2022

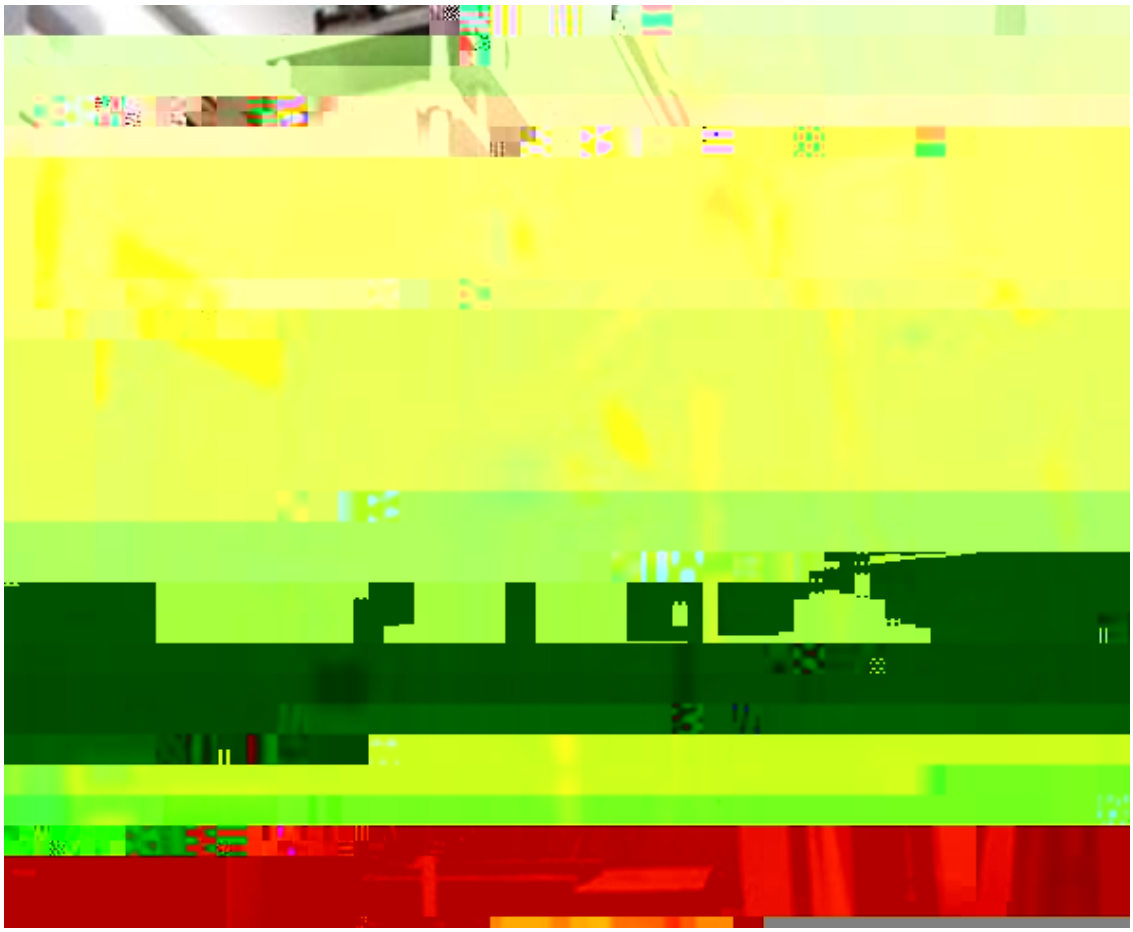
Annual Report of Collaborative Research Program

Illinois Institute of Technology (IIT)
Institute for Food Safety and Health (IFSH)
National Center for Food Safety and Technology (NCFST)

Processing Platform

Glenn Black, FDA and Jason Wan, IIT IFSH

The Processing Platform aims to provide a scientific basis for the processing and production of safe food, and support programs related to pasteurization, extended shelf life, sterilization, and package integrity and potential cross-contamination/contact issues.



