Risk Assessment

Modeling the Risk of Salmonellosis from Consumption of Peanuts in the United States

Significance: A quantitative microbial risk assessment of salmonellosis risk from peanuts is presented.

Peanut products were the target of the largest food recall in United States history from 2008 to 2009, with more than 3,200 products implicated, economic losses estimated at $1 billion, and more than 700 reported illnesses and 9 deaths. Predictive modeling tools such as quantitative microbial risk assessment can be used to aid processors in making risk management decisions that may reduce the chances of foodborne illness, but published risk assessment for peanuts is not currently available. A quantitative microbial risk assessment was performed to quantify salmonellosis risk from consumption of peanuts in the United States. Prevalence and concentration data for Salmonella on raw, shelled peanuts were used in combination with probability distributions of simulated log reductions achieved during production steps before consumption. Data for time-temperature combinations used in each step were obtained from published literature, industry surveys, or expert opinion, and survival data were obtained from the literature. A beta-Poisson dose-response model was used to predict probability of illness from ingestion of Salmonella cells. The model predicted 14.2 (arithmetic mean) or 0.0123 (geometric mean) illnesses per year. Sensitivity analysis showed that thermal inactivation log reductions applied had the biggest impact on predicted salmonellosis risk, followed by consumer storage time, Salmonella starting concentration, Salmonella starting prevalence, and number of originally contaminated 25-g servings per originally positive 375-g sample. Scenario analysis showed that increasing log reduction variability increased mean salmonellosis risk. Removing the effect of storage on Salmonella survival increased the arithmetic and geometric means to 153 and 0.598 illnesses per year, respectively. This study indicated that the risk of salmonellosis from consumption of peanuts can be lowered by reducing field contamination, control of storage steps, and monitoring of appropriate critical limits in peanut roasting.

Pathogen Detection

Evaluation of invA Diversity among Salmonella Species Suggests Why Some Commercially Available Rapid Detection Kits May Fail To Detect Multiple Salmonella Subspecies and Species

Significance: Considerations for improving the rate of false-negative test results in Salmonella detection assays are presented.

invA is a common molecular target for Salmonella-specific detection methods and is recommended by the U.S. Food and Drug Administration Bacteriological Analytical Manual as a target for PCR confirmation of putative Salmonella isolates. Novel assays designed for the rapid detection of foodborne pathogens are often validated according to guidelines provided by validation schemes, such as the AOAC International or the International Organization for Standardization. However, these validation guidelines allow for flexibility in the validation study experimental design, which may inflate the assay’s ability to detect foodborne pathogens, especially for foodborne pathogens such as Salmonella, exhibiting tremendous species diversity with >2,600 confirmed serovars. This study was conducted to (i) describe the sequence diversity of invA, across a diverse set of Salmonella serovars and (ii) evaluate the ability of two commercially available, AOAC International–validated rapid detection assays to detect a diverse collection of Salmonella spp. strains. In silico analyses identified 362 of 2,058 nucleotide sites that were variable among invA sequences from a diverse collection, representing 86 unique serovars spanning all species and subspecies. Not surprisingly, the majority of variable sites (308 of 2,058) occurred in non–Salmonella enterica subsp. enterica
strains, including *Salmonella bongori* and the other *S. enterica* subspecies. In vitro testing showed that both rapid detection assays, examined here, failed to detect all *Salmonella* strains at 1 log above the limit of detection, with assay A failing to detect *S. enterica* subsp. *salamae*, and assay B failing to detect *S. bongori*. Both strains were eventually detected at 100,000 times the limit of detection. Taken together, our study highlights the need to include non–subsp. *S. enterica* strains in the development and validation of rapid detection methods to limit false-negative test results.

**Mycotoxins**

**Cost-Effective Sampling and Analysis for Mycotoxins in a Cereal Batch**


**Significance:** An optimization model was constructed to identify the most cost-effective sampling and analysis plan for mycotoxins in a maize batch.

