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First evidence of SO_2-releasing bags controlling fungal growth, aflatoxins and cyclopiazonic acid contamination in unshelled peanuts.

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Running head title: SO_2 bags for fungal and mycotoxin control.

Impact statement: This study provides the first evidence that SO_2-releasing storage bags effectively control fungal growth and eliminate mycotoxin contamination in unshelled peanuts under poor storage conditions. Given the global importance of peanuts as a climate-resilient crop and cash crop, particularly in low- and middle-income countries, this application offers a practical and scalable solution to reduce post-harvest losses, enhance food safety, and improve market access. The findings support the adoption of SO_2-releasing bags to protect public health and strengthen food supply chains in regions most vulnerable to mycotoxin contamination.

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Abstract

Peanuts (also known as groundnuts; *Arachis hypogaea* L.) are a globally significant cash crop but are highly susceptible to fungal contamination, particularly by *Aspergillus* section *Flavi*, which can contaminate the product with mycotoxins, including aflatoxins. This contamination poses serious food safety concerns, especially in low- and middle-income countries, limiting access to international markets. This study assessed the efficacy of slow-release SO_2-bags compared to plastic bags (control) in reducing fungal growth and mycotoxin contamination in stored unshelled peanuts. Naturally contaminated and *Aspergillus flavus*-inoculated peanuts were stored at water activity (a_w) levels of 0.85 and 0.95 for 15 days at 25°C. Fungal populations were monitored, aflatoxins and cyclopiazonic acid were analysed using LC-MS/MS qTRAP. SO_2-releasing bags completely inhibited fungal growth in naturally contaminated peanuts and significantly reduced *A. flavus* population with effectiveness dependent on water activity. Additionally, SO_2-releasing bags suppressed mycotoxin production. This study demonstrates, for the first time, the effectiveness of SO_2-releasing bags in preventing fungal spoilage and mycotoxin contamination in post-harvest peanuts, offering a promising solution for enhancing peanut safety and quality.

Keywords: groundnuts, mycotoxins, *Aspergillus flavus*, applied mycology

Introduction

Peanuts is a cash crop and an excellent edible oil-seed legume (Akram et al., 2018). The world annual production of unshelled peanuts was 54M tonnes in 2023, with over 90% of peanuts produced in developing countries (FAOSTAT, 2023; FAO 2002). Peanuts are a valuable crop nutritionally as they are a very good source of vegetable protein and contain a significant amount of unsaturated fats and magnesium (Arya et al., 2016). They have been associated with various positive health effects (Parilli-Moser et al., 2022). The high levels of proteins in peanuts make them suitable to produce Ready-to-Eat Therapeutic Food (Diouf et al., 2016) which are used to support the eradication of malnutrition amongst children in developing countries (Schoonees et al., 2019).

Peanuts production is faced with several constraints including drought stress, fungal, bacterial, viral diseases, and insect pests (Kortei et al., 2022). Among these, colonisation of peanuts by mycotoxins-producing fungi within the *Aspergillus* section *Flavi* represents a food safety problem resulting in significant health and economic losses due to concentration of mycotoxins higher than maximum limits established by food safety authorities (Acur et al., 2020; Sultan et al., 2010). *Aspergillus flavus*, an opportunistic plant pathogen, is considered the main representative species within the *A.* section *Flavi* group. This species is able to produce aflatoxins (AFs) and cyclopiazonic acid (CPA). Among AFs, aflatoxin B_1 (AFB_1) is classified as a Group I human carcinogen according to the International Agency for Research on Cancer being associated with hepatocellular carcinoma (IARC, 1993). CPA is a neurotoxic compound often co-occurring with AFs, being produced by *A. flavus* and other species within the *Aspergillus* section *Flavi* (Ostry et al., 2018).

In many developing regions, inadequate agricultural practices and poor storage conditions exacerbate aflatoxin accumulation from harvest through to the final product (Pitt et al., 2013; Cervini et al., 2022), prompting many countries to establish maximum regulatory limits for

mycotoxins in food products, including peanuts and peanut-based products (Van Egmond et al., 2007). For instance, for raw peanuts entering the European Union (EU), the level of total AFs and AFB_1 must be less than 4 and 2 μg/kg, respectively (Commission Regulation (EU) 2023/915). On the contrary, no maximum regulatory limits exist for CPA in food products. Nevertheless, these restrictive levels are often impossible to achieve, especially in low- and middle-income countries, where lack of awareness of mycotoxins, poor regulatory framework and lack of good agricultural and storage practices are predominant conditions (Bereka et al., 2022; Bila et al., 2025; Tadesse et al., 2025). In many African countries, existing post-harvest interventions, such as the use of Purdue Improved Crop Storage bags, remain costly and inconsistently adopted (Nakoma Ngoma et al., 2025; Kamilu et al., 2025) despite there is evidence for their use to be more profitable for small-scale farmers as compared to traditional storage technologies, even in the short-term (Aker et al., 2023). As a result, significant food losses and exposure to these hazardous compounds continue to occur. Therefore, it is essential to continue developing and promoting the adoption of other effective mitigation strategies including post-harvest and storage technologies.

