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Rapid Detection of Aspergillus Contamination in Dried Figs Using Electronic Nose Technology and VOC Profiling

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Abstract

The presence of toxigenic *Aspergillus* strains in dried figs and the resulting risk of mycotoxin accumulation represent a major challenge for food safety and commercialisation. This study assessed the performance of an electronic nose (E-nose) for detecting *Aspergillus* spp. contamination in dried figs through changes in volatile organic compound (VOC) profiles. Nine *Aspergillus* strains, including *A. flavus*, *A. niger*, and *A. awamori*, were inoculated into separate dried fig batches, with VOCs characterized by GC-MS and sensor responses recorded. A strong correlation was observed between sensor signals and the distinct aromatic profiles associated with each *Aspergillus* species. For multi-class species discrimination, Linear Discriminant Analysis (LDA) and Partial Least Squares-Discriminant Analysis (PLS-DA) achieved accuracies of 84.5% and 79.8%, respectively. Furthermore, a simplified binary LDA model reached a 96.6% accuracy rate in distinguishing healthy from contaminated samples, a result supported by high PLS-DA Variable Importance in Projection (VIP) scores for several sensors. These findings highlight the potential of E-nose technology, coupled with chemometric modelling, as a robust and non-destructive solution for the early detection of *Aspergillus* contamination. This approach offers a promising solution for enhancing food safety and quality control in the dried fig industry, enabling early detection and mitigation of fungal and mycotoxigenic contamination.

1. Introduction

The consumption of figs (*Ficus carica* L.), both fresh and dried, has increased significantly in recent years. Turkey is the world's leading producer, while Spain ranks first in Europe, with a production of 43,500 tonnes in 2022 (FAOSTAT, 2024; Isa et al., 2020). Due to their high perishability and short shelf life of only 1–2 days at room temperature (Desa et al., 2019), figs are predominantly marketed in dried form. Drying usually occurs under natural sunlight, starting with fruit ripening and senescence on the tree and continuing after they have fallen to the ground (Flaishman et al., 2008). After harvesting, figs are further sun-dried, arranged in layers on the ground, until reaching moisture levels of approximately 26% or lower (Galván et al., 2022). This slow sun-drying process, carried out without controlled temperature or humidity, promotes the growth of a diverse fungal population, including moulds of the genera *Cladosporium*, *Fusarium*, *Penicillium* and *Aspergillus*, with *Aspergillus* being predominant (Galván et al., 2021). Several *Aspergillus* species, under favourable temperature and water activity conditions, can produce mycotoxins. Aflatoxins and ochratoxin A, produced by members of *Aspergillus* section *Flavi* and section *Nigri*, are the main mycotoxins found in dried figs (Aldars-García et al., 2018; Perrone et al., 2014). Their control relies on industrial screening using UV light, which allows for the removal of fruit contaminated with visible aflatoxins, although it does not detect ochratoxin A or low levels of aflatoxins (Abbas et al., 2004). According to Commission Regulation (EU) 2023/915 of 25 April 2023, the maximum limits are 6 µg/kg for aflatoxin B1 and 10 µg/kg for total aflatoxins. In the case of ochratoxin A, the maximum permitted level is 8 µg/kg.

To mitigate these risks, control strategies should focus on detecting fungal growth before secondary metabolite production.

Several non-destructive spectrometric techniques, such as near-infrared (NIR), mid-infrared (MIR), and Raman spectroscopy, have been increasingly investigated for the detection of fungal contamination in food products. These techniques rely on changes in the chemical composition of the matrix caused by fungal metabolism and have shown potential for rapid screening of cereals, nuts, and dried fruits (Ellis et al., 2018; Deng et al., 2025). In parallel, hyperspectral imaging (HSI), which combines imaging and spectroscopy to generate both spatial and spectral information, has demonstrated high accuracy in identifying fungal contamination by capturing subtle surface and compositional alterations before visible mould growth occurs (Feng & Sun, 2012; Qin et al., 2013; Xing et al., 2019).

However, the application of spectrometric and hyperspectral imaging techniques to dried figs presents significant limitations. Figs possess a unique anatomical structure characterized by the operculum, which acts as a natural entry point for fungal spores. Fungal colonisation often initiates internally, remaining concealed beneath the epidermis for extended periods. As a result, surface-oriented techniques such as NIR, Raman spectroscopy, and HSI may fail to detect early internal fungal growth in dried figs, particularly when contamination has not yet affected the outer layers (Li et al., 2025; Wan et al., 2025). Additionally, the heterogeneous composition, wrinkled surface, and variable water activity of dried figs can introduce spectral noise and reduce classification robustness, further constraining the practical implementation of these optical methods in industrial settings.

