September 2025
Annual Report of Research

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

IFSH INSTITUTE FOR FOOD SAFETY AND HEALTH ILLINOIS TECH
Innovation Through Collaboration

FDA U.S. FOOD & DRUG ADMINISTRATION

September 2025 IIT IFSH NCFST Annual Report of Research

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Research Activities

Research conducted at IFSH NCFST addresses key food safety issues facing the country and supports the development of safe food with health-promoting properties from farm to fork. This research forms a scientific basis for policy decisions affecting food safety and public health. Development and coordination of NCFST's scientific research programs are undertaken through the five science platforms: **Food Processing, Food Microbiology, Food Chemistry and Packaging, Nutrition, and Proficiency Testing and Method Validation**.

The **Food 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, package integrity, and potential cross-contamination/contact issues.

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.

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) contaminants in the food production environment. Another platform goal is to evaluate factors affecting migration of packaging constituents and contaminants into food.

The **Nutrition Platform** aims to contribute knowledge about food choice and intake behavior by consumers and their impact on nutrition and health.

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.

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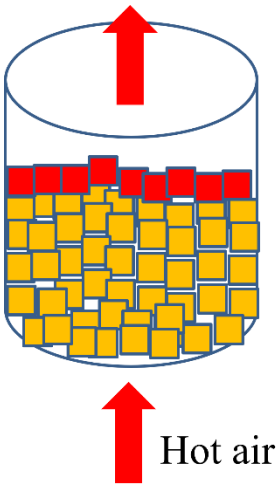
Processing Platform

Glenn Black, FDA and Jason Wan, Illinois Tech IFSH

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



Heat Transfer Challenges in Blanching for Microorganism Reduction: Complex Geometries and Heat Transfer Coefficients

Greg Fleischman
Food and Drug Administration

Blanching is a common pre-freezing treatment for vegetables. Its intended purpose is to inactivate enzymes that continue the ripening process during frozen storage. Though ripening continues at a slower rate, even at -18°C storage off-odor, off-color and off-flavor can develop. A potential added benefit of blanching is the inactivation of surface pathogens. Literature studies that have looked at specific blanching conditions have concluded that blanching can remove 5 logs of pathogens from vegetables surfaces. However, these blanching conditions often results in "over cooking" the vegetable and cannot be universally applied to all vegetables. Furthermore, the current used time and temperature combination are defined using internal temperatures based on seafood *Listeria monocytogenes* reduction guidance. Therefore, a more comprehensive approach with specificity to vegetables is needed.

The goals of the project for the final year were modified based on available equipment. In consultation with industry groups and American Frozen Food Institute (AFFI), the number of vegetables were consolidated from seven vegetables to four vegetables. Steam blanching was not conducted because the project team was not able to replicate industrial steam blanching in the laboratory setting with even distribution of steam and temperatures through the steam vessel and therefore decided to use modelling approaches to define steam blanching temperatures in collaboration with scientists from FDA DFPST.

The project team completed Goal 2 – Correlation of end-point temperature measurement as verification for a 5-log reduction of pathogens through the use of biotags, inert and non-infectious short DNA fragments. This involved the use of short inert DNA sequences, known as biotags, that were inoculated onto representative vegetables and comparison studies were conducted with representative vegetables inoculated with *L. monocytogenes* and exposed to water blanching process. The level of *L. monocytogenes* inactivation was correlated to peroxidase enzyme inactivation and detectability of biotags by real time PCR.

The experiments were performed and repeated using fresh peas initially washed with 0.03% sodium hypochloride solution to reduce background microflora and further rinsed in fresh sterile water to remove residual sodium hypochloride. The peas were then immersed in a 10-ppm biotag solution and allowed to dry before hot water blanching at 65ºC for up to 180 s. Biotags were consistently detected throughout the blanching process and there was a direct correlation (r2=0.83) to *L. monocytogenes* inactivation over time. At 65ºC, approximately 7 log cfu/g of *L. monocytogenes* were inactivated on fresh peas and the biotag Cq values from real-time PCR increased with blanching duration, indicating reduction in biotag signals with lower recovery of *L. monocytogenes*.

The experiment was similarly repeated with fresh corn and detection of biotag signals correlated with *L. monocytogenes* inactivation and recovery. Further collaboration will look to show efficacy

on carrots and broccoli and correlate the information to data generated by FDA DFPST on modelling.

Evaluation of Dry Cleaning for the Removal of Microbial Hazards from Food Contact Surfaces

Xiyang Liu[1] and Nathan Anderson[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration

In production of low-moisture foods, cleaning and sanitation are problematic as typical wet-cleaning and sanitation methods may create an additional hazard with the introduction of water. Dry-cleaning and sanitation methods are preferred; however, the efficacy of many such methods is unknown. One method involves the purging of product lines with uncontaminated material to remove any pathogens from food-contact surfaces. Evaluation of the efficacy of such a method *in situ* is difficult since only low levels of pathogen may be present and large quantities of uncontaminated material may be required even in smaller pilot plant scale experiments. Furthermore, the transfer rates from contaminated surfaces to clean material or from contaminated material to clean surfaces and/or adhesion of foodborne pathogens to food contact surfaces has not been established and may vary based on food type, surface material, and pathogen. The objective of this study was to compare the removal rate of *Salmonella* in two auger systems when un-contaminated wheat flour or corn meal was pushed through.

Two auger designs were evaluated: Design A featured a traditional configuration with two pipe linkage points and a longer dead-end length, while Design B employed a more sanitary layout with no linkage points and a shorter dead-end. In both systems, the auger shaft was driven by a gearmotor at approximately 17 rpm. First, 250 g of uncontaminated, heat-treated product were pushed through and discarded, followed by 100 g of *Salmonella*-inoculated product. Subsequently, 1000 g of uncontaminated, heat-treated product was pushed through the system while triplicate (~2 g) samples were collected at the outlet at various sampling points ($n \geq 8$). After each run, surfaces of the dead-end and product outlet were swabbed, and the plastic gasket from the linkage points was collected for Design A. All microbial enumerations are being conducted on modified TSAYE differential media.

Factors Affecting *Salmonella* Inactivation on Apples During Hot Air Drying

Xiyang Liu[1], Elizabeth Grasso-Kelley[2], Alvin Lee[1], Nathan Anderson[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration

Drying fruits has long been employed as a method to prolong their shelf life by reducing water activity (a_w), which inhibits the growth of microorganisms. While some drying methods prioritize expediting moisture removal from the fruit, the microbiological food safety requirements mandated by the Food Safety and Modernization Act were not always given top priority during the design phase. Research has indicated that a low moisture environment leads to increased thermal resistance of pathogens. This highlights the potential risks of foodborne illnesses associated with pathogen survival during the drying process, as it involves the creation of a low-moisture environment through thermal treatment. The objective of this study was to characterize the dynamic profile of *Salmonella* spp. reduction during hot air apple drying under various

temperatures, air velocities, and drying bed depths. Additionally, we aimed to explore the correlation between apple a_w and *Salmonella* spp. inactivation. Moreover, we then explored the use of a polynomial Response Surface Methodology (RSM) model to quantify the linear, interactive, and quadratic effects of the temperature, bed depths, and airflow setting on the *Salmonella* lethality. Finally, the developed RSM model was validated using three external validation datasets obtained separately.

A six-strain *Salmonella* cocktail (Agona 447967, Tennessee K4643, Montevideo 488275, Mbandaka 698538, Enteritidis PT30 ATCC BAA-1045, Reading Moff180418) was harvested from lawns cultured on tryptic soy agar with 0.6% yeast extract (TSAYE) and inoculated onto Gala apple cubes (6.40 mm) at 9.41±0.21 log CFU/4 cubes. Inoculated apple cubes were dried at low (L), medium (M), and high (H) conditions for temperature (T; 88, 104, 120°C), bed depth (B; 5.1, 8.9, 12.7 cm), and air velocity (A; 25, 37.5, 50.0%) respectively utilizing a Box Behnken Design. A total of 15 drying conditions were assessed. *Salmonella*-inoculated apple cubes were collected at various time points (n=6), measured for water activity, and enumerated on modified TSAYE. Additionally, three validation runs were performed with conditions randomly selected (Validation 1: T = 101°C, B = 6 cm, A = 20%; Validation 2: T = 97°C, B = 8.5 cm, A = 41%; Validation 3: T = 113°C, B = 12 cm, A = 32%).

This study showed that following this initial stage, a rapid decrease in both apple a_w and the *Salmonella* population was observed. In this study, although a 5-log reduction in *Salmonella* was achieved at the end of drying in most conditions (n = 14), the reductions were achieved at various endpoint water activities. Across all fifteen drying runs, *Salmonella* log reduction correlated linearly with the decrease in apple a_w, allowing a_w reduction to be used as a practical proxy for apple drying process lethality. Using linear regression, we predicted log reductions at a_w 0.60, a typical a_w level seen in the market. The highest *Salmonella* reduction was estimated for HT-LB-MA (4.97 ± 0.21 log CFU/4 cubes), HT-MB-HA (4.75 ± 0.23 log CFU/4 cubes), and MT-LB-HA (4.73 ± 0.17 log CFU/4 cubes), whereas the lowest *Salmonella* reduction was estimated for LT-MB-LA (2.25 ± 0.11 log CFU/4 cubes) and LT-HB-MA (2.50 ± 0.11 log CFU/4 cubes). Overall, higher temperature, higher airflow, and lower bed depth resulted in higher *Salmonella* reduction, estimated at a_w 0.60. An RSM model containing the linear effect of drying temperature, bed depth, and airflow as well as the interactive effect between the bed depth and airflow, was established for the prediction of *Salmonella* reduction when apples are dried to a_w of 0.60. The model confirmed that higher temperature and airflow both increased the *Salmonella* reduction, whereas higher bed depth negatively affected lethality, and that the benefit of extra airflow diminished as the bed depth increased. External validation runs produced experimental reductions that matched model predictions with no statistical difference. Model validation results showed that the model prediction had a 10.1% difference from the true value and was 7.3% higher than the true value. These findings demonstrated that the RSM model offered a reliable, easy-to-use tool for estimating *Salmonella* inactivation across the full range of practical drying conditions.

This work was supported by the Agriculture and Food Research Initiative, Sustainable Agricultural Systems Program Grant No. 2020-68012-31822 from the USDA National Institute of Food and Agriculture and in part through HFP's Cooperative Agreement with IFSH and the DFPST operating budget.

Isothermal Inactivation Kinetics of Salmonella Montevideo on Partially Dried Apple Cubes

Xiyang Liu[1], Elizabeth Grasso-Kelley[2], Alvin Lee[2], Nathan Anderson[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration

Thermal resistance of *Salmonella* is known to increase as the water activity decreases. The dynamic nature of apple drying from high to low water activity poses challenges in predicting microbial lethality. Additional data on the thermal inactivation kinetics of *Salmonella* on apple cubes can assist in predicting microbial inactivation during drying. In addition, a preliminary study has shown that *Salmonella* Montevideo appeared to be the most thermally resistant compared to five other *Salmonella* strains under isothermal treatments. Moreover, the suitability of the commonly used surrogate *Enterococcus faecium* (*E. faecium*) in dried fruit is unknown. The purpose of this study is to investigate the inactivation of *Salmonella* Montevideo and *E. faecium* on partially dried apple cubes with different a_w during isothermal treatment at various temperatures.

Gala apple cubes (6.40mm) were pre-dried to a_w of 0.45, 0.60, 0.75, or 0.90. *Salmonella* Montevideo 488275 or *Enterococcus faecium* NRRL B-2354 was harvested from lawn culture grown on tryptic soy agar with yeast extract (TSAYE) and inoculated onto the pre-dried apple cubes (3% v/w) to achieve ~8 log CFU/g population. After ~96 h re-equilibration (45, 60, 75, or 90% RH), inoculated apple cubes were packed into aluminum test cells under controlled RH and isothermally treated in a water bath. At various time points (n=6), triplicate samples were collected and cooled in an ice-water bath, and *Salmonella* or *E. faecium* was enumerated on modified TSAYE differential media.

Salmonella isothermal inactivation data were fitted into a first-order kinetic primary and secondary model combination through global model fitting. At a_w 0.45, D-values at 67.5, 75.0, and 82.5°C were 30.46, 8.19, and 2.20 min, respectively. At a_w 0.60, D-values at 60.0, 67.5, and 75.0°C were 31.90, 8.57, and 2.30 min, respectively. At a_w 0.75, D-values at 52.5, 60.0, 62.5, and 67.5°C were 33.42, 8.98, 5.80, and 2.41 min, respectively. At a_w 0.90, D-values at 50.0 55.0, 57.5, and 60.0°C were 18.49, 7.70, 4.97, and 3.21 min, respectively. Both higher temperatures and higher a_w accelerated *Salmonella* inactivation in partially dried apple cubes.

