IOCT 2025 Conference

The 3rd International Online Conference on Toxins

10–12 September 2025 | Online

MDPI

Discriminating geographical origin and detection of aflatoxins in pistachio seeds using FTIR spectroscopy

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INTRODUCTION & AIM

Food contamination by mycotoxins represents a serious threat to public health. These toxic substances are produced by filamentous fungi such as *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* under specific conditions of temperature and humidity. They can impact a wide variety of agricultural products (nuts, cereals, and their derivatives) during both pre- and post-harvest stages. Among these toxins, aflatoxin B1 (AFB1) poses a particularly serious health risk, being classified as a human carcinogen. European regulations have established maximum allowable levels of 2 µg/kg and 12 µg/kg of aflatoxin B1 (AFB1) for pistachios intended for direct human consumption and for those subjected to physical treatment prior to consumption, respectively. In this study, we evaluated the feasibility of detecting aflatoxins in pistachio seeds using Fourier-Transform Infrared (FT-IR) spectroscopy. As a rapid and non-destructive technique, FT-IR holds significant potential for identifying contaminants in food, including mycotoxins (Lee *et al*., 2015). In addition to aflatoxin detection, FTIR spectroscopy was used also to discriminate pistachio samples from different geographical areas. The interaction between the sample and the infrared light generates a unique spectral fingerprint based on the molecular vibrations of its components, enabling the identification of specific substances and the authentication of food products.

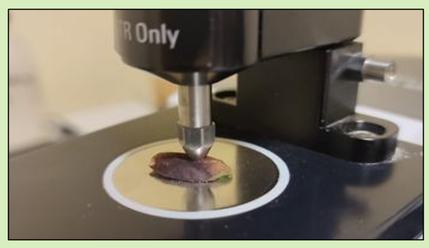
METHOD

FT-IR measurements were performed on 19 pistachio samples collected from 16 different orchards in Agrigento, Catania and Messina provinces (field sampling campaign of 2023; Fig. 1) and on 4 pistachio samples purchased from the market, of unknown origin. A sample certified with the "Pistachio Verde di Bronte DOP" label, purchased from the market, was used as control.

Origin	N. of samples	cultivar	ID
Agrigento†	5	Napoletana	C1-C5
Catania† (Bronte, Adrano)	12	Napoletana Larnaka	C6-C17
Messina† (Milazzo)	2	Napoletana Aegina	C18-C19
Control (Bronte)‡	1	Napoletana	A
Unknown origin‡	4	-	B, C, D, E

Fig. 1. Area of origin and pistachio samples collected for this study; † samples harvested from field (sampling campaing of 2023); ‡ samples purchased from the market

A portable spectrometer (Agilent Cary 630(R)) working in attenuated total reflectance (ATR) was used. For each sample, 4 seeds were considered and 16 measures were performed, aligning the seeds to the axis of the instrumental optical slit. FTIR-ATR spectra were collected averaging 64 scans in the 4000-650 cm^{-1} range, with a resolution of 4 cm^{-1}. After the data acquisition, the spectra were normalized for comparison.



Simultaneously, HPLC analysis was performed on the market-purchased samples to assess the possible presence of AFB1, AFB2, AFG1 and AFG2. Only the sample B was found to contain aflatoxin B1, at a concentration below the maximum limit established by current European regulations (detected concentration = **1.25 µg/Kg**).

RESULTS & DISCUSSION

The spectra of the samples found to be negative for aflatoxins by HPLC analysis were compared with the spectrum of the aflatoxin-positive sample (sample B) and with that of a sample certified with the label "Pistachio Verde di Bronte DOP", negative by HPLC (sample A) and used as a control (Fig. 2).

