative humidity (60–68%) varied only modestly across Gulu, Oyam, and Kamuli—conditions that all fall within the optimal growth range for A. flavus, thus supporting comparable CFU levels across sites. However, aflatoxin biosynthesis is more sensitive to microenvironmental shifts and is regulated by specific gene clusters (mainly aflR, aflS, pksA) that respond sharply to oxidative stress and marginal increases in temperature and humidity. Elevated temperatures and humidity, as observed in Gulu, are known to induce the expression of these genes, thereby enhancing AFB1 production even when fungal biomass remains constant (Magan et al., 2014; Schmidt-Heydt et al., 2017; Cáceres et al., 2020). While CFU levels remained stable, Gulu—being the hottest and most humid site—recorded the highest AFB1 levels, suggesting that climatic conditions stimulated aflatoxin biosynthesis without affecting fungal biomass. Recent studies confirm that even mild heat or moisture stress can upregulate toxin-related genes independently of visible fungal growth (Giorni et al., 2022; Medina et al., 2023). This highlights the need to monitor both fungal load and toxin levels, as the presence of mold does not always predict aflatoxin risk.

Thes biological findings have also have major economic implications. Uganda's grain exports have repeatedly faced rejection due to aflatoxin contamination, with Kenya's 2018 ban costing $121 million and South Sudan's 2023 seizure destroying nearly $270,000 worth of grain (The Independent, 2021a). The demonstrated ability of NaMBS + PICS to reduce AFB1 to undetectable levels offers a scalable solution to safeguard both domestic consumption and export markets. Beyond immediate efficacy, this study highlights the novelty of applying SO_2-releasing sheets to cassava storage. While NaMBS is widely used as a food preservative (E220 in the EU, GRAS in the USA), its use in dried root crops is unprecedented. Prior studies in grapes show residual SO_2 remains below legal thresholds (<10 ppm),

suggesting minimal consumer risk (https://www.tessara.co.za/uvasys/). Still, questions remain about organoleptic impacts. Some studies report no significant changes (Ćurko et al., 2021; Mlček et al., 2023), while others report significant changes (Guerrero-Chanivet et al., 2022). This warrants further sensomic evaluation in cassava, alongside cost-benefit analysis of deploying NaMBS sheets at scale.

Specifically, it will be necessary to monitor SO_2 concentrations throughout the storage period and to quantify residual levels in the product to confirm compliance with food safety standards. And finally, a cost benefit analysis as well as repeated experiments for consistent results for exploiting this innovation in reducing aflatoxins in storage conditions are required. This study provides the first field-based evidence that NaMBS sheets can control both aflatoxigenic fungi and AFB_1 in cassava chips stored under real-world conditions. While residual SO_2 monitoring, organoleptic testing, and repeated trials remain necessary, the findings establish NaMBS as a promising, scalable intervention for aflatoxin mitigation in staple foods.

Conclusion

The study provides compelling field-based evidence that sodium metabisulphite (NaMBS) sheets, particularly when combined with hermetic PICS bags, offer a highly effective postharvest solution for reducing aflatoxin B1 (AFB1) contamination in cassava under tropical storage conditions. The observed 94–99% reduction in AFB1 levels—including complete suppression below detectable limits in PICS bags—exceeds reductions reported for other interventions such as ozone, irradiation, and lime treatment. Notably, the synergistic interaction between sulfurdioxide vapours and the low-oxygen environment of hermetic storage appears to inhibit both fungal proliferation and toxin biosynthesis. This integrated innovation holds strong potential for improving food safety, safeguarding public health, and enhancing Uganda's credibility in regional and international markets. Importantly, this was the first study to investigate the application of SO_2-releasing sheets specifically for the preservation of cassava chips in field conditions. However, significant critical research questions remain unanswered, including a more detailed elucidation of the biochemical mechanisms through which SO_2 inhibits fungal growth and mycotoxin production. Equally important is the need to investigate the potential effects of this preservative on the nutritional and sensory (organoleptic) qualities of cassava chips. Furthermore, socio-economic analyses are essential to evaluate the cost-effectiveness, farmer acceptability, and scalability of this method under diverse real-world storage scenarios. Taken together,

these future investigations will be pivotal in determining whether this novel approach can be widely adopted to transform aflatoxin management in cassava and other staple crops across sub-Saharan Africa.