E. faecium isothermal inactivation data were fitted into a first-order kinetic primary and secondary model combination through global model fitting. At a_w 0.45, D-values at 67.5, 70.0, and 80.0°C were 12.16, 3.98, and 1.89 min, respectively. At a_w 0.60, D-values at 60.0, 67.5, and 75.0°C were 27.33, 8.94, and 2.92 min, respectively. At a_w 0.75, D-values at 52.5, 60.0, and 62.5°C were 61.39, 20.07, and 6.56 min, respectively. At a_w 0.90, D-values at 55.0, 60.0, and 65.0°C were 31.07, 14.74, and 7.00 min, respectively. Compared to *Salmonella*, *E. faecium* D-value was lower at a_w 0.45, similar at a_w 0.60, and higher at a_w 0.75 and 0.90. The suitability of the surrogate may vary as the product water activity changed. *E. faecium* could be a conservative surrogate for *Salmonella* in apples at higher aw (0.60, 0.75, and 0.90) but may not be suitable at lower a_w (0.45).

This work was supported by the Agriculture and Food Research Initiative, Sustainable Agricultural Systems Program Grant No. 2020-68012-31822 from the USDA National Institute of Food and Agriculture and in part through HFP's Cooperative Agreement with IFSH and the DFPST operating budget.

High Pressure Processing as an Alternative Pasteurization Process to Address Raw Milk

Lindsay Halik, Nicole Maks-Warren, Alvin Lee
Illinois Tech IFSH

There have been outbreaks within the dairy industry from pathogens not traditionally recognized to be transmitted via raw milk. One such microorganism is highly pathogenic avian influenza H5N1 (HPAI) strains transmitted by wild birds. Although the occurrence is rare, zoonotic transfer of pathogens could pose significant risks to the dairy industry and public health.

Current pasteurization conditions are based on thermal technologies and have been effective against several pathogens found in raw milk. Raw milk is pasteurized through a batch or continuous process as specified in the FDA Pasteurized Milk Ordinance (PMO) at the minimum conditions (71.7ºC for 15s) needed to inactivate *Coxiella burnetii*, which is the most heat-resistant pathogen currently recognized in the PMO. Initial pasteurization conditions prior to 1957, were calibrated to inactivate *Mycobacterium bovis*, a microorganism that causes tuberculosis, at 71.1ºC for 15s. In 1957, the pasteurization conditions were updated to 71.7ºC for 15s to inactivate *Coxiella burnetii* and these conditions are still used today.

High pressure processing (HPP) is a process that has been shown to be effective in inactivating vegetative cells of various pathogens and normally used in products with high water activities (approx. >0.97) and some countries have explored the use of HPP for the pasteurization of raw milk. The impact of HPP on dairy milk, dairy products and dairy components has been reviewed in an article by Chawla *et al.* (2011). However, the article does not address microbiological inactivation of microorganisms of concern listed in the PMO. Also, it is unknown if the most resistant microorganism for HPP treated milk would still be *Coxiella burnetii* or if *Coxiella burnetii* would be applicable for use as a microorganism to demonstrate process equivalency.

IFSH has previously conducted various HPP experiments to inactivate *Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7 and sporeformers including *Clostridium botulinum* and *Bacillus cereus* on both high and low a_w food products.

This project looks to demonstrate HPP efficacy to inactivate HPAI and demonstrate HPP process equivalency as described within PMO.

The objectives of the proposed work are to evaluate the equivalency of HPP to that of pasteurization for raw milk, specifically for HPAI strains through the following:

- Establish influenza A (H5N1) cell culture capability at IFSH
- Development of inoculation and HPP treatment protocols using low pathogenicity influenza A
- Screen HPAI H5N1 strain using sub-lethal dose of HPP for inactivation kinetics studies
- Establish inactivation kinetics using the most pressure resistant HPAI H5N1 strain in relation to temperature, HPP dwell time and pressure.

- Screen microorganisms e.g. *Coxiella burnetti* that could be used in HPP milk validation and to establish equivalency
- Demonstrate efficacy of HPP by comparison to thermal pasteurization

Microbiology Platform

Elizabeth Grasso-Kelley, FDA and Alvin Lee, Illinois Tech 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.

Evaluation of the Risk for *Clostridium botulinum* and Toxin Production in Commercial Plant-Based Meat Alternative Products

Catherine Felice (Rolfe)[2], Travis Morrissey[2], Viviana Aguilar[1], Guy Skinner[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration

Plant-based meat alternative (PBMA) products contain a mixture of plant-based ingredients with common protein sources of wheat gluten, soybean, and pea and are intended to replace traditional animal-based meats. These products appeal to a variety of consumers, from those who are strictly vegan or vegetarian to those who aim to reduce their red meat intake or have allergies such as alpha-gal syndrome. PBMAs are produced from plant-derived ingredients, with a variety of incoming raw material potentially containing spores of *C. botulinum*. Many PBMA products are produced through extrusion, a process in which spores, such as *C. botulinum*, may remain after processing. These products are frequently vacuum-packed or in modified atmosphere packaging and kept in refrigerated temperatures to mitigate the risk of growth and toxin production of *C. botulinum* throughout the shelf-life.

This research project is evaluating the risk of *C. botulinum* in plant-based meat alternative products through determining the prevalence of *C. botulinum* spores and whether PBMA products can support *C. botulinum* toxin production.

A total of 20 products were purchased from local grocery stores to survey matrix characteristics. Of the selected products, six are soy protein-based, eight are pea protein-based, three are wheat gluten-based, and three are mushroom based. The pH of the products was found to range from 5.78 to 7.35. The water activity was found to be 0.94 to 0.99. The salinity was found to range between 0.71 and 2.72 ppt. The endopeptidase-MS assay was verified for the use of botulinum toxin detection in PBMA products. Detection of botulinum toxin in PBMA product was comparable to that of botulinum toxin detection in TPGY media. A total of 18 purchased PBMA products surveyed for botulinum toxin production. Products were weighed into 25 g samples (in triplicate) and heat-shocked at 60ºC for 15 minutes prior to adding 225 mL TPGY broth. The PBMA products and TPBY broth were incubated anaerobically at 37°C for 7 days. Following incubation, the products were evaluated by Endopep-MS assay to determine if botulinum toxin production occurred. From the 18 PBMA products, no toxin production was recovered. Background aerobic and anaerobic plating were conducted for all available (17) PBMA products. Anaerobic plate counts were observed at <0.70 to 7.15 ± 0.36 log CFU/mL. Aerobic plate counts were observed at <0.70 to 7.17 ± 0.37 log CFU/mL. Additionally, shotgun sequencing is being performed on the surveyed PBMA products to understand the metagenomics of PBMA products. The sequencing trials should conclude in Summer 2025.

An inoculated challenge study was conducted on 19 purchased PBMA products. Samples were weighed into 50 g samples (in triplicate) and inoculated with the proteolytic *C. botulinum* spore cocktail at 103 spores/mL and incubated anaerobically at 37°C for 7 days. Following incubation, the PBMA samples were stomached with 50 mL of gel phosphate buffer and evaluated by Endopep-MS for botulinum toxin production. Challenge study results demonstrated positive toxin production from 13 PBMA inoculated products, with 11 positive products for Type A and B toxins and 2 positive products for Type A toxin only. Further challenge studies will be performed at 18°C

for the products which had positive toxin production at 37°C, completion of trials is expected by end of Summer 2025.

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

Efficacy of Dry-heat Treatment in Reducing *Salmonella* and *E. coli* O157:H7 Populations on Sprout Seeds

Tong-Jen Fu and Arlette Shazer
Food and Drug Administration

The Produce Safety Rule requires seeds for sprouting be treated by either sprout growers or seed suppliers to reduce pathogens. Dry-heat treatment can be an option for seed suppliers as it is scalable and avoids the need for a post-treatment drying step.

The efficacy of dry heat in reducing *Salmonella* and *E. coli* O157:H7 on alfalfa seeds, as affected by temperature (60, 70, 80°C), relative humidity (20-80%), time (6, 16, 24 h) and treatment scale (10 g, 1 kg) was examined. The impact of treatment on seed germination, sprout yield and pathogen re-growth during sprouting was also assessed.

Ten g of seeds inoculated with ~ 4 log CFU/g of *Salmonella* or *E. coli* O157:H7 were subjected to dry heat treatment in a humidity-controlled chamber. Treated seeds were analyzed for *Salmonella* or *E. coli* O157:H7 by plate count and culture enrichment. One hundred seeds were germinated in a petri dish and percent germination was recorded for 5 days. Sprout yields were determined after 7 days. For large-scale treatment, 1 kg seeds containing 10% of inoculated seeds were treated. Pathogen re-growth was examined by sprouting 200 g of treated seeds in glass jars for 5 days and pathogen levels were analyzed daily.

The results showed that greater log kills were achieved when treatment was conducted at higher temperatures, higher relative humidities (RH), or for longer time. Optimal conditions that reduced *E. coli* O157:H7 to below detection (< -0.3 log CFU/g) or *Salmonella* by > 3 logs while maintaining germination and sprout yield at > 90% were identified (70 °C/40%RH for 16 or 24h). A similar log kill was achieved whether 10 g or 1 kg of seeds were treated. Large-scale treatment under optimal conditions could reduce *Salmonella* and *E. coli* O157:H7 to below detection, but pathogen re-growth (by > 6 logs) was observed during sprouting. Overall, dry-heat treatment can be an effective means in reducing *Salmonella* and *E. coli* O157:H7 on alfalfa seeds but pathogen re-growth in treated seeds could occur, necessitating microbial testing of sprout production batches.

Findings from this research will provide the sprout industry and FDA with needed knowledge regarding the effectiveness of dry heat for seed decontamination as well as the factors to be considered when conducting seed treatment validation studies. An understanding of pathogen proliferation during sprouting will inform sprout production testing programs.

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

Clostridium botulinum Challenge Study in Cold Brew Coffee Part II

Travis Morrissey[2], Catherine Felice (Rolfe)[2], Emily Weyl[2], Viviana Aguilar[1], Guy Skinner[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration

Several studies have demonstrated the antimicrobial properties of hot brew coffee and certain compounds have been identified as exerting an inhibitory effect on Gram-positive and Gram-negative organisms. Brewing coffee is an extraction process that relies on several factors including water temperature and volume, diameter of the coffee grind particles, brewing time, and variety of coffee. Unlike traditional hot brew coffee, cold brew coffee is prepared by brewing the coffee grounds at ≤25°C for approximately 8 to 36 hours. Since temperature greatly affects the aqueous solubility of compounds, the chemical composition and antimicrobial activity of cold brew coffee extracts likely differs from that of traditionally hot brew coffee. Our previous study investigated *Clostridium botulinum* growth in commercially available cold brew coffee over a 5-month challenge study. One of the selected cold brew coffee products (shelf-stable) supported *C. botulinum* growth and toxin production of both Type A and B spores. Product characteristics of this shelf-stable product included high pH (>pH 6), and potassium phosphates added to the formulation. The previous work also showed *C. botulinum* spore inactivation when the cold brew coffee exposed to oxygen. This current study is 1.) investigating the factors that led to this cold brew coffee product to support the growth and toxin production of *C. botulinum*, and 2.) using an untargeted approach using HPLC-Q-TOF-MS to identify differences in coffee stored under aerobic and anaerobic conditions.

Cold brew coffee products that contain potassium phosphates were procured. Concurrently, we added potassium phosphate to commercial cold brew coffee not containing any additives to adjust the pH to ~7. These coffee products were independently inoculated with 10^3 *C. botulinum* spore cocktail and incubated at 27 ºC. At 3 and 5 months, coffees were negative for toxin production. The potassium phosphate study was repeated and confirmed the results from the first trial.

In June 2024 a voluntary recall of canned coffee products included cold brew coffee containing dairy creamer. Studies started by collecting time to toxin data for *C. botulinum* inoculated into cold brew coffee with added creamer. Three coffee products were selected with 5% and 20% added creamer for a total of 6 different coffee/cream samples. The coffee samples were inoculated with 10^3 *C. botulinum* spore cocktail and incubated at 27ºC. After 2 months of incubation, one of the coffee products with 20% creamer was positive for botulinum toxin, while the rest were negative. These samples will be tested again at 3, 4, and 5 months.

This research aims to fill knowledge gaps relating to cold brew coffee formulation and safety. The data provided will aid the FDA Food Processing Evaluation Team during the evaluation of cold brew coffee formulation control filings (2541f).

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

Evaluation of the Inactivation of *Listeria monocytogenes* and *Salmonella enterica* on Noodle Soup Garnishes Based on Broth Formulation and Temperature

Joelle Salazar, Megan Fay, Gregory Fleischman, Bashayer Khouja, Diana Stewart, David Ingram
Food and Drug Administration

The native microbiomes of leafy greens and herbs can provide information on the safety and quality of these foods. There have been many recent outbreaks of foodborne pathogens associated with leafy greens. Acquiring data on the native microbiota of these food products can provide insights into the survival of foodborne pathogens, appropriate enrichment techniques, and shelf life potential. Previous studies have assessed the resident microbiota of Romaine and iceberg lettuce, cilantro, spinach, and red and green leaf lettuce to further our understanding of the bacterial and viral communities in these food products. In this study, we aim to assess both the bacterial and viral microbiomes of various types of leafy greens and herbs. Leafy greens and herbs will be acquired from two sources: 1) collected from the field or packinghouse and 2) acquired from local retail grocers at the point of sale to consumers. Surface microbiomes (phyllosphere) and the microbiomes within these plant tissues (endophytes) will be determined.