Volatile organic compounds (VOCs)-low molecular weight (50–300 Da) compounds with high vapour pressure (≥ 0.01 kPa at 20°C) released by all living organisms through metabolic activity-can serve as indicators of food contamination or freshness (Singh et al., 2020; Spence, 2021; Lin et al., 2022). Analysing VOCs produced by *Aspergillus* spp. offers a promising approach for early detection of these moulds in various food matrices, thereby reducing mycotoxin contamination (Rizzi et al., 2007). Previous studies have shown that *Aspergillus flavus* emits specific volatile biomarkers during growth, enabling differentiation between toxigenic (aflatoxin-producing) and non-toxigenic strains. For instance, compounds such as epizonaren, heptadecane, 2-methylfuran, and toluene were detected exclusively in samples contaminated with toxigenic *A. flavus* strains (Josselin et al., 2021). Regarding *Aspergillus niger*, 1-octen-3-ol is notable for imparting the characteristic odour of this fungal genus (Pennerman et al., 2016; Jeleń & Grabarkiewicz-Szcz□sna, 2005).

The identification of VOCs is currently a time-consuming process, creating a need for faster and more efficient technologies. The electronic nose-a device composed of sensor arrays, data acquisition systems, and statistical processing tools-enables VOC profiling and the generation of characteristic aroma “fingerprints” (Liu et al., 2018; Hartyáni et al., 2013; Jiang & Wang, 2016). When combined with techniques such as gas chromatography-mass spectrometry (GC-MS), this approach facilitates the correlation of VOC patterns with fungal contamination. The integration of these methods has successfully identified fungal development, including *Alternaria, Aspergillus, Botrytis, Penicillium*, and *Rhizopus*, in diverse food matrices such as strawberries, apples, pomegranates, rice, and kiwifruit (Pan et al., 2014; Jia et al., 2019; Gu et al., 2020; Nouri et al., 2020; Haghbin et al., 2023; Martinez et al., 2025; Gu et al., 2021). Importantly, VOC-based approaches offer a key advantage for dried figs, as volatile

metabolites produced by internal fungal growth can diffuse through the fruit matrix and be detected externally, overcoming the intrinsic limitations of surface-sensitive optical methods.

Collectively, these studies demonstrate the effectiveness of this novel, non-destructive, and rapid technology for discriminating contaminated food matrices unfit for consumption. Therefore, the objective of this study was to identify volatile compounds serving as potential biomarkers of *Aspergillus* spp. development in dried figs and to implement a rapid detection method for contaminated figs using an electronic nose.

2. MATERIAL AND METHODS

2.1. Experimental design.

For this study, mould strains isolated from dried fig samples were selected from the Culture Collection of the CAMIALI research group (UEx, Badajoz, Spain) and based on previous work done by Galván et al. (2021, 2023). These included three strains of *Aspergillus flavus* (AF_42, AF_55, and AF_114), four strains of *Aspergillus niger* (AN_39, AN_50, AN_85, and AN_185), and two strains of *Aspergillus awamor*i (AW_338 and AW_402). Each strain was individually cultured on potato dextrose agar (PDA) at 25°C for 10 days. After cultivation, spores were collected by suspending them in sterile distilled water and adjusting the concentration to 10^6 spores/mL using a Neubauer chamber and additional sterile distilled water as needed.

Dried figs (*Ficus carica* L.) of the 'Calabacita' cultivar, the most representative variety for drying in the Extremadura region, were obtained from an industry in the southwestern region of Extremadura (Spain). To guarantee quality, the figs were stored at room temperature (20ºC) and in a place with low humidity (< 65% RH), with a maximum storage period of 5 days. As part of the standard processing of dried figs, the fruits were blanched at 80–90°C for 10 minutes and left to dry prior to inoculation. Subsequently, they were immersed in the *Aspergillus* spp. spore suspension (10^5 conidia/mL) for 1 minute. Ten batches were prepared: nine batches consisting of figs inoculated with individual spore suspensions of each previously mentioned *Aspergillus* strain, and one control batch, composed of blanched figs immersed in water for 1 minute. The figs were stored in plastic containers (25 x 25 x 11 cm; containing 8–12 figs each to ensure a representative environment) at 25ºC for 5 days, resulting in noticeable fungal development in the inoculated batches. This experimental design was replicated to ensure the reliability of the results.