The presence of hazards (e.g., contaminants, pathogens) in food/feed, water, plants, or animals can lead to major economic losses related to human and animal health or the rejection of batches of food or feed. Monitoring these hazards is important but can lead to high costs. This study aimed to find the most cost-effective sampling and analysis (S&A) plan in the cases of the mycotoxins deoxynivalenol (DON) in a wheat batch and aflatoxins (AFB1) in a maize batch. An optimization model was constructed, maximizing the number of correct decisions for accepting/rejecting a batch of cereals, with a budget as major constraint. The decision variables were the choice of the analytical method: instrumental method (e.g., liquid chromatography combined with mass spectrometry (LC-MS/MS)), enzyme-linked immuno assay (ELISA), or lateral flow devices (LFD), the number of incremental samples collected from the batch, and the number of aliquots analyzed. S&A plans using ELISA showed to be slightly more cost effective than S&A plans using the other two analytical methods. However, for DON in wheat, the difference between the optimal S&A plans using the three different analytical methods was minimal. For AFB1, in maize, the cost effectiveness of the S&A plan using instrumental methods or ELISA were comparable whereas the S&A plan considering onsite detection with LFDs was least cost effective. In case of nonofficial controls, which do not have to follow official regulations for sampling and analysis, onsite detection with ELISA for both AFB1, in maize and DON in wheat, or with LFDs for DON in wheat, could provide cost-effective alternatives.

**One-Step Ultrasensitive Bioluminescent Enzyme Immunoassay Based on Nanobody/Nanoluciferase Fusion for Detection of Aflatoxin B1 in Cereal**


**Significance:** Nanobody fragments fused with nanoluciferase were used to develop a rapid and sensitive immunoassay for detection of aflatoxin B1 in cereal.

Nanoluciferase (Nluc), the smallest luciferase known, was used as the fusion partner with a nanobody against aflatoxin B1, to develop a bioluminescent enzyme immunoassay (BLEIA) for detection of the aflatoxin B1 in cereal. Nanobody (clone G8) against aflatoxin B1 was fused with nanoluciferase and cloned into a pET22b expression vector, and then transformed into Escherichia coli. The nanobody fusion gene contained a hexahistidine tag for purification by immobilized metal affinity chromatography, yielding a biologically active fusion protein. The fusion protein G8-Nluc retained binding properties of the original nanobody. Concentration of the coelenterazine substrate and buffer composition were also optimized to provide high intensity and long half-life of the luminescent signal. The G8-Nluc was used as a detection antibody to establish a competitive bioluminescent ELISA for the detection of aflatoxin B1 in cereals successfully. Compared to classical ELISA, this novel assay showed more than 20-fold improvement in detection sensitivity, with an IC50 value of 0.41 ng/mL and linear range from 0.10 to 1.64 ng/mL. In addition, the entire BLEIA detection procedure can be completed in one step within 2 h, from sample preparation to data analysis. These results suggest that nanobody fragments fused with nanoluciferase might serve as useful and highly sensitive dual functional reagents for the development of rapid and highly sensitive immunoanalytical methods.

**A Rapid and Nondestructive Method for Simultaneous Determination of Aflatoxigenic Fungus and Aflatoxin Contamination on Corn Kernels**


**Significance:** A rapid and non-destructive method for detecting aflatoxinogenic fungus and aflatoxin contamination utilizing Vis–NIR spectroscopy is presented.
Conventional methods for detecting aflatoxigenic fungus and aflatoxin contamination are generally time-consuming, sample-destructive, and require skilled personnel to perform, making them impossible for large-scale nondestructive screening detection, real-time, and on-site analysis. Therefore, the potential of visible–near-infrared (Vis–NIR) spectroscopy over the 400–2500 nm spectral range was examined for determination of aflatoxigenic fungus infection and the corresponding aflatoxin contamination on corn kernels in a rapid and nondestructive manner. The two *A. flavus* strains, AF13 and AF38, were used to represent the aflatoxigenic fungus and nonaflatoxigenic fungus, respectively, for artificial inoculation on corn kernels. The partial least-squares discriminant analysis (PLS-DA) models based on different combinations of spectral range (I: 410–1070 nm; II: 1120–2470 nm), corn side (endosperm or germ side), spectral variable number (full spectra or selected variables), modeling approach (two-step or one-step), and classification threshold (20 or 100 ppb) were developed and their performances were compared. The first study focusing on detection of aflatoxigenic fungus-infected corn kernels showed that, in classifying the “control+AF38-inoculated” and AF13-inoculated corn kernels, the full spectral PLS-DA models using the preprocessed spectra over range II and one-step approach yielded more accurate prediction results than using the spectra over range I and the two-step approach. The advantage of the full spectral PLS-DA models established using one corn side than the other side were not consistent in the explored combination cases. The best full spectral PLS-DA model obtained was obtained using the germ-side spectra over range II with the one-step approach, which achieved an overall accuracy of 91.11%. The established CARS-PLSDA models performed better than the corresponding full-spectral PLS-DA models, with the better model achieved an overall accuracy of 97.78% in separating the AF13-inoculated corn kernels and the uninfected control and AF38-inoculated corn kernels. The second study focusing on the detection of aflatoxin-contaminated corn kernels showed that, based on the aflatoxin threshold of 20 and 100 ppb, the best overall accuracy in classifying the aflatoxin-contaminated and healthy corn kernels attained 86.67% and 84.44%, respectively, using the CARS-PLSDA models. The quantitative modeling results using partial least-squares regression (PLSR) obtained the correlation coefficient of prediction set (*R* *P*) of 0.91, which indicated the possibility of using Vis–NIR spectroscopy to quantify aflatoxin concentration in aflatoxigenic fungus-infected corn kernels.