Amongst these, sulphur dioxide (SO_2) and its sulphites (e.g. sodium and potassium sulphites) have been commonly used in a wide range of foods including fruits, vegetables, fruit juices and concentrates, syrups, wines and jams and to a lesser extent prawns, fish, minced meats, sausages, and mushrooms. In Europe, their maximum level is regulated by Commission Regulation (EU) No 1129/2011 where they are listed with E-numbers from E220 to E228 (EU, 1129/2011). They are known to exert antimicrobial and fungistatic actions on food during storage. Sodium metabisulphite (NaMBS) is often used as a post-harvest antifungal agent for table grapes' storage, during processing of raisins and grape juice, and during grape crushing for wine production (Magan and Aldred, 2007; Prabhakar and Reddy, 2000). NaMBS pads which release SO_2 have been widely used to control fungal spoilage caused by *Botrytis cinerea*

(grey mould) on grapes during storage (Domingues et al., 2018; Habib et al., 2021; Morgan et al., 2019; Youssef et al., 2020). They are composed of laminated plastic membranes containing NaMBS which is encapsulated in a wax matrix. These pads release SO_2 through a dual-phase mechanism: an initial burst within the first 48 hours provides a fungicidal effect, followed by a continuous low-level release that maintains a fungistatic action. The release of SO_2 is triggered by water vapour generated from the commodity's respiration and high relative humidity, which reacts with the NaMBS (Tessara, Berrysis, 2021). Despite their proven effectiveness in high-value horticultural commodities, the potential application of slow-release SO_2 for controlling fungal growth and mycotoxin contamination in raw commodities such as peanuts remains largely unexplored. Therefore, this study aimed to investigate whether NaMBS pads adapted into small bags which slowly release SO_2 could effectively control fungal spoilage, *A. flavus* colonisation and associated mycotoxin production in unshelled peanuts, particularly under high water activity conditions known to favour contamination.

Materials and methods

Peanut samples

Unshelled peanuts (*Arachis hypogaea* L.) from a local market in the UK were used as naturally contaminated peanuts for this study. The water activity (a_w) was 0.35 and they were kept at 4ºC in resealable zip-lock polyethene bags until use. For this experiment, both naturally contaminated (non-sterilised control group) and *A. flavus* inoculated unshelled peanuts were used.

Fungal isolate and inoculum production

The aflatoxin-producing *A. flavus* isolate (P40-2), previously isolated from Ethiopian peanut soil samples, was used to prepare the *A. flavus*-inoculated peanut samples. This isolate was characterised using a polyphasic approach (Table S1) following Frisvad et al. (2019) and is preserved at −20ºC in a glycerol-water solution (30:70, v/v) in the mycotheque of the Magan Centre of Applied Mycology, Cranfield University.

For inoculum preparation, the isolate was subcultured (3 µl centrally inoculated) on Malt Extract Agar (MEA, Oxoid Ltd., Fisher Scientific, Loughborough, UK) supplemented with 0.05% chloramphenicol at 30ºC for 7 days of growth in the dark. At the end of the incubation period, the surface of the fungal colony was gently scraped with a sterile spatula, and spores were transferred to sterile distilled water containing Tween80 (0.005%). The fungal spore concentration was adjusted to 1 x 10^5 spores/ml using a Thoma haemocytometer counting chamber and 1 ml of the determined concentration was used as inoculum of *A. flavus* and added to the unshelled peanuts.

SO_2-releasing bags

Slow-release laminated SO_2 pads (Tessara PTY, Ltd, Cape Town, South Africa) were used in this experiment to evaluate their effectiveness in controlling fungal growth and mycotoxin

production. The pads were converted into sealable mini-bags (11 cm × 15 cm) and stored at 4ºC until use. These pads are made of sodium metabisulphite (NaMBS; Na_2SO_5) encapsulated in a wax matrix. Water vapour that originates from commodity respiration reacts with NaMBS to release SO_2, which acts as a fumigator, according to the following reaction:

$$Na_2SO_5 + H_2O \rightarrow 2NaHSO_3 \text{ (intermediate product)} \rightarrow Na_2SO_3 + SO_2 \text{ (gas)} + H_2O$$

The SO_2 pads used in this experiment followed a dual-release principle, characterised by an initial rapid phase in which concentrations exceeding 100 ppm were released within the first 24 hours, followed by a sustained phase with lower emissions of approximately 3–5 ppm maintained for around 60 days. Plastic sealable bags of the same size served as controls.

