September 2024
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
Human Foods Program

September 2024 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 Nutrition Platform supports research needs of FDA Office of Nutrition and Food Labeling (ONFL).

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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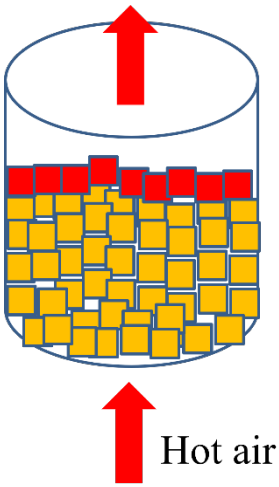
Processing Platform

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 support 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. Though its intended purpose is to inactivate enzymes that would otherwise lower the quality of frozen vegetables, it can also inactivate surface pathogens. Previous work on smooth surface vegetables (e.g., carrot cubes, peas, potato cubes, etc.) showed that heat transfer to them during blanching can sufficiently raise surface temperatures to levels that eliminate microorganisms residing there. The florets of cauliflower and broccoli were also examined to determine if their complex shape would interfere with heat transfer. In water blanching, the crown of the floret heated faster than the stalk in both broccoli and cauliflower florets, with cauliflower generally heating at a slower rate than broccoli. In steam this was reversed at the start of blanching, though crown temperatures did overtake stalk heating near the end. Present work is focused on measuring temperatures in those slowest heating regions to give a conservative evaluation of the heat transfer coefficient. Once established, it can be used to look at any set of blanching conditions to calculate the expected surface inactivation of microorganisms of interest.

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

Xiyang Liu[1], Elizabeth Grasso-Kelley[2], Alvin Lee1, Lilybell Warda[3], and Nathan Anderson[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration; [3]Illinois Tech FdSN

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 in pathogens. This highlights the potential risks of foodborne illnesses associated with the drying process, as it involves the creation of a low-moisture environment through thermal treatment. The objective of this study is to examine the reduction in *Salmonella* spp. during hot air apple drying under various temperatures, air velocities, and drying bed depths. Additionally, we aim to explore the correlation between apple aw and Salmonella spp. inactivation.

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 13 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.

Linear (R2 >0.86) relationships between *Salmonella* reduction and apple cube a_w were observed for all conditions (n=13). The lowest and highest *Salmonella* reduction when reaching the same apple aw was estimated for LTMBLA and HTLBMA. On a_w 0.60 apple cubes, estimated *Salmonella* reduction was 4.37 ± 0.18 and 8.88 ± 0.51 log CFU/sample for LTMBLA and HTMBLA, respectively. On a_w 0.30 apple cubes, estimated *Salmonella* reduction was 4.18 ± 0.21 and 8.93 ± 0.36 log CFU/sample for LTMBLA and MTLBHA, respectively. For apple cubes dried to the same aw, higher *Salmonella* reduction was estimated for LTMBHA than LTMBLA, and for HTMBLA than LTMBLA ($p<0.05$), respectively. The bed depth and airflow had a significant ($p<0.05$) interactive effect on *Salmonella* reduction.

This study shows that the water activity of the apple cubes during drying could be used as a predictor for *Salmonella* inactivation under tested drying conditions. A 5-log *Salmonella* reduction was not achieved in any tested conditions on intermediate moisture (0.6 a_w) apple cubes. Drying apples with higher temperatures, higher airflow, and lower bed depth could lead to higher *Salmonella* inactivation under the conditions tested. A beneficial effect of decreasing bed depth on microbial inactivation is more pronounced at certain airflow rates. Optimizing these two factors together could improve *Salmonella* inactivation efficiency.

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 CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

Isothermal Inactivation Kinetics of Salmonella Montevideo on Partially Dried Apple Cubes

Xiyang Liu[1], Alvin Lee[1], Elizabeth Grasso-Kelley[2], Suresh Vakkalagadda[3], and Nathan Anderson[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration; [3]Illinois Tech FdSN

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. The purpose of this study is to investigate *Salmonella* Montevideo inactivation 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 0.45, 0.60 or 0.75. *Salmonella* Montevideo 488275 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 ~96h re-equilibration (45, 60, or 75% 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* was enumerated on TSAYE with ammonium iron citrate and sodium thiosulfate.

Following post-inoculation equilibration at 45, 60, and 75% RH, the a_w of apple cubes was 0.44 ± 0.01, 0.61 ± 0.02, and 0.76 ± 0.01, respectively. *Salmonella* populations post-equilibration were significantly greater ($p<0.05$) on apple cubes at a_w 0.45 and 0.60 (8.46 ± 0.20 and 8.48 ± 0.23 log CFU/g, respectively) than 0.75 (7.89 ± 0.27 log CFU/g). At a_w 0.45, D-values were 12.93 ± 0.33, 4.70 ± 0.10, and 1.71 ± 0.07 min at 67.5, 75.0, and 87.5.°C, respectively. At a_w 0.60, D-values were 35.92 ± 1.36, 10.50 ± 0.26, and 3.07 ± 0.14 min at 60.0, 67.5, and 75.0°C, respectively. At a_w 0.75, D-values were 41.82 ± 0.39, 9.57 ± 0.36, and 2.19 ± 0.17 min at 52.5, 60.0, and 67.5°C, respectively. Highest and lowest ($p<0.05$) D-values were found on a_w 0.45 and 0.75 apple cubes, respectively. Similarly, the z-value was highest ($p<0.05$) for a_w 0.45 apple cubes (17.06 ± 0.43°C) and lowest for a_w 0.75 apple cubes (11.71 ± 0.38°C).

This study shows that when combined with the apple a_w and isothermal treatment temperature, the calculated D-values that represent the *Salmonella* thermal resistance could be used as a parameter in predicting *Salmonella* reduction during dynamic apple drying. In addition, the negative correlation found between apple a_w and z-values allows secondary modeling in which apple a_w is used to predict z-values which represent the change in the thermal resistance of *Salmonella* based on the change in temperature.

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 CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

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 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. A study conducted in Finland and Germany found a prevalence rate of 32% for *C. botulinum* in vacuum-packed vegetarian sausages, with the majority found in chilled products. This concern is associated with a poorly implemented cooling step following processing or with temperature abuse during storage by the consumer. Matrix characteristics of PBMA may include a range in pH (approximately 5.5 - 7.0) with high water activity (>0.98), potentially providing an environment that may support *C. botulinum*.

This research project will evaluate 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. The aim of this project is to survey a variety of brands and types of refrigerated, vacuum-sealed plant-based imitation meat alternative products for the presence of *C. botulinum* and determine if the matrix characteristics of these products provide an environment for *C. botulinum* toxin production under refrigerated or temperature abuse conditions. A challenge study with proteolytic and nonproteolytic *C. botulinum* strains will be performed to evaluate the potential for *C. botulinum* toxin production. This study will determine whether plant-based meat alternative products have the potential for *C. botulinum* outgrowth and toxin production under refrigerated and/or temperature abuse conditions.

A total of 12 products were selected and purchased for surveying matric characteristics. From the selected products four are soy protein-based, five are pea protein-based, and three are wheat gluten-based. The pH of the products was found to range from 5.656 to 7.299. The water activity was found to be 0.9451 to 0.9893. The salinity was found to range between 1.23 and 2.72 psu. 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. Current experiments include incubating the purchased PBMA products at 37°C to evaluate if botulinum toxin production occurs. Future studies will include toxin detection during refrigerated and abuse conditions for uninoculated and inoculated challenge studies.

This research was funded through CFSAN'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

Arlette Shazer, Tong-Jen Fu
Food and Drug Administration

The Produce Safety Rule requires that seeds used to grow sprouts be treated to reduce pathogens. Treatments may be applied at sprout operations or by seed suppliers. Numerous studies have been performed in search of effective seed treatments. Most of these studies focused on treatments applicable to sprout growers. Very few have examined treatments that may be applied by seed suppliers. Although chemical treatments are the most studied, their industrial use are limited due to the lack of EPA approved chemicals. Physical methods, such as dry heat, have increasingly been evaluated. Dry-heat treatments have the added advantages in that they are scalable and can avoid the need for a post-treatment drying step typically required for chemical treatments. These advantages make dry heat treatment a potential option for seed suppliers. The efficacy of dry heat for decontamination of seeds differed among published studies. For dry heat to be recommended as a seed treatment option, research is needed to better understand factors that may affect treatment efficacy and to identify conditions that can effectively decontaminate seeds while preserving their germination capability. In the previous study, we have evaluated the impact of dry heat in reducing *Salmonella* on inoculated mung beans. Factors such as treatment temperature, treatment time, relative humidity and treatment scale on pathogen reduction, seed germination and sprout yield were examined. In this study, experiments will be performed to examine the impact of various treatment conditions on inactivation of *Salmonella* and *E. coli* O157:H7 on other types of seeds (e.g., alfalfa) to understand if seed type affects potential inactivation. Conditions that can reduce the pathogen to below detection while maintaining germination and sprout yield will be identified. The extent of pathogen re-growth during sprouting of seeds treated under the optimal conditions will be examined.