The efficacy of different procedures for virus concentration from leafy greens and herbs was evaluated. The most efficient method, determined by RNA yield, used ultrasonication at 30,000 x g. The procedure used differential centrifugation to pellet debris, concentrate the bacterial fraction, and finally concentrate the viral fraction. Ultrasonication at 30,000 x g for 1 h was sufficient to recover the viral fraction. Using the viral fraction, DNA and RNA were extracted using the DNeasy Blood and Tissue Kit and the QIAamp Viral RNA Kit (Qiagen), respectively. Current work involves the processing of RNA to cDNA and targeted PCR amplification of select viral families. Both bacterial and viral DNA and RNA have been extracted from six types of leafy greens (green leaf lettuce, romaine lettuce, iceberg lettuce, kale, spinach, and cabbage) and five types of herbs (cilantro, basil, chives, dill, and parsley). From each leafy green or herb, triplicate samples were processed for surface microbiota and triplicate were processed for inside/endophyte microbiota. For the bacterial DNA, samples were subjected to PCR to amplify the V1-3 region of the 16S rRNA. Currently, all leafy green and herb samples are ready for targeted 16S sequencing. Libraries for metagenomic sequencing have been generated for two targeted metagenomic sequencing runs. Construction of a library of viral amplicons is currently underway.

The information generated will provide information on the bacterial and viral microbiomes of several leafy greens and herbs. The results of this project will pave the way for new research on bacterial pathogen survival in leafy greens and help to understand pathogen interaction with resident microbiota. Issues of importance include appropriate enrichment procedures for foodborne pathogens, spoilage, and shelf-life potential of leafy greens and herbs.

This research was funded through the HFP DFPST operating budget.

Evaluation of Strain-Specific Phenotypic and Genomic Differences on the Survival of *Listeria monocytogenes* on Selected Vegetables During Frozen and Thawed Storage

Joelle Salazar[2], Jasmin Phelps[2], Megan Fay[2], Bashayer Khouja[2], Xinyi Zhou[1], Diana Stewart[2], Vijay Juneja[3], Atin Datta[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration; [3]United States Department of Agriculture

Some frozen foods are ready-to-eat (RTE), while others must be cooked prior to consumption. Foodborne outbreaks caused by *Listeria monocytogenes* have recently been linked to frozen corn, peas, and vegetable mixtures in the U.S. and Europe. A better understanding of how *L. monocytogenes* survives and the molecular mechanisms it uses to persist in this harsh environment will aid in the development of prevention-based mitigation strategies for frozen vegetables.

This study examined the survival of different serotypes of *L. monocytogenes* on frozen mixed vegetables during long-term frozen storage (-18, -10ºC; 12 mo) and during storage once thawed at different temperatures (5, 10, 25ºC; 14 d), mimicking retail and consumer use, respectively. It was determined that *L. monocytogenes* survived on frozen vegetables for 12 mo with minimal population reductions; no difference in pathogen populations were observed between the two storage temperatures (-18 and -10ºC) or between the different serotypes of *L. monocytogenes* evaluated (1/2a, 1/2b, 4b). *L. monocytogenes* survived but did not grow on frozen vegetables after thawing and storing at 5ºC; at 10ºC, populations increased by 2.47 log CFU/g (serotype 4b) and 4.58 log CFU/g (serotype 1/2b) after 14 d. At 25ºC, pathogen increases of 3.27 log CFU/g (serotype 1/2a) and 4.68 log CFU/g (serotype 1/2b) were observed after 14 d. The highest growth rates were observed by serotypes 4b and 1/2a. The growth kinetics were then compared to those of *E. coli* and *S. enterica* on thawed vegetables. While *E. coli* and *S. enterica* cocktails survived on frozen vegetables once thawed and stored at 5, 10, or 25ºC, neither pathogen proliferated at any temperature tested; these results are in stark contrast to the high growth rates of *L. monocytogenes* at 10 and 25ºC. Additionally, the differential gene regulation of *L. monocytogenes* on frozen vegetables during thawing (25ºC; 1 h) and storage (5, 10, 25ºC; 24 h) was determined via transcriptomic sequencing. Transcriptomic profiling uncovered differential gene regulation and biological pathway enrichment during freezing, thawing, and storage stress which suggests that *Listeria* employs various survival mechanisms to adapt to the changing environmental stress.

Results of this study can aid in the development of guidelines for the safe storage and handling of frozen vegetables.

This research was funded through HFP's Cooperative Agreement with IFSH, the DFPST operating budget, and Oak Ridge Institute for Science and Education.

Evaluation of the Microbiome of Powdered Infant Formula and Assessment of the Response of *Cronobacter sakazakii* to Desiccation and Sanitizer Stress

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Powdered infant formula (PIF) is not a sterile food product. Understanding the microbiome of PIF products is essential and will aid in understanding pathogen interaction and survival and dynamics during enrichment. A few published studies have identified the microbiomes of PIF production facilities or the PIF manufacturing process in Asian countries, however, no study has identified the microbiomes of commercially available PIF in the U.S. Furthermore, *C. sakazakii* has a high tolerance to desiccation and other stressors. On abiotic surfaces, *C. sakazakii*, especially in its sessile form, also displays a high tolerance to sanitizers. However, the molecular mechanisms by which *C. sakazakii* responds to these harsh conditions are not well understood. Understanding how *C. sakazakii* overcomes the stress associated with PIF manufacturing plants and the sanitizers used in these environments will aid in creating science-based preventive controls. The main objectives of this study are to 1) identify the microbiome of PIF and assess the population dynamics of *C. sakazakii* and the native microbiota in PIF during standard enrichment, and 2) assess the survival and inactivation of *C. sakazakii* to desiccation stress in PIF and on food-contact surfaces with and without sanitizers. Results of the first objective identified a PIF core microbiome, which was dominated by *Bacillus*, *Lactococcus*, *Streptococcus*, and *Actinoalloteichus*. When inoculated at 1 log CFU/g into PIF, the relative abundance (RA) of *C. sakazakii* after BAM, ISO, and R&F enrichment methods was 9, 94, and 93%, respectively; *Bacillus* also dominated the enrichments, regardless of what method was used. For the second objective, the inactivation of sessile and planktonic *C. sakazakii* was evaluated on three food contact surfaces (stainless steel, high-density polyethylene (HDPE), and rubber). Sessile cells survived with no significant decrease in population on all surfaces at 33 and 53% relative humidity (RH) for 90 days. For planktonic cells, populations decreased significantly on all surfaces, however greater decreases were observed on HDPE and rubber at 53% (>4.5 log CFU/coupon) after 90 days. Transcriptomic studies with sanitizers are currently in process.

This research will fill data gaps on the microbiomes of PIF products and the population dynamics of *C. sakazakii* and native microbiota during enrichment. The study will also evaluate the survival mechanisms of *C. sakazakii* in response to PIF production environmental conditions.

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

Assessment of Population Dynamics of *Cronobacter sakazakii* and *Salmonella enterica* in Powdered and Reconstituted Infant Formula During Storage

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In 2021-2022, the U.S. Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) investigated consumer complaints and reports of ill infants who had consumed powdered infant formula (PIF). The incident resulted in four infant cases and two deaths possibly due to *Cronobacter sakazakii,* and one linked investigation of an illness due to *Salmonella enterica*. The implicated manufacturing company identified *C. sakazakii* in the plant in non-product contact areas. Multiple published studies have identified *C. sakazakii* in commercially available PIF and in the PIF-processing environment. *C. sakazakii*, like *S. enterica*, is well-known for its ability to survive in dry environments and low moisture food matrices and has a high

tolerance for desiccation and other stressors. In addition, the WHO has specific instructions for the safe preparation, storage, and handling of PIF in care settings and in the home, which includes using 70°C water for reconstitution. Published studies examining the survival and growth of *C. sakazakii* in reconstituted PIF have determined that water temperatures ranging from 52-58°C result in an approximate 1 log reduction in less than 1 h, and that water at >70°C can achieve >4 log reduction. The main objectives of this project are to 1) evaluate the survival of *C. sakazakii* and *S. enterica* in PIF during long-term storage at different relative humidity (RH) levels and 2) determine the survival or inactivation of *C. sakazakii* and *S. enterica* during reconstitution of PIF. Results for the first objective indicated that both pathogens survived in PIF during storage at 23, 33, 43, 53, and 75% RH for up to 360 days. While minimal reduction (1-2 log CFU/g) was observed at the lower RH levels, *C. sakazakii* and *S. enterica* population reductions were >5 log CFU/g at 75% RH after 360 days. For the second objective, PIF was reconstituted with water at 25, 45, 70, 85, or 100°C. No pathogen inactivation was observed when 25 or 45°C water was used, and minimal reduction (1-2 log CFU/mL) was observed when 70°C water was used. The higher temperatures (85 and 100°C) resulted in 3-5 log CFU/mL pathogen reductions.

This study will fill data gaps pertaining to *C. sakazakii* and *S. enterica* survival and persistence in PIF during storage and in reconstituted PIF. From this research, time/temperature/humidity parameters will be established for retail and consumer storage of PIF, as well as appropriate temperatures for the reconstitution of PIF products.

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

Efficacy of Chlorine Dioxide in Controlling *Cronobacter sakazakii* in Powdered Infant Formula Production

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Cronobacter sakazakii is an environmental pathogen capable of surviving in low-moisture foods, including powdered infant formula (PIF). It poses a serious health risk to infants under two months of age, especially those born prematurely or with weakened immune systems. Therefore, effective control measures to prevent *C. sakazakii* contamination in PIF manufacturing are essential to ensure product safety. Chlorine dioxide (ClO_2) gas is a powerful oxidizer with broad-spectrum antimicrobial properties. Its ability to penetrate surface irregularities makes it effective against pathogens on fresh produce and low-moisture foods. ClO_2 has been successfully evaluated for decontaminating products such as almonds, mung bean seeds, radish seeds, and spices. The current project includes industry funding support and is designed to explore the use of ClO_2 gas for inactivating *C. sakazakii* and *Salmonella* spp. on stainless steel surfaces (SS) and assessing its potential application as a sanitation break in PIF manufacturing.

The ClorDiSys ClO_2 equipment has been installed, and preliminary trials with a non-pathogenic *E. coli* strain (K-12) have been conducted to confirm equipment readiness, achieve the desired relative humidity levels, and test ClO_2 concentration levels for bacterial inactivation/ reduction. *E. coli* (K-12) was inoculated onto stainless steel (SS) coupons (1.40×10^8 CFU/coupon). The

coupons were subjected to ClO_2 treatments (180 ppm for 4 h, 360 ppm for 2 h, and 2,000 ppm for 5.5 h at 20–23% RH), and any surviving cells were recovered using membrane filtration. The results were used to further optimize the equipment. The team is currently re-running the trial to ensure accurate ClO_2 treatment and effective inactivation/reduction of *E. coli*. The next steps involve repeating the tests with pathogens (*C. sakazakii* and *Salmonella* spp.). A composite powder infant formula will be mixed from various off the shelf product with similar nutrient content.

This research was funded through HFP's Cooperative Agreement with IFSH and industry funding.

Evaluation of Highly Pathogenic Avian Influenza (H5N1) Survival in Raw Milk Yoghurt

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The goal of the project will be to evaluate the behavior of HPAI (H5N1) in yoghurts made from raw milk.

To date, protocols have been established for the making of traditional set yoghurt, Greek-style yoghurt and soft drinkable yoghurt using a variety of starter cultures. Data on pH and titratable acidity were collected during the yoghurt incubation period (up to 8 h) at 42ºC. Cell culture capabilities are being established at IFSH in Building 91E. The study will begin with the use of Low Pathogenic Avian Influenza (LPAI) and attenuated LPAI strains from St. Jude Hospital. These LPAI strains require USDA-APHIS transport permit and application has been submitted and waiting for USDA approval.

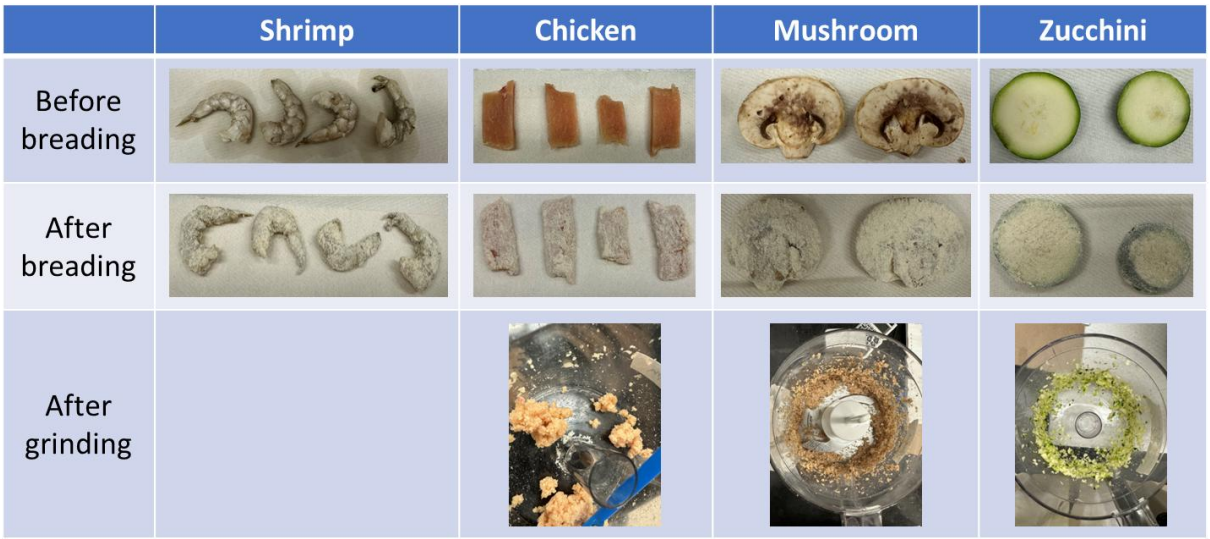
The purpose of using LPAI and attenuated strains will be to establish reproducible and reliable inoculation and recovery procedures from raw milk and yoghurts and to establish optimized enumeration methods. With these methods, the project team will be able to screen various Influenza A strains, assess its characteristics and select appropriate strains for studies.