Peanut water activity adjustment and experimental design

A water adsorption curve (Figure S1) was initially developed by adding different volumes (0 – 1.8 ml) of distilled water to 6 g of sub-samples (x 3 replicates) of unshelled peanuts in 50 ml Duran glass bottles. These were shaken vigorously and stored overnight at 4ºC to allow water adsorption. Samples were then placed at room temperature to equilibrate for 30 minutes, and the a_w was determined using the portable HygroPalm HP23-A device (RoTronic, Bassersdorf, Switzerland). Water adsorption curve was determined by plotting the amount of added water (ml) against the a_w values measured. This curve was used to determine the exact amount of water needed to reach the target a_w levels set for the experiments. Per each a_w, 600 g of peanuts were adjusted to target levels of 0.85 and 0.95 by adding sterile water based on the adsorption curve. Half of them (300 g) were kept as naturally contaminated and the other half (300 g) were inoculated with *A. flavus* and then split into plastic bags (3 replicates, 50 g each) and SO_2-releasing bags (3 replicates, 50 g each) and incubated at 25ºC for 15 days (Figure S2). For the *A. flavus*–inoculated peanuts, the total amount of added water was reduced by 1 ml to account for the volume of the *A. flavus* inoculum (1×10^5 spores ml^{-1}). The samples were kept at 4°C

overnight for equilibration. 0.85 and 0.95 a_w were chosen to simulate moderately and highly unsafe conditions of storage which are conducive for aflatoxin production.

Quantification of the fungal community and *Aspergillus flavus* populations

The efficacy of SO_2 releasing bags was tested on total fungal populations following the serial dilution method described by Pitt and Hocking (2009). Two grams of unshelled peanuts from each of the three replicates, were weighed and transferred into sterile stomacher bags containing 18 ml of sterile 0.1% peptone water to obtain initial 10^{-1} dilution. After 30 minutes of soaking, samples were placed into the stomacher blender (Lab-Blender 400; Seward Medical, UK) and homogenised for 4 minutes. Afterwards, 10-fold serial dilutions were prepared and 100 µl were plated with a sterile Drigalski spreader in triplicates on Dichloran Rose-Bengal Chloramphenicol (DRBC, Oxoid Ltd., Fisher Scientific, Loughborough, UK). Inoculated plates were incubated at 25ºC for 5 days after which the Colony Forming Units (CFUs) were determined. Total colonies growing on the plates in a range of 5 to 50 colonies (Al-Jaza et al., 2022) were counted using a colony counter (Gallenkamp CNW 325 010X Colony Counter Lab) and their numbers expressed as Log_{10} of CFUs per grams of dry mass of sample (Log_{10} CFU g^{-1} dry mass). Moreover, *A. flavus* colonies were specifically enumerated, alongside the total fungal counts, by comparing their morphological characteristics with those of the reference isolate (P40-2) used in this study.

Multi-mycotoxins analysis by LC-MS/MS

Naturally contaminated and *A. flavus*-inoculated unshelled peanuts were stored at -20ºC after the experiment and then dried at 60ºC for 24 hours in an oven (Gen Lab Ltd., Model: OU/100/F). Each treatment and its biological replicates were individually ground into a fine powder using a Waring commercial blender at maximum speed for 30–35 seconds and stored at -20ºC until analysis.

Mycotoxin extraction followed the method reported by Crosta et al. (2025). In total, 72 samples were analysed, including three technical replicates per treatment. For LC-MS/MS analysis, 5 µl of each sample was injected into the sampling port of the qTRAP 6500+ LC-MS/MS system (SCIEX instrument, Foster city, CA, USA) equipped with an electrospray ionisation (ESI). Data acquisition was conducted with Analyst® Data Acquisition version 1.6.3, and quantification through MultiQuant™ version 3.0.3. Quantification was performed using a standard curve from standard stock solutions of aflatoxins B_1, B_2, G_1, G_2 (AFB_1, AFB_2, AFG_1, AFG_2) and cyclopiazonic acid (CPA) provided by Romer labs, Austria. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on (Malachová *et al.*, 2014) and they are included in table S2.

Data analysis

Statistical analyses were performed using Statistica (version 14; SAS Institute Inc., Cary, NC, USA). The analysis of variance (ANOVA) was conducted to evaluate the effect of storage bag type (plastic bags vs SO_2-releasing bags), water activity (0.85 vs 0.95 a_w), and contamination status (natural contamination vs inoculation), as well as their interactions on total fungal count, *A. flavus* count and mycotoxin production. Significant differences between means were determined using Tukey's HSD post-hoc test ($p \leq 0.05$). Graphs were generated using GraphPad Prism (version 8.0.2; GraphPad Software, Inc., San Diego CA, USA).

