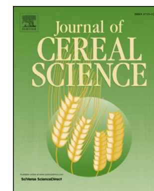
Journal of Cereal Science 59 (2014) 70–78

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs



Application of Raman spectroscopy for qualitative and quantitative analysis of aflatoxins in ground maize samples

Kyung-Min Lee [a,*], Timothy J. Herrman [a], Unil Yun [b]

[a] Office of the Texas State Chemist, Texas A&M AgriLife Research, Texas A&M University System, College Station, TX 77841, USA
[b] Department of Computer Science, School of Electrical and Computer Engineering, Chungbuk National University, Cheongju, Republic of Korea

ARTICLE INFO

Article history:
Received 8 July 2013
Received in revised form
26 September 2013
Accepted 1 October 2013

Keywords:
Aflatoxin
Raman spectroscopy
Chemometrics

ABSTRACT

The applicability of Raman spectroscopy combined with chemometrics using different preprocessed spectra data was examined to develop fast, low-cost, and non-destructive spectroscopic methods for classification and quantification of aflatoxin-contaminated maize samples within the aflatoxin concentration range of 0–1206 μg/kg. This technique will find useful application in evaluating large numbers (e.g. >2000) of samples from maize hybrid performance trials and breeding programs. The best discriminant models were obtained from the linear discriminant analysis (LDA). The LDA models on validation samples showed correct classification rates in the range of 94–100% and did not misclassify any aflatoxin contaminated samples as aflatoxin negative. Of the models for predicting aflatoxin concentration, the partial least squares regression (PLSR) models showed the best quality of regression (slopes of 0.939–0.990) and highest coefficient of determination ($r^2 = 0.941$–0.957). The models provide limited applicability to quantify aflatoxin concentration below 20 μg/kg. No significant difference was observed between predicted values using Raman spectroscopy and reference values using high-performance liquid chromatography (HPLC) ($p > 0.05$), indicating the suitability of Raman spectroscopy to rapidly screen large numbers of maize samples for aflatoxin contamination.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

The contamination of food and feed grains and oilseeds by fungal mycotoxins has raised concerns about animal and human health, worldwide. The most commonly occurring mycotoxins in food and feed grains are aflatoxins and fumonosins, produced by the *Aspergillus* and *Fusarium* fungal strains, respectively. Mycotoxin contaminated grains and oilseeds are toxic and carcinogenic to humans and animals. As a result, they are devaluated in the markets for food and feed and can result in economic loss for growers, handlers, and food and feed processors (Robens and Cardwell, 2003). Therefore, food safety authorities and agencies such as United States Food and Drug Administration and European Commission (EC Commission Regulation 165/2010) have established permissible levels of some mycotoxins in grains and oilseeds for their use in major human foods and animal feeds.

To evaluate the toxicity of mycotoxins in grains and oilseeds, various analytical methods have been used to directly classify and quantify mycotoxin levels both in the laboratory and in the field. These analytical methods include thin layer chromatography (TLC), gas chromatograph (GC), high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), enzyme-linked immunosorbent assay (ELISA), immune-affinity column assay, and biosensors (Zheng et al., 2006). Although these methods have been widely used for short or long time periods, there are some limitations and constraints for their application (Table 1). The development of spectral analysis technology for mycotoxin detection allows for rapid screening of a large number of samples (>2000). The rapid screening of large samples is necessary in plant breeding programs, hybrid performance trials, or during harvest in the field (Herrman et al., 2002).

Spectroscopic techniques, including near-infrared reflectance (NIR), Fourier transform infrared spectroscopy (FTIR), and Raman spectroscopy, can provide qualitative and quantitative information pertaining to mycotoxin components and its structures with a

Abbreviations: FTIR, Fourier transform infrared spectroscopy; HPLC, high-performance liquid chromatography; LDA, linear discriminant analysis; LOD, limit of detection; LOQ, limit of quantitation; MLR, multiple linear regression; NIR, near-infrared reflectance; PCA, principal component analysis; PCDA, principal component discriminant analysis; PCR, principal components regression; PLSDA, partial least squares discriminant analysis; PLSR, partial least squares regression; PRESS, predicted residual sum of squares; r^2, correlation coefficient of determination; RMSEC, root-mean-square error of calibration; RMSEP, root mean standard error of prediction; RPD, ratio of the standard deviation of the reference data to the standard error of cross-validation.

* Corresponding author. Tel.: +1 979 845 1121; fax: +1 979 845 1389.
E-mail address: kml@otsc.tamu.edu (K.-M. Lee).

0733-5210/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jcs.2013.10.004

Table 1
Analytical methods commercially available for mycotoxin analysis.

