Proposal No. 2011_001_RC

The Andersons Research Grant Program

Project Title:

Application of Raman Spectroscopy for Detection of Aflatoxins and Fumonisins in Maize, Cottonseed, and Peanut meal

Principal Investigator(s)

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Year 1: $25,000 Year 2: $24,700

1. PROBLEM IDENTIFICATION AND RELATED RESEARCH

1.1. Problem Identification

Contamination of food and feed grains and oilseeds with fungal mycotoxins by the *Aspergillus* strains producing aflatoxins and the *Fusarium* strains producing fumonisins has been a major problem in US agriculture and a health hazard to humans and animals. Contaminated grains and oilseeds with mycotoxins, the secondary metabolites of fungi, can be extremely toxic and carcinogenic to esophagus and liver in humans and animals. In addition to the health hazard, mycotoxin contaminated grains and oilseeds are devaluated in the markets for food and feed, resulting great economic loss for growers, animal protein producers, handlers, and food and feed processors (Robens and Cardwell 2003). Therefore, authorities in many countries and food safety agencies such as United States Food and Drug Administration and European Commission (EC Commission Regulation 165/2010) have legally enforced limits of some mycotoxins in grains and oilseeds for their use in major human foods and feeds.

Because microbial mass is not always related to mycotoxin content, a direct quantification of mycotoxin is desirable when it is required to evaluate a toxicity of grains and oilseeds. Mycotoxin analysis has been well managed using diverse mycotoxin analytical methods available in laboratory and non-laboratory locations, including bright greenish-yellow fluorescence (BGYF) test, thin layer chromatography (TLC), gas chromatograph (GC), high performance liquid chromatography (HPLC), mass spectrometry (MS), enzyme-linked immunosorbent assay (ELISA), immune-affinity column assay, and recently biosensors (Zheng et al 2006). Despite the advantages and accuracies of such methods, the methods have certain drawbacks. They are expensive, complex, labor-intensive, and time consuming techniques, being difficult to make rapid screening for a large number of samples and to use at the collection point of contaminated grains and oilseeds. For example, chromatographic methods provide great accuracy and reproducibility, but require a complex and time-consuming steps for separation, extraction, purification, detection and sample clean-up. Although ELISA requires less sample extract clean-up procedure compared to chromatographic methods, in addition to mycotoxins, non-mycotoxin compounds can also interact with the antibodies. Therefore, the development and validation of rapid, sensitive, and accurate methods with minimum effort and cost for early screening of mycotoxin contaminated grains and oilseeds are highly needed to provide real-time monitoring for mycotoxin levels at receiving point of the samples and consistent repeatable performance and automatic data management.

A variety of emerging methods based on novel technologies such as lateral flow devices (LFD), molecularly imprinted polymers (MIP), surface plasmon resonance (SPR) biosensors, and infrared spectroscopy have been reported for the rapid analysis of mycotoxins (Pascale 2009). Of these techniques, spectroscopic techniques are attractive because with a single scan, they can provide a plenty of qualitative and quantitative information pertaining to mycotoxin components and structures. Infrared spectroscopic techniques such as near-infrared reflectance (NIR), Fourier Transform Infrared spectroscopy (FTIR), and Raman spectroscopy which require no or little sample preparation and pretreatments are an excellent candidate for a rapid and low cost method for detection of mycotoxins in grains and oilseeds and have been used for rapid identification and classification of fungal and mycotoxin contaminated grains and oilseeds (Kos et al 2002, 2004, & 2007, Delwiche and Gains 2005, Gordon et al 1997 & 1999, Wheeler et al 1993, Greene et al 1992). These techniques use different physical processes to measure sample

spectra and thus expected to provide specific complementary information about mycotoxins in samples.

In spite of the rapid measurements and good sensitivity, their applications sometimes are not very successful due to difficult interpretation and spectrum overlapping. With advent of modern interferometric and fourier transform techniques, NIR method has been widely used for a variety of purposes in the grain and oilseed industry including mycotoxin detection, quantification of grain components, classification, sorting, on-line process control, end-use quality tests, hardness measurement, and milling yields. However, in general NIR absorption bands are not well resolved and overlapped with other components and strong water absorption bands, which may greatly influence some important bands associated with mycotoxin molecules. FTIR has been proposed by an alternative vibration spectroscopic technique for rapid characterization of mycotoxins in grains and oilseeds due to its higher sensitivity and similar features with NIR. However, due to strong HOH bending absorption of water molecules throughout the range of FTIR wavelengths, component bands of interest are overlapped with water absorption bands and often distorted due to residual features of water bands even after subtraction or differentiation (Byler and Susi 1988). On contrary, Raman technique which is based on the polarity of chemical bonds has been not fully explored in cereal science and in investigation and detection of mycotoxins in grains and oilseed despite of its great possibilities and advantages over other spectroscopic techniques. Raman as an analytical tool can provide a molecular level insight into mycotoxin and is highly expected to substitute time-consuming and destructive mycotoxin analytical methods.