Microbiology Platform

Elizabeth Grasso-Kelley, FDA and Alvin Lee, IIT IFSH

The Food Microbiology Platform aims to contribute knowledge about the characteristics, survival, and inactivation of hazardous microorganisms in foods and processing environments in support of food contamination risk assessment and management.

(mung bean, alfalfa) and treatment scale (10 g and 1 kg). The impact of treatment on seed germination, sprout yield and the extent of *Salmonella* re-growth during sprouting was also examined.

A greater log kill was observed when treatment was conducted at higher temperatures, under a higher relative humidity (RH), or for a longer time. Treatment at 60 C/80%RH or 70 C/60%RH for 16 h reduced *Salmonella* by > 3 logs to below detection (< -0.3 log CFU/g) while maintaining germination and sprout yield at > 90% of that of untreated controls. A similar log kill was achieved whether 10 g or 1 kg of beans were treated. *Salmonella* re-growth was observed during sprouting of treated beans, although could be delayed. Dry-heat treatment can be an effective means in reducing *Salmonella* on mung beans, but pathogen could re-grow during sprouting. The potential delay in pathogen re-growth during sprouting of dry-heat treated seeds needs to be considered when conducting microbial testing of sprout production batches.

Research findings will provide the sprout industry and FDA with a better understanding of the efficacy of dry heat for treatment of seeds for sprouting and the factors to consider when conducting seed treatment validation studies.

This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget and supports CFSAN's 2020-25 Science and Research Strategic Plan by addressing Strategic Goal 1.

Impact of temperature on pathogen proliferation during sprouting and postharvest storage

Tong-Jen Fu[2], Deena Awad[1], Chih-Tso Lin[1]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration

Sprouts pose a particular food safety concern as conditions that promote seed germination also promote pathogen growth. Developing ways to minimize proliferation of pathogens, if present, during sprouting is crucial in the overall approach to reduce public health risks of sprouts. This study examined how sprouting temperature (4, 10, 20, 30 C) may affect pathogen proliferation

of *Salmonella* increased. For seeds inoculated at the low level and treated with 20,000 ppm $Ca(OCl)_2$, no *Salmonella* was detected in the treated seeds. However, re-growth of the pathogen was observed during sprouting at 30 C or 20 C. Experiment is on-going to determine the behavior of *Salmonella* in harvested sprouts stored at different temperatures.

These findings suggest that sprouting at 4 C could reduce *Salmonella* population in sprouts. Combining seed treatment with sprouting at 4 C could reduce *Salmonella* to below detection, which can make production batch testing ineffective. The pathogen, however, could re-grow during postharvest storage if cold chain is not maintained.

Examination of power ultrasound and organic acid-based hurdle technology to reduce foodborne pathogens on select produce matrices

evaluated in fresh and fresh-cut enoki and wood ear mushrooms. Both mushroom types were inoculated at 3 log CFU/g and stored for 7 d at 5, 10, or 25°C. For 5 and 10°C, both pathogens survived on whole and cut enoki and wood ear mushrooms with no significant change in population during storage. At 25°C, significant increases in populations were observed for both pathogens on both mushroom varieties. For whole and cut wood ear mushrooms, *L. monocytogenes* increased by 2.24 and 1.08 log CFU/g during storage at 25°C, respectively; *S. enterica* increased by 3.68 and 4.71 log CFU/g, respectively. The results of this study will aid in informing guidelines on proper time and temperature control for safety for mushrooms.

Clostridium botulinum challenge study in commercially prepared cold brew coffee

Chemistry and Packaging Platform

Lauren Jackson, FDA

The Food Chemistry and Packaging Platform aims to investigate approaches to prevent, reduce or mitigate the formation of hazardous chemical contaminants during processing, and to prevent the cross-transfer of pre-formed natural toxins, allergens or man-made (environmental)

Systematic approaches for sampling foods for allergens and gluten

Lauren Jackson[2], Binaifer Bedford[2], Girdhari M. Sharma[2], Shizhen S. Wang[2], Travis Canida[2], Stuart Chirtel[2], Marion Pereira[2], Paul Wehling[3], Mark Arlinghaus[3], Josh Warren[1], Thomas B. Whitaker[4]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration; [3]General Mills; [4]NC State University

Agricultural commingling of grains with other raw commodities can occur during harvest, transportation, storage, processing, and packaging. Limited information exists on approaches for sampling grain that contains allergens due to agricultural commingling or cross-contact. This study investigated wheat kernels contaminated with two allergenic legumes, peanuts and soybeans. Since the milling process can alter the distribution of peanut and soybean in wheat flour, this study evaluated the impact of a discrete sampling method on peanut protein (P) and soy protein (S) quantitation in flour.