Results and discussion

Fungal spoilage of peanuts is initiated during the pre-harvest phase, if favourable conditions (e.g. insect damage, pods breakage, abundant rainfall) are encountered. Occurrence of toxigenic fungal species may lead to the production of mycotoxins that, if no mitigation strategies are applied, will accumulate during post-harvest processing and storage resulting in mycotoxins contamination exceeding the legislative limits (Cervini et al., 2022).

A combination of good agricultural practices, pre-harvest and post-harvest controlling strategies would mitigate fungal growth and mycotoxins occurrence in peanuts. In the present study we have shown, for the first time, that NaMBS immobilised storage bags which release SO_2 may provide a possible solution for storage of unshelled peanuts. Indeed, such food preservation treatment has been mainly used to control post-harvest decay by *B. cinerea* rot in grapes during post-harvest stages. Usually, the NaMBS immobilised sheets are placed over, but not touching the grapes during shipping to allow release of SO_2 and to avoid or limit damage. The amount of SO_2 released is affected by the temperature; therefore, the efficiency of these sheets depends on good temperature chain management (Ahmed et al., 2018; Habib et al., 2021).

Total fungal count and *A. flavus* count were significantly affected ($p < 0.001$) by a_w, storage bag type, contamination status and their three-way interaction (Table S3). In naturally contaminated peanuts stored in SO_2-releasing bags, total fungal growth was completely inhibited at both a_w levels. In contrast, naturally contaminated peanuts stored in plastic bags exhibited measurable fungal growth, with significantly higher counts at 0.95 a_w ($\log_{10}$ 7.43 ± 0.11 CFU g^{-1}) compared with 0.85 a_w ($\log_{10}$ 2.95 ± 0.08 CFU g^{-1}). In *A. flavus*-inoculated peanuts, storage in SO_2-releasing bags resulted in a significant reduction in total fungal counts compared with plastic bags with the efficacy varying according to a_w (Figure 1A).

A similar pattern was observed for *A. flavus* counts. In naturally contaminated peanuts, *A. flavus* growth was significantly inhibited when samples were stored in SO_2-releasing bags, while in plastic bags *A. flavus* was detected only at 0.95 a_w ($\log_{10}$ 6.52 ± 0.74 CFU g^{-1}). In inoculated peanuts, SO_2-releasing bags significantly reduced viable *A. flavus* counts compared with plastic bags, with higher counts consistently observed at the higher a_w level (Figure 1B). These data suggested that at high fungal loads, favoured by high a_w conditions (0.95 a_w), the immobilised preservative bag system, may result in reduction of fungal populations with *A.*

flavus being more resilient to it at higher a_w (0.95 a_w) than at moderately dry conditions (0.85 a_w). This finding was also supported by visual observations: no visible fungal growth was detected in peanuts stored in SO_2-releasing bags, whereas control samples showed moderate spoilage at 0.85 and heavy spoilage at 0.95 a_w (Figure 2). Water activity and the type of storage bags significantly influenced mycotoxin production ($p < 0.001$, Table S4). With respect to mycotoxins, in our study AFB_1, AFB_2 and CPA were mainly detected at 0.95 a_w in both naturally contaminated and *A. flavus*-inoculated peanuts while no mycotoxins were detected at 0.85 a_w, in control samples, at 25ºC (Table 1). The inhibitory effect of low a_w on mycotoxin production in peanuts at 25ºC has been previously documented. Garcia-Cela et al. (2020), in a study investigating CO_2 production as an indicator of *A. flavus* colonisation and associated AFs and CPA contamination in shelled peanuts, reported that AFB_1, AFB_2 and CPA were not detected at 0.90 a_w after 7 days of incubation at 25ºC, whereas they were detected at 0.95 a_w. Liu et al. (2017) also found that AFB_1 was absent at 0.85 a_w but it could be detected at water activities above 0.90. Also, Abdel-Hadi et al. (2010) observed higher AFB_1 at 0.90-0.95 a_w but couldn't detect any at 0.85 a_w, consistent with our findings. Previously, Passone et al. (2010) found that AFB_1 was produced at 0.85-0.88 a_w but only after 1-month storage.

The application of storage bags which released SO_2 reduced the levels of AFs and CPA below the LOD and LOQ in both naturally contaminated peanuts and *A. flavus*-inoculated samples (Table 1). No previous studies have examined the use of SO_2 for minimising mycotoxins contamination of raw commodities such as unshelled peanuts. In a study by Al-Jaza et al. (2022) slow release SO_2 laminated sheets (40 and 70% NaMBS) were tested to control *A. flavus* spoilage and AFB_1 contamination in *A. flavus*-inoculated whole red stored chillies. They showed that AFB_1 was completely inhibited using both concentrations of NaMBS immobilised on the laminated sheets.