The efficacy of dry heat treatment in reducing *Salmonella* on alfalfa seeds as affected by treatment time (6, 16, 24 h), temperature (60, 70, 80°C), relative humidity (20-80%) was examined. The impact of treatment on seed germination and sprout yield was also examined. Ten g of seeds inoculated with ~4 log CFU/g of Salmonella were subjected to dry heat treatment in a humidity-controlled chamber. Treated seeds were analyzed for *Salmonella* by plate count and culture enrichment. One hundred treated or control seeds were germinated in a petri dish and percent germination was recorded for 5 d. Sprout yields were determined after 7 d. A greater log kill was observed when treatment was conducted at higher temperatures, under higher relative humidities (RH), or for longer time. Heat treatment can negatively affect germination and sprout yield. Optimal treatment conditions that reduced *Salmonella* by > 3 logs or to below detection (< -0.3 log CFU/g) while maintaining germination at > 90% and sprout yield at > 85% were identified (60C/80%RH/6h, 60C/60%RH/24h or 70C/40%RH/16h).

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 CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

Clostridium botulinum challenge study in cold brew coffee Part II

Travis Morrissey[2], Catherine Rolfe[2], Viviana Aguilar[1], Guy Skinner[2]
[1]Illinois Tech; [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 characteristic 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 will 1.) investigate the factors that led to this cold brew coffee product to support the growth and toxin production of *C. botulinum*, and 2.) use 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. Concurrent, 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 27ºC. At 3 months, the coffees were negative for toxin production. Coffee samples will be assessed throughout 5 months. Additional cold brew coffee was inoculated with 10^3 *C. botulinum* spores/mL and incubated at 27ºC aerobically and anaerobically for 28 d. Every 7 d the pH of the cold brew coffee was measured, and *C. bot* was enumerated via MPN. pH dropped slightly for both aerobic and anaerobic coffee samples. Spore enumeration was stable at 10^3 in the anaerobic coffee. Spore enumeration dropped from 10^3 to almost 0 over the 28-d incubation under aerobic conditions.

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 CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

Evaluation of Viral and Bacterial Microbiomes of Leafy Greens and Herbs

Joelle Salazar[2], Megan Fay[2], Wei Zhang[1]
[1]Illinois Tech IFSH; [2]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 CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

Evaluation of Foodborne Pathogen Survival on Dehydrated and Rehydrated Enoki and Wood Ear Mushrooms

Joelle Salazar, Megan Fay, Diana Stewart, Bashayer Khouja
Food and Drug Administration

Specialty mushrooms have been the subject of foodborne illness outbreaks, recalls, and import alerts in the U.S. in recent years. A multistate salmonellosis outbreak linked to dried wood ear mushrooms occurred in 2020, resulting in 55 cases and six hospitalizations. Additionally, two multistate listeriosis outbreaks linked to imported fresh enoki mushrooms occurred in 2020 and 2023, resulting in 41 cases, 36 hospitalizations, and four deaths combined.

This study examined the fate of *Listeria monocytogenes* and *Salmonella enterica* on different preparations of enoki and wood ear mushrooms. First, the survival and growth of the two foodborne pathogens were evaluated on fresh and fresh-cut mushrooms during storage at different temperatures (5, 10, 25ºC; 14 d). It was determined that both *L. monocytogenes* and *S. enterica* survived on fresh and fresh-cut enoki and wood ear at all temperatures examined. Both pathogens proliferated at 25ºC, with higher growth rates observed on enoki. Pathogen inactivation was evaluated on fresh mushrooms (70, 80, 90ºC; 24 h). Heat dehydration of fresh mushrooms at 90ºC resulted in a 4 log CFU/g reduction of both pathogens after 2-4 h. At 70 and 80ºC, reductions of 4 log CFU/g occurred on wood ear after 4-8 h; a tailing effect was observed on enoki with 2 log CFU/g residual populations after 24 h. Following dehydration, it was found that both pathogens survived on dehydrated enoki and wood ear during long-term storage at 25ºC. Population reductions of 4 log CFU/g were observed for both pathogens on enoki; however, storage alone did not eliminate either pathogen, during storage for up to 6 mo. Additionally, pathogen survival was determined on dehydrated mushrooms during rehydration (5, 25ºC; 2 h) and subsequent storage (5, 10, 25ºC, 14 d). Both pathogens survived on dehydrated mushrooms during rehydration at 5 or 25ºC for 2 h. During subsequent storage at 5, 10, or 25ºC, neither pathogen proliferated on wood ear. Both pathogens proliferated on enoki during storage; the highest growth rate was observed by *S. enterica* when enoki were rehydrated at 25ºC and stored at 25ºC. Last, pathogen inactivation was evaluated on fresh mushrooms after submerging in ramen broth at different initial temperatures (60, 70, 80, 90, 100ºC; 1 h) to mimic retail/home use. When mushrooms were submerged in ramen broth at an initial temperature of 60 or 70ºC, both pathogens reduced by 1-2 log CFU/g. At 80 and 90ºC, reductions of 4 log CFU/g were observed; at 100ºC, reductions up to 5 log CFU/g were observed. However, ramen broth did not completely inactivate either pathogen on either mushroom.

This study has filled research data gaps associated with pathogen survival on specialty mushrooms. The results suggest that both enoki and wood ear support the growth of *L. monocytogenes* and *S. enterica*. The two heat treatments examined (heat dehydration of mushrooms in household dehydrators and submerging the mushrooms in hot ramen broth) did not completely inactivate either pathogen on either mushroom type. Results of this study can aid in the development of guidelines for the safe storage, dehydration, and rehydration of specialty mushrooms.

This research was funded through CFSAN's Cooperative Agreement with IFSH and the 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, Diana Stewart, Megan Fay, Bashayer Khouja, Atin Datta
Food and Drug Administration

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 were 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; 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 CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

Examination of *Listeria monocytogenes* Survival in Refrigerated Hard-Boiled Egg-Based Deli Salads Depending on Egg Treatment and Ingredients

Megan Fay, Diana Stewart, Bashayer Khouja, Joelle Salazar
Food and Drug Administration

Deli salads with chopped hard-cooked eggs are commonly purchased from retail stores, prepared by restaurants, or prepared by consumers. Pre-packaged hard-cooked eggs have been a source of foodborne illness due to contamination with *Listeria monocytogenes.* Due to outbreaks and recalls

associated with hard-cooked eggs and previous research suggesting that citric acid at pH 2.5 may not be effective at reducing *L. monocytogenes* on hard-cooked eggs, it is important to understand how the addition of treated or untreated contaminated hard-cooked eggs into deli salads affects the survival and/or growth of *L. monocytogenes*.

Hard-boiled eggs (HBEs) were submerged in 2% citric acid or water at 5°C for 24 h as a pre-treatment. The HBEs were then inoculated with a high inoculation level of *L. monocytogenes* for modeling studies (4 log CFU/HBE) or at a low level for survival studies (1 log CFU/HBE). The inoculum was allowed to dry and the eggs were chopped. Chopped HBEs were stored at 5, 10 or 25ºC for up to 14 days. It was determined that there was no significant difference between the growth rates of *L. monocytogenes* on citric acid treated and untreated chopped HBEs when stored at 5ºC. However, there was a significant difference between growth rates at 10 and 15ºC between the HBE treatments; longer lag phases were observed in the citric acid treated HBEs, along with lower growth rates. Inoculated, chopped HBEs were then incorporated into commercial deli salad recipes including potato, chicken, macaroni, tuna and egg salad. Deli salads were then stored for up to 1 month at 5, 10, or 15ºC. Minimal differences in survival in deli salads was observed at 5 and 10°C regardless of if the salads were made with treated or untreated HBEs. At 15ºC storage, potato salad had the highest population after storage.

The results indicate that lower temperature storage greatly impacts the effectiveness of the citric acid treatment of the HBEs, resulting in increased lag phases and lower *L. monocytogenes* populations. Refrigerated storage of HBEs and deli salads is essential to mitigate microbial growth in post-process contaminated deli salads. The information from this study could be used to inform guidance on the safe storage of chopped HBEs and deli salads containing chopped HBEs.

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

Factors Affecting Growth and Survival of *Salmonella* on Packaged Fresh Peaches

Diana Stewart, Joelle Salazar, Bashayer Khouja, Megan Fay
Food and Drug Administration

Following the 2020 *Salmonella* Enteritidis outbreak linked to peaches, FDA issued an investigative report detailing several factors potentially contributing to contamination of the peaches at the farm and packinghouse levels. The implicated peaches from the large grower/packer were available in bulk as well as prepacked bags and were purchased from wholesale sources and many retail supermarkets. Unfortunately, the outbreak strain of *Salmonella* was not found on fruit or leaves in the implicated grower's orchard. However, other *Salmonella* strains were found on the peach tree leaves; the strains identified were associated with nearby poultry farming operations. Together, analyses of the geographic surveys and WGS analysis of isolates informed the idea that airborne transmission of fugitive dust from these farms could be the source of the contamination. As *Salmonella* and peaches are considered a novel pathogen/commodity pair linked to this outbreak, an understanding of the ability of Salmonella to survive and potentially proliferate in pristine and blemished fresh peaches in package microenvironments at temperatures seen during post-packing and retail display is needed to better implement preventive controls and mitigate consumer risk.

Additionally, assessment of the differences and similarities of the peach leaf and fruit microbiomes from various geographical locations may provide some indication that peach tree leaves could be used as a surrogate for peach fruit when determining the influence of nearby dust-generating farming operations.