Method	Strength	Weakness
Thin layer chromatography (TLC)	Simple, rapid, inexpensive, robust, widely used in various matrices	Requiring clean-up, not precise, laborious
Gas chromatograph (GC)	Selective, sensitive, accurate	Requiring clean-up, time consuming, laborious, expensive
High performance liquid chromatography (HPLC)	Selective, sensitive, accurate	Requiring clean-up, time consuming, laborious, complex, expensive
Liquid chromatography-mass spectrometry (LC-MS)	Selective, sensitive, accurate	Time consuming, laborious, complex, expensive, requiring skilled and experienced staff to operate
Enzyme-linked immunosorbent assay (ELISA)	Requiring low sample volume, rapid, simple, specific, sensitive, portable	Matrix dependent, laborious, application-limited
Immunoaffinity column assay	Easy to use, rapid, simple, specific, selective, sensitive, portable	Matrix dependent, laborious, application-limited
Biosensors	Rapid, specific, real-time monitoring, adaptable to remote sensing	Cross-reactive, insensitive

single scan. In addition, these techniques require little or no sample preparation and pretreatments. Therefore, they are ideal candidates for a low cost method for rapid identification and classification of fungal and mycotoxin contaminated grains and oilseeds (Delwiche and Gaines, 2005; Greene et al., 1992; Kos et al., 2002, 2007). Raman spectroscopy relies on a scattering effect of the molecules and record a loss of energy in an incident light whereas infrared spectroscopy obtains spectral information derived from an absorption process based on the ratio of transmitted to incident radiation. Due to different intensity of the vibration and polarization of the molecule by the absorbed energy, Raman and infrared spectroscopies produce the different shapes and positions of the bands for the identical sample. So each technique can provide complementary information about mycotoxins in samples.

However, the application of spectroscopic technology has been limited due to difficulties in interpretation of spectral data and spectra overlapping. The advent of modern spectral amplification and enhancement techniques has enabled the use of NIR and FTIR to detect and identify fungal species and mycotoxins in grains and oilseeds. The NIR method has been used to identify or predict concentrations of mycotoxins in wheat and maize with high accuracy using calibration or classification models through chemometrics (Delwiche and Hareland, 2004; Fernández-Ibañez et al., 2009). FTIR spectroscopy offers a good signal-to-noise ratio and high resolution of spectra in the mid-infrared region that efficiently detects and identifies fungal species and mycotoxins in grains and oilseeds (Abramović et al., 2007; Greene et al., 1992; Kos et al., 2002; Mirghani et al., 2001). However, both NIR and FTIR absorption bands are not well resolved and superimposed with other components. In addition, due to strong HOH bending absorption of water molecules throughout the range of spectroscopic wavelengths, component bands of interest are overlapped with water absorption bands and are often distorted due to residual features of water bands even after subtraction or differentiation (Byler and Susi, 1988).

Despite its great possibilities and advantages over other spectroscopic techniques, Raman spectroscopy has received remarkably little attention in cereal science and for investigation and detection of mycotoxins in grains and oilseed. The Raman spectroscopy technique is based on the polarisability of chemical bonds and is more sensitive to the symmetrical vibrations of covalent bonds in the non-polar group. Due to its insensitivity to water and fewer overlapped bands, Raman spectroscopy provides more useful qualitative and quantitative information on chemical functional groups of mycotoxin compounds and its derivatives than the conventional spectroscopic technique by providing a molecular level insight into mycotoxins. Several studies have shown promising results for rapid screening of mycotoxin contaminated grains and oilseeds and their products by the use of this technique (Golightly et al., 2009; Liu et al., 2009; Sohn et al., 2004).

Therefore, the objective of this research was to explore the possibility of the Raman spectroscopy technique combined with chemometrics using different preprocessed spectra data to develop a rapid, inexpensive, and convenient spectroscopic method for classification and quantification of aflatoxin contaminated maize samples. This proposed method as a first screening step for aflatoxin detection in samples could provide a high-throughput analysis platform that could improve food and feed safety at grain elevators, serving as the first collection point during harvest and for breeding/hybrid performance programs testing thousands of samples.

2. Experimental

2.1. Materials

Aflatoxin standards (B1, B2, G1, and G2) were obtained from Sigma Chemical Co. (St. Louis, MO). The Aflatest immunoaffinity column was purchased from VICAM (Watertown, MA). All reagents and organic solvents were of analytical grade and used as received.

2.2. Sample preparation

Maize samples for aflatoxin tests were a representative subsample from Office of the Texas State Chemist (OTSC) regulatory samples collected by Texas Feed and Fertilizer Control Service investigators in conformance with the state's statistically derived risk-based plan of work using official sampling procedures and chain-of-custody. Of the collected maize samples for aflatoxin inspection, a total of 132 naturally contaminated samples with aflatoxin concentration range of 0–1206 μg/kg were selected for the present study. Each sample, of approximately 4.5 kg, was thoroughly ground using a Retsch Ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany) with a 0.075 mm diameter screen. The ground samples were stored at 4 °C in a refrigerator prior to HPLC and Raman spectral analysis and between experiments. Before recording Raman spectra, the ground maize samples were tested for moisture content according to AACC method 44-15A (AACC, 2000). The samples with higher moisture content were dried to below 15% at 40 °C for 12 h to stop fungal growth in samples and then stored in plastic bottles in a refrigerator (4 °C) and equilibrated to room temperature for at least 1 h before analysis.

2.3. Raman spectroscopy

Approximately 5 g of sample was removed from each ground sample and directly analyzed by Raman spectroscopy (RamanStation 400F, Perkin–Elmer, Beaconsfield, Buckinghamshire, U.K.). A total of 132 samples were measured in quadruplicate. The Raman system was equipped with a 256 × 1024 pixel CCD detector and 350-mW near-infrared 785 nm laser. About 30-mW laser power at which Raman spectra are reproducible and stable for laser radiation was delivered to the sample with focus to a 5-mm × 5-mm area.

Download English Version:

https://daneshyari.com/en/article/4515940

Download Persian Version:

https://daneshyari.com/article/4515940

Daneshyari.com