**Heavy Metals**

**Development of a Sensitive and Specific Whole-Cell Biosensor for Arsenic Detection**


**Significance:** An arsenic whole-cell biosensor able to detect arsenic below the WHO limit level was developed.

Whole-cell biosensors (WCBs) have been designed to detect As(III), but most suffer from poor sensitivity and specificity. In this paper, we developed an arsenic WCB with a positive feedback amplifier in *Escherichia coli* DH5α. The output signal from the reporter mCherry was significantly enhanced by the positive feedback amplifier. The sensitivity of the WCB with positive feedback is about one order of magnitude higher than that without positive feedback when evaluated using half-saturation As(III) concentration. The minimum detection limit for As(III) was reduced by one order of magnitude to 0.1 μM, lower than the World Health Organization standard for the arsenic level in drinking water, 0.01 mg/L or 0.13 μM. Due to the amplification of the output signal, the WCB was able to give detectable signals within a shorter period, and fast response is essential for in-situ operations. Moreover, the WCB with the positive feedback amplifier showed exceptionally high specificity toward As(III) when compared with other metal ions. Collectively, the designed positive feedback amplifier WCB meets the requirements for As(III) detection with high sensitivity and specificity. This work also demonstrates the importance of genetic circuit engineering in designing WCBs and the use of genetic positive feedback amplifiers is a good strategy to improve the performance of WCBs. Importance: Arsenic poisoning is a severe public health issue. Rapid and simple methods for the sensitive and specific monitoring of arsenic concentration in drinking water are needed. In this study, we designed an arsenic WCB with a positive feedback amplifier. It is highly sensitive and able to detect arsenic below the WHO limit level. In addition, it also significantly improves the specificity of the biosensor toward arsenic, giving a signal about 10-20 times stronger in response to As(III) than to other metals. This work not only provides simple but effective arsenic biosensors but also demonstrates the importance of genetic engineering, particularly the use of positive feedback amplifiers, in designing WCBs.

**Food Packaging**

**Styrene Monomer Migration From Polystyrene Based Food Packaging Nanocomposite: Effect of Clay and ZnO Nanoparticles**


**Significance:** In this study, organoclay and zinc oxide nanoparticles reduced migration of styrene monomers into food simulants. Inhibition from migration of plastic ingredients such as styrene monomer (SM) is very important in food packaging industry. Styrene monomer is one of the substances which can potentially migrate from polystyrene based packaging. In the present study,
organoclay and zinc oxide nanoparticles (ZnO-NPs) were used for decreasing of the SM migration into food simulants (10 and 50% ethanol (v/v)). A used GC-FID method for measuring of the migrated SM showed good precision and accuracy. Maximum reduction of SM migration into 10% and 50% ethanol (24 h storage at 40 °C) were observed in the polystyrene/nanoclay and polystyrene/ZnO samples, respectively. The SM migration data in 50% ethanol at 5 °C followed from Fickian diffusion law and the lowest diffusion coefficient (2.89 × 10^{-14} cm²/s) was observed in the polystyrene/ZnO/nanoclay samples.

**Effects of Package Atmosphere and Storage Conditions on Minimizing Risk of Escherichia coli O157:H7 in Packaged Fresh Baby Spinach**