Comparisons of *Salmonella s*urvival on surrogate dust media were conducted. Silica was chosen as the preferred surrogate over sand and finely ground corn cob animal litter. Inoculated silica was used to contaminate intact and blemished retail and orchard sourced peaches. Inoculated peaches were stored in five different retail display/storage methods including produce boxes, cardboard trays, plastic clamshells, vented Ziploc-style produce bags, and thin produce bags closed with twist ties for up to one month. Storage conditions included 1-2 and 5ºC with 80-90% humidity for up to 28 d as well as 18ºC and 25C at 40-50% humidity for up to 14 d. At least three trials with triplicate samples were conducted for each condition and storage type. There was little difference between intact and blemished peaches regardless of temperature or packaging type. Though the data is still undergoing analysis, there did appear that there was growth (~1 log) for peaches stored at 18 and 25ºC in the thin produce bags and produce boxes. Interestingly, the blemished peaches did not appear to allow more growth than the intact peaches under any of the tested conditions. Higher temperature storage allowed growth in same packaging types, however these temperatures also promoted fruit degradation which reduced the length of time we could measure changes in *Salmonella* populations of the fruit. In the majority of trials, regardless of fruit condition, *Salmonella* populations were still present at the end of the storage trial period.

The microbiome of various variety of peaches and peach tree leaves grown or sourced from different geographical locations using targeted 16S sequencing was used to determine differences and similarities between peaches and leaves from the same orchard, between orchards, and between varieties. At least 15 samples with paired peach and tree leaf samples were obtained from various orchards. In addition, 20 peach samples from various sources and locations. Overall, at least 25 different peach varieties are represented. These samples were washed with buffer and frozen future DNA extraction and 16S sequencing. At this time, a comparison of Red Haven peaches from six locations and 7 other peach varieties across a variety of locations is underway.

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

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

Joelle Salazar[2], Diana Stewart[2], Ravinder Reddy[2], Megan Fay[2], Wei Zhang[1]
[1]Illinois Tech IFSH; [2]Food and Drug Administration

In 2022, a foodborne illness outbreak due to consumption of contaminated powdered infant formula (PIF) occurred in the U.S., resulting in at least four cases of hospitalizations and at least two infant deaths linked to C*ronobacter sakazakii* or *Salmonella enterica* serovar Newport. The manufacturing company identified *C. sakazakii* in the plant in non-product contact areas. PIF is not a sterile food product due to the need to include micronutrients which are heat liable. Therefore, PIF has the potential to become contaminated with foodborne bacterial pathogens after heat

treatment. Understanding the microbiome of these non-sterile 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, 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 aims of this study are therefore 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 inactivation and molecular response of *C. sakazakii* to desiccation stress in PIF and on food-contact surfaces with and without sanitizer treatment.

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 CFSAN'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 2022, a foodborne illness outbreak due to consumption of contaminated powdered infant formula (PIF) occurred in the U.S., resulting in at least four cases of hospitalizations and at least two infant deaths linked to *Cronobacter sakazakii* or *Salmonella enterica* serovar Newport. The outbreak resulted in the implicated manufacturing company recalling several brands of powdered infant formula, creating a severe nationwide shortage for more than three months. The manufacturing company identified *C. sakazakii* in the plant in non-product contact areas. PIF products are not sterile due to the need to include micronutrients which are heat-liable. These micronutrients must therefore be added after the pasteurization or heat treatment stage to ensure the nutritional value of the PIF is in compliance with regulatory standards. 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 to reconstitute PIF. 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 aims of this study are therefore to 1) evaluate the survival of *C. sakazakii* and *S. enterica i*n PIF during long-term

storage when inoculated at different levels and stored at different temperatures and humidity levels and 2) model the survival or inactivation of *C. sakazakii* and *S. enterica* during reconstitute of PIF and during storage of reconstituted PIF.

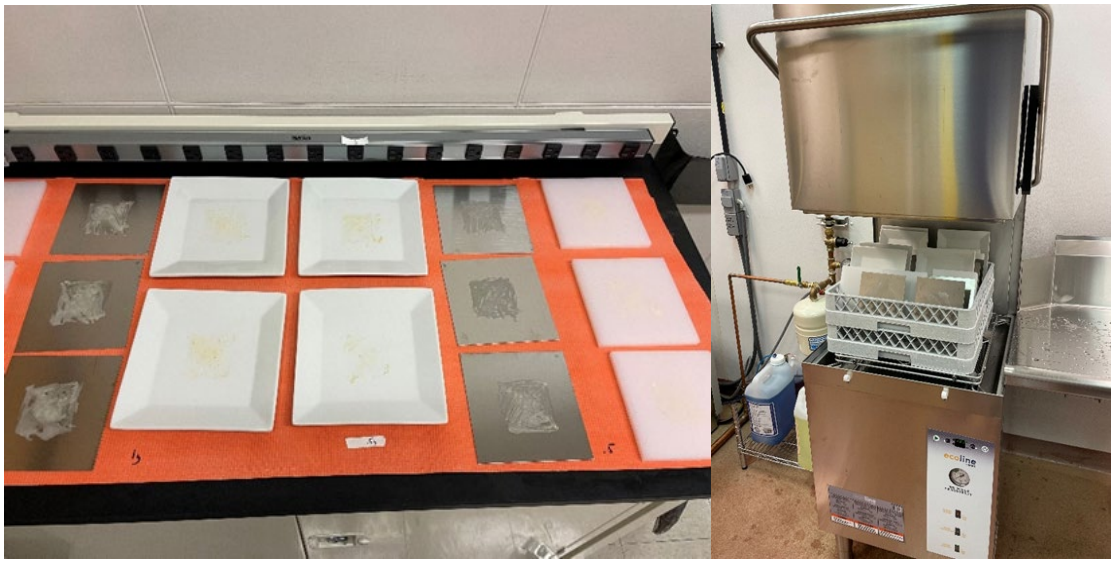
This study will fill data gaps pertaining to *C. sakazakii* and *S. enterica* survival and persistence in PIF during storage and also in reconstituted PIF. From this research, time/temperature/humidity parameters will be established for retail and consumer storage of PIF, as well as appropriate temperatures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

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

Chemistry and Packaging Platform

Lauren Jackson, FDA and Brian Schaneberg, 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, Lauren Jackson
Food and Drug Administration

Oil roasting is a common method for roasting peanuts and tree nuts. During the roasting process, peanut particulates can contaminate the roasting oil. When the oil is reused for roasting or frying other products with different allergen profiles, there is a potential for allergen cross-contact. It is not well understood how the oil roasting process and oil regeneration treatments such as filtering affect peanut detection and peanut levels in roasting 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.

To develop a quantitative proteomics method for peanut in oil, lightly roasted peanut flour (PF) was mixed into oil at concentrations of 1000, 500, 250, 100, and 20 µg/mL in triplicate. These samples were analyzed in untargeted mode on an Agilent 6550 Q-TOF mass spectrometer. Peptides were identified from the spectra using SpectrumMill software and a database of all known peanut allergen proteins. The list of peptides was narrowed down to eight candidate proteotypic peptides based on high overall abundance, consistency across replicates, and detectability across all analyzed concentrations.

The method was used to quantify PF in oil samples that were heated for 0, 1, 3, and 10 minutes and filtered through sieves and paper filters ranging in pore size from 2 mm to 10 µm. These samples were also quantified with ELISA. While the ELISA method consistently showed decreases in detectable peanut protein with respect to increasing heating time and decreasing filter size, the proteomics method was inconsistent between the target peptides regarding the effect of heat on protein detectability. This inconsistency led to the hypothesis that heat affected peanut proteins differently as they were extracted and digested. To confirm, standards of PF in oil at 1,000, 100, and 10 µg/mL were prepared and heated for 0, 1, 3, and 10 minutes. Results showed that 6/8 peptides underestimated the amount of peanut protein in the standards as heat was added.

The previous quantitative method was developed on target peptides identified from a list of peptides from unheated oil samples. To account for the effect of heat in peptide selection, new samples were prepared using a blanched peanut butter (PB) mixed into oil at concentrations of 1,000, 100, and 10 µg/mL and heated for 0, 1, 5, and 10 min. These samples were prepared in duplicate and analyzed in duplicate in untargeted mode. Peptides were identified as above, and additional criteria was applied to candidate peptide selection based on showing no reduction in abundance from 0 to 10 min heating. This list was narrowed down to 28 peptides, and the samples were re-analyzed in targeted mode targeting the candidate peptides for increased accuracy and sensitivity. From this analysis, 18 of the peptides showed a linear decrease in abundance from 0 to 10 min and so were removed from consideration. A nine-point calibration curve was constructed from 1,000 to 5 µg/mL PB in oil and analyzed targeting the remaining ten peptides, which all showed good linearity and replicability.

This updated targeted method will be used to quantify peanut protein in oil after roasting successive batches of peanuts. As our understanding of allergen thresholds improves, allergen analysis will move towards not just detection, but quantitation. Having a multitude of methods available to quantifying peanut allergen in common food matrices will be critical to assessing food risk and reducing unintended allergen exposure.