1.2 Related Research

FTIR and NIR applications for mycotoxin analysis

Some previous studies have demonstrated that FTIR spectroscopy could be a useful instrumental method for detecting and identifying fungal species and mycotoxins in grains and oilseeds. FTIR produces a relatively good signal-to-noise ratio and high resolution of spectra in the mid-infrared region and also is fast and easy to manipulate data by software. Therefore, it has been considered as an alternative to the standard wet chemical methods for rapid determination of mycotoxin concentration, offering the possibility of bulk automation. Fourier-transform infrared photoacoustic spectroscopy (FT-IR-PAS), diffuse reflection spectroscopy (DRS), and transient infrared spectroscopy (TIRS) methods have been used to differentiate uninfected maize from infected maize by fungi (Gordon et al 1997 & 1999, Greene et al 1992). The application of FTIR attenuated total reflection (ATR) spectroscopy was also examined for the detection of fugal and mycotoxin contamination on maize (Kos et al 2002), wheat (Abramovic et al 2007), and ground nut (Mirghani et al 2001). In their studies, the researchers employed chemometrics techniques such as principal component analysis (PCA), cluster analysis (CA), discriminant analysis (DA), and partial least squares (PLS) for evaluation of the spectra and classification of samples based on the level of mycotoxin contamination.

NIR spectroscopy has been widely used to detect mycotoxins in naturally or artificially contaminated grains and oilseeds. NIR technique was applied to identify or predict concentrations of deoxynivaleneol (DON), aflatoxins, and fumonisins in wheat and maize using the calibration or classification models with high coefficients of determination (R^2) (Fernandez-Ibanez et al 2009, Delwiche and Hareland 2004). In their studies, chemometrics, mainly PLS

algorithm, needed to be employed for data analysis and calibration because NIR spectra cannot be used for direct quantitation of mycotoxins due to highly overlapped and broad bands. FT-NIR was faster and more sensitive with higher resolutions than conventional dispersive NIR in detecting mycotoxins and considered as a promising technique for rapid and quantitative determination of mycotoxins (Fernandez-Ibanez et al 2009).

Raman applications for mycotoxin analysis

For last decades, Raman spectroscopy have been modified to increase an instrumental sensitivity and applied to a variety of food and feed samples (Ma and Philips 2002). Raman spectroscopy has more sensitivity to the symmetrical vibrations of covalent bonds in nonpolar groups than FTIR which works better with asymmetrical vibrations in polar functional groups. Although NIR has been found to be a useful analytical tool for mycotoxin detection, NIR spectra are based on absorption of only a limited number of chemical functional groups and consist of relatively broad spectral bands. In previous comparative studies with grain samples, Raman spectroscopy exhibited much higher spectral resolution and more distinctive bands than NIR spectra (Sohn et al 2004, Ma and Phillips 2002).

Several studies reported the use of Raman spectroscopy in the detection and identification of mycotoxin compounds. Several limited studies showed promising results for rapid screening of mycotoxin contaminated grains and oilseeds and their products by Raman. Liu et al (2009) applied Raman spectroscopy with simple algorithm and principal component analysis (PCA) to distinguish between low and high level DON-contaminated grains. Grow et al (2003) used a biochip and a variant of surface enhanced Raman spectroscopy (SERS) to detect aflatoxin B1 and aflatoxin G1. Aflatoxin B1 in peanut butter was detected using the SERS magnetic bead-based assay after extracting samples with methanol (Glightly et al 2009).