For the VOC study, three representative samples were randomly selected from the incubated batches and placed individually inside glass jars with plastic lids, each with a capacity of 780 cm^3. Prior to sealing, the jars were conditioned by flushing the headspace with GC-grade air for 2 minutes to eliminate any residual volatile compounds from previous samples.

2.2. Volatile compound analysis

2.2.1. Volatiles extraction

Volatile extractions were conducted by directly inserting the headspace solid-phase microextraction (HS-SPME) fiber through the jar lid. The fiber used is made of Supelco (USA) and has the following characteristics: 75 µm Carboxen/polydimethylsiloxane (CAR/PDMS). The extraction process took place at 25ºC for 40 minutes. These extraction parameters were established according to the methodology described by Martínez et al. (2025a). These parameters were selected to preserve the natural volatile composition of the samples and to ensure reproducible extraction under conditions close to those encountered during dried fig storage. Gas chromatography/mass spectrometry analyses were carried out as detailed in section 2.2.2. Before their initial use, fibres were conditioned following the specified conditioning guidelines. Each batch (AF_42, AF_55, and AF_114, AN_39, AN_50, AN_85, and AN_185, AW_338, AW_402 and control) was analysed in triplicate.

2.2.2. Gas chromatography/mass spectrometry (GC/MS) analysis.

Volatile compounds were identified and quantified through gas chromatography/mass spectrometry (GC/MS) following the methodology outlined by Martínez et al. (2025a). The analysis was conducted on an Agilent 6890 GC system coupled with a 5973 mass spectrometer (Agilent Technologies, Little Falls, DE, USA). Separation was achieved utilizing a capillary column measuring 50 m in length, with an inner diameter of 0.32 mm and a film thickness of 1.05 µm, coated with a composition of 5% phenyl and 95% polydimethylsiloxane (equivalent to HP-5MS, mod. 19091J-215, Agilent Technologies).

Most relevant volatile compounds were identified using authentic reference standards obtained from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany), all with a declared purity of at least 98%. The remaining compounds were identified based on a combination of Kovats retention indices, calculated utilizing n-alkanes (R-8769, Sigma Chemical Co., St. Louis, MO, USA), and comparison with the NIST/EPA/NIH mass spectral library, with a comparison quality threshold exceeding 90%. Additionally, confirmation of specific compounds was attained by aligning retention times and mass spectra with a laboratory-built library derived from analyses of pure standards under identical conditions. A semiquantitative approach was adopted to evaluate the volatilome profile. The relative abundance of each VOC was determined based on the total volatile fraction. Specifically, the area of each individual peak, expressed in Arbitrary Area Units (AAU), was normalized against the total sum of all identified peak areas within the same sample, with results expressed as a percentage of the total area. The use of relative peak areas normalized to the total volatile fraction is a common strategy in comparative volatilomics and aroma fingerprinting studies when the aim is to characterize compositional changes in the volatilome rather than obtain absolute concentrations (Dursun et al., 2021; Santos et al., 2020)..

2.4. E-nose analysis.

2.4.1. E-nose system

This study employed a portable electronic nose (E-nose) developed by the Sensory Systems research team at the University of Extremadura (Arroyo et al., 2020). This E-nose consists of an array of 11 metal oxide semiconductor (MOX) sensors sourced from various manufacturers, a technology that has been previously applied for the analysis of different food products, including fruits, to monitor quality and detect spoilage (Martínez et al., 2025a, b). The sensor array includes: (i) Bosch BME680, primarily designed for environmental monitoring, which measures temperature (°C), pressure (hPa), humidity (%RH), and gas resistance (Ω); (ii) Sensirion SGP30, which detects equivalent CO_2 (eCO_2) concentration (ppm), total volatile organic compound (TVOC) concentration (ppb), and provides raw resistive values indicating the presence of hydrogen (H_2) and ethanol; (iii) ScioSense CCS811, designed to measure eCO_2 (ppm), TVOC (ppb), and sensor resistance (Ω); and (iv) iAQ-Core, which similarly measures eCO_2 (ppm), TVOC (ppb), and sensor resistance (Ω). By utilizing these sensors, the E-nose offers comprehensive detection capabilities essential for the study's objectives.

These sensors incorporate analogue conditioning circuits, A/D converters, a microcontroller, communication modules, and a heated microplate into a single package, consolidating all necessary sensing elements onto a compact chip design. The system operates on a 3.7 V lithium battery and transmits data wirelessly via Bluetooth to a mobile application.