Conclusions

The results of this study suggest that slow-releasing SO_2 bags may represent a promising mitigation strategy for reducing fungal spoilage and mycotoxin contamination during the storage of unshelled peanuts. To confirm their effectiveness, further pilot-scale trials using larger quantities of peanuts are now warranted. These studies should also include shelled peanuts, as they are the preferred form of storage in many African countries.

In addition, fundamental research is needed to assess the safety of this intervention, particularly the potential formation of secondary metabolites resulting from interactions between SO_2 and mycotoxins, which appear to be effectively suppressed by this treatment. It is also essential to evaluate the impact of SO_2 application on the nutritional quality and palatability of peanuts to ensure that this approach supports both food safety and nutrition security in relevant contexts.

Dedication

The authors dedicate this paper to the memory of Prof. Naresh Magan, who initiated and led this work at Cranfield University before his untimely passing.

Authors contribution declaration

Carla Cervini: writing – original draft, supervision, conceptualization, methodology, writing – review and editing, data curation, formal analysis, investigation; Vaibhav Verma: writing – original draft, investigation; Carol Verheecke-Vaessen: funding acquisition, writing – original draft, supervision, methodology, formal analysis; Angel Medina: funding acquisition, resources, methodology; Naresh Magan: funding acquisition, conceptualization, methodology.

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Conflict of interest

The authors declare that there is no conflict of interest.

Data availability statement

All data is incorporated into the article.

References

Abdel-Hadi A, Carter D, Magan N. Temporal monitoring of the *nor-1* (*aflD*) gene of *Aspergillus flavus* in relation to aflatoxin B_1 production during storage of peanuts under different water activity levels. *J Appl Microbiol.* 2010;109(6):1914–1922. https://doi.org/10.1111/J.1365-2672.2010.04820.X

Acur A, Arias RS, Odongo S, *et al.* Genetic diversity of aflatoxin-producing *Aspergillus flavus* isolated from selected groundnut growing agro-ecological zones of Uganda. *BMC Microbiol.* 2020;20:46. https://doi.org/10.1186/s12866-020-01924-2

Ahmed S, Roberto SR, Domingues AR, *et al.* Effects of different sulfur dioxide pads on *Botrytis* mold in 'Italia' table grapes under cold storage. *Horticulturae.* 2018;4(4):29. https://doi.org/10.3390/horticulturae4040029

Aker JC, Dillon B, Welch CJ. Demand, supply and long-term adoption: Evidence from a storage technology in West Africa. *J Dev Econ.* 2023;165:103129. https://doi.org/10.1016/j.jdeveco.2023.103129

Akram NA, Shafiq F, Ashraf M. Peanut (*Arachis hypogaea* L.): A prospective legume crop to offer multiple health benefits under changing climate. *Compr Rev Food Sci Food Saf.* 2018;17(6):1325–1338. https://doi.org/10.1111/1541-4337.12383

Al-Jaza D, Medina A, Magan N. Efficacy of sodium metabisulphite for control of *Aspergillus flavus* and aflatoxin B_1 contamination *in vitro* and in chilli powder and whole red chillies. *Food Control.* 2022;135:108786. https://doi.org/10.1016/J.FOODCONT.2021.108786

Arya SS, Salve AR, Chauhan S. Peanuts as functional food: A review. *J Food Sci Technol.* 2016;53(1):31–41. https://doi.org/10.1007/s13197-015-2007-9

304 Bereka TY, Kuyu CG, Tolera KD, Addis EM. Current postharvest practices and aflatoxin
305 contamination awareness amongst maize producers in Jimma Zone, Southwest of Ethiopia.
306 *World Mycotoxin J.* 2022;15(1):35–44. https://doi.org/10.3920/WMJ2020.2642

307 Bila J, Macuamule C, Bombe A, *et al.* Assessment of farmers' knowledge and attitudes toward
308 fungi and mycotoxin contamination in staple crops in southern Mozambique. *Front Sustain*
309 *Food Syst.* 2025;9:1622532. https://doi.org/10.3389/fsufs.2025.1622532

310 Parilli-Moser I, Hurtado-Barroso S, Guasch-Ferré M, Lamuela-Raventos RM. Effect of peanut
311 consumption on cardiovascular risk factors: A randomized clinical trial and meta-analysis.
312 *Front Nutr.* 2022;1:853378. https://doi.org/10.3389/fnut.2022.853378

313 Cervini C, Verheecke-Vaessen C, He T, *et al.* Improvements within the peanut production
314 chain to minimize aflatoxins contamination: An Ethiopian case study. *Food Control.*
315 2022;136:108622. https://doi.org/10.1016/J.FOODCONT.2021.108622

316 Crosta M, Cervini C, Medina A, Battilani P. Impact of simulated climate change conditions on
317 *Aspergillus flavus* biocontrol effectiveness in peanut-based medium and peanut seeds. *Int J*
318 *Food Microbiol.* 2025;428:110981. https://doi.org/10.1016/j.ijfoodmicro.2024.110981