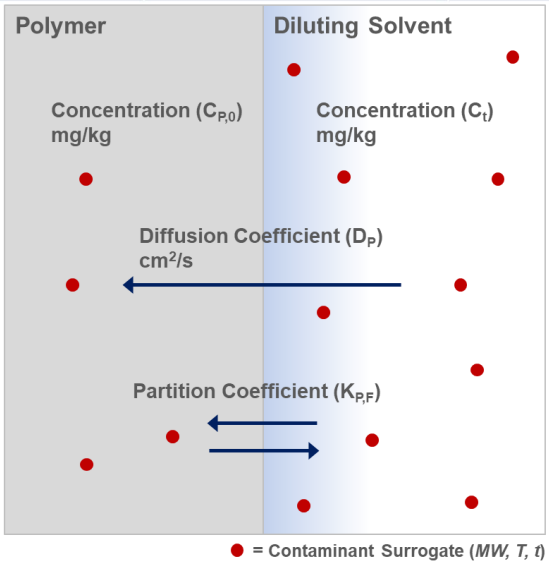
This research was funded through HFP's Cooperative Agreement with IFSH.

Chemistry and Packaging Platform

Lauren Jackson, FDA and Aman Sandhu, Illinois Tech IFSH

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) contaminants in the food production environment. Another platform goal is to evaluate factors affecting migration of packaging constituents and contaminants into food.





Assessment of Allergen Cross-Contact Risk Associated with Production of Oil-Roasted Nut and Peanut Products

Robert Beverly[2], Xingyi Jiang[2], Lauren Jackson [2]
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A common method to roast peanuts is oil-roasting, wherein batches of whole peanuts are immersed in hot oil in a batch frier or conveyed on a belt through the oil in a continuous fryer. While peanut-allergic populations know to avoid oil-roasted peanut products, the reuse of the roasting oil to roast or fry other foods such as tree nuts, seeds, or other snack foods, can pose a cross-contact risk as particulates or fragments from the whole peanuts are shed into the oil during roasting and may transfer to non-allergenic foods. Preventive controls to minimize cross-contact risk can include passive and active filtration of the oil to remove particulates before reuse. To ascertain the success of these measures, accurate quantitative methods are needed to measure peanut protein concentrations in oil. Immunochemical methods such as ELISA have reduced efficacy when the target protein is denatured, as occurs during heating, and can underestimate the amount of allergen present. Quantitative proteomics methods utilizing mass spectrometry may be able to overcome the deficiencies of ELISA, as both unheated and heated peanut proteins can be digested into peptides for detection.

Over the past year, a targeted proteomic method was developed to quantify thermally denatured peanut proteins in frying oil. A matrix-matched calibration curve utilizing unheated cooking oil spiked with peanut butter prepared from blanched, unroasted peanuts (BPB) was created for the method and its accuracy was tested across different peanut concentrations, heating durations, and standardized peanut sources. The method was then applied to quantifying peanut protein in oil used to batch-roast whole peanuts and to evaluate the efficacy of filtration for removing peanut proteins from oil.

A three-phased approach was used to identify candidate peptides. In the first phase, non-targeted proteomics were performed on oil samples spiked with BPB to achieve 1,000 µg peanut protein/mL oil that were unheated and heated for 1, 5, and 10 min, as it was critical to find peptides that performed similarly well in both unheated and heated samples. These samples generated 242 unique tryptic peptides, and the list was narrowed down to 28 candidate peptides to proceed into the second phase. In the second phase, oil samples spiked to 1,000, 100, and 10 µg peanut protein/mL were analyzed in targeted MS/MS mode using the m/z and retention time values for the candidate peptides generated in phase one. The aim of this phase was to confirm that in the targeted data, when the peptides and their fragment ions were specifically monitored by the mass spectrometer, there was no significant reduction in abundance from the unheated samples to the heated samples. The list of candidate peptides was narrowed down to ten based on visual inspection of the data and calculated reductions of <20% in abundance from unheated to 10-min heated samples. Finally, in phase three, calibration curves were prepared to assess the linearity of the response for each peptide based on R^2>0.95. All peptides from phase two had good linearity and so were chosen for synthesis as heavy-labeled internal standards for quantitative analysis.

To assess the accuracy of the method, BPB was dispersed in oil at 900, 90, and 9 µg peanut protein/mL, and the mixtures heated for 0, 1, 5, and 10 min. In the unheated samples, the method was accurate across the marker peptides at, with an average measured concentration of 881.8

µg/mL (97.9% recovery). At 90 µg/mL, the average concentration was again accurate at 87.5 µg/mL (97.2. At 9 µg/mL, several peptides were no longer detectable, but the remaining peptides measured an average concentration of 9.52 µg/mL (105.8%). There were significant decreases in concentration after 10 min of heating at 190℃. At 900 µg/mL and 90 µg/mL across all ten peptides the average recoveries were 77.3% and 78.6%, respectively. At 9 µg/mL, three of the peptides were no longer detectable after 1, 5 or 10 min of heating and two more peptides were not detectable after 10 min of heating. Of the remaining peptides, the average recovery was 94.7% and the maximum was 110.3%.

The targeted proteomic method was used to quantify peanut protein in oil (400 mL) that was used to roast ten, 20 g batches of peanuts and examine the effects of filtration treatments on removal of peanut protein from the roasting oil. The method was able to show increasing peanut protein levels with each batch of peanuts roasted, and after 10 batches, peanut protein levels in oil reached 463.1 µg/mL. Filtration of the peanut roasting oil revealed that levels of peanut protein decreased with filter pore size and that only filter papers with pore sizes ≤11 µm reduced peanut protein below the detectable limit. This project will aid in determining the risk of allergen cross-contact during production of oil-roasted/fried snack foods and for developing effective allergen preventive controls.

This research was funded through the DFPST operating budget.

Transfer of Seafood Allergens to Frying Oil and Subsequent Fried Products

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Part 1. In retail and food service operations, the reuse of frying oil is a common practice for economic and operational efficiency. However, this raises concerns regarding the unintended transfer of food allergens, to allergen-free foods – a critical issue for individuals with food allergies. This study addressed these concerns by focusing on gluten with three objectives: (1) evaluate the effect of frying on gluten solubility, (2) quantify gluten accumulation with oil reuse and its transfer to subsequently prepared gluten-free foods, and (3) investigate passive treatments for removing gluten from reused oil.

Gluten-spiked oil was heated at 180 °C for 1, 3, 6, and 10 min, and proteins from oil were extracted using PBS-urea-βME. Par-fried breaded shrimp (one to ten batches) were fried at 180 °C for 3 min per batch. The oil was then used to fry French fries, tater tots, and chicken breast bites. Both gluten-spiked oil and breaded shrimp frying oil were passively filtered under gravity through sieves and cellulose filter papers with pore sizes ranging from 2 mm to 25 µm and 25 µm to 11 mm, respectively. As for active filtration, response surface methodology was employed to identify optimal conditions that minimized gluten content, and these conditions were then validated for oil used to fry breaded shrimp. Gluten levels were measured using ELISAs.

The optimized extraction method recovered over 98% of gluten from oil. Frying significantly decreased gluten detectability, with about 75% recovered after 1 min. Gluten levels in oil increased with more frying batches and transferred to subsequently prepared gluten-free foods, exceeding the 20 mg/kg threshold for “gluten-free” labeling. Passive filtration showed promise in minimizing

cross-contact risk. The residual gluten protein content in the oil decreased by over 80% when passed through a filter paper with a pore size of 25 µm. Active filtration using 0.5% (w/w) filter aids with a 30 min mixing time further reduced gluten residues by at least 99.7% compared to untreated oil, and by 78.8% relative to oil filtered through a 0.15 mm pore size sieve. However, the efficiency varied among filter aids, with synthetic magnesium silicate and diatomaceous earth-based filter aids demonstrating the better performance.

In conclusion, this study developed an effective extraction method to recover gluten proteins from frying oil and demonstrated the potential cross-contact risk associated with reused oil. These findings highlight the need for oil treatment strategies to mitigate protein cross-contact.

Part 2. Meat and seafood products such as chicken, fish, shrimp are often breaded before deep frying to add a desirable texture, improve flavor, and enhance visual appeal. In retail and food service operations, it is common practice to reuse the same breading mixture across different batches and types of food products. For example, breading may be used to coat raw seafood products as well as raw chicken prior to cooking. There are also concerns that this practice may pose risks by transferring seafood allergens to other foods. This study aims to determine whether seafood allergens, particularly from shrimp and fish, can be transferred to other foods when breading mixtures are reused.

Twenty batches of shrimp and cod (25-30 g/batch) were prepared separately in a mixing bowl containing 200 g of breading mixture. After the 1st, 5th, 10th, 15th, and 20th batches, the breading mixture was manually stirred, and 0.5 g samples were collected from three distinct locations in the bowl. Residual shrimp protein concentration was quantified using a Crustacean Residue ELISA kit (ELISA Systems), while cod protein concentration was determined using AlerTox ELISA Fish Kit (Hygiena).

Shrimp proteins were not detected in the breading mixture after a single batch of shrimp was breaded. However, shrimp protein concentrations progressively increased with repeated use, reaching approximately 1.0 ± 0 ppm, 5.5 ± 0.4 ppm, and 10.6 ± 0.2 ppm after 10, 15, and 20 batches, respectively. For cod, a similar trend of increasing fish protein concentrations with repeated reuse of the breading mixture was observed. After 20 batches, cod protein concentrations reached 11,981 ± 156.2 ppm in the breading mixture.

This project directly addresses food safety and allergen management in the retail/food service setting. It helps to better understand allergen cross-contact risks associated with reusing breading systems.

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

Assessment of Undeclared Allergens in Peanut, Nut, and Seed Butters and Pastes

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Plant-based butters, such as peanut and sunflower butters, are rich in protein and have seen a steady increase in demand. This increase has been accompanied by a greater variety of products with different allergen profiles, increasing risk for allergen cross-contact to occur during manufacturing if shared equipment is not effectively cleaned. This study performed sanitization trials on a nut/seed mill to determine the effectiveness of dry-cleaning methods at minimizing cross-contact.

Milling experiments used a commercial nut butter mill to process ~220 g of whole roasted almonds into almond butter. The mill received the following treatments: no cleaning, flushing with vegetable oil (500 mL), cleaning inner surfaces with a brush/scraper, and cleaning inner surfaces with a brush/scraper followed by use of an alcohol-based sanitizer. Following sanitation, ~6.5 kg of roasted peanuts was milled into peanut butter and samples were collected every 60 s. For pipe experiments, interior surfaces of a 3.8 cm diameter x 30.5 cm long stainless-steel pipe were coated with heated (55°C) almond butter and then connected to a pump via a butterfly valve. The pipe was either left uncleaned or flushed with 18.9 L vegetable oil (55 °C). Subsequently, 31.8 kg peanut butter (55 °C) was pumped through the pipe and samples were collected every 10 s. Sanitation trials were conducted in triplicate. Almond protein levels in peanut butter samples were measured with an almond-specific ELISA kit.

When the mill was not cleaned, 343 ± 371 ppm almond protein was detected in peanut butter after 1.3 kg peanuts were processed in the mill. Flushing the mill with oil, using a brushing/scraping treatment, and using brushing/scraping and an alcohol-based sanitizer resulted 118 ± 35.1, 29.1 ± 11.1 and 0.31 ± 0.29 ppm almond protein, respectively in peanut butter after 1.3 kg peanuts were processed. Almond protein levels in peanut butter were ≤ 2.41 ppm when ~12 and ~2.9 kg of peanut butter was pumped through the almond butter-contaminated pipe after no cleaning and after an oil flush, respectively. Dry sanitation methods differ in their ability to remove nut butter residues from equipment surfaces. This project will aid in identifying effective dry sanitation procedures for preventing or minimizing allergen cross-contact.