**Significance:** Temperature and package atmosphere storage practices to limit growth of *E. coli* are presented.

Packaged fresh spinach has been associated with outbreaks of illness caused by *Escherichia coli* O157:H7. The purpose of this study was to assess the behavior of *E. coli* O157:H7 in packaged baby spinach in response to storage conditions of temperature and package atmosphere and including effects of inoculation level, spinach leaf damage (cut leaves), internalized or leaf surface contamination, exposure to hypochlorite sanitizer, and package size. Behavior of *E. coli* O157:H7 inoculated at 2 and 4 log CFU/g on spinach packaged in polymer bags composed of a two-layer laminate (polypropylene and polyethylene) and stored under atmospheres of 20% O₂-3% CO₂ and 0% O₂-15% CO₂ (aerobic and anaerobic, respectively) was assessed at 5, 7, 12, and 15°C for up to 14 days. Growth kinetics were calculated using DMFit software. Temperature decreases progressively diminished growth or survival of the pathogen, and an aerobic package atmosphere resulted in longer lag times (4 to 6 days) and lower population levels (0.2 to 1.4 log CFU/g) compared with the anaerobic atmosphere at 15°C. Internalized contamination, leaf cuts, or exposure to 100 ppm of hypochlorite did not result in changes in pathogen behavior compared with controls; however, a growth minimization trend consisting of longer lag times and lower population levels was repeatedly observed in the aerobic compared with the anaerobic package atmospheres. In contrast, growth of indigenous mesophiles and Enterobacteriaceae was unaffected by package atmosphere. Spinach stored at 5 to 7°C in two sizes (5 and 16 oz) of polyethylene terephthalate clamshell packages with ambient air atmospheres was more likely to progress to lower-oxygen conditions in 16-oz compared with 5-oz packages after 7 days of storage (*P* < 0.05). Practices to maintain aerobic conditions within the package, as well as storage of the package at low temperature, are ways to limit growth of *E. coli* O157:H7 in packaged spinach.

**Caffeine**

**Caffeine and Caffeine Metabolites in Relation to Hypertension in U.S. Adults**


**Significance:** Caffeine metabolites, but not caffeine, are associated with reduced risk of hypertension in a population of U.S. adults.

**Background/Objectives:** Most studies assessing the association between coffee consumption and hypertension ascertained caffeine intake in terms of number of cups per days, and yield mixed results. Although the inter-individuals variability in the caffeine metabolism is known, the relation of caffeine metabolites with hypertension remains unsettled. We examined the association of caffeine and 13 direct and indirect caffeine metabolites with hypertension in U.S. adults. **Methods:** Using data from 2009–2010 National Health and Nutrition Examination Survey, we included 2278 individuals aged 18 to 80 years. Urinary methyluric acids (MU) and methylxanthines (MX) products of caffeine metabolism were measured using high performance liquid chromatography-electrospray ionization-tandem quadrupole mass spectrometry. We used multivariate logistic regression to model hypertension (systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥80 mmHg) as functions of urinary coffee metabolites. **Results:** The odds of hypertension decreased across quartiles of 3-MU, 7-MU, 3-MX and 7-MX, with 7-MU being the more powerful metabolite. Compared with adults in the bottom quartile of 7-MU, the odds of hypertension decreased by 81% (95% CI: −90 to −22%) in those in the upper quartile. In contrast, the odds ratio for being hypertensive from the bottom to the upper quartile were 4.47 (95% CI: 1.21–16.50) for 1,3-dimethyluric acid, 4.45 (95% CI: 1.48–13.39) for 1,3-dimethylxanthine, and 5.08 (95% CI: 1.11–23.36) for 1,7-dimethylxanthine. Neither insulin resistance nor abdominal obesity were moderators in these associations. **Conclusions:** Final metabolites of caffeine (namely 3-MU, 7-MU, 3-MX and 7-MX), but not caffeine, significantly reduce the odds for hypertension in this population.
Food Allergy

Maternal Triacylglycerol Signature and Offspring Risk of Food Allergy

Significance: Maternal plasma triacylglycerols may influence offspring risk of food allergy.

Background: The prevalence of IgE-mediated food allergy (FA) is rising worldwide but the underlying mechanisms are poorly understood. Objective: To examine the role of maternal lipidomic profiles in offspring risk of FA development; and to investigate the potential modification effects by timing of first solid food introduction. Methods: This report included 1,068 mother-child dyads from the Boston Birth Cohort. Maternal lipid metabolites in plasma were assessed using liquid chromatography tandem mass spectrometry. Food sensitization (FS) was defined as specific IgE ≥ 0.35 kU/L to any of the 8 common food allergens using ImmunoCAP. FA was defined based on FS, clinical symptoms and food avoidance. Logistic regression was applied to analyze the associations between maternal metabolites and offspring risk of FS and FA, and to explore the potential effect modifications. Results: Of the 1,068 children, 411 had FS and 132 had FA. Among the 209 metabolites, maternal triacylglycerols (TAGs) of shorter chain carbons and fewer double bonds were associated with higher risk of FA, whereas TAGs of longer chain carbons and more double bonds were significantly associated with lower risk of FA in offspring. These associations were stronger in children with delayed solid food introduction (≥7 months of age) than those with earlier solid food introduction (P=0.010 for interaction between the maternal TAG score and timing of solid food introduction). No significant association was found for FS. Conclusion: This is the first study to demonstrate a link between maternal TAGs and offspring risk of FA and potential risk modification by timing of solid food introduction.