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

Transfer of Seafood Allergens to Frying Oil and Subsequent Fried Products

Xingyi Jiang, Lauren Jackson
Food and Drug Administration

Breaded and battered shrimp accounted for 21% of the major types of seafood purchased in the U.S. Batch and continuous fryers are commonly used to produce par-fried battered or breaded seafood, which is subsequently frozen. Some manufacturers of breaded and battered seafood also use the same fryers and oil to produce other par-fried foods such as French fries, raising concerns about potential allergen cross-contact due to oil reuse. The major goals for this project during the past year are: (1) Develop shrimp proteins and gluten extraction methods from oil matrices, (2) Validate protein extraction efficacy under both unheated and fried conditions, (3) Investigate the transfer of shrimp proteins and gluten to the frying oil and to the subsequently prepared food products, and (4) Evaluate oil post-treatments on protein removal from frying oil.

For shrimp proteins and gluten extraction from oil, the selection of defatting reagents, extraction buffer ingredients, extraction time and temperature were optimized. The developed method included the following three key steps: (1) centrifugation to separate oil phase and particulates; (2) defatting using an acetone:hexane mixture to reduce oil interference; and (3) protein extraction using 10 mM phosphate buffered saline (PBS, pH 7.4) containing 6 M urea and 2% β-mercaptoethanol (PBS-urea-βME). This strategy was developed based on the understanding that proteins existed as micro-particulates in oils. Compared to previous reported methodologies, this strategy requires only small amounts of defatting and extraction reagents, significantly simplifying the extraction procedure.

To validate protein extraction efficacy and study the effect of frying on protein immunodetectability, 1000 ppm shrimp powder or gluten-spiked oil underwent a frying process at 180 °C for 1, 3, 6 and 10 min, respectively. The oil residual protein content, protein profile and allergen concentration were determined using bicinchoninic acid (BCA) assay, gel electrophoresis and enzyme-linked immunosorbent assay (ELISA), respectively. For shrimp proteins, frying significantly reduced their solubility and immunoreactivity. After 3 min of frying, more than 80% of tropomyosin, the major shrimp allergen, was undetectable by ELISA. On the contrary, gluten demonstrated better recovery after frying, with recoveries of 73%, 65%, 53%, and 44% after 1, 3, 6, and 10 min, respectively. Due to the thermostability of gluten, we further examined its transfer from breaded shrimp to the frying oil and subsequently prepared food products.

For quantification purpose, gluten-spike oil underwent a frying process at 180 °C for 3 min. Subsequently, the oil was utilized to prepare 12 g of fries (180 °C for 5 min). The results showed that around 250 ppm of gluten were presented in oil after frying, and about 4%, i.e., 109 ppm, was transferred to fries. In a real-world scenario, around 75 g/batch of breaded shrimp were fried in 750 g oil at 180 °C for 2.5 min. In total, ten batches were prepared. The oil was collected for protein extraction after the 1st, 3rd, 6th, and 10th batch. Subsequently, the oil from after completing 10 batches was utilized to prepare fries, chicken nuggets, and tarter tots following the instructions provided on their labels. Our results showed that the oil residual gluten level increased as a function of frying batch. The amount of gluten transferred was dependent on the food product, with a hierarchy of cross-contaminated gluten concentration in food products: tarter tots (262 ppm), fries (175 ppm), and nuggets (38 ppm). Considering the serving size of each product, this concentration significantly exceeds the allergen threshold and gluten-free requirements, surpassing the threshold of 5 mg for gluten-sensitive individuals.

The study also examined oil post-treatments for gluten removal from gluten-spiked oil and breaded shrimp frying oil. The untreated oil was filtered through metal sieves or cellulose filter paper of various pore sizes. The total protein content, protein profile and tropomyosin concentrations of frying oil were determined using the BCA assay, gel electrophoresis and by ELISA, respectively. Post-treatment such as sieving and filtration reduced oil residual gluten content. However, even passing through the smallest pore size, ppb levels of gluten could still be quantified.

This study demonstrated that both shrimp proteins and gluten can be transferred to frying oil and a subsequent prepared food. However, the amount of allergenic protein transferred were likely underestimated due to the decreased extractability and detectability of proteins, especially shrimp proteins, after frying. Future research will involve (1) improving recovery of shrimp proteins in reused frying oil; (2) performing shrimp tropomyosin quantification using alternative methods; and (3) determining the transfer of allergens to frying oil and another food product in a continuous fryer.

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

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

Hilary Green[2], Jeremiah Kidd[2], Josh Warren[1], Lauren Jackson[2]
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Plant-based butters (PBB) and spreads made from peanuts, soy, tree nuts and seeds (sunflower, sesame, etc.) have become popular due to their high nutritional value and the shift from animal-protein based diets to those rich in plant protein sources. In recent years, the variety of PBB available on the market has increased, which can give individuals with food allergies more food options. Many PBB are processed on the same equipment or in facilities that produce a variety of products with different allergen profiles, and cross-contact can occur if equipment is not effectively cleaned. In 2021, there were three different recalls in the U.S. due to the presence of undeclared peanut in almond butter and baked snack bars that contained almond butter. Similarly, Canada also saw three recalls in 2021 due to

undeclared peanut, almond, and cashew in various PBB. The higher frequency of allergen related recalls in recent years suggests that more information is needed about unintended allergen presence (UAP) in PPB and the effectiveness of cleaning and other allergen control practices used by firms manufacturing these products. This project is assessing peanut, tree nut and seed butters (peanut, almond, hazelnut, cashew, soy, sunflower, sesame, etc.) for the presence and concentrations of unintended allergens (especially various tree nuts, peanuts, soy, wheat/gluten, and sesame) and determine how these results relate to allergen advisory statements found on each product. A variety of major seed and nut butters will be purchased, and different lots of each product will be assessed to determine whether allergen concentrations vary between batches/lots. The xMAP food allergen detection assay (FADA) is being used to quantify the allergens present in the products. The second part of this study aims to evaluate cleaning procedures at a pilot-plant scale to determine their effectiveness at reducing cross-contact when manufacturing different PBB products on shared equipment.

In the past year, a study evaluated the effectiveness of a variety of cleaning methods for removing almond and almond butter residue from a pilot-scale nut butter grinder. Almond butter was manufactured from whole, roasted almonds using a nut butter mill. Subsequently, the mill was cleaned with a designated method and then used to produce peanut butter from whole, roasted, blanched peanuts. Samples of peanut butter were collected at varying timepoints, and each sample was tested for almond protein using a quantitative, almond-specific ELISA. The most effective cleaning method involved scrubbing the mill with hot water and detergent. However, wet cleaning cannot easily be utilized by industry as water can allow microbial pathogens to grow and contaminate nut and seed butter products. Therefore, this treatment was used as a control and other dry-cleaning methods more commonly used by industry were evaluated. It was found that using peanut butter as a push-through material was the least effective method while manually cleaning using scrapers/brushes and flushing with oil were more effective at removing almond residue from the mill. These results demonstrate it is imperative for industry to test the effectiveness of cleaning methods, particularly when using push-through, and that a combination of methods may be the best way to minimize allergen cross-contact. The results of this project will enable assessment of allergen control procedures used during the manufacture of peanut, nut and seed butters.

This research was funded through CFSAN'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], and John Koontz[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration

FDA's surrogate testing protocol using hexane or heptane as a diluent has successfully evaluated the efficiency of industrial recycling processes in removing contaminants from reclaimed polyethylene terephthalate (PET) for many years, but recently there has been strong industry interest in improving recycling rates of polypropylene (PP). Due to the greatly different sorption behavior of PP relative to PET, however, new conditions will likely be required to optimize the surrogate testing protocol specific to recycled PP. This study examines how the interaction

between the surrogate contaminant, diluting solvent, and polymer type impacts the sorption of surrogate contaminants into PP.

Three categories of PP and its copolymers used in food contact materials were selected. The homopolymer- (PP-h), random- (PP-r), and heterophasic- (PP-heco) cast PP films with a thickness of 10 mil were fabricated on a pilot-scale film line at Amcor. Higher concentration of a sorbed compound may alter the chemical properties of the PP polymers by plasticization and swelling. Thus, preliminary soaking experiments at 40°C for up to 14 days were also performed to determine the effects of various surrogates and diluting solvents on swelling of the polymers. In general, n-hexane induced the largest degree of swelling in terms of % weight gain and diameter change in all polymer samples, followed by heptane. The alcohols (isopropanol and ethanol) resulted in the least amount of swelling. PP-heco swelled the most in all solvents, followed by PP-r, and PP-h. The impact of solvent during surrogate sorption into the polymer cannot be overlooked when determining realistic initial contamination levels in PP. To study any changes in physical morphology of PP samples, the overall enthalpy change (ΔH) and the % crystallinity have been determined before and after soaking experiments using DSC and XRD techniques, respectively. Results showed that when compared to hexane, 2-propanol didn't seem to affect both the overall enthalpy and the crystallinity for PP-h samples.

Based on volatility, polarity, Hansen solubility parameters, and preliminary swelling experiments, we have successfully determined potential non-volatile surrogates (methyl salicylate and phenylcyclohexane) and diluting solvents (hexane, 2-propanol, and ethanol) suitable for surrogate sorption testing of PP polymers, The PP-h film, typically used for rigid containers, was the first type of PP tested. The sorptions of 1% methyl salicylate (MS) from each diluting solvent into the PP-h at 40°C for 10 days were successfully quantified by using GC-MS coupled with polymer swelling/ultrasonic extraction techniques, and the maximum equilibrium sorptions of MS into PP-h were determined by nonlinear regression curve fitting of the sorption data using Fick's Second Law of diffusion equations. Results showed that MS equilibrium sorption into PP-h is attained at or before 8 hours from n-hexane, while sorption equilibrium is reached at 2 days and 3 days from ethanol and 2-propanol, respectively. At equilibrium, average sorption of MS into PP-h from 2-propanol and n-hexane is about 55% and 140% higher, respectively, compared to ethanol. N-hexane also caused increased swelling and crystallinity in the polymer. Therefore, traditional diluting solvents may not appropriate for use during surrogate testing in PP.

