2021
Annual Report of Collaborative Research Program

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

2021 IIT IFSH NCFST Annual Report of Collaborative Research

Interim Director, IFSH
Jason Wan, Ph.D.

Illinois Institute of Technology
6502 South Archer Road
Bedford Park, IL 60501-1957
708.563.1576 (main)
ifsh@iit.edu

www.iit.edu/ifsh

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, IIT IFSH

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

Determining bacterial inactivation in food powders using the fluidized bed resistometer

Gregory Fleischman[2], Kaiping Deng[1,2],
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration

Pathogen contamination in food powders is a problem of increasing concern to the FDA. The measurement of pathogen inactivation kinetics in powders is therefore an important endeavor. Yet, there are no measurement devices for accurate kinetics measurement that specifically accommodate powders. Rather, devices that were designed for liquids or pastes have been used. As such, these devices do not uniformly expose powder to obtain inactivation kinetics under various conditions of interest. Furthermore, relative humidity of the environment is an additional factor to temperature in powder inactivation kinetics; this is not a factor in liquids and pastes. For these reasons, in a previous project (CARTS IF01357), a device was developed to fluidize powder with an air stream having independently controlled temperature and relative humidity. The present project has looked at the performance of this device. Two problems arose, however. The first was with hygroscopic powders that clumped from absorption of the ambient humidity. The second was containment of powder fines. Even with two-stage containment, fines were nevertheless escaping. It was decided to temporarily shelve this approach and pursue tumbling powders under an infrared source to focus on uniformity under dry heat. An applicator was developed with an in-house constructed infrared line source placed along the axis of a rotating horizontal custom-designed bottle which incorporated baffles to encourage powder tumbling during rotation.

Enhancing the safety of high pressure processed (HPP) juices

Alvin Lee[1], Catherine Rolfe[1,2], Nathan Anderson[2], Glenn Black[2]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration

HPP juice manufacturers are required to demonstrate a 5-log reduction of the pertinent microorganism to comply with FDA Juice HACCP. There is currently no consensus on validation approaches for shelf-life studies in terms of storage conditions, sampling times, or temperature abuse. Little is known regarding the shelf-life and stability of HPP treated juices. Shelf-life studies involving prolonged storage at one temperature is not a realistic example of a manufacturer to consumer path. FDA Juice HACCP indicates shelf-life analysis should include storage conditions of possible temperature abuse a product might be subjected to. However, the level of abuse, whether moderate or severe, is not well defined and there is no consensus on abuse temperature or time parameters to assess.

In the last quarter the aim was to compare HPP inactivation and post-HPP survival of acid-adapted *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* in apple and orange juice during a 75-day shelf-life using quantitative and qualitative assessment.

Bacterial strains *E. coli* O157:H7 TW14359, *Salmonella* Cubana and *L. monocytogenes* MAD328, which were deemed most barotolerant among screened bacterial strains, were acid-adapted in intermediate pH 5.0 TSBYE using conditions previously developed during the course of the project. Individual strains were inoculated into apple juice (pH 3.50±0.20) and orange juice (pH 3.88±0.10) at approximately 6.00 log CFU/mL, pressure treated at 586 MPa (180s, 4°C

initial) and stored at 4°C for remainder of shelf-life. Inoculated, untreated controls were included. Triplicate samples were analyzed in duplicate with quantitative plating and qualitative enrichments conducted on days 1, 2, 3, 4, 5, 10, 15, 35, 55, and 75 with temperature abuse conducted on days 3 (30°C, 2h) and 12 (30°C, 2h and room temperature, 1h).

Significantly ($p<0.05$) greater log reduction occurred for *S.* Cubana (approximately 3 logs) and *L. monocytogenes* MAD328 (>5 logs) compared to *E. coli* O157:H7 TW14359 (<1 log) following initial post-HPP analyses. While complete inactivation was not observed, bacterial inactivation exceeded 5-log in both HPP-treated juices immediately following pressure treatment for *L. monocytogenes* MAD328, after 24 hours in cold storage for *S.* Cubana, and after 4 days of cold storage for *E. coli* O157:H7 TW14359. Contradictory positive quantitative and negative qualitative analyses resulted for *E. coli* O157:H7 TW14359, i.e. *E. coli* O157:H7 typical colonies were observed on microbiological agar plates but qualitative enrichments were negative. Qualitative enrichments of *L. monocytogenes* MAD328 in orange juice were observed during cold storage time on days 35 and 75 but >5 log reduction was maintained.