2.4.2. Measurement Process and Data Analysis

E-nose analyses were conducted concurrently with VOC determinations to ensure consistency across experimental conditions (Figure S1). The measurement process of the E-nose comprises two distinct phases: (i) Adsorption phase (120 seconds): During this phase, the sensors are directly exposed to the headspace of the samples, allowing for the adsorption of odorant molecules; (ii) Desorption phase (120 seconds): Here, the sensors are only exposed to air, establishing a baseline for subsequent odour differentiation. Each measurement was obtained from five consecutive readings, interspersed with blank intervals. In total, 116 measurements were recorded, with the number of individual fruits measured per batch ranging from 4 to 12 (as detailed in Table S1), depending on the specific experimental group.

Each measurement cycle produces a characteristic value crucial for data processing. To extract this feature, a baseline manipulation algorithm is utilized. This algorithm calculates the characteristic value proportionally to the difference between the baseline value (*Vref*), representing the mean of the last 5 values recorded during the sensor's exposure to the air phase, and the value recorded during exposure to volatiles (*Vodor*), also the mean of the last 5 values. The final value (*Vf*) was calculated using the following expression:

$$Vf = (Vref - Vodor) * 100 - 1$$

2.5. Statistical Analysis.

The data underwent statistical analysis using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL) and R software (v. 4.3.x; R Core Team) primarily utilizing the mixOmics package. Prior to analysis, VOC data

were normalized to relative abundances (percentage of total peak area) to ensure comparability. Descriptive statistics were computed, and the impact of inoculation on individual compounds was explored through univariate one-way ANOVA. When significant differences were detected, Tukey's Honestly Significant Difference (HSD) test was performed as a post hoc analysis to identify specific group differences. The effectiveness of the E-nose sensors in discerning various volatile profiles of dried figs was assessed via Pearson correlation coefficients and grouped using hierarchical cluster analysis (HCA). Principal component analysis (PCA) was conducted utilising the signal values of E-nose sensors and most relevant VOCs (with significant differences between control and inoculated batches ($p < 0.05$) and relative abundance greater than 0.25% of total VOCs) to ascertain their discriminatory capacity among the samples under scrutiny.

Linear Discriminant Analysis (LDA) and Partial Least Squares-Discriminant Analysis (PLS-DA) were employed to classify fig batches based on E-nose sensor data. LDA was specifically selected for its ability to provide a stable and interpretable classification framework given the study's sample-to-variable ratio, effectively mitigating the risk of overfitting often associated with more complex non-linear machine learning algorithms. To identify the most influential sensors and optimize the model, a stepwise algorithm based on the Wilks' lambda method was applied. Furthermore, to ensure a robust evaluation and account for the unbalanced sample distribution (24 control vs. 92 contaminated samples), the performance of the classification models was assessed using metrics insensitive to class prevalence, including Likelihood Ratios, Balanced Accuracy, Cohen's Kappa (κ), and the Matthews Correlation Coefficient (MCC). The resulting models were depicted graphically by projecting the fig groups onto the space of the discriminant functions (DF).

In the case of PLS-DA, to evaluate the predictive performance and robustness of the model, Leave-One-Out (LOO) cross-validation was performed. This validation approach was chosen to ensure a reliable estimation of the Overall Error Rate and the Balanced Accuracy, which accounts for any potential imbalance between groups. The stability of the model's predictive ability was further assessed to ensure that the reported discrimination was not a result of overfitting. The importance of individual variables in the discrimination process was assessed through the Variable Importance in Projection (VIP) scores, considering variables with VIP > 1.0 as significant contributors to the models.

3. RESULTS AND DISCUSSION

3.1. Identification of VOCs released by healthy and inoculated dried figs.

Headspace solid-phase microextraction (HS-SPME) combined with gas chromatography–mass spectrometry (GC–MS) analysis identified 52 volatile organic compounds (VOCs) emitted by control and *Aspergillus*-inoculated dried figs (Table 1). Alcohols (48.82%), hydrocarbons (26.20%), and aldehydes (16.72%) represented the most abundant chemical classes. Regarding compound diversity, aldehydes were the most numerous (14), followed by hydrocarbons (13), alcohols (8), esters (5), ketones (5), and

furans (3). Most of these compounds have previously been reported as VOCs in both fresh and dried figs (Villalobos et al., 2018). In dried figs, Russo et al. (2017) identified 42 and 55 volatile compounds in samples from Italy and Turkey, respectively. Mujić et al. (2014) reported 60 compounds in two Croatian varieties, while Yao et al. (2021) detected 75 compounds in figs from Xinjiang (China). Consistent with the present findings, aldehydes and alcohols were among the most diverse functional groups in these studies.