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 CFSAN'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],
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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 two primary areas. In the first, we have explored migration of Ag from packaging containing Ag-modified zeolites. Zeolites are inorganic scaffolds that facilitate incorporation of large amounts of Ag or AgNPs into polymers. We synthesized Ag-modified zeolites, incorporated them into polymers, characterized them using electron microscopy and atomic force microscopy, and performed migration tests. This work resulted in one publication that is currently under review at a peer-reviewed journal. In the second, we have explored the impact of milkfat content on migration of Ag from AgNP-containing polymeric dairy packaging. This work is still on-going but we are presenting preliminary work at the upcoming Food and Agricultural Nanotechnology Gordon Research Conference and expect to draft a manuscript by the end of the calendar year.

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 CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

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

John Koontz[2], Huayi Wang[2], Senthamizh Senthilkumaran[1], and Yoon Song[2]
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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 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. 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 polyethylene.

HDPE (0.943 g/cm3), LDPE (0.921 g/cm3), and LLDPE (0.914 g/cm3) resins used in food contact were selected and manufactured into cast films with a 10-mil thickness on a pilot-scale extrusion line at Amcor for our studies. PE relative molar mass distributions were measured using high-temperature size exclusion chromatography with refractive index detection and calculated using polystyrene column calibration standards. HDPE and LDPE were high-polydispersity polymers with Mw/Mn values of 9.9 and 8.8, respectively. LLDPE was a low-polydispersity polymer with an Mw/Mn value of 2.8. PE crystallinity is currently being evaluated by differential scanning calorimetry (DSC) and X-ray diffraction (XRD), with DSC also providing the melt temperature of each PE type.

Due to the greatly different sorption behavior of PE expected relative to PET, new conditions will be required to optimize the surrogate testing protocol specific to recycled PE, namely selection of an appropriate diluting solvent. HDPE, LDPE, and LLDPE film mass was measured gravimetrically after swelling test with diluting solvents of hexane, isopropanol, and ethanol at 40°C. The three PEs appear to have considerably less interaction with the diluting solvents than polypropylenes, which have relatively close Hansen solubility parameter distances to PE. Methylene chloride as extraction solvent resulted in 8–13% weight increase of PE films due to polymer swelling. A liquid injection GC-MS method was developed for quantification of the sorption of two non-volatile surrogates of different polarities into PE. A nonlinear curve fitting procedure of test experimental sorption data by variation of the diffusion coefficient and the partition coefficient has been successfully performed within the fitting module of AKTS-SML migration software.

PEs have specific considerations of unique surrogate contaminants (high MW, heavy metal), unique diluting solvents, different time to equilibrium sorption, and refinement of sorption modeling using experimental sorption data. 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 producing material suitable for food-contact applications.

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

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

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The 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. Provisions in the Food Code (in Chapters 3 & 4) pertain to washing, rinsing and sanitization of equipment, food contact surfaces and utensils as well as the use limitations for wiping cloths. These provisions were developed to reduce microbiological risks associated with food. Until recently, little was known about the effectiveness of these provisions in preventing allergen cross-contact. Our previous study investigated manual washing of allergen-contaminated surfaces using a wash-rinse-sanitize-air dry procedure (3-sink washing method). This study used different physical forms of peanut-, milk- and egg-containing foods to contaminate stainless steel, plastic and wood surfaces. A common household liquid detergent and chlorine bleach solution were used for the washing and sanitizer treatments, respectively. Although the study yielded much needed information on the effectiveness of some of the Food Code provisions related to allergen control, further studies are needed to determine the impact of detergent type and concentration, different types of food soils (applied to a surface under ambient conditions, heated/cooked on a surface), other types of food contact surfaces (ceramic/porcelain, different finishes of stainless steel, etc) and use of mechanical ware washing machines on cleaning efficacy. Furthermore, since gluten cross-contact is also a concern in retail and food service operations, studies that evaluate cleaning on gluten removal are also warranted. Additional work is also needed to determine the effectiveness of different types of wipes (dry and wet) on allergen and gluten removal from commonly used surfaces found in retail and food-service operations. It is anticipated that this work will yield useful 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.

Over the past year, two separate studies were completed. The first study evaluated the use of wet and dry wipes on their ability to remove allergens from food-contact surface, while the study determined the effectiveness of manual and mechanical washing methods at removing allergen-containing foods from food-contact surfaces. For the wiping study, three stainless steel (SS) and three textured white polyethylene (PE) coupons were contaminated with 0.5 g or 1 g of egg-based (powdered whole egg; reconstituted whole egg powder), gluten-containing (wheat flour; wheat-containing batter), or sesame-based (sesame flour; tahini) foods. After drying for 30 min at ambient temperatures (23 ± 2°C), coupons were wiped with either a dry paper wipe, a dry terry cloth, a wet terry cloth soaked in 200 ppm quat solution, or one, two, or three sanitizing wipes for 5 sec. A second type of sanitizing wipe was also evaluated for tahini-contaminated surfaces. After wiping treatments, coupons were tested for residual allergens with lateral flow devices (LFDs). Three independent trials were conducted for each experimental variable. For

the washing study, coupons made of PE (n=3), SS (n=3), and ceramic (CE, n=3) were contaminated with 0.5 g or 1 g of egg-based (egg powder; reconstituted egg powder), gluten-containing (wheat flour; batter), or sesame-based (sesame flour; tahini) foods. After drying for 30 min at 23±2°C, coupons were subjected to a manual or mechanical washing treatment. The manual treatment used 3, 18.9 L buckets containing wash solutions at 43°C. Coupons were washed (10 sec), rinsed (10 sec), and then sanitized (60 sec), in neutral detergent solution, water, and 200 ppm quat solution, respectively. Mechanical treatments involved washing contaminated coupons in commercial warewashing machines. Residual allergens on coupons were detected with LFDs. Washing trials were done in triplicate.

Results from the wiping study indicate that dry wiping methods were not effective at removing allergen-containing foods from the SS and PE coupons. The wet terry cloth was effective, and the sanitizing wipes were effective when more than one wipe was used. PE was more difficult to clean than SS, and foods in paste form were more difficult to remove than powdered forms. There were no observable differences in the ability to remove 0.5 g than 1.0 g of food soils. LFDs were able to detect allergens on some surfaces that were visually clean. The manual washing treatment removed gluten-containing foods on SS, but mixed results were seen for PE and CE. Egg-based foods were removed from all surfaces during manual washing. Mixed results were found when the manual method was used to remove sesame flour from SS and CE coupons, while complete removal was found for PE. Tahini was detected on all three surfaces after manual washing. Mechanical washing treatments were effective at removing gluten-containing foods, egg powder and sesame powder from all surfaces while reconstituted egg and tahini were challenging to remove.

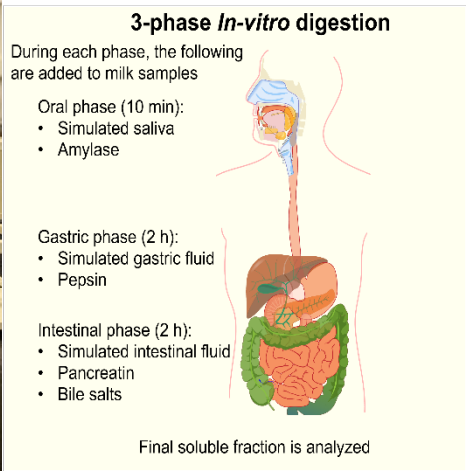
Overall, the results indicate that wet wipes are more effective for removal of allergens than dry wipes. Using multiple wet wipes facilitates removal of allergenic foods. The results from the washing study indicate that the nature of the food soil and surface impacted the effectiveness of washing treatments. More extensive washing treatments were needed for difficult-to-clean soils such as food pastes.

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

Nutrition Platform

Lauren Jackson, FDA and Britt Burton-Freeman, IIT IFSH

The Nutrition Platform aims to contribute knowledge about food choice and intake behavior by consumers and their impact on nutrition and health. The Nutrition Platform supports research needs of FDA Office of Nutrition and Food Labeling (ONFL).



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], Amandeep Sandhu[1], Indika Edirisinghe[1], Britt Burton-Freeman[1], Lauren Jackson[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration

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 will be used as a positive control through a randomized controlled trial with a parallel study design in 48 healthy subjects (n=12 per arm). Plasma/serum and urine samples will be 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). The data from these analyses will influence the understanding of the relative absorption (bioavailability) of certain nutrients from PBMAs. This information will be useful 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.

Accomplishments include designing the human subjects trial, obtaining IRB approval, conducting the human trial during the winter months of 2023/2024 in Chicago, IL. Current activities include specimen analysis.