Due to its insensitivity to water and fewer overlapped bands, Raman spectroscopy can be an alternative to FTIR and NIR and provide more accurate qualitative and quantitative information on mycotoxins in high moisture containing samples like grains and oilseeds with minimizing inferences from residual components. However, Raman spectroscopy has received remarkably little attention as a spectroscopic technique for the detection of mycotoxin. Raman spectroscopy is sensitive enough to detect chemical functional groups of mycotoxin compounds and derivatives. Therefore, we expect able to characterize mycotoxin molecules through the molecular fingerprint of Raman and further correlate Raman information with the levels of mycotoxin contamination. As demonstrated in quality control of cereal products (Sohn et al 2004), Raman spectroscopy also has a great potential to classify grains and oilseeds based on mycotoxin contamination level with higher accuracy. The resolution of Raman spectra can be greatly improved using SERS and hyperspectral imaging technique which may allow us to determine multiple mycotoxins in samples simultaneously since each mycotoxin produces a unique SERS fingerprint and hyperspectral imaging pixel.

Spectroscopic amplification and Enhancement techniques

Nonuniform samples (e.g. intact grain kernels) are easy to produce spectra irrelevant chemical information and thus it is necessary to have mathematical treatments to reduce scatter effects and extract only meaningful information from collected spectra. Chemometrics used as a tool for the complex data treatment are often applied to obtain characteristic and additional information from large data sets of the sample spectra. In chemometrics, spectral absorbance

values at many wavelengths over the peaks of interest are used as inputs in chemometric methods. Before applying chemometrics, resolution-enhancement techniques such as derivatives (curve-fitting) and deconvolution are useful for the original spectra, particularly weak Raman spectra, to visualize overlapping and superimposed bands. Both techniques are suitable to identify component frequencies in complex spectra and resolve the broad band into its original components (Byler and Susi 1988). The chemometric model would provide rapid and reliable prediction of the level of contamination and presence or absence of mycotoxins in grains and oilseeds with relatively high accuracy (Kos et al 2002, 2004, & 2007, Wheeler et al 1993).

Hyperspectral imaging, known as chemical or spectroscopic imaging, is an emerging technique that can obtain both spatial and spectral information from the sample (Gowen et al 2007). In hyperspectral image, each pixel contains the spectrum of each specific spot, which enables to characterize the composition of that particular pixel. Hyperspectral imaging carrying out in reflectance, transmission or fluorescence modes has been used to detect defects, infections and quality attributes of agricultural products including grain and oilseed products (Cogdill et al 2004). It is believed that through the application of appropriate chemometric methods, hyperspectral imaging becomes a powerful technique for mycotoxin analysis. It could be particularly useful at the place where the standard wet chemical methods for certain mycotoxin are not available or difficult.

As mentioned above, surface-enhance Raman spectroscopy (SERS) is an attractive method which enhances scattering by six orders of magnitude or more and can improve detection limits up to the parts-per-billion range. Nevertheless, the ability of SERS to detect trace biochemical agents in grains and oilseeds hasn't been fully exploited and very little research has been reported in mycotoxin analysis.

2. OBJECTIVES

Our proposed research is to assess the applicability of Raman spectroscopy as a rapid, inexpensive, and convenient analytical methods for determining mycotoxins in naturally and artificially contaminated grains and oilseeds by combining Raman spectroscopy and chemometrics techniques. The approach to achieve this object includes two tasks:

1. Investigate the possibility of Raman spectroscopy as a first screening method to detect mycotoxins in maize samples. This task includes the use of SERS and chemometrics techniques to obtain precise band measurements and good-quality Raman spectra for the development of calibration models to effectively distinguish contaminated from uncontaminated maize samples.
2. Extend Raman spectroscopic techniques developed for maize samples to detect the presence of mycotoxins in cottonseeds and peanut meals. This task will focus on the classification, detection, and quantification of mycotoxins in two oilseed products and explore the possibility of techniques as a potential tool for automatic diagnosis of mycotoxin contamination.

Our research approach will primarily address the following NC-213 objectives:

Objective 1: Characterize quality attributes and develop systems to measure quality of cereals, oilseeds, and bioprocess coproducts.

Objective 2: Develop methods to maintain quality, capture value, and preserve food safety at key points in the harvest to end product value chain.

3. PROCEDURES

Task 1: Investigate the possibility of Raman spectroscopy as a first screening method to detect mycotoxins in maize samples

In the beginning of this project, we will chose maize as the target commodity focusing on its contamination with aflatoxins (B1, B2, G1, and G2) and fumonisins (B1, B2 and B3) since maize is most commonly contaminated with these mycotoxins under hostile environments and stress conditions and the two mycotoxins are also major and common contaminants of grains and oilseeds.