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Author Contributions

Elias Oyesigye: Conceptualization, Data collection, curation, Formal analysis, Funding acquisition, Validation, Visualization, writing original draft, Writing review & editing, Resources. Carla Cervini: Conceptualization, Investigation, Supervision, Writing review & editing. George Mahuku: Conceptualization, Supervision, Writing – review & editing. J.B Nkurunungi: Field lay out advise, field supervision, editing. Angel Medina: Conceptualization, Supervision, Writing – review & editing.

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Table 1: Average climatic conditions of experimental sites.

Location	Elevation (m) (± SD)	Av. Rainfall (mm) (± SD)	Av. Relative Humidity (%) (± SD)	Av. Temperature (°C) (± SD)
Gulu	1078±21	1303±43	68±2.11	31.3±0.89
Oyam	1090±32	1135±24	60±1.12	30.7±0.41
Kamuli	1092±24	1448±61	61±2.11	28.4±0.21

Table 2: The effect of NaMBS, type of storage bags, location and their interaction on fungal load (cfu/g) after 30 days of storage.

Variable	Mean (cfu/g ×10^5) without NaMBS	Mean (cfu/g) with NaMBS	df.	F-value	p-value
***Aspergillus* Section**			3	23.1	0.0001**
A. Section *Flavi*	35.7	4.38			
A. Section *Aspergillus*	3.17	3.07			
A. Section *Nigri*	12.6	2.67			
A. Section *Cremei*	1.78	0.5			
District			2	2.80	0.0643
Bag Type			1	18.3	0.0006**
Traditional gunny bag	20.4	3.47			
PICS bag	6.14	1.89			
District × Bag Type			2	0.27	0.7613
NaMBS Treatment	213.3	2.68	1	35.2	0.003**
District × NaMBS			2	1.45	0.2377
Bag Type × NaMBS			1	11.6	0.0009**
District × Bag × NaMBS			2	0.16	0.8522
Model			14	10.45	0.0001
Residual			126	-	-
Total (Model Fit: R^2 = 0.5361, Adj R^2 = 0.4845)			140	-	-

Legend: ** $p < 0.05$ (significant). Variables and (or) interactions that are significant have been bolded for a clear understanding.

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Table 3: Effect of NaMBS treatment, storage bag type, district and their interactions on AFB1 (µg/kg) after 30 days of storage.

Source of Variation	Mean AFB1 (µg/kg) ± SD	df	η^2 (effect Size)	F-value	p-value
District		2	0.164	9.19	0.0002
Gulu	69.3±3.5[a]				
Kamuli	50.3±3.1[b]				
Oyam	67.6±1.1[a]				
Bag type		1	0.413	66.17	0.003
PICS bag	45.9±2.9[a]				
Traditional bag	78.7±2.3[b]				
NaMBS Treatment		1	0.894	794.07	0.001
Without, NaMBS	119±2.2[a]				
With, NaMBS	5.6±2.3[b]				
District × NaMBS		2	0.122	6.53	0.0022
Gulu, without NaMBS	132.1±6.9[b]				
Gulu, with NaMBS	6.43±1.2[a]				
Kamuli, without NaMBS	96.7±9.6[c]				
Kamuli, with NaMBS	3.62±0.5[a]				
Oyam, without NaMBS	128.5±12[b]				
Oyam, with NaMBS	6.63±1.1[a]				
NaMBS × types of storage bag		1	0.243	30.13	0.0001
District × types of storage bag		2	0.009	0.44	0.6436
District × Storage Bag Type × NaMBS		2	0.008	0.39	0.68
Store (Blocking Factor)		2	0.019	0.92	0.4004
Residual (Error Term)		94	-	-	-
Model			0.908		
Total		107		-	-

Means followed by the same letter within a column are not significantly different from each other at the 95% confidence level (Tukey's HSD test). Different letters indicate significant differences among treatment means. Letter assignment is arbitrary and does not imply ranking; it only denotes statistical groupings. η^2 is the effect size analysis.