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

Quantification of Sorption Behavior of Polypropylene Towards Various Chemical Contaminants Under FDA Surrogate Testing Protocol for Use in Recycled Plastics

Yoon Song[2], Huayi Wang[2], Senthamizh Senthilkumaran[1], John Koontz[2]
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FDA's current guidance to industry for testing the efficiency of industrial recycling processes in removing contaminants from recycled plastics intended for use in food contact applications is predominately based on known processes reclaiming polyethylene terephthalate (PET). FDA's surrogate contaminant testing protocol for PET employs hexane or heptane as an inert diluting solvent. However, there is growing interest in using other recycled plastics beyond PET in food

contact applications. As such, there is growing need for appropriate surrogate testing protocols specific to recycled polyolefin material feedstocks, including polypropylene (PP). The sorption behavior of PP must be investigated to offer recommendations on the conditions required to optimize the surrogate testing protocol for recycled PP. This study examines how the interaction between the surrogate contaminant, diluting solvent, and polymer type impacts the sorption behavior of homopolymer PP (h-PP).

The test surrogate contaminants [methyl salicylate (MS) and phenylcyclohexane (PCH)] and diluting solvents (n-hexane, 2-propanol, and ethanol) were selected based on the current FDA protocol and Hansen solubility parameter calculations. Swelling of h-PP films was determined gravimetrically as 0.7, 1.8, and 13.8% (w/w) in ethanol, 2-propanol and n-hexane, respectively. The h-PP films swollen in n-hexane followed by drying exhibited higher crystallinity by XRD than neat h-PP. Sorption experiments were performed at 40°C for up to 14 days on 10-mil thick monophasic h-PP cast film immersed in different diluting solvents. Sorption of MS (non-volatile, polar surrogate) and PCH (non-volatile, nonpolar surrogate) from each diluting solvent at 1% (v/v), respectively, into PP films was quantified by GC-MS. The equilibrium sorption of both MS and PCH into h-PP from n-hexane was rapidly attained within 12 h, while the sorption equilibrium of MS and PCH in the alcohols was reached at 2-3 and 7-10 days, respectively. MS equilibrium sorption concentrations into h-PP from 2-propanol and ethanol were 35% and 58% lower, respectively, compared to the sorption from n-hexane (2816 mg/kg). However, PCH equilibrium sorption concentrations into h-PP from 2-propanol and ethanol was 11-12% higher compared to the sorption from n-hexane (3821 mg/kg). This study demonstrates that n-hexane is inappropriate for use as a diluting solvent in surrogate contaminant testing of PP and the effects of solvent swelling cannot be overlooked when determining realistic initial contamination levels in PP.

For further evaluation of the sorption behavior, eight nonvolatile compounds were selected as potential contaminant surrogates for h-PP based on MW and polarity. Low MW surrogates (152-184 g/mol) were methyl salicylate, benzophenone, phenylcyclohexane, and n-tridecane. High MW surrogates (275-391 g/mol) were diphenyl phthalate, bis (2-ethylhexyl) terephthalate, tetradecylbenzene, and bis (2-ethylhexyl) adipate. 2-Propanol was selected as the most appropriate diluting solvent for use during surrogate testing of h-PP. The sorption of the 8-surrogate mixture, each surrogate at 1% concentration, from 2-propanol into h-PP film at 40°C was sampled at 12 time points over a total of 28 days and quantified by GC-MS. Equilibrium sorption of all surrogates into h-PP can generally be categorized into polar and nonpolar surrogate groups at 14 days. The polar surrogates (methyl salicylate, benzophenone, diphenyl phthalate, terephthalate) resulted in lower sorption in the range of 500-1700 mg/kg. While most nonpolar surrogates (phenylcyclohexane, tridecane, and tetradecylbenzene) had higher sorption in the range of 3400-4200 mg/kg. Within the nonpolar group, the more intermediate polarity bis (2-ethylhexyl) adipate experienced very low sorption (800 mg/kg). Diffusion coefficients (D_P) and partition coefficients ($K_{P,F}$) of surrogate contaminants into h-PP from 2-propanol were determined by nonlinear curve

fitting optimization of experimental sorption data. Migration modeling of surrogate sorption into h-PP highlighted the critical importance of using experimental $K_{P,F}$ values for accurate estimation of surrogate sorption into polyolefins.

This research will generate data that will assist OFAS in updating the 2006 Recycled Plastics Guidance for Industry and enhance FDA's ability to fulfill its mission of protecting and promoting public health as well as in evaluating premarket notification consultation (PNC) submissions on surrogate testing protocols for demonstrating the efficacy of industry's polyolefins recycling process.

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

Metal Ion Transport from Food Contact Materials Manufactured with Nanostructured Materials

Timothy Duncan[2], Laxmi Adhikari[2],
[1]Illinois Tech IFSH; [2]Food and Drug Administration

Polymer nanocomposites (PNCs) may be used in FDA regulated products like food contact materials and medical devices after premarket authorization. As such, FDA is interested in studying the release behavior of PNC components from PNC-containing food contact materials or medical devices that may potentially impact their safety. One critical aspect is being able to predict exposure to PNC components during product lifecycles.

This study uses a model system based on silver nanoparticles (AgNPs) incorporated into low density polyethylene (LDPE) to study the extent to which food chemistry and nanoparticle surface treatment impacts the amount and form of nanoparticles released from PNC packaging. (Silver nanoparticles are not currently authorized for use in food contact materials in the United States.) In the last year we have focused on three primary areas. In the first, we have explored migration of Ag from packaging containing AgNPs into solid foods. We synthesized AgNPs, incorporated them into model polymer-based food packaging, and performed migration tests to several foods including milled rice, bleached wheat flour, cheese slices, and fresh spinach. This work resulted in one publication that was chosen for an Editor's Choice award at the American Chemical Society. In the second focus area, we explored the impact of fats, proteins, and lactose content on migration of Ag from AgNP-containing packaging to different bovine milks. This work was completed in early 2025 and is currently under review at a peer-reviewed journal. The final focus area is the impact of capping agent on AgNP migration from food contact polymers. Capping agents are chemicals used to treat the surfaces of nanoparticles and assist in their incorporation within polymers. This work is ongoing, but we have observed that capping agents play a significant role in nanoparticle migration. We anticipate that this work will result in another peer-reviewed publication by the end of 2025 or early 2026.

This project will have two primary outcomes. Outcome 1 will be an improved understanding of how polymer polarity, nanoparticle capping agent, and food chemistry contribute to the quantity

and form of nanoparticle-derived material that consumers may be exposed to from PNC-containing products. Outcome 2 will be an assessment of the suitability of FDA's currently recommended migration protocols for food contact substances to PNCs. For instance, if food 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 the DFPST operating budget.

Sorption Behavior of Surrogate Chemical Contaminants in Polyethylenes for Use as Post-Consumer Recycled Food Contact Materials

Yoon Song[2], Huayi Wang[2], John Koontz[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration

The use of recycled materials for food packaging is restricted due to the possibility of contaminants migrating into the food. FDA operates a review process for recycled plastics which primarily focuses on experimental data to demonstrate that the recycling process results in material of a purity suitable for the intended use. Surrogate testing is employed to challenge recycling technologies with respect to their ability to reduce possible contamination. The potential fast diffusion of large molecular weight (MW) contaminants into polyethylene (PE) materials in comparison to other plastic resins requires additional safety evaluation since considerable sorption is expected in PE materials. This study's objective is to evaluate the sorption behavior of various chemical contaminant surrogates in polyethylene (HDPE, LDPE, LLDPE) and optimize FDA surrogate test conditions that are specific to PE.

Eight nonvolatile compounds were selected as potential contaminant surrogates for PE based on MW and polarity. Low MW surrogates (152-184 g/mol) were methyl salicylate, benzophenone, phenylcyclohexane, and n-tridecane. High MW surrogates (275-391 g/mol) were diphenyl phthalate, bis (2-ethylhexyl) terephthalate, tetradecylbenzene, and bis (2-ethylhexyl) adipate. When stored in diluting solvents of n-hexane, 2-propanol, and ethanol, high-density polyethylene (HDPE) films swelled 5.3%, 1.1%, and 0.4% w/w, respectively, at 40°C for 14 days. 2-Propanol was selected as the most appropriate diluting solvent for use during surrogate testing of PE. The sorption of the 8-surrogate mixture, each surrogate at 1% concentration, from 2-propanol into HDPE film at 40°C was sampled at 12 time points over a total of 28 days and quantified by GC-MS.

Equilibrium sorption of all surrogates into HDPE occurred by 14 days. The polar surrogates (methyl salicylate, benzophenone, diphenyl phthalate, bis (2-ethylhexyl) terephthalate) resulted in lower sorption in the range of 300-1100 mg/kg, while most nonpolar surrogates (phenylcyclohexane, n-tridecane, and tetradecylbenzene) had higher sorption in the range of 2200-3300 mg/kg. Within the nonpolar group, the more intermediate polarity bis (2-ethylhexyl) adipate experienced very low sorption (400 mg/kg). Diffusion coefficients (D_P) and partition coefficients

($K_{P,F}$) of surrogate contaminants into HDPE from 2-propanol were determined by nonlinear curve fitting optimization of experimental sorption data. Migration modeling of surrogate sorption into HDPE using partition coefficient estimation ($K_{P,F}$=1) highlighted the critical importance of using experimental $K_{P,F}$ values for accurate evaluation of surrogate sorption into polyolefins.

This study will identify chemistry issues that FDA will recommend in updated industry guidance that a recycled plastics manufacturer should consider during their evaluation of a recycling process for its decontamination efficacy in producing material suitable for food-contact applications.

This research was funded through the DFPST operating budget.

Evaluation of Wiping and Washing Treatments for Removal of Allergens and Gluten from Food-Contact Surfaces

Jeremiah Kidd[2], Lauren Jackson [2], Amandeep Sandhu[1]
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Preventing food allergen and gluten cross-contact is an important aspect of food safety for retail and food-service establishments. The U.S. FDA Food Code represents FDA's best advice for a uniform system of provisions that address the safety and protection of food offered at retail and in food service. The Food Code contains provisions for the washing, rinsing and sanitization of equipment, food-contact surfaces and utensils. Although these provisions were originally developed to reduce microbiological risks associated with food, their effectiveness in protecting food against allergen and gluten cross-contact has not been well established. Research is needed to identify the impact of different washing methods (i.e. manual vs. mechanical) and the influence of food type and form on allergen and gluten removal from food-contact surfaces. Furthermore, information is needed on the effects of precleaning steps used prior to manual and machine washing on enhancing the effectiveness of washing treatments.

The main objective of this project was to evaluate the effectiveness of washing treatments for removing commonly used allergen- and gluten-containing foods or ingredients from a variety of common food-contact surfaces used in retail and food service establishments. Specific goals were to (i) evaluate the effectiveness of the manual wash-rinse-sanitize-air dry cleaning method on removing various forms of egg-, gluten-, sesame-, and almond-containing foods from stainless steel, textured white polyethylene, ceramic and wood surfaces; (ii) examine the ability of high and low temperature mechanical warewashing treatments for removing food soils from a variety of surfaces; and (iii) study the use of precleaning steps on enhancing food soil removal during manual and mechanical warewashing treatments.

Coupons made of stainless steel (SS) polyethylene (PE), and ceramic (CE) were contaminated with 0.5 g or 1 g of egg-based (egg powder; reconstituted egg powder), gluten-containing (wheat flour; batter), sesame-based (sesame flour; tahini) or almond-based (almond flour, almond butter) foods. Maple hardwood (W) coupons were contaminated with only egg- and gluten-containing foods. Wood coupons were then subjected to only the manual wash treatment while SS, PE, and CE coupons were washed using manual and mechanical washing treatments.

Coupons were manually washed (10 sec), rinsed (10 sec), and then sanitized (60 sec), in neutral detergent solution (43°C), water (43°C), and 200 ppm quat solution (43°C), respectively. Mechanical treatments involved washing coupons in low and high temperature warewashing machines using standard washing cycles. Food residues on coupons after washing treatments were detected with allergen- or gluten-specific qualitative lateral flow devices (LFDs). A second set of experiments evaluated the use of precleaning such as scraping the coupons with a paper wipe or rinsing them with a ~56°C water spray in combination with manual and mechanical washing for removing tahini soils from SS, PE and CE coupons. Another precleaning treatment investigated the use of multiple low temperature mechanical wash cycles on tahini removal from the coupons. Experimental trials were done in triplicate.