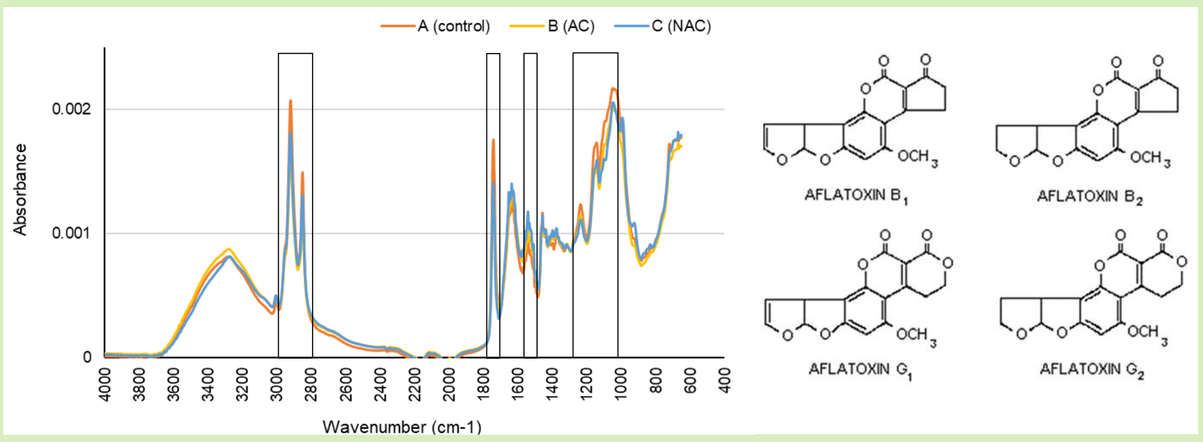


Fig. 2. FT-IR spectra of one aflatoxin-contaminated (sample B) and one non-contaminated pistachio sample (sample C) compared with the spectrum of a sample certified with the label "Pistacchio di Bronte DOP" (sample A - control). The spectra were normalized by dividing the single absorbance values by their sum.

The functional groups present in aflatoxins include benzene rings, carbonyl groups (from both ketones and lactones), alkenes and methoxy groups. These groups are responsible for characteristic infrared absorption bands in the spectra, typically observed within the spectral regions of 3035-2820, 1775-1720, 1570-1481, and 1270-1060 cm^{-1} (Valasi *et al*., 2021). These spectral regions were selected and compared in order to distinguish pistachio samples contaminated with aflatoxins from uncontaminated ones (Fig. 3).

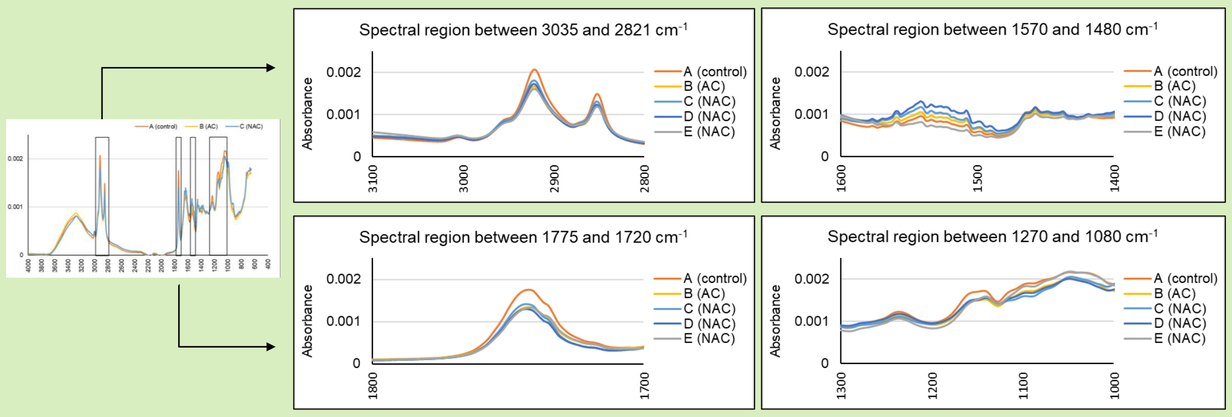
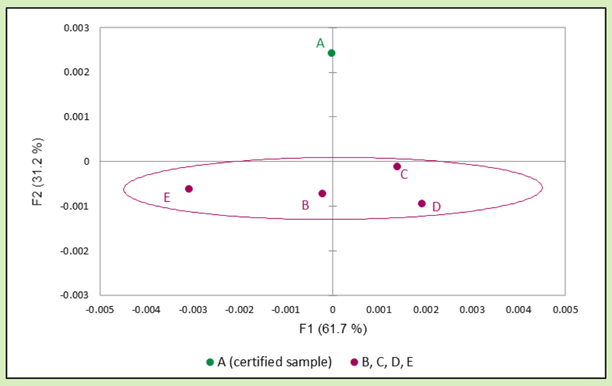


Fig. 3. Characteristic spectral regions of aflatoxin absorption used to compare aflatoxin-contaminated (sample B) and non aflatoxin-contaminated samples (samples C, D, E)