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Figure 1: Field experiment at one of the whole sale stores in Gulu district (2°51'36.0"N 32°25'48.0"E).

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Figure 2a: Cassava chips in traditional storage bags at the end of 30 days with (A) and without (B) sodium metabisulphite (NaMBS) sheets. Fungal spoilage is evident in (B), in contrast to absence of evidently grown fungi on cassava chips which were stored with NaMBS (A).

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Figure 2b: Cassava chips collected from storage bags after the experiment. Chips stored without NaMBS treatment (C and E), whether in traditional gunny bags or hermetically sealed PICS bags, exhibited mycelial growth. In contrast, chips treated with NaMBS sheets (D) showed little to no visible mycelial growth (F), indicating the effectiveness of the treatment in suppressing fungal contamination.

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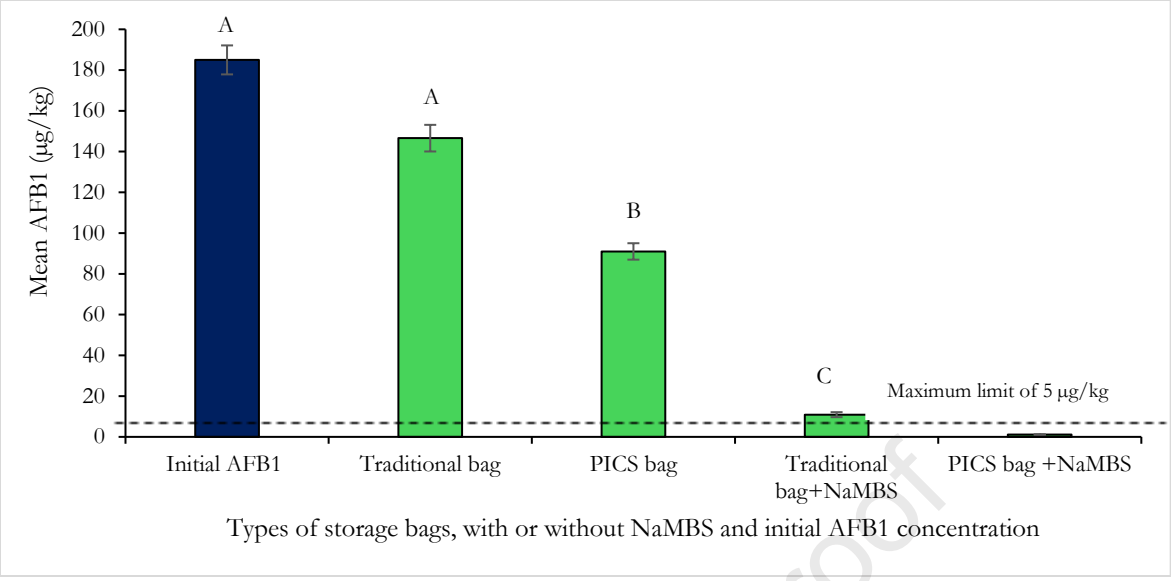


Figure 3: AFB_1 concentrations (µg/kg) in cassava chips stored after a month in traditional bags and hermetic PICS bag, with and without NaMBS sheet. Letters: A, B, C indicate significant differences between treatments at ($P < 0.05$). The P value of the model =0.0001. Since cassava is not regulated by the European Union, the maximum limit of 5 µg/kg is set for a close related product (maize) placed on market for human consumption. The bar in deep blue colour represents the initial AFB1 concentration.

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1. Sodium metabisulphite sheets reduced Aflatoxin B1 by 99.9%.
2. Fungal load dropped significantly with treatment of Sodium metabisulphite sheets
3. Purdue Improved Crop Storage bags outperformed traditional storage bags.
4. Climate in traders stores significantly influenced aflatoxin accumulation.
5. Sodium metabisulphite offers scalable toxin control.

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Declaration of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Sincerely,
(on behalf of all authors)



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Field experiment in Ugandan cassava stores reveals that slow release SO2 sheets suppress aflatoxigenic fungi, resulting in undetectable aflatoxin B1 levels

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