Aldehydes, representing 16.72% of the total VOC fraction, were the chemical class most affected by fungal inoculation, showing an overall decrease in compounds such as 3-methyl-2-butenal, hexanal, furfural, heptanal, and benzaldehyde (Table 1). These aldehydes are among the key aroma contributors in healthy dried figs (Russo et al., 2017; Zidi et al., 2021). Hexanal (7.95%) and benzaldehyde (5.25%) were particularly abundant in non-inoculated samples and have been associated with pleasant sensory notes described as green, grassy, apple, citrus, orange, and almond (Shi et al., 2013; Palassarou et al., 2017). Other aldehydes linked to herbal and fresh aromatic notes, such as furfural (0.96%) and heptanal (0.13%), also decreased in inoculated samples. Beyond aldehydes, minor compounds were similarly reduced by mould activity, including octanoic acid ethyl ester, associated with fruity notes like apple, pineapple, and brandy (Mujić et al., 2014), and 2(3H)-furanone, 5-ethyldihydro (provisionally identified), linked to honey-like flavours (Aprea et al., 2012). Overall, these results indicate that *Aspergillus* spp. development diminishes the relative abundance of compounds that define the characteristic flavour of dried figs.

Conversely, three compounds consistently increased in all batches of figs inoculated with *Aspergillus* spp. (Table 1). These compounds have previously been associated with mould growth and are generally described as off-odours. The most prominent was 1-octen-3-ol, accounting for 39.21% of the total volatile fraction. This compound, characterized by a distinct mouldy odour, has been proposed as a marker of indoor fungal contamination (Fischer et al., 1999; Wålinder et al., 2008) and originates from the biodegradation of lipid-containing substrates mediated by microbial lipoxygenases. Styrene, representing 5.42% of the total VOCs, is linked to an intense “chemical” odour and is produced through the metabolic transformation of cinnamic acid by various moulds, including *A. niger* (Lafeuille et al., 2009; Pinches & Apps, 2007). Finally, phenylethyl alcohol (2.68%) results from the bioconversion of phenylalanine by different *Aspergillus* species (Lomascolo et al., 2001; Masuo et al., 2015).

In addition to the three previously mentioned compounds produced by *Aspergillus* spp. in dried figs, potential species-specific biomarkers were also identified. Specifically, 15 compounds showed a significant increase in samples inoculated with *A. awamori*, while 5 compounds increased in figs inoculated with *A. flavus* (Table 1). Inoculation with *A. niger* did not result in the production of unique VOCs. Most of these compounds accounted for less than 1% of the relative abundance, except for 3-methylbutanol (3.51%), which increased in *A. awamori*-inoculated samples, and (Z)-2-octen-1-ol (2.11%; tentatively identified), which increased in *A. flavus*-inoculated samples. It is noteworthy that some volatile compounds were common to multiple *Aspergillus* species; for example, E-2-octenal and 1-hexanol were detected in both *A. flavus* and *A. awamori*.

Previous studies have reported that the growth of *Aspergillus* spp. in food leads to the production of volatile compounds that can serve as biomarkers, in some cases specific enough to differentiate between toxigenic and atoxigenic strains (de Luca et al., 2010; Jurjevic et al., 2008). Among the biomarkers associated with *A. awamori*, the most abundant was 3-methylbutanol, a compound previously linked to fungal growth in environmental contexts (Gao et al., 2002) and described as having medicinal or glue-like odours (Thomsen et al., 2018). For *A. flavus*, the most prominent biomarker was (Z)-2-octen-1-ol, associated with mushroom-like aromas (Cheng et al., 2018). Other biomarker compounds included hydrocarbons with descriptors related to petrol-like aromas, such as heptane, 1-octene, and ethylbenzene (Sun et al., 2025). Additionally, compounds imparting grassy or green notes, such as 2-pentylfuran, 1-hexanol, and (E)-2-octenal, were detected (Salas et al., 2005). High molecular weight aldehydes predominantly increased by *A. awamori* contributed fatty or waxy aromas (e.g., 2-octenal, (E,E)-2,4-octadienal, and decanal) and fruity or floral notes (e.g., nonanal and 2-undecenal) (Fickert & Schieberle, 1998; Mostafa et al., 2022). Finally, 2,5-methylfuran and 1-penten-3-one added meaty and pungent notes, respectively (Vilar et al., 2022).

The identification of these specific VOCs as potential biomarkers is relevant for characterizing *Aspergillus* populations in dried figs and other dried products. Particularly noteworthy are the differences in volatile profile between *A. awamori* and *A. niger*, two species within the *A. niger* aggregate that pose taxonomic challenges (Perrone et al., 2011). Both species are commonly found in dried figs and other dried fruits (Heperkan et al., 2012; Varga et al., 2010). However, it should be noted that fungal VOC emissions are dynamic and may vary according to the stage and severity of infection. Therefore, the volatile profiles reported in this study correspond to a specific incubation period and should be interpreted within this experimental context. The standardized incubation conditions allowed species-related differences to be evaluated while minimizing variability associated with infection progression.