Sample preparation

Maize samples will be obtained at 10 kg from Office of the Texas State Chemist (OTSC) routine surveillance samples that are usually submitted to the Office for analytical test based on the OTSC inspection and sampling program. Of ~850 maize samples for mycotoxin inspection, we will select a total of 150 naturally contaminated samples which will be composed of genetically and environmentally diverse maize hybrids.

One hundred fifty maize samples thoroughly mixed for HPLC mycotoxin analysis for the OTSC inspection will be dried at 40 °C and stored at 4 °C in a refrigerator prior to spectral analysis. For spectroscopy test, the individual kernels will be drawn from each maize sample. Since maize samples are ground for HPLC analysis, the same ground samples can be used for spectroscopy measurement. However, since the concentration of mycotoxins in maize samples varies with particle sizes and the repeatability of spectral measurements improves with a narrowing of particle size distribution (Kos et al 2007), particles with the size range of 200-250 um will be taken for all subsequent Raman spectroscopic measurements after particle size analysis. All maize kernels and ground samples will be tested for moisture content before the recording of the Raman spectra to ensure to stop fungal growth in samples during storage and test. Maize samples stored at a refrigerator will be equilibrated to room temperature for at least 1 hr before test.

To obtain a calibration between the band intensity and mycotoxin content for quantification, uninfected maize samples will be spiked with pure aflatoxin standards B1, B2, G1, and G2 in concentrations of 0–100 ppb and fumonisin standards B1, B2 and B3 in concentrations of 0-50 ppm. Raman spectra of pure mycotoxins will be recorded and included in a library that may also contains signatures of maize samples and other interferent materials for identification of mycotoxins from infected maize products.

Spectroscopic measurements

Raman spectra for maize kernels and ground samples will be acquired on RamanStation 400F (Perkin-Elmer, Beaconsfield, Buckinghamshire, U.K.) interfaced with Spectrum (v. 6.3) software in the Raman shift range from 200 to 3500 cm-1 using a near-infrared laser light source of 785 nm at 350 mW and a 256 x 1024 pixel CCD detector. We will use commercially available 96-well SERS-active microtiter plates (Real-Time Analyzers, Inc., Middletown, Connecticut) as a sample holder consisted of ~1-mm thick coating of a silver-doped sol-gel to

enhance Raman scattering for detection of multiple mycotoxins. Maize kernels and ground samples will be placed on the multi-well plate, and their Raman spectra will collected at a resolution of 8 cm^{-1} from the large sample spot consisting of six locations around the centered location with exposure times of 1 sec and 10 scans at the sample location. The spectra from seven locations will be co-added to give a single spectral file. Before measuring spectra for infected maize samples, different sound maize varieties will be analyzed to evaluate maize variety variation in spectra. Prior to the spectral analysis, the collected spectra will be baseline corrected and normalized to reduce variation in Raman signal due to subtle changes in experimental conditions. Hyperspectral images for transversely cut maize kernel and surface of ground samples will be obtained to identify different areas and the distribution of chemical functional groups and components. These images might provide spatial information on mycotoxin distribution on a sample spot by different colors as well as spectral information associated with mycotoxins.

To compare Raman with NIR and FTIR, mycotoxin contaminated and uncontaminated samples will be scanned using NIR and FTIR spectrometer. In NIR method, the original spectra will be processed using interfaced software with mathematical treatment functions (derivative, gap, smooth, and normalization) and scatter correction of the spectra to select best combinations for the development of a calibration model. The FTIR analysis will be performed on an IlluminatIR (Smiths Detection, Danbury, CT) attached with diamond attenuated total reflection (ATR) objective and all reflective objective (ARO). The spectra will be recorded at 4 cm^{-1} resolution with a total of 128 co-added scans. The spectrum will be corrected using same mathematical treatments used for NIR spectrum to remove effects of CO_2, contaminant, scatter, and inference fringe on the spectrum.

Spectral data processing and analysis

Some spectra regions are known to be associated with biochemical components in maize products which should be useful for this study in detecting and quantifying fungal mycotoxins. It is anticipated that mycotoxin production in maize products affects other peaks of biochemical components. Therefore, one should be aware that collected spectra are a sum of fungi, mycotoxins, and maize spectral regions. Besides, spectral difference between pericarp and inside endosperm needs to be considered in spectral evaluation of whole kernels and ground samples. Therefore, band features such as peak shift, ratio of peak heights, slope line, and signal elevation will be carefully examined to compare changes of target compounds in the spectra of between contaminated and uncontaminated maize and also exclude spectral regions unrelated to mycotoxin contamination. The spectral subtract procedure will be used to differentiate between infected and uninfected maize spectra. Normalized peak ratios and other spectral features will be also used to correlate spectra information with levels of mycotoxin infections. Spectra calibration will be carried out on a regular basis.