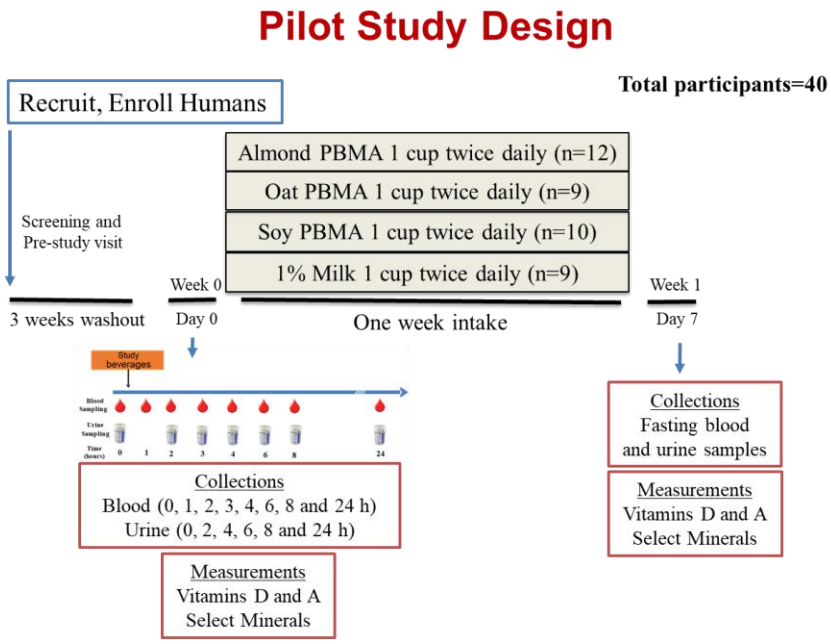
Mechanical washing treatments were significantly ($p<0.05$) more effective at removing the allergen- and gluten-containing foods from the coupons than the manual treatment. Although there were no significant differences ($p>0.05$) between coupon type with respect to cleanability, tahini and almond butter were significantly ($p<0.05$) more difficult to remove from coupons than the other food soils. Precleaning tahini soiled coupons with a prescrape or a water rinse did not improve the effectiveness of the manual washing treatment, while the prescrape improved tahini removal during mechanical washing. Multiple wash cycles in the low temperature warewasher improved tahini removal from coupons. The results indicate that the nature of the food soil, the type of washing method (manual vs mechanical), and use of precleaning methods impacted washing treatment effectiveness. More extensive or specialized washing treatments are needed for some allergenic food soils, particularly food pastes such as tahini and almond butter. This research yields important and practical information for the retail and food-service industry on ways to prevent or minimize allergen and gluten cross-contact when using shared food-contact surfaces.

This research was funded through HFP's Cooperative Agreement with IFSH, the DFPST operating budget, and industry funding.

Nutrition Platform

Lauren Jackson, FDA and Britt Burton-Freeman, Illinois Tech IFSH

The Nutrition Platform aims to contribute knowledge about food choice and intake behavior by consumers and their impact on nutrition and health.



Relative Absorption of Fat-Soluble Vitamins D and A and Minerals from Select Plant-Based Milks in Human Subjects: A Pilot Trial

Joseph Zuklic[1], Indika Edirisinghe[1], Amandeep Sandhu[1], Britt Burton-Freeman[1], Cole Carter[2], Lauren Jackson[2], Katherine Pett[3], Tanishq Pardesi[3], Sharvari Sanjay Satam[3]
[1]*Illinois Tech IFSH;* [2]*Food and Drug Administration;* [3]*Illinois Tech Department of Food Science*

Plant-based milk alternatives (PBMAs) now account for around 10% of the total milk market and the sales of PBMA are predicted to increase. The different types of PBMAs on the market shelf include almond, oat, soy, coconut, cashew, pea, hemp, and rice. Among these, PBMA made from almonds, oats and soy are the most popular in North America. PBMAs are intended to resemble bovine milk in terms of color and texture; however, they are often nutritionally unequal and have unacceptable flavor profiles. As a result, most of the PBMAs are fortified with minerals and vitamins to mimic bovine milk composition with added sugars and flavorings to mask off-flavors. The Dietary Guidelines, 2020-2025 identify the Dairy Group, which includes milk, as a key contributor of calcium, protein, vitamin A, vitamin D, magnesium, phosphorus, potassium, riboflavin, vitamin B-12, zinc, choline, and selenium. The bioavailability of these nutrients (inherent and fortified) in PBMAs has not been investigated in humans. However, due to the presence of some components (i.e., oxalates, phytates, tannins) and the processing methods used in production, the bioavailability of nutrients from PBMAs is believed to be low. Moreover, there are no standards of identity for PBMAs in the US and the nutritional quality of different types of PBMAs is quite variable.

The study analyzed the relative absorption of vitamin D and minerals after acute and short-term intake (up to 24 h and 1 week) of almond, oat and soy PBMAs in humans. Bovine milk is used as positive control. The study was a randomized controlled trial with a parallel study design in 48 healthy subjects (n=12 per arm). Plasma/serum and urine samples were collected after 3-week washout from milk products (baseline, 0 h), and then periodically at 1 h, 2 h, 4 h, 6 h, 8 h, 10 h and 24 h, and 1 week post daily consumption for the analysis of vitamin A, D, and select minerals (calcium, potassium, magnesium). On completion of the data from these analyses it will help influence the understanding of the relative absorption (bioavailability) of certain nutrients from PBMAs. This information will help in the formulation and fortification of products as well as in providing regulatory agencies with a better understanding of these products for setting up the dietary guidelines.

Human trials were conducted during the winter months of 2023/2024 in Chicago, IL. Clinical trial began in December 2023 and was completed in Feb 2024 with some modifications. A total of 62 participants were screened for the study with 44 participants randomized for each study arm. There was a total of 36 completers with all postprandial blood draws. Current activities include specimen analysis. The digestion and analysis methods were developed and validated for calcium (Ca), magnesium (Mg) and potassium (K) in human plasma and urine using microwave assisted digestion followed by Inductively coupled plasma optical emission spectrometry (ICP-OES). These minerals (Ca, Mg and K) have been analyzed in over 500 plasma samples, and the collected data is being assessed. The urine samples (~ 450) are being analyzed and estimated to finish the

analysis by the end of August 2025. The method development for the quantification of vitamin A and D in human plasma and urine is ongoing.

This research was funded by FDA CFSAN Office of Nutrition and Food Labeling through HFP's Cooperative Agreement with IFSH and the DFPST operating budget.

Estimation of the Intestinal Bioaccessibility of Vitamin D and Minerals Across Different Types of Plant-Based Milk Using an In Vitro Model

Cole Carter[2], Amandeep Sandhu[1], Britt Burton-Freeman[1], Lauren Jackson[2]
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Plant-based milk alternative (PBMA) consumption has been increasing due to factors such as bovine milk protein allergy, taste preference, religious reasons, and environmental awareness. Due to a lack of key micronutrients naturally present in the base ingredients used for the production of PBMAs, they are often fortified with micronutrients to mimic those naturally occurring in fluid bovine milk ("milk").

In the previous project (CARTS#: IF01782), the analysis of target nutrient elements (calcium, magnesium, phosphorous, potassium, selenium, and zinc) and vitamins (vitamin A, vitamin B complex, vitamin D and choline) present in the most commonly consumed PBMA types (i.e., almond, coconut, cashew, oat, pea, hemp, rice, and soy) was performed. The effect of thermal processing (high temperature short time pasteurization; HTST) on micronutrient retention/variability in a model almond PBMA was also assessed.

Work by Zhou et al. (2021) using *in vitro* models demonstrated that interactions between minerals and lipids/lipid-soluble components of PBMAs can influence the intestinal bioaccessibility of vitamin D. We used an *in vitro* model of digestion to assess the relative intestinal bioaccessibility of vitamin D and key minerals across commonly consumed PBMA types. The fatty acid composition (saturated and unsaturated fatty acids) of PBMAs is also being determined. Associations between amounts of minerals, fatty acid composition, and vitamin D bioaccessibility are being assessed using the standardized INFOGEST *in vitro* digestion method. Particular attention is given to amounts of divalent mineral ions (e.g., Zn^{2+}, Ca^{2+} and Mg^{2+}) due to the potential for these ions to form insoluble complexes, especially in the presence of plant based phytate. Vitamin D is quantified using an AOAC official method for LC-MS/MS. Mineral content is determined using an ICP-MS method adapted from the FDA's elemental analysis manual. A GC-MS method from AOAC to characterize the fatty acid composition is being used. Once all of the results are determined from this study it enables consumers to better understand the nutritional qualities of PBMAs to make informed food choices, provide opportunities for better product formulation, and to help inform regulatory agencies' understanding of these products. The study also is helpful for use as a screening tool to identify specific PBMA types for use in animal or human nutrient bioavailability trials. Activities and accomplishments include the following to date: the experimental design of study has been developed. Target plant-based milk alternatives (PBMAs) have been identified and acquired (soy, almond, and oat, as well as 1% dairy for comparison), and experimental procedures for both mineral and vitamin D content have been

established and validated using a NIST reference material (adult nutritional formula 1896). Additionally, The ICP analysis for minerals has been completed for both the *in vitro* digested and undigested samples, where the results indicate that PBMAs have lower mineral bioaccessibility than dairy milk, but that high fortification levels may compensate for this effect (e.g. the 150% fortification for calcium in PBMAs results in similar bioaccessible calcium content as dairy). LC-MS/MS analysis for vitamin D has been completed for undigested samples, and analysis for *in vitro* digested samples is ongoing. Method development for fatty acid analysis using GC-MS is also ongoing.

This research was funded by FDA CFSAN Office of Nutrition and Food Labeling through HFP's Cooperative Agreement with IFSH and the DFPST operating budget.

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 Moffett Proficiency Testing Laboratory (MPTL) at the Institute of Food Safety and Health, Illinois Tech, and the FDA Division of Food Processing Science and Technology, is an ISO/IEC 17043 accredited proficiency test provider. The MPTL plays a crucial role in enhancing analytical capacity and capability across federal, state, and local laboratories involved in food safety and public health initiatives. Primary function of MPTL is development and implementation of microbiological and chemical proficiency testing (PT) programs designed to assess and improve laboratory performance. These programs support multiple FDA centers, including the Human Foods Program (HFP), the Center for Veterinary Medicine (CVM), Office of Chief Scientist (OCS) and USDA-FSIS (Food Safety & Inspection Service). The MPTL inter-laboratory studies are critical part of national networks of laboratories supporting laboratory capacity and capability building such as the Food Emergency Response Network (FERN), the Veterinary Laboratory Investigation and Response Network (Vet-LIRN), and the DHS Integrated Consortium of Laboratory Networks (ICLN).

Summary of proficiency testing events offered by the Moffett Proficiency Testing Laboratory (Fiscal year 2025)

Target Analyte or testing protocol	Proficiency Test Matrix
Quality Indicators; *B. cereus; S. aureus; S. Typhimurium; C. saksazakii*	Infant Formula, Powdered
Vitamin A; Vitamin D3	Milk, Finished
Aerobic Plate Count; Coliforms; Alkaline Phosphatase	Milk, Finished
Vitamin A; Vitamin D3	Milk, Finished
Drug Residues; Somatic Cells; Added Water	Milk, Raw
Aerobic Plate Count; Coliforms	Growing Area and Depuration Water WWater, Oyster
V. parahaemolyticus; V. cholerae; V. vulnificus	Oyster, Puree
Mycotoxins	Pet Food, Canine
Salmonella	Feces, Canine
Whole Genome Sequencing	Culture Slants
HPAI	Milk, Finished
B. anthracis Sterne; *Y. pestis* A1122; Ricin	Egg Product, Liquid
Patulin	Juice, Fruit, Apple
Metals	Chili, Meat-based
Glyphosate	Barley
Terbuphos; Propoxur; Amanitin	Egg Product, Liquid
L. monocytogenes; L. welshimeri; L. innocua; L. ivanovii	Chicken, Cooked, Shredded
BoNT	Infant Formula, Liquid
Cyclospora	Spinach
B. anthracis Sterne; *Y. pestis* A1122	Egg Product, Liquid
1,3-dimethylamylamine; Sildenafil; Tadalafil; Nortadalafil; Sibutramine; Orlistat	Shrimp
pH; Water Activity	Soy Sauce
Sulfamerazine; Flumequine; Ciprofloxacin	Botanical Supplement
C. sporogenes; Short Bodyhook; Insufficient Overlap (low base plate pressure); Loose Seam; Insufficient Overlap (2nd operation	Soup, Chicken, Rice; Metal Cans Retort Integrity/Defects
C. sporogenes; S. epidermidis; E. cloacae; Non-bonding weak seal; Pinhole leak	Soup, Chicken, Rice; Retort Pouches Integrity/Defects

Detection of Highly Pathogenic H5N1 Avian Influenza (HPAI) Milk

Emily Smith[2], Jenny Jin[1], Jodie Ulaszek[1], Ravinder Reddy[2]
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In response to the emergence of Highly Pathogenic H5N1 Avian Influenza (HPAI) in dairy cattle, the FDA's Veterinary Laboratory Investigation and Response Network (Vet-LIRN), IFSH NCFST, FDA's Human Foods Program (HFP) Moffett Center, and USDA's National Animal Health Laboratory Network (NAHLN), are conducting an Inter-laboratory Comparison Exercise (ICE) scheduled for July 2025. This exercise aims to evaluate the proficiency of participating laboratories in detecting HPAI virus RNA in milk samples using various PCR-based methods. The ICE will involve 16 test samples of inactivated virus spiked into milk at different concentrations, to be analyzed by pre-registered laboratories under BSL-2 conditions. Results will be comparatively evaluated to identify opportunities for improving participants' performance and providing information on detection levels. This initiative supports the critical need for reliable HPAI testing in milk samples, contributing to animal and human health protection, and food safety assurance.