Wheat kernels may be contaminated with soybeans and peanuts due to agricultural commingling. The milling process can alter their distribution in wheat flour, which can impact allergen quantitation. This project measured the variance associated with analyses of peanut protein (P) and soy protein (S) in wheat flour samples obtained by discrete sampling and predict total variance (Vt) at P or S concentrations (mg/g) depending on test portion size (Ns; grams), and number of aliquots analyzed (Na). Ten wheat kernel lots (45 kg each) were mixed with varying amounts of crushed, raw peanut, and dried soybean, followed by milling using a hammer mill configured with 0.6 mm outlet screen. From each lot, 32 flour samples (200 g each) were collected during milling and each randomly split (two, 100 g samples) to be used in discrete and composite sampling. One (1) g and five (5) g test samples were taken from each of the 32 discretake

Variance equations can be used to predict sampling dependent variability in peanut and soy test results. Peanut measurement variability was nearly 10 times greater than that of soy, possibly due to the differences in their composition and physical properties. Work is underway to analyze composite flour samples and establish the relationship between variance and P or S concentrations in the composite samples.

This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget and supports CFSAN's 2020-25 Science and Research Strategic Plan by addressing Strategic Goal 1.

biologically active botulinum neurotoxin in complex media

Yun Wang[2], H. Christopher Fry[3], Chenglong Lin[1], Kristin M. Schill[2,4], Timothy V Duncan[2]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration; [3]Argonne National Laboratory; [4]University of Wisconsin-Madison

Clostridium botulinum is a foodborne pathogen that produces the most potent toxin known: botulinum neurotoxin (BoNT). Current methods to detect BoNT, though reliable, are time consuming and expensive. In a previous project, we used quantum dots (QDs) and intelligently designed peptides to develop molecular probes that can rapidly quantify levels of biologically active BoNT in liquid media and discriminate the A and B serotypes (2017) and A, B, and E serotypes (2020). This detection strategy differs from many other toxin detection strategies in that it does not rely on antibodies for detection, and it also can quantify biologically active toxin – that is, toxin that is able to harm human beings if ingested. The current project extends this nanosensor to detect additional BoNT serotypes of relevance to food safety, and it aims to translate the technology to a microfluidic chip-based platform for rapid field-based detection.

In the last year, we have continued to develop an analogous nanosensor for detection of BoNT serotype F. A biorecognition peptide that is specific for the F serotype has been ordered and conjugation chemistry with 800 nm emitting QDs has been optimized. We verified that this peptide-QD complex is able to detect the F-type light chain in buffer solution in under 2 hours total detection time and have preliminary limits of detection and sensitivity benchmarks, which compare favorably to the mouse bioassay for this toxin serotype. Currently we are optimizing the sensor performance and performing the selectivity tests.

In addition to the solution-based work, we are translating the technology to a microfluidic chip platform. This work is being done in collaboration with FDA's Center for Biologics Evaluation and Research (CBER) and San Jose State University. The polymer substrate has successfully been functionalized with BoNT-selective peptides and cleavage in the presence of BoNT light chain has been demonstrated using a fluorimeter equipped with a plate reader. Currently we are evaluating detection thresholds and selectivity.

The most important deliverable of this project is a reliable method that can detect harmful toxins in food substances quickly, accurately, and with high selectivity. The solution sensor shows good performance for BoNT rapid detection and can discriminate between three serotypes (A, B and E), with a fourth serotype (F) forthcoming. The outcome of the microfluidic portion of the project will be a facile, hand-held technology that can quickly and accurately detect BoNT or other proteolytic food toxins in the field.