Chemometrics including principal component analysis (PCA), cluster analysis (CA), discriminant analysis (DA), partial least squares (PLA), and pattern recognition techniques (e.g. artificial neural network) using some spectral features will be performed to extract characteristic information from spectra. Of multivariate techniques in chemometrics, the technique that gives a high correct classification rate with easy application will be selected in determining presence and absence of infection and levels of mycotoxins in maize products. In addition, the results obtained from chemometrics will be compared with those from multiple linear regressions with

the same input variables for chemometrics. The data from HPLC conventional methods for aflatoxin and fumonisin determination will be compared with the results from spectral measurements.

As a first step in development of a calibration model, data will be split into a calibration (75% samples) and a validation (25% samples) set. For unbiased comparison of calibration models, the spectra data of NIR, FTIR, and Raman will be obtained from the same calibration set and processed using the same mathematical treatments. These preprocessed spectra data then will be exported to another spectroscopic software and/or SAS (Release 9.2, Cary, NC) to allow the same data treatment prior to building a calibration model. The model's prediction ability will be validated using a cross-validation method. The best model will be selected based on the correlation coefficients, standard error of calibration, and standard error of prediction. The results of NIR, FTIR, and Raman will be compared to obtain complementary information to each other. The statistical or chemometrices models will be integrated into computer program. Hyperspectral images obtained from maize whole kernels or ground samples will be exported to SAS (v. 9.2, Cary, NC) for multivariate statistical analysis. The hyperspectral data will be another set of wealthy spectra data that can provide significant information about mycotoxins and may be quite useful for building a robust calibration model.

Classification of mycotoxin contaminated maize grains

We will test if spectroscopic data from Raman, FTIR, and NIR measurements can be used to classify maize samples using multivariate statistical techniques. Two types of statistical procedures, unsupervised (hierarchical cluster analysis) and supervised methods (discriminant analysis), will be employed to compare spectra. We anticipate that each spectroscopic technique would produce different set of classified groups in terms of the number of groups and their members, which may allow us to evaluate a suitable spectroscopic technique for classification of mycotoxin contaminated samples. The data will be projected onto principal component axes to visualize classified groups. Through this procedure, the spectral regions most influencing the classification of groups will be determined. Using more important and reliable spectral features will make it possible to construct a more robust classification scheme. The best model for maize grains will be selected based on the standard error of cross-validation (SECV) and highest coefficient of determination (R^2). The external validation results will be evaluated using the correct classification rate (%).

Task 2: Extend Raman spectroscopic techniques developed for maize samples to detect the presence of mycotoxins in cottonseeds and peanut meals

With the development of mycotoxin detection method for maize grains, we will explore the ability of spectroscopic techniques to detect aflatoxins and fumonisins in two oilseed feed products, cottonseeds and peanut meals. Many studies and cases have showed these two commodities are highly and frequently contaminated with mycotoxins. The OTSC has also monitored mycotoxin levels in these two commodities as they are the major source of mycotoxin contamination in feed products. We anticipate that the approaches developed for maize samples can be easily extended to the characterization of mycotoxins in cottonseeds and peanut meals. However, similar difficulties (sampling variances, diversity of genotypes and environments, and very low mycotoxin concentration in samples) encountered during developing the method for

maize grains are also expected, requiring more critical and careful examination of sample spectra.

For this task, a total of 50 cottonseed and 50 peanut meal samples will be involved. Due to lack of samples collected based on the OTSC mycotoxin sampling plan, we will obtain additional samples from retailers or commercial aggregators in Texas. The whole cottonseed and peanut meal samples will be thoroughly mixed, ground, and prepared for HPLC and spectroscopy measurements using the same procedure as described for maize samples. Likely, the same considerations about particle size will apply to these samples.

Mycotoxin free samples will be then spiked with defined amounts of mycotoxins to different concentrations. All three spectroscopic techniques will be employed to measure changes in the spectra of mycotoxin contaminated oilseed samples, but we will more focus on practical application of Raman using SERS method as described for maize samples since this high-sensitive and specific technique is more promising to handle mycotoxin contaminated samples at low concentrations than other spectroscopic methods. We will also develop and train appropriate chemometrics in combination with specific band features and hyperspectral imaging to effectively determine mycotoxin contamination of two oilseed products.