319 Diouf A, Badiane A, Manga NM, *et al.* Daily consumption of ready-to-use peanut-based
320 therapeutic food increased fat-free mass, improved anemic status but had no impact on the zinc
321 status of people living with HIV/AIDS: A randomized controlled trial. *BMC Public Health.*
322 2016;16:1. https://doi.org/10.1186/s12889-015-2639-8

323 Domingues AR, Roberto SR, Ahmed S, *et al.* Postharvest techniques to prevent the incidence
324 of *Botrytis* mold of 'BRS Vitoria' seedless grape under cold storage. *Horticulturae.*
325 2018;4(3):17. https://doi.org/10.3390/horticulturae4030017

European Food Safety Authority (EFSA), Arcella D, Gómez Ruiz JA. Use of cut-off values on the limits of quantification reported in datasets used to estimate dietary exposure to chemical contaminants. *EFSA Supporting Publications.* 2018;15:1452E.

European Commission. Commission Regulation (EU) No 2023/915 of 25 April 2023 on maximum levels for certain contaminants in foodstuffs and repealing Regulation (EC) No 1881/2006. *Off J Eur Union.* 2023;L119.

European Commission. Commission Regulation (EC) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 by establishing a Union list of food additives. *Off J Eur Union.* 2011;L295.

FAO. Groundnut, post-harvest operations. *FAO.* 2002. https://www.fao.org/fileadmin/user_upload/inpho/docs/Post_Harvest_Compendium_-_Groundnut.pdf

FAOSTAT. Groundnut world production. *FAOSTAT.* 2023. http://www.fao.org/faostat/en/#home (Accessed 06 August 2025).

Frisvad JC, Hubka V, Ezekiel CN, *et al.* Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins and other mycotoxins. *Stud Mycol.* 2019;93:1–63. https://doi.org/10.1016/j.simyco.2018.06.001

Garcia-Cela E, Gari Sanchez FJ, Sulyok M, *et al.* Carbon dioxide production as an indicator of *Aspergillus flavus* colonisation and aflatoxins/cyclopiazonic acid contamination in shelled peanuts stored under different interacting abiotic factors. *Fungal Biol.* 2020;124:1–7. https://doi.org/10.1016/j.funbio.2019.10.003

Habib W, Khalil J, Mincuzzi A, *et al.* Fungal pathogens associated with harvested table grapes in Lebanon, and characterization of the mycotoxigenic genera. *Phytopathol Mediterr.* 2021;60:427–439. https://doi.org/10.36253/phyto-12946

International Agency for Research on Cancer (IARC). *Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins.* Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 56. Lyon: IARC; 1993.

Kamilu M, Muhammad Abdul I, Kongvum WE, *et al.* Assessment of the use and determinants of adoption of PICS bag technology among grain merchants in Dawanau Market, Kano State. *Southeast Asian J Agric Allied Sci.* 2025;4(1):44–57. https://doi.org/10.63943/sajaas.vol4iss1art41pp44-57

Kortei NK, Tetteh RA, Wiafe-Kwagyan M, *et al.* Mycobiota profile, phenology, and potential toxicogenic and pathogenic species associated with stored groundnuts (*Arachis hypogaea* L.) from the Volta Region, Ghana. *Food Sci Nutr.* 2022;10:888–902. https://doi.org/10.1002/fsn3.2719

Liu X, Guan X, Xing F, *et al.* Effect of water activity and temperature on the growth of *Aspergillus flavus*, the expression of aflatoxin biosynthetic genes and aflatoxin production in shelled peanuts. *Food Control.* 2017;82:325–332. https://doi.org/10.1016/J.FOODCONT.2017.07.012

Magan N, Aldred D. Post-harvest control strategies: Minimizing mycotoxins in the food chain. *Int J Food Microbiol.* 2007;119:131–139. https://doi.org/10.1016/J.IJFOODMICRO.2007.07.034

368 Malachová A, Sulyok M, Beltrán E, *et al.* Optimization and validation of a quantitative LC–
369 MS/MS method covering 295 bacterial and fungal metabolites including all regulated
370 mycotoxins in four model food matrices. *J Chromatogr A.* 2014;1362:145–156.
371 https://doi.org/10.1016/J.CHROMA.2014.08.037

372 Morgan SC, Tantikachornkiat M, Scholl CM, *et al.* The effect of sulfur dioxide addition at
373 crush on the fungal and bacterial communities and the sensory attributes of Pinot gris wines.
374 *Int J Food Microbiol.* 2019;290:1–14. https://doi.org/10.1016/J.IJFOODMICRO.2018.09.020

