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Research Paper

Detection of fungal infection in almond kernels using near-infrared reflectance spectroscopy

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Aspergillus flavus and *Aspergillus parasiticus* are ubiquitous in natural environments and have the potential to produce aflatoxins that can cause both chronic and acute diseases in humans and animals. The near-infrared (800–2500 nm) reflectance spectra of almonds from the 2012 and 2013 harvest seasons inoculated with one selected isolate of either *A. flavus* or *A. parasiticus* were analysed to determine if the spectra could be used to distinguish infected almonds from uninfected control almonds. A canonical classifier was developed that could discriminate infected almonds from the uninfected control kernels with a total cross-validation error rate of 0.26% and zero false negative errors. Additionally, a follow-up canonical classifier was developed to evaluate the potential to discriminate between infections by the two *Aspergillus* species, once infection was detected. Spectral analysis indicated that the NIR wavebands associated with lipid content were the most indicative factors in both identifying the infected almonds as well as discriminating between almonds infected by the *A. flavus* or the *A. parasiticus* isolate. Further comparison of the canonical discriminant analysis results from the 2nd derivative spectra and the original spectra also suggested that the chemical differences caused by the fungal metabolism had a much greater contribution to the discriminant function than the structural changes caused by the fungal invasion. The wavelengths identified in this study could be helpful in developing non-destructive multispectral sensing systems to effectively scan and detect almonds with fungal infection.

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1. Introduction

Many substrates support the growth of *Aspergillus flavus* and *Aspergillus parasiticus*, the two most common aflatoxin-producing species. In particular, *A. flavus* is a common agricultural contaminant in warm regions across the globe (Bennett & Klich, 2003; Patterson, 1973). Fungal contamination occurs both in pre- and post-harvest stages of cereals, nuts, cottonseeds and other agricultural commodities (Rai & Varma, 2009; Wilson & Payne, 1994). Contamination that happens in the field before harvest is usually associated with drought stress and insect damage; however, after harvest, fungal infection can occur when the crop storage conditions are favourable for its growth (i.e. high moisture content and warm temperatures).

Aflatoxins are hepatotoxic, mutagenic, carcinogenic, difuran-containing, polyketide-derived mycotoxins, which can cause hepatitis, liver cancer, and other health threats, including death, to both humans and animals (Bennett & Klich, 2003). Most developed countries enforce aflatoxin tolerance thresholds to prevent consumption and marketing of contaminated commodities. In the United States, the average economic loss due to mycotoxin (toxin produced by fungi) contamination is estimated at approximately one billion dollars, with aflatoxins representing a large portion of this loss (Amaike & Keller, 2011).

A survey of aflatoxins in California tree nuts between 1972 and 1975 showed that the average probability of contamination in almonds is one infected kernel in 26,500 with an upper confidence limit of one in 14,700 (Fuller, Spooncer, King, Jr., Shade, Mackey, 1977). As found in grains, the sampling variability is large because such a small percentage of kernels are contaminated. The level of contamination on a single kernel can also be very large, and thus a few contaminated kernels can result in an actionable level of aflatoxin in a whole lot (Whitaker, 2006). Because both the probability of contamination and the concentration of aflatoxin among infected almonds are highly variable, the current method of analysing a small subsample of almonds from a potentially contaminated lot produces an imprecise estimate of the true aflatoxin concentrations in infected individual kernels.

Once the samples are collected from a lot (or a truckload), the most common analytical methods currently used to determine the aflatoxin concentration in subsamples are thin-layer chromatography (TLC) based methods, high-performance liquid chromatography (HPLC) coupled methods, and immunochemical methods (e.g. Enzyme-Linked Immunosorbent Assay, ELISA). All these standard methods require destroying the samples (to create homogeneous particulate sub-samples), and most of these techniques also require highly trained personnel, specialised laboratory facilities, and costly instrumentation.

Several methods have been explored during the past two decades to help reduce the cost and risk of current processes; in particular, optical detection methods have good potential because of their non-destructive nature. Starting in the early 90s, researchers suggested using ultraviolet (UV) light to identify the presence of bright greenish-yellow fluorescence (BGYF), which is emitted from kojic acid, another secondary metabolite produced by both *A. flavus* and *A. parasiticus*, to suggest the presence of aflatoxins. However, it has also been documented in corn kernels that over half of the contaminated kernels only exhibit internal BGYF, which can only be observed after a kernel is ground or broken (Pearson, Wicklow, Maghirang, Xie, & Dowell, 2001).

Near-infrared (NIR) spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the near-infrared range and is widely used for sorting and detecting chemical attributes in grain and nut products for quality and process control (Armstrong, 2006; Campbell, Mannis, Port, Zimmerman, & Glover, 1999; Delwiche, 1998; de Mello & Scussel, 2009). The absorption bands in this region are frequently used to identify different properties of foods (e.g., moisture, protein, and starch contents). More recent investigations have studied the possibility of using NIR to detect fungal infection in maize samples and results showed good discrimination performance (Berardo et al., 2005; Pearson & Wicklow, 2006; Pearson et al., 2001; Tallada, Wicklow, Pearson, & Armstrong, 2011).

Previous studies also suggest that almonds that are broken, split, or ground have a higher chance of being contaminated with aflatoxin, indicating that wounds provide entry routes for contamination (i.e. insect infestation with the possible carryover of aflatoxin-producing fungi) (Schade, McGreevy, King Jr., Mackey, Fuller, 1975; Schatzki, 1996). Removing these broken and wounded almonds by using a standard colour and defect sorter can be relatively easy; however, almonds that are contaminated without leaving visually obvious wounds or discolouration may still pose a risk to public health in the visibly defect-free final product.

The primary objective of this study was to investigate the feasibility of using NIR spectroscopy to non-destructively distinguish individual, whole almond kernels infected with *A. flavus* or *A. parasiticus* from uninfected kernels in cases where there are not obvious visual external differences between them. Since the target of this study was detection of fungal infection and not the final aflatoxin concentration level, this study aimed to improve the performance of the early steps of the postharvest culling process by providing a way to quickly and non-destructively screen for individual almond kernels that are infected by the two main *Aspergillus* species and are therefore at higher risk of being contaminated by aflatoxin than uninfected kernels. A secondary objective was to explore the potential for discrimination between the two *Aspergillus* species (with one isolate each) among the artificially infected almond kernels using non-destructive NIR sensing methods and canonical discriminant analysis.

2. Material and methods

2.1. *Sample preparation*

The bulk pasteurised and shelled Nonpareil Californian almond kernels used in this study were collected from the 2012 and 2013 harvest seasons and obtained directly from local almond processing facilities in California (kernel size 22/24). Individual almonds that had no visible signs of damage or markings on the surface were manually selected from the