This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget and supports CFSAN's 2015-18 Science and Research Strategic Plan by addressing Strategic Goal 1.

ingredients/food simulants alter the form or amount of mass transferred from PNCs from a dissolved ionic state to a particulate state, this information would be critical to draw upon when manufacturers consult FDA about how to perform safety assessments on PNC-containing products. A related outcome will be standardized analytical methods to detect, quantify, and characterize substances released from PNCs to environmental media.

This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

Assessment of variability in target nutrients in a market basket of plant-based milk alternatives

Shalaka Shetge[1], Lillian Wang[1], WenYen Juan[2], Joseph Zuklic[1], Jason Wan[1], Jeanmaire Hryshko[2], Pat Hansen[2], Marc Boyer[2], Lauren Jackson[2], Aman Sandhu[1], Benjamin Redan[2]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration

Information is critically needed on the nutrient profile of plant-based milk alternatives (PBMAs) and the variability in micronutrient levels in such products. This project has the goal of filling these data gaps by performing a market basket analysis to assess select micronutrient levels in different brands and types of PBMAs, including those made from almond, coconut, cashew, oat, pea, hemp, rice, and soy.

AOAC method 2012.10 was used to analyze PBMA samples for vitamin A (as retinyl palmitate) using HPLC-DAD. The results revealed that the highest mean vitamin A amounts were in coconut beverages (93.2 µg vitamin A equivalents/100 g portion), while the lowest amounts were in cashew beverages (27.2 µg vitamin A equivalents/100 g portion). AOAC method 2016.05 was used for the analysis of vitamins D2 and D3 in PBMA samples using LC-MS/MS. Mean vitamin D levels were highest in rice beverages (4.33 µg vitamin D2/100 g portion) and lowest in cashew beverages (0.73 µg vitamin D2/100 g portion). FDA EAM 4.7 was adapted for analysis of key elements in the PBMA samples, including calcium, potassium, phosphorous, magnesium, and zinc. Results for calcium showed that the highest mean value was in coconut beverages (180 mg Ca/100 g portion), while the lowest levels of calcium was found in rice

This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget and supports CFSAN's 2020-25 Science and Research Strategic Plan by addressing Strategic Goal 1.

Assessment of undeclareeTE ETQEMC P MCID 4BC q0.00000areeTE ETQEMC P MCIEMC rC gMCID 4in

This research was funded by FDA CFSAN Office of Nutrition and Food Labeling through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget and supports CFSAN's 2020-25 Science and Research Strategic Plan by addressing Strategic Goal 5.

Proficiency Testing Programs

Ravinder Reddy, FDA and Jason Wan, IIT IFSH

The Proficiency Testing and Method Validation Research Platform aims to provide underpinning science for the development of food microbiological and chemical inter-laboratory studies and proficiency testing programs.

The Proficiency Testing (PT) program at the FDA/IFSH Moffett campus has the unique capability of developing and validating test methods for microbiological and chemical agents, as well as providing proficiency testing samples to FDA (including CFSAN, CVM, ORA), USDA, State government laboratories and the Food Emergency Response Network (FERN) laboratories for laboratory performance evaluations. The microbiological agents (bacteria and viruses) for proficiency testing include: *Bacillus anthracis* Sterne, *Campylobacter* spp., *Cronobacter sakazakii*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Vibrio cholerae*, and *Yersinia pestis*. Chemical contaminants for proficiency testing include: aflatoxins, drug and pesticide residues (such as flunixin, monocrotophos, scopolamine and strychnine), arsenic, copper, lead, and more recently, allergens. In addition, the program also provides proficiency testing for nutritional supplements, including vitamins A and D. Relevant food matrices include: produce, food ingredients, milk, dairy, shellfish, egg, water, infant formula and baby foods, beef, turkey, liver. ISO 17043 accreditation was awarded to the FDA/IFSH joint PT program in January 2017, recertified in 2019, and in 2021. This is the first

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