4. ANTICIPATED RESULTS, PRODUCTS, AND IMPACTS

At the end of this research we expect that the use of SERS technique of Raman spectroscopy combined with chemometrics improves the discrimination performance and provides much of desired sensitivity and specificity over existing spectroscopic approaches, which ultimately helps reduce the degree of contamination of grains and oilseeds by mycotoxins. Anticipated specific results are as follows:

- This research will provide accurate and low-cost analytical method over conventional spectroscopic and standard wet chemical methods for quick estimation of true mycotoxin concentration at busy locations where a high throughput is preferred.
- This research will offer spectroscopic methods able to allow more rapid qualitative and quantitative characteristics of mycotoxin compounds, providing real-time monitoring for mycotoxin levels in grains and oilseeds at receiving points.
- Successful implementation of a robust model for rapid, low-cost, and convenient detection of mycotoxin levels help prevent risks to human health, provide high value of safe grains and oilseeds for domestic and global markets, and improve economic benefits.
- This research will integrate spectra features into chemometrics designed for recognition of Raman spectra by computer, which will be able to provide a great potential for automatic detection of mycotoxin contamination and online-monitoring quality control.

5. LEVERAGING RESOURCES

Raman spectroscopy has already demonstrated its superiority, or at least equality to IR and other spectroscopic techniques in some food areas. However, its novel and unique properties haven't been paid attention by the grain and oilseed industry. This spectroscopic technique as a first screening of mycotoxin contaminated samples should be attractive and useful to breeders,

growers, processors, and plant pathologists. The OTSC has close relationships with feed firms in Texas. The results of proposed research using regulatory samples out of their products will be interesting to Texas feed manufacturers and can help enhance feed safety and high-throughput screening, improving economic benefits for the Texas grain and feed industry. We predict that Raman spectroscopic technique will feasibly expand its application to other grain and oilseed commodities and their processes through the skills and technologies developed from this project. Based on data and outcomes obtained during this project, we will submit a proposal for a larger grant opportunity with the Agricultural and Food Research Initiative (AFRI) grant program as well as local funds to test feasibility of mycotoxin detection technique by Raman at commercial storage and processing facilities.

6. PROJECT TIMETABLE

The expected timetable for the proposed research activities is presented below. The project start relies on the availability of maize grains and two oilseed samples during the year.

Activities	**Year 1** (***Task 1: maize***)				**Year 2** (***Task 2: oilseeds***)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Sample preparation	**X**				**X**			
Spectra data collection		**X**	**X**			**X**	**X**	
Spectra data processing and analysis		**X**	**X**	**X**		**X**	**X**	**X**
Annual report and journal article preparation				**X**				**X**

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W.B. Johnson, W.S. Ratnayake, D.S. Jackson, **K.M. Lee**, T.J. Herrman, S. R. Bean, S.C. Mason. 2010. Factors affecting the alkaline cooking performance of selected corn and sorghum hybrids. Cereal Chem. 87:524–531.

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RESEARCH INTERESTS

Structural and biochemical properties of grain macromolecules, grain tracing and recall system, development and standard of grain quality tests, multivariate statistical techniques, statistical sampling design, LC-MS and GC-MS analyses, spectroscopic techniques (NIR, IR, and Raman) for rapid screening and quantification, risk assessment and management of microbiological contamination, development of snack and bakery products, minimal processing of fruits and vegetables.

AWARDS AND HONORS

Admission as a top seat and graduation as valedictorian at Kyonggi University
Full time chancellor scholarship during four years of the undergraduate study
Full time scholarship for the master's degree study from STINT (The Swedish Foundation for International Cooperation in Research and Higher education)

PROFESSIONAL AFFILIATIONS AND ACTIVITIES

American Association of Cereal Chemists (AACC)
Association of American Feed Control Officials (AAFCO)
- Laboratory Methods and Services, Committee Member, 2007–Present

Multistate Project NC–213, Marketing and Delivery of Quality Grains and BioProcess Coproducts
- Objective co-chair, 2008–Present

TECHNICAL REVIEWER

Cereal Chemistry, Transactions of the ASABE, and Journal of Agricultural and Food Chemistry