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

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]
[1]Illinois Tech IFSH; [2]Food and Drug Administration

Plant-based milk alternative (PBMA) consumption has been increasing due to factors such as bovine milk 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 our previous project (CARTS#: IF01782), analytical methods were developed and levels of targeted 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) were measured. The effect of thermal processing (high temperature short time pasteurization; HTST) on micronutrient retention/variability in a model almond PBMA was also reported.

The present project aims to expand this research and investigate *in vitro* bioaccessibility of nutrients in PBMA to complement our assessment of nutrient bioavailability in human subjects (CARTS 1695). Interactions between minerals and lipids/lipid-soluble components of PBMAs can influence the intestinal bioaccessibility of vitamin D. Using this as a starting point we propose to use an *in vitro* model of digestion to evaluate 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 will also be determined. Associations between amounts of minerals, fatty acid composition, and vitamin D bioaccessibility will be identified. Particular attention will be given to amounts of divalent mineral ions (e.g., Ca2+ and Mg2+) due to the potential for these ions to form insoluble complexes. Vitamin D will be quantified using an AOAC official method for LC-MS/MS. Mineral content will be determined using an ICP-MS method adapted from the FDA's Elemental Analysis Manual (EAM). We will use a GC-MS method to characterize the fatty acid composition. The *in vitro* model protocol has been developed and is being used to conduct preliminary analyses verifying parameters for the final protocol.

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

Proficiency Testing Programs

Ravinder Reddy, FDA and Jason Wan, IIT IFSH

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

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

program within FDA CFSAN and IIT which has received an ISO accreditation, demonstrating a high-level of quality control system in laboratory management and operations.

Proficiency test of SARS-CoV-2 omicron variant detection in diagnostics samples by veterinary diagnostic laboratories

Neha Singh[1], Megan R. Miller[4], Sarah M. Nemser[4], Andriy Tkachenko[4], Steffen Uhlig[3], Kirstin Frost[3], Karina Hettwer[3], Jodie Ulaszek[1], Matthew Kmet[2], Leyi Wang[5], Matthew C. Allender[5,6], Ravinder Reddy[2]
[1]Illinois Tech IFSH; [2]Food and Drug Administration; [3]QuoData- Quality & Statistics, Germany; [4]Center for Veterinary Medicine, Food and Drug Administration; [5]Department of Veterinary Clinical Medicine and Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Illinois at Urbana; [6]Brookfield Zoo Chicago

Veterinary diagnostic laboratories (VDLs) play a critical role in screening both human and animal samples for SARS-CoV-2. To evaluate the SARS-CoV-2 detection methods used by VDLs, a proficiency test (PT) was performed by the U. S. Food and Drug Administration's (FDA) Veterinary Laboratory and Investigation and Response Network (Vet-LIRN) in collaboration with two other organizations. Thirty-two sets of 12 blind-coded samples were prepared by fortifying Molecular Transport Medium (MTM) or feline feces with non-COVID coronavirus RNA or COVID coronavirus at various levels and shipped to 32 participants for blinded (unbiased) analysis. Results were analyzed according to the principles of International Organization for Standardization 16140-2:2016 using two approaches such as establishing the rate of detection (ROD) and the success rate by applying the analysis of binary outcome by Logit approach. ROD provided the overall assessment of laboratories performance whereas the novel Logit approach provided an insight to more specific analysis based on the complexity of each sample. The ROD was 83% and 98% for MTM samples at 200 and 20,000 genome copies per 100 µL respectively. Fecal samples were classified as challenging exploratory and results were not included in the assessment of performance but discussion purposes only. Fecal samples exhibited matrix interference impacting the performance. The ROD was 44% and 89% for fecal samples at 2,000 and 20,000 genome copies per 100 µL respectively. The non-COVID coronavirus RNA, which was used to address the specificity, didn't interfere with methods used. Establishing the success rate by evaluating the qualitative results (detected/not detected) applying a Logit approach revealed that, out of thirty-two participants, twenty-eight had satisfactory results, one participant had unsatisfactory results, and three participants had questionable results for MTM samples. For fecal samples, three participants out of thirty-two did not meet the expectations at higher concentrations. Lower concentrations of fecal samples were excluded from this analysis. Again, the fecal samples were considered as challenge samples and the results were provided to assist participants in their continuous efforts to improve their performance and not to evaluate their performance.

Analysis of method performance for quantitative assessment of *Listeria monocytogenes* in queso fresco cheese

Neha Singh[1], Ravinder Reddy[2], Karina Hettwer[3], Kirstin Frost[3], Matthew Kmet[2], and Steffen Uhlig[3]
[1]Illinois Tech IFSH; [2]Food and Drug Administration; [3]QuoData-Quality & Statistics, Germany

Quantitative assessment of *Listeria monocytogenes* (*Lm*) is important to analyze its level in a food product. FDA BAM method is commonly used for detecting and enumerating *Lm* in cheese; however, performance characteristics of the enumeration method is not widely available in the literature.

In 2023, the Moffett Proficiency Testing Laboratory (MPTL) organized a PT for Food Emergency Response Network laboratories to determine the ability of participants to enumerate *Lm* in queso fresco cheese and evaluate the performance of the enumeration method.

Mean recovery rate in preliminary studies was 114%, and the confidence interval included the value of 100% indicating no evidence of systematic deviations. Samples tested at pre- and post-shipment stages were within tolerance range of 2.10 CFU/g and 24.00 CFU/g. MPN data obtained by the participants had discrepancies; therefore, the tube readings were used to recalculate MPN values. Based on the Q/Hampel method (ISO 13528:2022), the repeatability standard deviation was 0.308 $\log_{10}$ MPN and the reproducibility standard deviation was 0.444 $\log_{10}$ MPN. The resulting laboratory standard deviation was 0.320 $\log_{10}$ MPN indicating the extent of systematic deviations among participants similar to the intrinsic random variation of the MPN procedure. Overall, the procedure had an acceptable performance and the participants were able to perform MPN method for the given inoculation level.

APPENDIX

Refereed Publications Calendar Year 2023-2024

1. Acuff, J.C., Dickson, J.S., Farber, J.M., Grasso-Kelley, E.M., Hedberg, C. Lee, A. and Zhu, M.J. **2023**. Practices and progress: updates on outbreaks, advances in research, and processing technologies for low-moisture food safety. *J Food Prot.* 86(1),100018. https://doi.org/10.1016/j.jfp.2022.11.010

2. Camacho, J. C., Shieh, Y. C., Redan, B. W., Koontz, J. L. **2023.** Antiviral activity of copper contact surfaces against MS2 coliphage and hepatitis A virus. *J Appl Microbiol*, 134(8), 1-9. https://doi.org/10.1093/jambio/lxad160

3. Chen, Y., Lopez, S., Reddy, R.M., Wan, J., Tkachenko, A., Nemser, S.M. Smith, L., Reimschuessel, R. **2023**. Validation and interlaboratory comparison of anticoagulant rodenticide analysis in animal livers using ultra-performance liquid chromatography–mass spectrometry. *Journal of Veterinary Diagnostic Investigation*, 35(5), 470. https://doi.org/10.1177/10406387231178558

4. Deng, K., Shizhen, S.W., Kiener, S., Smith, E., Chen, K.S., Ruiqing, P., Laasri, A., Pelaez, C., Ulaszek, J., Kmet, M., De Jesus, A., Hammack, T. Reddy, R., and Wang, H. **2023**. Multi-laboratory validation study of a real-time PCR method for detection of *Salmonella* in baby spinach, *Food Microbiology*, 114 (2023), 104299, https://doi.org/10.1016/j.fm.2023.104299

5. Deng, K., Nemser, S., Frost, K., Goodman, L.B., Ip H.S., Killian, M.L., Ulaszek, J., Kiener, S., Kmet, M., Uhlig, S., Hettwer, K., Colson, B., Nichani, K., Schlierf, A., Tkachenko, A., Miller, M.R., Reddy, R., and Tyson, G. H. **2023**. Successful detection of delta and omicron variants of SARS-CoV-2 by veterinary diagnostic laboratory participants in an interlaboratory comparison exercise. *The Journal of Applied Laboratory Medicine*, 8(4), 726. https://doi.org/10.1093/jalm/jfad018

6. Fay, ML, Salazar, JK, Ren, Y, Wu, Z, Mate, M, Khouja, BA, Lingareddygari, P, Liggans, G. **2023**. Growth kinetics of *Listeria monocytogenes* and *Salmonella enterica* on dehydrated vegetables during rehydration and subsequent storage, *Foods*. 12(13), 2561. https://doi.org/10.3390/foods12132561

7. Fay, M.L., Salazar, J., Chavda, N.J., Patil, G.R., Ingram, D.T. **2023**. Survival kinetics of *Listeria monocytogenes* and *Salmonella enterica* on dehydrated enoki and wood ear mushrooms during long-term storage. *Food Microbiol*., 114, 104304. https://doi.org/10.1016/j.fm.2023.104304

8. Fay, M.L., Salazar, J.K., Zhang, X., Zhou, X., Stewart, D.S. **2023**. Long-term survival of *Listeria monocytogenes* in nut, seed, and legume butters. *Journal of Food Protection*, 86(6), 100094. https://doi.org/10.1016/j.jfp.2023.100094

9. Fay, M.L., Salazar, J.K., George, J., Chavda, N.J., Lingareddygari, P, Patil, G.R., Juneja, V.K., Ingram, D.T. **2023**. Modeling the fate of *Listeria monocytogenes* and *Salmonella enterica* on fresh whole and chopped wood ear and enoki mushrooms. *J. Food Prot*., 86(5), 100075. https://doi.org/10.1016/j.jfp.2023.100075