These results suggest in certain juice types a cold holding time following pressure treatment may be required to achieve a 5-log reduction. Also, certain juice matrices may interfere with qualitative enrichments of bacterial targets.

Temperature redistribution in food during the post-microwave stand-time

Gregory Fleischman
Food and Drug Administration

A two-minute stand-time has been recommended in the FDA Food Code as part of a recommendation for safe cooking of meat in a microwave oven. A single study in 1956 on commercial microwave ovens showed the importance of stand-time, up to 40 minutes, to achieve complete heating of large cuts of meat such as roasts. However, since that time the size and cost of the ovens have dramatically decreased, their numbers dramatically increased, and meat prepared in them now represent smaller single-serving portions. In 1992 the first foodborne illness outbreak linked to microwave undercooking appeared, and since then more, some of which attributable to insufficient stand-times. But no studies have examined stand-time to determine if the current recommendation is sufficient for temperature redistribution to complete under-cooked regions of a meat portion. This project aims to examine the stand-time in microwave-cooked meat using both modeling and experiment. Modeling examines the effect of stand-time on temperature distribution across the meat width (the thickness in a slab of meat or the diameter across a cylindrical or spherical meat portion). Experimentally, surface temperatures are observed after microwave heating to examine the effect of stand-time along the meat length (the surface of a slab of meat or the length of a cylindrical portion). The modeling showed that there were limits to what a two-minute stand-time could achieve, depending on the width of the portion and the average temperature of the meat upon completion of heating. This occurred regardless of shape. Surface temperature distribution is currently ongoing.

An assessment of blanching efficacy in inactivating pathogens on the surface of foods

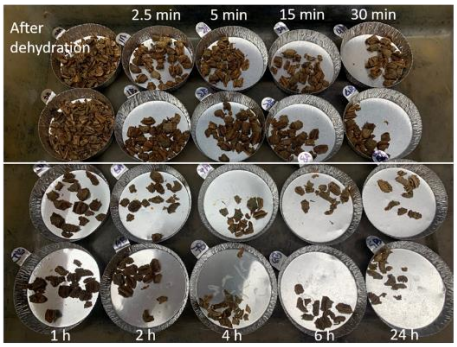
Gregory Fleischman
Food and Drug Administration

Blanching is a common pre-freezing treatment for vegetables. Its intended purpose is to inactivate enzymes that continue the ripening process during frozen storage. Though ripening continues at a slower rate, even at -18°C storage off-odor, off-color and off-flavor can develop. A potential added benefit of blanching is the inactivation of surface pathogens. Literature studies that have looked at specific blanching conditions have concluded that blanching can remove 5 logs of pathogens from vegetables surfaces. However, a broader range of conditions are encountered in industrial blanching than those seen in the literature. Therefore, the present work examines blanching efficacy using mathematical modeling based on heat transfer to the vegetable surface from the blanching medium, water or steam, and combining it with known kinetics of pathogen inactivation. These two different media are simply represented by different heat transfer coefficients in the model. The results from the model using literature conditions examined for pathogen reduction (as opposed to just enzyme inactivation) agreed with those studies; time and temperature did produce greater than a 5 log reduction in the pathogen examined. Still to be examined are unusual surfaces as exhibited by, for example, broccoli, and spinach leaves. One additional, and counterintuitive, phenomenon will also be addressed. This is the longer time used for blanching the same product in steam than that in water. Steam has a much greater heat transfer coefficient, yet recommended times are notably greater than those in water. This has been attributed to the entrapped air in vegetable tissue that is released during blanching. This will be examined experimentally.