375 Nakoma Ngoma T, Leslie JF, Katengeza SP, *et al.* Barriers to hermetic bag adoption among
376 smallholder farmers in Malawi. *Sustainability.* 2025;17(3):1231.
377 https://doi.org/10.3390/su17031231

378 Ostry V, Toman J, Grosse Y, Malir F. Cyclopiazonic acid: 50th anniversary of its discovery.
379 *World Mycotoxin J.* 2018;11(1):135–148. https://doi.org/10.3920/WMJ2017.2243

380 Passone MA, Rosso LC, Ciancio A, Etcheverry M. Detection and quantification of *Aspergillus*
381 section *Flavi* spp. in stored peanuts by real-time PCR of *nor-1* gene, and effects of storage
382 conditions on aflatoxin production. *Int J Food Microbiol.* 2010;138:276–281.
383 https://doi.org/10.1016/J.IJFOODMICRO.2010.01.003

384 Pitt J, Hocking A. *Fungi and food spoilage.* 3rd ed. London: Blackie Academic and
385 Professional; 2009. http://dx.doi.org/10.1007/978-0-387-92207-2

386 Pitt J, Taniwaki M, Cole M. Mycotoxin production in major crops as influenced by growing,
387 harvesting, storage and processing, with emphasis on the achievement of food safety
388 objectives. *Food Control.* 2013;32:205–215. https://doi.org/10.1016/j.foodcont.2012.11.023

389 Prabhakar K, Reddy KS. Permitted preservatives – sulphur dioxide. In: Batt CA, Tortorello
390 ML, editors. *Encyclopedia of Food Microbiology.* Vol. 3. London: Academic Press; 2000.
391 p.1750–1754.

392 Schoonees A, Lombard MJ, Musekiwa A, *et al.* Ready-to-use therapeutic food (RUTF) for
393 home-based nutritional rehabilitation of severe acute malnutrition in children aged six months
394 to five years. *Cochrane Database Syst Rev.* 2019;2019.
395 https://doi.org/10.1002/14651858.cd009000.pub3

396 Sultan Y, Magan N. Mycotoxigenic fungi in peanuts from different geographic regions of
397 Egypt. *Mycotoxin Res.* 2010;26:133–140. https://doi.org/10.1007/s12550-010-0048-5

398 Tadesse K, Fenta L, Habtamu A, *et al.* Assessments of farmers' knowledge and management
399 of mycotoxin contamination in stored sorghum grains in North West Ethiopia. *Mycotoxin Res.*
400 2025;41:485–497. https://doi.org/10.1007/s12550-025-00598-x

401 Tessara. *Berrisys, post-harvest innovation.* 2021. https://www.berriesza.co.za/wp-
402 content/uploads/2021/06/6-Berries-ZA-Presentation-Tessara-28-May-2021-Final.pdf

403 Van Egmond HP, Schothorst RC, Jonker MA. Regulations relating to mycotoxins in food:
404 Perspectives in a global and European context. *Anal Bioanal Chem.* 2007;389:147–157.
405 https://doi.org/10.1007/s00216-007-1317-9

406 Youssef K, Junior OJC, Mühlbeier DT, Roberto SR. Sulphur dioxide pads can reduce gray
407 mold while maintaining the quality of clamshell-packaged 'BRS Nubia' seeded table grapes
408 grown under protected cultivation. *Horticulturae.* 2020;**6**(2):20.
409 https://doi.org/10.3390/HORTICULTURAE6020020

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Figure 1. Effect of SO_2-releasing bags on A) total fungal population (log_{10} CFU g^{-1} dry peanuts) and B) *A. flavus* counts (log_{10} CFU g^{-1} dry peanuts) at 0.85 and 0.95 a_w in naturally contaminated and *A. flavus*-inoculated unshelled peanuts assessed on DRBC after 5 days of incubation at 25°C. Different letters within each panel indicate significant differences (Tukey test, $p \leq 0.05$).