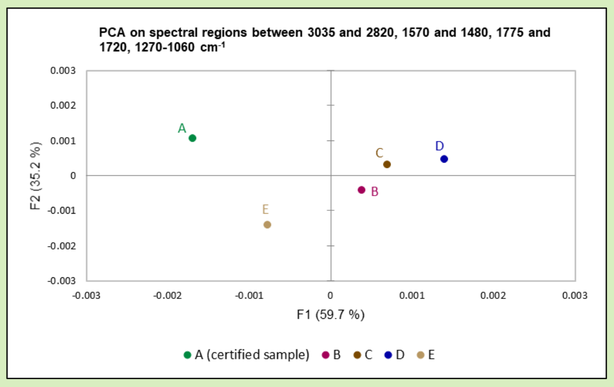


Fig. 4. PCA performed on the FTIR spectra (on the left) and on the spectral regions associated with aflatoxin absorption (on the right) extracted from the aflatoxin-contaminated (sample B) and non aflatoxin-contaminated pistachio samples (samples C, D, E). The sample A, certified with the label "Pistachio Verde di Bronte DOP", was used as a control. PCA was applied to the mean-centered data matrices.

PCA highlighted differences between the sample A, collected in the Bronte district with PDO certification, and the foreign samples purchased on the market. On the opposite, no significant differences were found between the sample B, contaminated with aflatoxins, and the uncontaminated ones, most likely because the contamination level in B was below the instrument's detection threshold (Fig. 4).
These findings suggest that differences in the pistachio spectral profiles are mainly attributable to environmental factors (cultivation area, harvest time, storage conditions), highlighting the potential of FTIR spectroscopy to authenticate foodstuffs based on their provenance. This potential was further confirmed by comparing the spectra of the field-collected samples with that of the certified control sample (the latter had been collected and stored under different conditions with respect to the first group (Fig. 5).

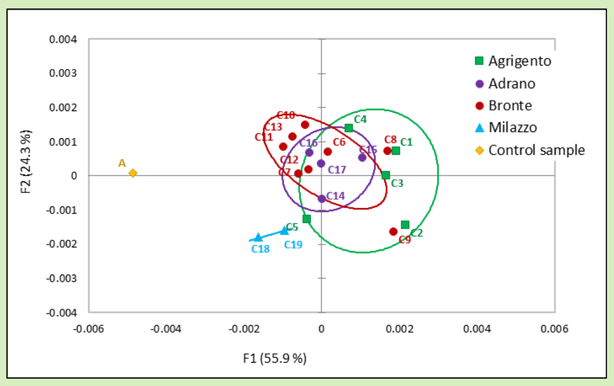


Fig. 5. PCA performed on the FTIR spectra of pistachio samples harvested in the provinces of Agrigento, Catania and Messina (field sampling campaign of 2023), using the spectrum of the certified control sample purchased from the market as a reference (sample A). PCA was applied to the mean-centered data matrix.

CONCLUSION

Results demonstrated the ability of the technique to discriminate samples of different geographical origin or collected at different times, highlighting the strong relationship between terroir and the chemical composition of vegetal crops. On the opposite, the presence of aflatoxins was not detected in the contaminated sample, likely due to the low contamination level being below the instrument's detection threshold.

Further studies are currently underway to assess the potential of the technique to distinguish between contaminated and uncontaminated seeds through the detection of aflatoxins AFB1, AFB2, AFG1, and AFG2. At present, pistachio samples are being inoculated with an aflatoxin-producing isolate of *Aspergillus flavus* in order to monitor toxin production at different time intervals after inoculation, simulating natural contaminations. In addition, pistachio seeds will be inoculated with increasing known concentrations of aflatoxins, in order to determine the detection limit of the technique.

References Lee *et al*., 2015. *Food Chem*. 173: 629-639; Valasi *et al*., 2021. *J. Food Sci. Technol*. 58(1): 356-365

This research was financially supported by the European Union - Next Generation EU - Mission 4 Component 1 CUP E53D23015570001 (project PRIN 2022 PNRR "P20223P48S" entitled "*Implementing advanced elemental and chemical analysis for quality, safety and traceability assessment of PGI and PDO agri-food products*").

Finanziato dall'Unione europea NextGenerationEU · Ministero dell'Università e della Ricerca · Italiadomani Piano Nazionale di Ripresa e Resilienza · Università degli Studi di Catania · INFN Catania Istituto Nazionale di Fisica Nucleare Sezione di Catania