3.2. Relationship between VOCs and signals from the E-nose sensors.

To assess the potential of these results for developing predictive models, a principal component analysis (PCA) was performed using the most relevant volatile organic compounds (VOCs) identified across the dried fig batches and the signals from E-nose sensors. Correlation coefficients between VOCs and sensor responses were subsequently used to conduct a hierarchical cluster analysis (HCA). This analysis allowed the identification of five main clusters, as shown in Table 2, grouping volatile organic compounds according to their correlation patterns with the sensors. Figure 1 shows the scores (A) and the loadings (B) within the plane defined by principal component 1 (PC1) and principal component 2 (PC2). PC1 and PC2 explained 47.84% and 24.71% of the variance, respectively, accounting for a cumulative 72.55% of the total variability.

The analysis revealed that compounds such as toluene (v10), 3-methyl-2-butenal (v12), hexanal (v14), furfural (v17), benzaldehyde (v27), and nonanal (v42) were predominantly associated with control fig samples on the positive axis of PC1. These VOCs, grouped in cluster 2, exhibited a significant positive

correlation with CO_2iAQ and TVOCiAQ sensors and a significant negative correlation with RBME680, H_2SGP30, EtanolSGP30, ResohmCC811, and RiAQCore sensors (Table 2; Fig. 1A). Several of these compounds, including hexanal, furfural, 2-octenal, 2-pentylfuran, benzaldehyde, and nonanal, are known contributors to the characteristic aroma of fresh and dried figs, imparting notes of freshness, green, sweetness, woodiness, rose, and cherry (Oliveira et al., 2010; Mujić et al., 2014; Yao et al., 2021). This aromatic profile explains their association with non-inoculated samples.

Conversely, VOCs grouped in cluster 4 (styrene (v23), 1-octen-3-ol (v28), and (Z)-2-octen-1-ol (v39)) located on the negative axis of principal component 1, were positively associated with inoculated dried figs (Fig. 1). These compounds are well-documented products of fungal metabolism and have been reported in various *Aspergillus* species, including *A. flavus* (Jelen et al., 2005; Josselin et al., 2021; Zhang et al., 2017), *A. niger* (Jelen et al., 2005; Lafeuille et al., 2009), and *A. awamori* (Villasante et al., 2025). Their biosynthesis, linked to mouldy odour formation (Gong et al., 2024), explains the lack of clear association with any single inoculated strain.

Styrene exhibited a significant positive correlation with the ResohmCCS811 sensor and negative correlations with TVOCCCCS811, CO_2iAQ, and TVOCiAQ (Fig. 1A; Table 2). 1-Octen-3-ol, a well-known fungal growth marker, showed positive correlations with most sensors, with significant associations for RBME680, H_2SGP30, EthanolSGP30, ResohmCCS811, and RiAQCore. Similarly, (Z)-2-octen-1-ol demonstrated a significant positive correlation with H_2SGP30 and negative correlations with CO_2SGP30 and TVOCSGP30. Overall, these three compounds shared positive correlations with RBME680, H_2SGP30, EthanolSGP30, ResohmCCS811, and RiAQCore, and negative correlations with CO_2SGP30, TVOCSGP30, CO_2CCS811, TVOCCCS811, CO_2iAQ, and TVOCiAQ (Table 2).

Conversely, VOCs grouped in cluster 1-3-methylbutanol (v7), 1-hexanol (v22), and 2-octenal (v38)-were associated with the growth of *A. flavus* strains (144 and 55) and *A. awamor*i strains (402 and 338), positioned on the positive axis of PC2 (Fig. 1). These VOCs exhibited significant negative correlations ($p < 0.05$) with the TVOCSGP30, TVOCCCS811, CO_2iAQ, and TVOCiAQ sensors (Table 2). Additionally, p-xylene (v21) and 2-pentylfuran (v30), which belong to cluster 3, were located between clusters 1 and 2 (Fig. 1).

The association of phenylethyl alcohol (v44) with the growth of *A. niger* strains (AN39, AN50, AN85, and AN185) and *A. flavus* 42 is also illustrated in Fig. 1. This compound, uniquely classified in cluster 5, is positioned on the negative axis of PC2 and is produced through the bioconversion of L-phenylalanine-a metabolic pathway previously described for *Aspergillus* species such as *A. oryzae* (Masuo et al., 2015) and *A. niger* (Lomascolo et al., 2001).