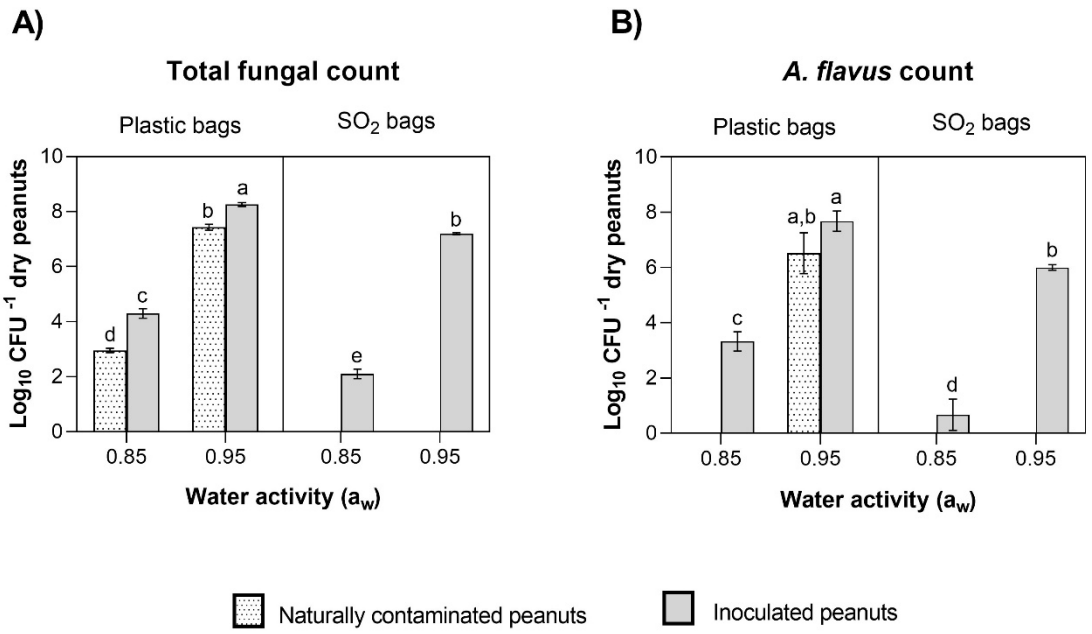
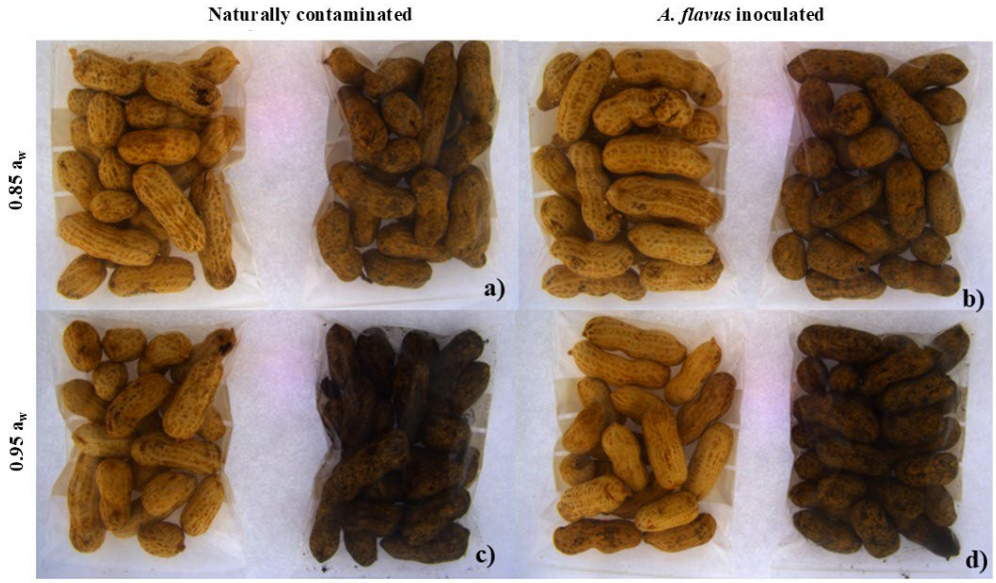


Figure 2. Visual appearance of unshelled peanuts after 15 days of incubation at 25°C. The pictures represent: 0.85 a_w (a) naturally contaminated and (b) *A. flavus*-inoculated; 0.95 a_w (c) naturally contaminated and (d) *A. flavus*-inoculated peanuts. In each panel, peanuts stored in SO_2-releasing bags are shown on the left, and those stored in plastic bags (control) on the right.



433 **Table 1.** Mean values (ng g^{-1}) of aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2) and cyclopiazonic acid (CPA) in naturally contaminated and *A. flavus*

434 inoculated unshelled peanuts. Different letters indicate significant difference (Tukey test, $p \leq 0.05$) within each mycotoxin.

Contamination	a_w	AFB_1 (ng g^{-1})		AFB_2 (ng g^{-1})		CPA (ng g^{-1})	
		Plastic bags	SO_2 bags	Plastic bags	SO_2 bags	Plastic bags	SO_2 bags
Naturally contaminated peanuts	0.85	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	0.95	94.94 ± 54.81 [b]	< LOD	83.70 ± 16.04 [a]	< LOD	112.71 ± 65.12 [b]	< LOD
A. flavus inoculated peanuts	0.85	1.58 ± 1.37 [b]	< LOD	< LOD	< LOD	< LOD	< LOD
	0.95	585.4 ± 186.39 [a]	< LOD	113.53 ± 33.83 [a]	< LOD	699.29 ± 283.15 [a]	< LOD

435 LOD: Limit of detection.

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First evidence of SO2-releasing bags controlling fungal growth, aflatoxins and cyclopiazonic acid contamination in unshelled peanuts

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