Real-Time PCR Assay for Detection of *Cyclospora cayetanensis* on Fresh Produce

Jodie Ulaszek[1], Robert Newkirk[2], Jenny Jin[1], Ravinder Reddy[2]
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Cyclospora cayetanensis is an emerging parasitic protozoan pathogen causing cyclosporiasis, a gastro-intestinal disease in humans. Laboratory methods that can be used to detect parasites in food and water are essential to identify potential sources of infection and provide critical support for outbreak investigations. The FDA developed a real-time PCR method (qPCR) to detect *C. cayetanensis* based on a new molecular mitochondrial gene (Mit1C). The new Mit1C target on the mitochondrial genome of *C. cayetanensis* is specific to *C. cayetanensis* and is very sensitive. In collaboration with FDA MOD1 parasitology team, IFSH NCFST, and FDA HFP Moffett Center is conducting a *C. cayetanensis* Inter-laboratory Comparison Exercise (ICE) scheduled for July 2025. Participants will be evaluated on detection of unsporulated oocysts in spinach. The objective of the proficiency study is to evaluate the proficiency of the laboratories as well as proof of a Verification study to demonstrate laboratory and analysts' ability to implement and conduct an analytical method.

APPENDIX

Publications Calendar Year 2024-2025

1. Adhikari, L., Sayeed, M., Mudireddy, R., Villalon, K., Shekhawat, G., Bleher, R. Duncan, T.V. **2024.** Surface heterogeneity at the polymer-food interface influences Ag migration from plastic packaging incorporating Ag-exchanged zeolites. *ACS Appl. Mater. Interfaces*, 16(36), 48163-48175. https://doi.org/10.1021/acsami.4c05581

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11. Jiang, X., and Jackson, L.S. **2024**. Food allergens. Encyclopedia of Food Safety, 2nd Edition. Pages 295-308, G. Smithers (Ed), Elsevier, London, UK. https://doi.org/10.1016/B978-0-12-822521-9.00233-1

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31. Tkachenko, A., Chen, Y., Petrey, M., Fritz, S., Walsh, T., Rotstein, D., Miller, M.R., Williams, B., Dark, M., Kmet, M., Reddy, R., Tyson, G., Nemser, S.M. **2025**. A novel proficiency test to assess the animal diagnostic investigation process in identifying an unknown toxicant. *Toxicology Reports*. 14, 101925.

32. Wang, H., Kiener, S., Wang, S.S., Deng, K., Smith, E., Chen, K-.S., Pamboukian, R., Laasri, A., Pelaez, C., Ulaszek, J., Kmet, M., De Jesus, A., Zhang, G., Hammack, T., and Reddy, R. **2024**. Multi-laboratory validation study of a real-time PCR methods for detection of *Salmonella* in frozen fish. *Food Microbiology*. 5061916. https://dx.doi.org/10.2139/ssrn.5061916

33. Wong, C.W.Y., Burton, T., Montoya, J.C., Birje, N., Zhou, X., Salazar, J.K., Mackenzie, J.M., Rau, T.F., Teplitski, M., and Zhang, W. **2024**. Antimicrobial efficacy of GS-2 on reusable food packaging materials for specialty crops. *MDPI Foods*. 13(21), 3490. https://doi.org/10.3390/foods13213490

34. Zhang, L., Bedford, B., Warren, J., Sharma, G., Brown, A.L., Hopfer, H., Ziegler, G.R., and L.S. Jackson. **2024**. Effectiveness of dry cleaning treatments for removing milk chocolate from valve/pipe assemblies and pilot-scale chocolate processing equipment. *Journal of Food Protection*, 87(10), 100346. https://doi.org/10.1016/j.jfp.2024.100346

35. Zuklic, J.F., Cai, J., Jackson, L., Redan, B.R., Sandhu, A., Wan, J. **2024**. Determination of Choline and B Vitamins in a Market Basketof Eight Types of Plant-Based Milk Alts Using Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry. *Current Developments in Nutrition,* 8(2), 102903. https://doi.org/10.1016/j.cdnut.2024.102904

Presentations:

1. Adhikari, L., Todorov, T.I., Yang, T., Hornick, J., Sawant, R., Paulose, T., Duncan, T.V. **2024**. Contact transfer of Ag to solid foods and abiotic surfaces from model AgNP-containing polymer nanocomposite packaging. FDA NanoDay, October 9, 2024, Virtual, Poster.

2. Adhikari, L., Mudireddy, R.R., Duncan T.V. **2024.** Milk proteins and fat influence Ag migration from model dairy packaging containing silver nanoparticles. FDA NanoDay, October 9, 2024, Virtual, Poster.

3. Adhikari, L., Todorov, T.I., Yang, T., Hornick, J., Sawant, R., Paulose, T., Duncan, T.V. **2024**. Contact transfer of Ag to solid foods and abiotic surfaces from model AgNP-containing polymer nanocomposite packaging. Nanoscale Science and Engineering for Agriculture and Food Systems Gordon Research Conference, June 23-28, 2024, Manchester, New Hampshire. Poster.

4. Adhikari, L., Mudireddy, R.R., Duncan T.V. **2024.** Milk proteins and fat influence Ag migration from model dairy packaging containing silver nanoparticles. Nanoscale Science and Engineering for Agriculture and Food Systems Gordon Research Conference, June 23-28, 2024, Manchester, New Hampshire. Poster.

5. Adhikari, L., Sayeed, M., Mudireddy, R.R., Duncan T.V. **2024.** Transport of silver from silver zeolite-enabled packaging. ACS Spring National Meeting, March 18, 2024, New Orleans, Louisiana. Poster.

6. Anant, S. and Fu, T.-J. **2024**. Growth profiles of *Salmonella* in broccoli sprouts during sprouting and postharvest storage at different temperatures. IFT FIRST Annual Meeting and Expo, July 14-17, Chicago, Illinois. Poster.

7. Beverly, Robert. **2025**. A targeted proteomics method to assess allergen content of oil after roasting peanuts. HFP Allergen Committee and Technical Team (ACTT) Research Meeting. Virtual, April 18, 2025. Oral.

8. Chen, B., Deng, K., Hettwer, K., Uhlig, S., Singh, N., Reddy, R., Wan, J. **2024**. Factors Influencing the Level of Detection of *Listeria monocytogenes* in Ice Cream. International Association for Food Protection Annual Conference, July 14-17, 2024, Long Beach, California. Poster.

9. Duncan, T.V. **2025**. Regulatory science perspective on the analysis of microplastics and nanoplastics in human food. Northwestern Medical School Seminar, March 4, 2025, Chicago, Illinois, Oral.

10. Duncan, T.V. **2025**. Regulatory science perspective on the analysis of microplastics and nanoplastics in human food. NJ Association for Food Protection Seminar Series, January 18, 2025, Virtual, Oral.

11. Duncan, T.V. **2024**. Regulatory science perspective on the analysis of microplastics and nanoplastics in human food. MSU Food Aid Sustainable Packaging Solutions Workshop, November 13-15, 2024, Lansing, Michigan, Oral.

12. Duncan, T.V. **2024**. Regulatory science perspective on the analysis of microplastics and nanoplastics in human food. Fall Phi Tau Sigma Webinar, November 12, 2024, Virtual, Oral.

13. Duncan, T.V. **2024**. Metal ion transport from model food packaging containing engineered nanomaterials. Illinois Tech Department of Chemistry Seminar, October 8, 2024, Chicago, Illinois, Oral.

14. Duncan, T.V. **2024**. Ag Migration from Model Food Contact Materials Incorporating Ag Nanoparticles. IFSH Annual Meeting, September 5-6, 2024, Rosemont, Illinois, Oral.

15. Duncan, T.V. **2024**. Regulatory science perspective on the analysis of microplastics and nanoplastics in human food. Fed Only Nanoplastics Interest Group Meeting, April 10, 2024, Virtual. Oral.

16. Fay, M.L., Marathe, A.N., Khouja, B.A., Salazar, J.K., and Stewart, D.S. **2024**. P2-179: Growth kinetics of *Listeria monocytogenes* on chopped citric acid-treated hard-boiled eggs. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

17. Fay, M.L., Salazar, J.K., Fleischman, G., Khouja, B.A., Stewart, D.S., and Ingram, D.T. **2024**. Inactivation of *Listeria monocytogenes* and *Salmonella enterica* on specialty mushrooms in ramen soup based on broth temperature. FDA Foods Program Regulatory Science Conference, September 10-11, Washington, D.C. Poster.

18. Fu, T.-J. **2024**. CARTS 1777 - Impact of temperature on pathogen proliferation during sprouting and postharvest storage. CARTS Close Outs, February 7, Virtual. Oral.

19. Fu, T.-J. **2024**. Decontamination of sprout seeds by chemical and dry-heat treatments. North Central Region FSMA Conference, April 3, Burr Ridge, IL. Oral.

20. Fu, T.-J. **2024**. S12: Global recommendations on prevention and control of microbiological hazards in fresh fruits and vegetables from the Joint FAO/WHO Expert Meeting. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Oral.

21. Fu, T.-J. **2024**. Ensuring the safety of seeds for sprouting and other applications. Seed Safety Conference, August 13, Burr Ridge, IL. Oral.

22. Grasso-Kelley, E. **2024**. WS2: Selecting and validating pathogen reduction processes for low-moisture foods and ingredients. International Association for Food Protection Annual Meeting, Pre-Meeting Workshop, July 12-13, Long Beach, CA. Oral.

23. Grasso-Kelley, E. **2024**. Low water activity food safety. Illinois Tech Food Microbiology Lecture, November 18, Virtual. Oral.

24. Jackson, Lauren. **2024**. FDA regulatory update on food allergens, ACS Spring National Meeting, New Orleans, Louisiana, Virtual, March 17-21, 2024.

25. Jackson, Lauren. **2024**. Codex request and FAO/WHO's mandate on food allergens: the updated global priority food allergen list. International Association for Food Protection Annual Meeting. Long Beach, California. July 14-17, 2024. Oral presentation.

26. Jackson, Lauren. **2024**. Importance of analytical methods for food allergen compliance and enforcement activities. AOAC Annual Meeting, Baltimore, Maryland, August 23-28, 2024. Oral presentation.

27. Jackson, Lauren S. **2024**. Transfer of toxic elements to beverages from processing aids used during filtration treatments. ACS Fall 2024 National Meeting, Denver, Colorado, August 18-22, 2024. Oral presentation.

28. Jackson, Lauren S. **2024**. My career as a chemical food safety research scientist at the U.S. food and drug administration. University of Nebraska Seminar Series, Lincoln, Nebraska, October 31, 2024. Oral presentation.

29. Jackson, Lauren S. **2024**. Evaluation of manual and mechanical warewashing and wiping treatments for removal of allergens from food-contact surfaces. FAACT Food

Industry & Research Summit, Oak Brook, Illinois, November 12, 2024. Oral presentation.

30. Jackson, Lauren S. **2025**. Analytical methods for verifying the effectiveness of allergen cleaning procedures. American Chemical Society Fall Meeting, Washington, DC, August 19, 2025. Oral presentation.

31. Jiang, X., Beverly, R., Kidd, J.A., Cluster, J., Swajian, K., Jackson, L.S. **2024**. Transfer of shrimp proteins in shared batch fryers. ACS Spring Meeting, March 17-21, New Orleans, LA. Oral.

32. Jiang, X., Beverly, R., Kidd, J.A., Cluster, J., Swajian, K., Rao, Q., Jackson, L.S. **2024.** Assessment of gluten transfer and removal in frying oil. AOAC Annual Meeting, August 23-28, Baltimore, MD. Oral.

33. Jiang, X., Beverly, R., Kidd, J.A., Cluster, J., Swajian, K., Rao, Q., Jackson, L.S. **2024.** Assessment of gluten transfer and removal in frying oil. IFSH Annual Meeting, September 12-13, Rosemont, IL. Oral.

34. Jiang, X., Beverly, R., Kidd, J.A., Cluster, J., Swajian, K., Bingi, A.K., Rao, Q., Jackson, L.S. **2025**. Optimization of active filtration to reduce gluten cross-contact in reused frying oil. AOCS Annual Meeting, April 27-30, Portland, OR. Oral.

35. Jiang, X., Beverly, R., Kidd, J.A., Cluster, J., Swajian, K., Bingi, A.K., Rao, Q., Jackson, L.S. **2025**. Transfer of gluten proteins in shared batch fryers. FDA Science Forum, June 11-12, virtual. Poster.

36. Jiang, X., Warren, B., Remington, B., Luccioli, S., Williams, L., Moore, V., Jackson, L.S. **2025.** Seafood allergen cross-contact risk associated with reuse of shared breading mixtures. IAFP Annual Meeting, July 27-30, Cleveland, OH. Poster.

37. Jiang, X., Beverly, R., Kidd, J.A., Cluster, J., Swajian, K., Bingi, A.K., Rao, Q., Jackson, L.S. **2025.** Evaluation of passive and active filtration to reduce gluten cross-contact in reused frying oil. IFT FIRST Annual Meeting, July 13-16, Chicago, IL. Poster.

38. Jiang, X., Beverly, R., Kidd, J.A., Cluster, J., Swajian, K., Bingi, A.K., Rao, Q., Jackson, L.S. **2025.** Transfer of gluten proteins in shared batch fryers. ACS Fall Meeting, August 17-21, Washington, DC. Oral.