The combined PCA and HCA analyses highlight the potential of MOX sensors as effective tools for developing discriminative models based on their relationship with VOC profiles emitted by dried figs during storage.

3.3. Determination of fungal infection of dry figs during storage.

3.3.1. Discriminant analysis for multi-class classification of *Aspergillus* species in dried figs

Linear Discriminant Analysis (LDA) and Partial Least Squares-Discriminant Analysis (PLS-DA) models were applied to classify batches of dried figs based on volatile profiles captured by E-nose sensors. Initially, a leave-one-out cross-validation scheme with four cancellation groups (Control, *A. awamori*, *A. flavus*, and *A. niger*) was employed to assess the predictive capability of both frameworks. Table 3 summarizes the classification results by fungal inoculation type, including the sensors selected for the LDA model and the Variable Importance in Projection (VIP) scores for the PLS-DA model, along with their corresponding validated accuracy rates.

The LDA model underscored the discriminative power of specific sensors in differentiating fungal contamination patterns. Among these, ResohmCCS811, TVOCSGP30, H2SGP30, EtanolSGP30, and RiAQCore emerged as the most influential variables. The model achieved an overall prediction success rate (Accuracy) of 84.5% under LOOCV. Class-specific accuracy revealed that *A. niger* samples exhibited the highest precision (94.4%), while *A. flavus* presented the lowest (65.6%), suggesting a more complex volatile signature for the latter. The robustness of the LDA approach was supported by an overall balanced accuracy of 89.8% and a Cohen's Kappa (Δ) of 0.792, with a low overfitting rate of 4.3 percentage points (Table 3).

Complementing this analysis, the PLS-DA model provided an alternative classification framework based on latent variables. The most significant contributors to this model, identified by VIP scores > 1.0, were ResohmCCS811, RiAQCore, TVOCCCS811, RBME680, and CO2CCS811. Overall, the PLS-DA model achieved a validated accuracy of 79.8% and a balanced accuracy of 86.4%. Similar to the LDA results, *A. niger* and *A. awamori* showed high classification success (97.2% and 95.8%, respectively). However, the PLS-DA model faced significant challenges in identifying *A. flavus*, reaching an accuracy of only 34.4% under LOOCV, which impacted the overall Kappa value (0.714).

Diagnostic reliability metrics further characterized the performance of both models. In the LDA framework, *A. awamori* and *A. niger* achieved high specificity (> 94%) and strong positive likelihood ratios (LR + of 42.17 and 25.19, respectively), indicating robust separation from other classes. The PLS-DA model showed exceptionally high specificity for *A. flavus* (97.6%) and *A. niger* (98.8%), although its sensitivity for *A. flavus* was limited. For the Control group, both models exhibited reliable performance, with LDA showing a Negative Predictive Value (NPV) of 96.6% and PLS-DA reaching 97.5%, ensuring that healthy samples were correctly identified with high confidence.

In summary, while both discriminant methods proved effective for the early detection of Aspergillus species, the LDA model demonstrated superior generalizability and a better balance in classifying A.

flavus compared to PLS-DA. The consistency of macro-level metrics, such as balanced accuracy and Kappa coefficients across both schemes, reinforces the structural soundness of using E-nose technology for fungal screening in dried figs, despite the inherent metabolic overlap between certain toxigenic species.

3.3.2. Simplified binary classification for detecting *Aspergillus* contamination in dried figs

From a quality and safety perspective, detecting contaminated figs as a pool can be as valuable as differentiating between the species involved. In this context, as shown in Table 4 and Fig. 2B, a classification accuracy of 96.6% was achieved using only the ResohmCCS811, TVOCSGP30, and H_2SGP30 sensors as discriminant variables. The simplified LDA model proved highly stable under leave-one-out cross-validation (LOOCV), exhibiting a robustness (overfitting) value of only 0.8 and indicating excellent generalization. The agreement beyond chance was strong, as evidenced by a Cohen's Kappa of 0.893 and a Matthews Correlation Coefficient (MCC) of 0.893.

The diagnostic performance of the LDA classifier was further confirmed by high likelihood ratios (Total LR + = 84; LR− = 0.035) and a balanced accuracy of 93.2%. Despite the prevalence asymmetry in the dataset (*Aspergillus* spp. representing approx. 79% of samples), the model maintained high reliability in its predictions. Specifically, positive predictions for *Aspergillus* presence were highly trustworthy (PPV = 96.8%), while the negative predictive value (NPV) reached 96.8% for the control group, suggesting a very low probability of false negatives.