2011_001_RC

CURRENT & PENDING SUPPORT

Name: Kyung-Min Lee

NAME	SUPPORTING AGENCY/ SPONSOR AND AGENCY ACTIVE AWARD/PENDING PROPOSAL NUMBER	TOTAL $ AMOUNT	EFFECTIVE AND EXPIRATION DATES	% OF TIMECOMMITTED	TITLE OF PROJECT
Lee	(Pending) The Andersons, Inc.	$50,000	Jan 1, 2012 – Dec 31, 2013	15	Application of Raman spectroscopy for detection of aflatoxins and fumonisins in maize, cottonseed, and peanut meal
Lee	(Current)				

BUDGET
Overall Budget

Category	Year 1 Amt. requested from Andersons	Year 2 Amt. requested from Andersons	Total
Salaries and Wages*			
Post-Ph.D. research associate(s)	$4,000	$12,000	$16,000
Graduate assistant			
Stipend			
Tuition and fees			
Hourly wage			
Other (specify in Budget Narrative)			
Total	$4,000	$12,000	$16,000
Fringe Benefits			
Post-Ph.D. research associate(s)			
Graduate assistant			
Hourly wage			
Other			
Total			
Materials and Supplies	$19,500	$11,200	$30,700
Equipment (List individual pieces of equipment that are essential to the project in the Budget Narrative.)			
Travel	$1,000	$1,000	$2,000
Publication charges	$500	$500	$1,000
Indirect costs**			
Total (Max. $25,000/yr from Andersons Research Grant Program)	$25,000	$24,700	$49,700

*Andersons funds cannot be used for faculty salaries, departmental space, or facilities.

**The Andersons Research Grant Program policy specifies that no indirect costs can be charged to this project.

Budget Narrative

Salaries and wages

The personal cost includes 4-month salary for a graduate student work on the project, $4,000 in year 1 and $12,000 in year 2. The graduate student will perform sample preparation and spectroscopic measurements and assist Dr. Lee on spectroscopic data analysis and preparation of scientific manuscripts.

Materials and Supplies:

All maize and oilseed sample will be estimated at the OTSC laboratories except for NIR measurements. The cost of $7,500/2-years for Raman spectroscopy includes the purchases of SERS-active microtiter plates and spectrometer accessories. The cost of $ 5,000 per year for HPLC test is made toward the purchases of chemicals, reagents, and lab supplies. FTIR cost and NIR outside laboratory service at $7.00 per sample will cost $3,500/2 years of each. A request of $3,000 is made for collection, purchase, and shipping of maize and oilseed samples. Pure mycotoxins and fumonisins kits are estimated at $1,300 and 1,100, respectively.

Table1. Estimated itemized costs

Activity	Fees	Total cost
Moisture content	$4.0	$1,000
HPLC	$50.0	$5,000
NIR	$7.0	$3,500
Raman	$15.0	$7,500
FTIR	$7.0	$3,500
Sample collection, purchase, and shipping		$3,000

Travel:

Travel cost of $2,000 is requested to attend professional meetings and the NC-213 annual meeting for presentation of research results.

Publication costs:

The publication cost is requested at ~$500 per article in refereed profession journals.

Available facilities and equipment:

The Office of the Texas State Chemist (OTSC) has joined the Food Emergency Response Network (FERN) since 2005 as a sentinel chemical lab and supported a strong food-safety and bio-security emphasis within the network. Recently, the OTSC responsible for regulating the distribution feed launched a “One Sample Strategy” for aflatoxin to improve the performance of aflatoxin measurement accuracy within the Texas grain and feed industry.

The research for this proposal will be mostly performed at Office of the Texas State Chemist (OTSC) except for NIR measurement at outside grain quality laboratory. The OTSC laboratories are equipped with advanced analytical instruments and facilities adequate to perform spectroscopic and biochemical analysis for this project. The facilities and equipment include Perkin Elmer Raman spectrometer, Smiths Detection FTIR spectrometer, a Thermo Surveyor

HPLC-LTQ, Agilent and Waters HPLCs, Waters UPLC Quattro Premier XE, Agilent GC-MS, Varian ICP, Perkin Elmer ICP-MS, LECO nitrogen analyzers, Bioveris M1M analyzer, Varian UV-VIS, air/water purification systems, and many other miscellaneous equipment. At present, faculties and staffs in other departments at Texas A&M University are using the OTSC's analytical instruments and facilities for their researches.