39. Joshi, M., Salazar, J.K., Korade, S., Fay, M.L., Khouja, B.A., and Stewart, D.S. **2024**. P2-142: Combination use of power ultrasound and organic acids to reduce *Listeria monocytogenes* populations on peaches and apples. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

40. Khouja, B.A., Serra-Cordero, D.M., Green, K., Fay, M.L., Salazar, J.K., Stewart, D.S. **2024**. P2-133: Survival of *Salmonella enterica* on dry-inoculated fresh peaches during retail and consumer storage conditions. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

41. Khouja, B.A., Fay, M.L., Salazar, J.K., and Stewart, D.S. **2024**. Profiling the microbiomes of different varieties of peaches and peach tree leaves. FDA Foods Program Regulatory Science Conference, September 10-11, 2024, Washington, D.C. Poster.

42. Khouja, B.A., Fay, M.L., Salazar, J.K., and Stewart, D.S. **2024**. Profiling the microbiomes of different varieties of peaches and peach tree leaves. ASM Conference on Rapid Applied Microbial Next-Generation Sequencing and Bioinformatic Piplines, October 13-16, 2024, Washington, D.C. Poster.

43. Kidd, J., Sandhu, A., Gettis, T., Buckley, D., Teska, P., Jackson, L. **2024**. Evaluation of wiping treatments for removal of allergens from food-contact surfaces. International Association for Food protection Annual Meeting, July 14-17, 2024, Long Beach, California, Poster.

44. Kidd, J., Sandhu, A., Gettis, T., Buckley, D., Teska, P., Jackson, L. **2024**. Evaluation of manual and mechanical warewashing treatments for removal of allergens from food-contact surfaces. International Association for Food Protection Annual Meeting, July 14-17, 2024, Long Beach, California, Poster.

45. Kidd, J. **2024**. Evaluation of manual and mechanical warewashing treatments for removal of allergens from food-contact surfaces, IFSH Annual Meeting, September 25, 2024, Rosemont, Illinois, Oral Presentation.

46. Kidd, J., Sandhu, A., Gettis, T., Buckley, D., Teska, P., Jackson, L. **2024**. Evaluation of manual and mechanical warewashing treatments for removal of allergens from food-contact surfaces, FDA Science Forum, June 11-12, 2024, Virtual. Poster.

47. Kidd, J. **2025**. Evaluation of manual and mechanical warewashing treatments for removal of allergens from food-contact surfaces. HFP Allergen Committee and Technical Team (ACTT) Research Meeting. Virtual, April 18, 2025. Oral.

48. Kiener, S., Singh, N., Smith, E., Nemser, S., Hettwer, K., Uhlig, S., and Reddy, R. **2024**. Determination of LOD and RLOD of *Salmonella* in raw pet food matrices. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

49. Kiener, S., Singh, N., Smith, E., Nemser, S., Hettwer, K., Uhlig, S., and Reddy, R. **2024**. Determination of LOD and RLOD of *Salmonella* in raw pet food matrices. FDA Foods Program Regulatory Science Conference, September 10-11, Washington, D.C. Poster.

50. Lee, A., Maks-Warren, N., Aguilar, V., Piszczor, K., Swicegood, B., Ye, M., Elston, K., Warren, J., O'Neill, E., Fleck, M., Tejayad, S. **2024**. Inactivation of *Salmonella*, Shiga Toxin-producing *E. coli* and *Listeria monocytogenes* in raw diet petfoods using high pressure processing. International Association for Food Protection 19th European Symposium on Food Safety, April 30-May 2, Geneva, Switzerland. Poster.

51. Lee, A. **2024**. Using big data and AI to advance food safety. Food Safety Summit, May 6-9, Rosemont, Illinois. Oral.

52. Lee, A. **2024**. The genomic and environmental stressors that impact microbial pathogenesis. Food Safety Summit, May 6-9, Rosemont, Illinois. Oral.

53. Lee, A. **2024**. Sampling and testing in verification studies – A reality check. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Oral.

54. Lee, A. **2024**. Key benefits of sample pooling strategies. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Oral.

55. Lee, A. **2024**. The role and purpose of processing in food production. JIFSAN-CFS3 Advisory Council Annual Symposium, October 29-30, College Park, Maryland. Oral.

56. Lee, A. **2025**. Use of high pressure processing to control pathogens in seafood. Hiperbaric invited webinar, March 12. Oral.

57. Lee, A. **2025**. Is exact colony counting overkill or just right? International Association for Food Protection 19th European Symposium on Food Safety, May 6-8, Madrid, Spain. Oral.

58. Lee, A. **2025**. Innovative processing technologies for challenging food products. Food Safety Summit, May 12-15, Rosemont, Illinois. Oral.

59. Lee, A. **2025**. Emerging and new pathogens: What's old is new again. Institute of Food Technologists Annual Scientific Meeting, July 13-16, Chicago, Illinois. Oral.

60. Lee, A. **2025**. Antimicrobial use in raw diet pet foods to enhance HPP efficacy. International Association for Food Protection Annual Meeting, July 27-30, Cleveland, Ohio. Oral.

61. Liu, X., Grasso-Kelley, E.M., Lee, A., and Anderson, N.M. **2024**. P3-75: Factors affecting *Salmonella* inactivation on apples during hot air drying. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

62. Liu, X., Grasso-Kelley, E.M., Lee, A., and Anderson, N.M. **2024**. P3-74: Isothermal inactivation kinetics of *Salmonella* Montevideo on partially dried apple cubes. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

63. Liu, X., Grasso-Kelley, E.M., Lee, A., Anderson, N.M. **2025**. The effect of temperature, bed depth, and air velocity for the prediction of Salmonella inactivation during hot-air apple drying. International Association for Food Protection Annual Meeting, July 27-30, Cleveland, Ohio. Poster.

64. Koontz, J.L., Wang, H., Song, Y.S. **2025**. Sorption of recycling contaminant surrogates into high-density polyethylene (HDPE) food contact materials. American Chemical Society Fall Meeting, August 17-21, Washington, DC. Poster.

65. Maks-Warren, N., Srinivasan, M., Swicegood, B., Halik, L., Lee A. **2024.** Evaluation of various sanitizers and additives to reduce *Listeria monocytogenes* on RTE fishery

products. International Association for Food Protection 19th European Symposium on Food Safety, April 30-May 2, Geneva, Switzerland. Poster.

66. Maks-Warren, N., Srinivasan, M., Swicegood, B., Halik, L., Lee A. **2024.** Evaluation of various sanitizers and additives to reduce *Listeria monocytogenes* on RTE fishery products. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

67. Marathe, A.N., Fay, M.L., Khouja, B.A., Salazar, J.K., and Stewart, D.S. **2024**. P2-180: Survival of *Listeria monocytogenes* in deli salads containing hard-boiled eggs depending on egg treatment. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

68. Morrissey, T., Rolfe, C., Aguilar, V., and Skinner, G. **2024**. P2-112: Inactivation of *Clostridium botulinum* spores in commercial cold brew coffee stored under aerobic conditions. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

69. Nemser, S.M., Kiener, S., Singh, N., Smith., E., Hettwer, K., Kmet, M., Miller, M.R., Tkachenko, A., Uhlig, S., Reddy, R., and Tyson, G. **2024**. Proficiency Test offered by Vet-LIRN for the detection of *Salmonella* in companion animal food. FDA Foods Program Regulatory Science Conference, September 10-11, Washington, D.C. Poster.

70. Nemser, S.M., Kiener, S., Singh, N., Smith., E., Hettwer, K., Kmet, M., Miller, M.R., Tkachenko, A., Uhlig, S., Reddy, R., and Tyson, G. **2024**. Proficiency test offered by Vet-LIRN for the detection of *Salmonella* in companion animal food. American Association of Veterinary Laboratory Diagnosticians Annual Meeting, October 10-16, Nashville, TN. Poster.

71. Rolfe, C. **2024**. CARTS 1773 - Study on correlation between dipicolinic acid (DPA) release and heat resistance of *Clostridium botulinum* spores during thermal and pressure-assisted thermal processing. CARTS Close Outs, February 7, Virtual. Oral.

72. Rolfe, C. **2024**. HPP juice safety. FDA CFSAN Division of Plant Products and Beverages Seminar, March 14, Virtual. Oral.

73. Rolfe, C. **2024**. FDA regulatory introduction. Better Process Cheese School, March 26, Madison, WI. Oral.

74. Rolfe, C. **2024**. A food safety perspective on cold brew coffee. Illinois Tech Graduate Seminar, April 18, Virtual. Oral.

75. Rolfe, C., Morrissey, T., Aguilar, V., and Skinner, G. **2024**. P2-300: Evaluation of the risk for *C. botulinum* outgrowth and toxin production in commercial plant-based meat alternative products. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

76. Rolfe, C., Aguilar, V., Skinner, G., and Morrissey, T. **2024**. Evaluation of the risk for *C. botulinum* outgrowth and toxin production in commercial plant-based meat

alternative products. FDA Foods Program Regulatory Science Conference, September 10-11, Washington, D.C. Poster.

77. Rolfe, C. **2024**. Factors contributing to foodborne disease outbreaks. Illinois Tech Food Microbiology Lecture, September 25, Virtual. Oral.

78. Rolfe, C. **2024**. Metabolic injury and repair. Illinois Tech Food Microbiology Lecture, October 30, Virtual. Oral.

79. Salazar, J. **2024**. CARTS 1908 - Examination of power ultrasound and organic acid-based hurdle technology to reduce foodborne pathogens on select produce matrices. CARTS Close Outs, February 7, Virtual. Oral.

80. Salazar, J. K., **2024**. Growth kinetics of *Listeria monocytogenes* and *Salmonella enterica* during rehydration and subsequent storage of dehydrated vegetables. Conference for Food Protection's Rehydration Committee Meeting, February 12, Virtual. Oral.

81. Salazar, J. **2024**. Enoki published research. U.S. FDA | China-CFSA, May 15, Virtual. Oral.

82. Salazar, J., Carrol, L., Zhou, X., Solaiman, S., Hines, I., and Wang, L. **2024**. S48: From pathogen transcriptomics to prevention strategies. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Oral.

83. Salazar, J.K., Fay, M.L, Carstens, C.K., Khouja, B.A, Stewart, D.S., and Ingram, D.T. **2024**. Metataxonomic profiling of enoki mushrooms available at retail reveals diversity in the bacterial consortia of mushroom body and roots. ASM Conference on Rapid Applied Microbial Next-Generation Sequencing and Bioinformatic Piplines, October 13-16, Washington, D.C. Poster.

84. Shazer, A. and Fu, T.-J. **2024**. P3-189: Dry heat treatment in reducing *Salmonella* and *E. coli* O157:H7 contamination on alfalfa seeds. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, CA. Poster.

85. Song, Y.S., Wang, H., Senthilkumaran, S., Koontz, J. **2025**. Investigation of diluent effect on sorption behavior of polypropylene towards polar and nonpolar recycling contaminant surrogates. ACS Fall 2025, August 17-21, 2025, Washington, DC, Poster.

86. Stewart, D. **2024**. Overview of research related to process-contaminated ice cream. CORE/DFPST CoLLAB Session, April 26, Virtual. Oral.

87. Taneja, N.K., Kaushik, A., Juneja, V.K., and Salazar, J.K. **2024**. P3-26: Enhancing microbial safety and quality of milk with ultrasonication: kinetics modeling of pathogenic bacteria and milk characteristics. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, California. Poster.

88. Vakkalagadda, S., Liu, X., Lee, A., Grasso-Kelley, E., Anderson, N. **2025**. Isothermal inactivation of *Enterococcus faecium* on apple cubes at different temperatures and

water activities. International Association for Food Protection Annual Meeting, July 27-30, Cleveland, Ohio. Poster.

89. Wan., J. **2024**. Preventive Controls and HACCP, Principles and Synergies. International Union of Food Science and Technology (IUFoST) 22nd World Congress of Food Science and Technology, September 8-12, Remini, Italy. Oral.

90. Wan, J. **2025**. FSPCA FSMA Food Safety Training Programs. North Central Regional FSMA Center of Excellence Annual Meeting, April 23-24, Kansas City, Kansas. Oral.

91. Wan, J. **2025**. FSPCA Training Programs Update. Western Regional Center to Enhance Food Safety (WRCEFS) Annual Meeting, May 13-15, Oracle, Arizona. Oral.

92. Wan., J. **2025**. FSPCA Preventive Controls and Food Traceability Training Programs. Lead Regional Coordination Center Food Safety Outreach Program National Project Directors Meeting, May 28-29, Tampa, Florida. Oral.

93. Wan., J. **2025**. FSPCA Food Traceability training curriculum update. Southern Center for FSMA Training Annual Meeting, July 27, Cleveland, Ohio. Oral.

94. Wan. J. **2025**. Food Traceability Rule Training Curriculum for Industry – Objectives, Key Components, and Q&A. International Association for Food Protection Annual Conference, July 27-30, Cleveland, Ohio. Oral.

95. Yang, Y. and Fu, T.-J. **2024**. P3-192: Factors affecting *E. coli* O157:H7 proliferation in alfalfa sprouts during sprouting and postharvest storage. International Association for Food Protection Annual Meeting, July 14-17, Long Beach, California. Poster.

96. Zuklic, J.F., Cai, J., Wan, J., Jackson, L., Sandhu, A., Redan, B.W. **2024**. Determination of Choline and Target B Vitamins in a Market Basket of Commercial Plant-based Milk Alternatives (PBMAs) using Ultra-high performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS). American Society for Nutrition Annual Meeting. June 29-July 2, Chicago, Illinois. Poster.
36.