10. Fay, M. L., Salazar, J.K., Stewart, D.S., Khouja, B.A., Zhou, X., Datta, A.R. **2024**. Survival of *Listeria monocytogenes* on frozen vegetables during long-term storage at -18 and -10°C. *J. Food Prot*. 87(3),100224. https://doi.org/10.1016/j.jfp.2024.100224

11. Green, H.S. and Jackson, L.S. **2024**. Dry cleaning and sanitization technologies. Encyclopedia of Food Safety, 2nd Edition. Pages 732-738, G. Smithers (Ed), Elsevier, London, UK. https://doi.org/10.1016/B978-0-12-822521-9.00225-2

12. Green, H.S., Kidd, J. and Jackson, L.S. **2024**. Novel and emerging cleaning and sanitization technologies. Encyclopedia of Food Safety, 2nd Edition. Pages 739-745, G. Smithers (Ed), Elsevier, London, UK. https://doi.org/10.1016/B978-0-12-822521-9.00231-8

13. Huang, Y., Edirisinghe, I., Burton-Freeman B. M., Sandhu, A.K. **2023**. Characterization and pharmacokinetic profile of herbs and spices' phytochemicals over 24 h after consumption in overweight/obese adults. *Mol Nutr Food Res*., 67(14), 2200785. https://doi.org/10.1002/mnfr.202200785

14. Jiang, X., 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

15. Lee, A., Maks-Warren, N., Aguilar, V., Piszczor K, Swicegood B, Ye M, Warren J, O'Neill E, Fleck M and Tejayadi S. **2023**. Inactivation of *Salmonella*, shiga toxin-producing *E. coli*, and *Listeria monocytogenes* in raw diet pet foods using high-pressure processing. *J. Food Prot*., 86(9), 100124. https://doi.org/10.1016/j.jfp.2023.100124

16. Lu, Y., Fu, T.J., **2024**. Evaluation of ELISA test kits for detection of milk protein in frying oil treated at different temperatures. *J. Food Prot*. 87(2),100211. https://doi.org/10.1016/j.jfp.2023.100211

17. Pires, V.S., Zuklic, J., Hryshko, J., Hansen, P., Boyer, M., Wan, J., Jackson, L.S., Sandhu, A., and Redan, B.W. **2023**. Market basket survey of the micronutrients vitamin A, vitamin D, calcium, and potassium in eight types of commercial plant-based milk alternatives from United States markets. *ACS Food Sci. Technol*., 3(1), 100. https://doi.org/10.1021/acsfoodscitech.2c00317

18. Redan, B.W., Zuklic, J, Hryshko, J., Boyer, M., Wan, J., Sandhu, A. and Jackson, L.S. **2023**. Analysis of eight types of plant-based milk alternatives from the United States market for target macrominerals and trace elements. *Journal of Food Composition and Analysis*, 122, 105457. https://doi.org/10.1016/j.jfca.2023.105457

19. Rolfe, C.A., Anderson, N.M., Black, D.G., and Lee, A. **2023.** Barotolerance of acid-adapted and cold-adapted bacterial isolates of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* in an acidic buffer model. *Journal of Food Protection*, 86(8), 100116. https://doi.org/10.1016/j.jfp.2023.100116

20. Salazar, J.K., Fay, M.L., Khouja, B.A., Chavda, N.J., Patil, G.R., Ingram, D.T. **2023**. Effect of dehydration on the inactivation of *Listeria monocytogenes* and *Salmonella enterica* on enoki and wood ear mushrooms. *Frontiers*, 14:1257053. https://doi.org/10.3389/fmicb.2023.1257053

21. Sandhu, A.K., Islam, M., Edirisinghe, I., Burton-Freeman, B. **2023.** Phytochemical composition and health benefits of figs (fresh and dried): a review of literature from 2000 to 2022. *Nutrients*. 15(11), 2623. https://doi: 10.3390/nu15112623

22. Swinehart, M., Harris, L.J., Anderson, N.M., Feng, Y. **2023**. US consumer practices of homemade nut-based dairy analogs and soaked nuts. *J. Food Prot*., 86(9),100132. https://doi.org/10.1016/j.jfp.2023.100132

23. Tian, R., Imanian, B. **2023**. PlasmidHunter: accurate and fast plasmid prediction based on gene content using machine learning (preprint). bioRxiv 2023.02.01.526640. https://doi.org/10.1101/2023.02.01.526640

24. Tian, R., Imanian, B. **2023.** VBCG: 20 validated bacterial core genes for phylogenomic analysis with high fidelity and resolution. *Microbiome*, 11(247). doi: https://doi.org/10.1186/s40168-023-01705-9

25. Yang, T., Adhikari, L., Paulose, T., Bleher, R., and Ducan, T.V. **2023**.Titanium dioxide and table sugar enhance the leaching of silver out of nanosilver packaging. *Environ. Sci.: Nano*. 10(6), 1689 https://doi:10.1039/D3EN00098B

26. Yang, C., Salvador, L., Reddy, R.M., Wan, J., Tkachenko, A., Nemser, S.M., Smith, L. and Reimschuessel, R. **2023.** Validation and interlaboratory comparison of anticoagulant rodenticide analysis in animal livers using ultra-performance liquid chromatography–mass spectrometry. *Journal of Veterinary Diagnostic Investigation*, 35(5), 470. https://doi.org/10.1177/10406387231178558

27. Zhou, X, Salazar, JK, Fay, ML, Zhang, W. **2023.** Efficacy of power ultrasound-based hurdle technology on the reduction of bacterial pathogens on fresh produce. *Foods*, 12(14), 2653. https://doi.org/10.3390/foods12142653

28. Zhou, X., & Zhang, W. **2023.** CH02 - Advances in understanding and presenting contamination of fresh produce by *L. monocytogenes*. In K. R. Matthews (Ed.), Advances in ensuring the microbiological safety of fresh produce. Burleigh Dodds Science Publishing, Cambridge, UK, 2023, (ISBN 978 1 80146 268 6). https://shop.bdspublishing.com/store/bds/detail/workGroup/3-190-125637

Presentations:

1. Adhikari, L., Yang, T., Sayeed, M., Paulose, T., Bleher, R., Duncan T. V. **2023.** The effect of food components on migration of nanoparticulate additives out of plastic packaging. Pittcon 2023 Conference and Expo, March 20, 2023, Philadelphia, Pennsylvania. Oral.

2. Anderson, N. **2023**. Roundtable - RT11 - An ever-changing landscape: can using indicator organisms and run time validation studies allow industry to demonstrate process control while maintaining product safety in low-moisture foods? International Association for Food Protection (IAFP) Annual Meeting, July 16-19, Toronto, Canada. Panelist.

3. Anderson, N. **2023**. IFSH/FRI Data Sharing for Food Safety Symposium, September 14, Rosemont, IL. Organizer and Moderator.

4. Beverly, R. **2023**. Factors impacting the extent of peanut allergen cross-contact from reused frying oil. IFSH Annual Meeting. September 12, Rosemont, IL. Oral.

5. Biswas, P., Fay, M.L., Thatavarthi, J., Zhou, X., Salazar, J.K. **2023.** Determining the efficacy of power ultrasound combined with organic acid treatment for the reduction of foodborne pathogens on romaine lettuce. International Association for Food Protection (IAFP) Annual Meeting, July 16-19, 2023, Toronto, Canada. Oral.

6. Biswas, P., Fay, M.L., Thatavarthi, J., Zhou, X., Salazar, J.K. **2023.** Evaluating the use of power ultrasound coupled with organic acid treatment for the reduction of foodborne pathogens on cucumbers. Institute of Food Technologists (IFT) Annual Meeting, July 16-19, 2023, Chicago, Illinois. Oral.

7. Fay, M.L. Salazar, J.K. George, J., Chavda, N., Lingareddygari, P., Patil, G.R., Ingram, D.T. **2023.** Modeling the fate of *Listeria monocytogenes* and *Salmonella enterica* on fresh whole and chopped wood ear and enoki mushrooms. International Association for Food Protection (IAFP) Annual Meeting, July 16-19, 2023, Toronto, Canada. Oral.

8. Fu, T.-J. **2023.** Impact of temperature on pathogen proliferation during sprouting and postharvest storage. IAFP Annual Meeting, July16-19, Toronto, Canada. Oral.

9. Grasso-Kelley, E. March 14, **2023.** FDA Introduction. Better Process Cheese School. Madison, WI. Oral.

10. Grasso-Kelley, E. **2023**. FDA perspective on approaches for treating spices to increase safety. American Spice Trade Association Annual Meeting, May 9, San Diego, CA. Oral.

11. Imanian, B. **2023.** How to respond to the barrage of new discoveries and technologies, and how not to. The 11th Annual American Food Sure Summit, March 30 – 31, 2023, Chicago. Oral.

12. Jackson, L.S. **2023**. FDA research on allergen-cross contact during chocolate manufacture. Midwest Is Best. FDA Emergency Response Coordinators and State Rapid Response Teams Meeting. November 14, Peoria, IL. Oral.

13. Jackson, L.S. **2023**. Allergen cross-contact risk due to the use of shared frying oil. American Chemical Society Fall Meeting, August 13-16, San Francisco, CA. Oral.