Complementarily, a binary PLS-DA model was developed to further explore the data structure (Fig. 3B). The most influential sensors for this model, ranked by VIP scores > 1.0, were ResohmCCS811, CO2CCS811, RiAQCore, EtanolSGP30, and RBME680 (Table 4). The PLS-DA model achieved a validated total accuracy of 86.2% and a balanced accuracy of 85.1%, with a Cohen's Kappa of 0.626. While these results confirm the effectiveness of the E-nose for fungal detection, the LDA model outperformed PLS-DA in terms of both sensitivity (98.9% for *Aspergillus* spp. in LDA vs. 87.0% in PLS-DA) and overall predictive power in this binary scenario.

The accuracy levels achieved in this research align with previous studies on predicting fungal infections in fruits (Martínez et al., 2025a; Haghbin et al., 2022; Jia et al., 2019) and cereals using E-nose technology and various models for qualitative classification and quantitative regression. For instance, Gu et al. (2019) identified *Aspergillus* presence in rice grain, demonstrating a positive correlation between electronic nose signals and volatile components (GC-MS). Similarly, Liu et al. (2018) investigated the presence of *Botrytis cinerea*, *Monilinia fructicola*, and *Rhizopus stolonifera* in peaches, achieving a discrimination accuracy of 90% among the three fungal species after 48 hours of storage. Overall, both classifiers, especially the LDA model, provide robust and generalizable discrimination for fungal contamination by *Aspergillus* in dried figs.

Conclusions

In summary, this study demonstrates that *Aspergillus* spp. contamination of dried figs induces significant alterations in their VOC profile, characterized by a notable reduction in aldehydes and the emergence of species-specific volatile as potential biomarkers. Specifically, 15 compounds were indicative of *A. awamori* infection, while 5 were associated with *A. flavus*. *A. niger* inoculation did not yield unique volatile biomarkers. Notably, 1-octen-3-ol, styrene, and phenylethyl alcohol were identified as general biomarkers for *Aspergillus* spp. development.

This research highlights the effectiveness of E-nose technology, coupled with chemometric tools, for fungal screening. The multi-class LDA model successfully discriminated between *Aspergillus* species with an average accuracy of 84.5%, outperforming the PLS-DA model (79.8%). Furthermore, a simplified binary classification achieved a remarkable 96.6% discrimination rate between contaminated and healthy samples using only three key sensors (ResohmCCS811, TVOCSGP30, and H2SGP30), while the PLS-DA model confirmed the robustness of these results through high specificity and VIP scores. These findings validate the potential of E-nose technology as a rapid, non-destructive tool for detecting fungal contamination in dried figs.

Future work will focus on validating these findings through expanded assays, including evaluations of the discrimination capability for aflatoxin and ochratoxin A contamination and the assessment of VOC profile evolution during different stages of fungal growth. Since fungal volatile emissions may vary according to the degree and duration of infection, further studies will investigate the temporal stability of the identified biomarkers and their influence on classification performance. These efforts will support the integration of this technology into industrial quality control protocols, where further research will specifically address analysis time, cost-effectiveness, and the feasibility of real-time or online monitoring. In addition, we propose exploring non-linear machine learning algorithms, such as Support Vector Machines, Random Forests, or Artificial Neural Networks, to develop classification models for comparison with the current linear discriminant models. Conducting a comparative evaluation of these models will provide valuable insights into whether non-linear approaches offer superior discriminatory power for differentiating *Aspergillus* species or distinguishing contaminated from healthy samples, thereby ensuring compliance with stringent food safety regulations and improving the overall quality of dried fig products.

Declarations

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Author Contribution

Conceptualization: A. Martín and A. Hernández; Methodology: A. Martín, A. Hernández, A. Martínez, J. Lozano, and P. Arroyo; Validation: A. Martín and A. Hernández; Formal Analysis: A. Martínez and P. Arroyo; Investigation: A. Martín, A. Hernández, and A. Martínez; Resources: M. G. Córdoba, A. Martín, J. Lozano, and A. Hernández; Data Curation: A. Martínez and P. Arroyo; Writing – Original Draft: A. Martín and A. Martínez; Writing – Review & Editing: A. Martín and A. Hernández; Funding Acquisition: A. Hernández and A. Martín; All authors have read and approved the final version of the manuscript.

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Data Availability

Most of the data generated or analyzed during this study are included in this published article and its Supplementary Information. Any additional data supporting the conclusions of this study are available from the corresponding author upon reasonable request.

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Tables

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