14. Jackson, L.S. **2023**. Factors influencing formation of process contaminants and transfer of toxic elements to food and beverages. American Chemical Society Fall Meeting, August 13-16, San Francisco, CA. Oral.

15. Jackson, L.S. **2023.** Importance of cleaning in controlling allergens in retail and food-service operations. Conference for Food Protection Workshop, March 22. Oral.

16. Jackson, L.S. **2023.** Recommendations from the FAO/WHO expert consultation on food allergen risk assessment. ACS Spring Meeting, March 27, Indianapolis. Oral.

17. Jiang, X., Beverly, R., Kidd, J.A., Cluster, J., Karen, S., and Jackson, L.S. **2023**. Transfer of shrimp proteins in shared batch fryers. IFSH Annual Meeting, September 12-13, Rosemont, IL. Poster.

18. Khouja, B.A., Salazar, J.K., Stewart, D.S., Fay, M.L. **2023.** Assessment of population stability of *Salmonella enterica* in matrices for use in dry inoculations. International Association for Food Protection (IAFP) Annual Meeting, July 16-19, Toronto, Canada.

19. Khouja, B.A., Zeng, H., Fay, M.L., Salazar, J.K., Stewart, D.S. **2023.** Fate of *Listeria monocytogenes* during storage of hard-boiled eggs following treatment with organic acids. International Association for Food Protection (IAFP) Annual Meeting, July 16-19, Toronto, Canada. Poster.

20. Lee, A. **2023.** Evaluation of sanitizers and additives to control *Listeria monocytogenes* on RTE fishery products. National Fisheries Institute Seafood Science and Regulatory Forum. Palm Springs, CA. January. Oral.

21. Lee, A. **2023.** Roundtable – When opposites and peers come together in a round table: zero-tolerance versus microbial risk assessment within a poultry case study. Aberdeen, United Kingdom. May. Panelist.

22. Lee, A. **2023.** What is a Pathogen? Food Safety Summit, May, Rosemont, IL. Presenter, Moderator and Session Organizer.

23. Lee, A. **2023.** Challenges and opportunities navigating requirements of ready-to-eat and not ready-to-eat for refrigerated and frozen foods. International Association for Food Protection Annual Meeting, July, Toronto, Canada. Moderator and Session Organizer.

24. Lee, A. **2023.** Inactivation of foodborne viruses and their surrogates in industry related projects. International Association for Food Protection Annual Meeting, July, Toronto, Canada. Presenter.

25. Mathias, H., Fay, M.L., Zhou, X., Salazar, J.K. **2023.** Examination of power ultrasound and acid-based hurdle technology for the reduction of *Salmonella enterica* on peaches. Institute of Food Technologists (IFT) Annual Meeting, July 16-19, 2023, Chicago, Illinois.

26. Morrissey, T., **2023.** *Clostridium botulinum* challenge study in commercial cold brew coffee. IFSH Annual Meeting, September 12-13, Rosemont, IL. Oral.

27. Rolfe, C. **2023.** What pitfalls could lead to lawsuits from voluntary labeling requirements. IFT Annual Meeting. July, Chicago, IL. Moderator.

28. 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, March 12, Virtual.

29. Sandhu, A.K. **2023.** Relative absorption of fat-soluble vitamins D and A and minerals from select plant-based milks in human subjects: a pilot trial. CFSAN Regulatory Research Lecture Series, March 16, Virtual. Oral.

30. Sandhu, A.K. **2023.** Bio-active components research at the illinois institute of technology. Food Research Institute, University of Wisconsin-Madison 2023 Spring Meeting, May 16-17. Oral.

31. Sandhu, A.K. **2023.** Phytochemicals from herbs and spices are metabolized with cardiometabolic benefits. American Society for Nutrition, Dietary Bioactive Components and Nutrient-Gene Interactions Joint GEM Forum, July 24, Boston, MA. Oral.

32. Schaneberg, B. **2024**. Biotics – their future & challenges. AOAC Midwest Section Meeting and Exposition, June 3-5, Oak Brook, IL. Oral.

33. Schaneberg, B.T. **2023**. Managing a crisis: product recall best practices. Chicagoland Food & Beverage Network, September 20, Chicago, IL. Panelist.

34. Shazer, A.; Fu, T.-J. **2023.** Efficacy of dry heat treatment in reducing *Salmonella* contamination on sprout seeds. FDA Science Forum, June 13-14, Poster.

35. Biswas, P., Fay, M., Thatavarthi, J., Zhou, X., Salazar, J.K. **2023**. Determining the efficacy of power ultrasound combined with organic acid treatment for the reduction of foodborne pathogens on romaine lettuce. International Association for Food Protection Annual Meeting, July 16-19, Toronto, Canada. Poster.

36. Deng, K., Nemser, S., Frost, K., Goodman, L.B., Ip H.S., Killian, M.L., Ulaszek, J., Kiener, S., Kmet, M., Uhlig, S., Hettwer, K., Colson, B., Nichani, K., Schlierf, A., Tkachenko, A.,

Miller, M.R., Reddy, R., and Tyson, G. H. **2023**. Interlaboratory comparison exercises (ILC) of SARS-CoV-2 molecular detection method used by veterinary diagnostic laboratories. Association of Public Health Laboratories ID Lab Con, March 13-15, Atlanta, GA. Poster

37. George, J., Fay, M., Salazar, J. and Stewart, D. **2023**. Population dynamics of *Salmonella enterica* and *Listeria monocytogenes* during rehydration of dehydrated enoki mushrooms and subsequent storage. IAFP Annual Meeting, July 16-19, Toronto, Canada. Poster.

38. Gurtler, J.B., Grasso-Kelley, E., Xuetong, F., Jin, T. and Garner, C. **2023**. Inactivation of desiccation-resistant *Salmonella* on apple slices following treatment with epsilon-polylysine, sodium bisulfate or peracetic acid. International Association for Food Protection Annual Meeting, July 16-19, Toronto, Canada. Poster.

39. Huang, Y., Freeman, M., Edirisinghe, I., Burton-Freeman, B. and Sandhu, A.K. **2023**. Characterization and stability of a formulated mixed berry beverage (red raspberry and strawberry) over 3-year storage. 9th Biennial Berry Health Benefits Symposium, January 31-February 2, Tampa, Florida. Poster.

40. Huang, Y., Freeman, M., Edirisinghe, I., Burton-Freeman, B., and Sandhu, A.K. **2023**. Characterization and stability of a formulated mixed berry beverage (red raspberry and strawberry) over 3-year storage. IFSH Annual Meeting, Rosemont, IL September 12-13. Poster.

41. Josephina, G., Fay, M.L., Salazar, J.K., Stewart, D.S. **2023**. Population dynamics of *Salmonella enterica* and *Listeria monocytogenes* during rehydration of dehydrated enoki mushrooms and subsequent storage. International Association for Food Protection (IAFP) Annual Meeting, July 16-19, Toronto, Canada. Poster.

42. Korade, S., Jen, M., Parker, E., Lowe, M., Sandhu, A.K. and Krishnamurthy, K. **2023**. Effect of pulsed uv light treatment on vitamin c content of red raspberries. IFT FIRST Annual Meeting and Expo, July 16-19, Chicago, IL. Poster.

43. Liu, X., Grasso-Kelley, E. M., Anderson, N. M. **2023**. Inactivation of *Enterococcus faecium* NRRL B-2354 in apple cubes during hot air drying with different temperatures and airflow. IAFP Annual Meeting, July 16-19, Toronto, Canada. Poster

44. Patras, B., Pendyala, Krishnamurthy, K., Balamurugan, S., Maks, N. and Aguilar, V. **2023**. UV-C inactivation of *Clostridium botulinum* type B strain in opaque coconut water. International Association for Food Protection, July 16-19, Toronto, Canada. Poster.

45. Rolfe, C., T. Morrissey, V. Aguilar, B. Redan, G. Skinner, and N.R. Reddy. **2023**. Correlation between dipicolinic acid (DPA) release and heat resistance of *C. botulinum* type A and *C. sporogenes* spores during thermal processing. FDA Science Forum, June 13-14. Virtual.

46. Rolfe, C.T. Morrissey, Aguilar, V., Redan, B., Skinner, G. and Reddy, N.R. **2023**. Dipicolinic acid (DPA) release and heat resistance of nonproteolytic *C. botulinum* type B and type F spores during thermal processing. IFT First Annual Meeting, July 16-19, Chicago, IL. Poster.

47. Smith, E., Kaiping, D., Wang, H., Kiener, S., Wang, S.S., Chen, K.S., Pamboukian, R., Laasri, A., Kmet, M., Ulaszek, J., Hammack, T. and Reddy, R. **2023**. Multi-laboratory validation study of a real-time PCR method for detection of *Salmonella* in frozen fish. International Association for Food Protection, July 16-19, Toronto, Canada. Poster.

48. Tian R., Imanian, B. **2023**. PlasmidHunter: accurate and fast prediction of plasmid sequences using gene content profile and machine learning. IFSH Annual Meeting, September 12-13, Rosemont, IL. Poster.

49. Tian, R., Imanian, B. **2023**. VBCG: A new tool for phylogenomic analysis with higher fidelity and resolution using validated bacterial core genes. IFSH Annual Meeting. September 12-13, Rosemont, IL. Poster.