Microbiology Platform

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

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



Qualitative comparison of devices for environmental sampling of *Listeria monocytogenes* from various food contact surfaces

Diana Stewart, Joelle Salazar, Megan Fay, Arlette Shazer
Food and Drug Administration

This project is a continuation of Qualitative Comparison of Devices for Environmental Sampling of *Listeria monocytogenes* from Food Contract Surfaces, in which various sponges, wipes and swabs were used to recover *Listeria monocytogenes* from 8" x 8" stainless steel plates and high density polyethylene (HDPE) cutting boards in the presence of various matrices found in food processing facilities. This project expands on this previous work by comparing four environmental swabbing devices for recovery of *Listeria monocytogenes* from smaller 4" x 4" areas of the same surface types using buffer, Romaine lettuce wash or raw milk cheese whey as matrices. As in the previous project, *Listeria monocytogenes* was inoculated onto the surfaces at various levels in each matrix, dried overnight, and swabbed with the devices followed by *Listeria* detection using the FDA Compliance Document's 2-step enrichment method. The four swab devices that were evaluated include a nylon flocked swab, an oversized polyurethane swab, and cotton and polyester bud-style swabs. Results were compared between devices within each matrix as well as between the surface types for each matrix. Limits of Detection at 95% (LOD_{95}) were determined for all devices to compare *Listeria* detection on surfaces which may have quite disparate results at the tested inoculation levels. Significant differences between devices were determined using Fisher's Exact Test with significance at 0.05. Overall, regardless of matrix or surface type, the flocked swabs were generally least effective for recovery of *Listeria monocytogenes* and had the lowest detection limits for all matrices. There was variability for the other devices within each matrix/surface, however no device was consistently better than the others. Between surfaces, the LODs were nearly the same or slightly worse for HDPE than stainless steel which is probably due to the slightly bumpy texture of the cutting boards. The LODs for samples containing Romaine lettuce wash and raw milk cheese whey were better than for the no matrix samples which correlates well with the results found in the previous project. The reduced LODs for HDPE samples are presumed to be due to differences in drying rate and the lack of a protective effect from the presence of the food matrices. Overall, this information should be useful for food safety personnel evaluating or implementing a *Listeria* environmental sampling program.

Survival of *Salmonella enterica* on cut melons and transcriptomic response of the pathogen on melon treated with organic acid

Wei Zhang[1], Xinji Zhou1, Joelle Salazar[2]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration

Foodborne outbreaks of *Salmonella enterica* have been associated with fresh-cut melons in the U.S. including cantaloupe, watermelon, and honeydew. Research has shown that the antimicrobial activity of organic acids, such as malic acid and citric acid, may reduce pathogen populations on fresh produce, including melons. This study was divided into two objectives: 1) to examine the survival of *Salmonella* on melon flesh treated with organic acids, and 2) to determine the differential gene regulation of the pathogen on the treated flesh. For objective 1, different serotypes of *Salmonella* (Typhimurium, Enteritidis, and Newport) were evaluated for

their abilities to survive on cut cantaloupe and honeydew flesh treated with or without malic or citric acid. Cut melon flesh was treated by submersion in either 2% malic or citric acid for 1 min or left untreated. Flesh was then inoculated with 4 or 7 log CFU/g of one of the *Salmonella* serotypes and stored at 4°C for 7 days. All tested *Salmonella* strains survived on untreated and citric acid-treated cantaloupe and honeydew flesh with no significant decrease in population during 7-day storage at 4°C, regardless of initial inoculation level. On malic acid-treated melon flesh, the average *Salmonella* population decreased by <1 log CFU/g over 7-day storage for both initial inoculation levels (4 and 7 log CFU/g). For objective 2, the differential gene regulation of serotypes Newport and Typhimurium on malic acid-treated cantaloupe flesh was examined using RNA-seq. RNA was extracted at time 0 (after inoculation and prior to storage) and after 1 day storage of cantaloupe flesh at 4°C. Samples for sequencing were prepared using the TruSeq Stranded mRNA Kit and run on a MiSeq. After 1-day storage, a total of 169 and 492 genes were differentially regulated in Newport and Typhimurium on malic acid-treated cantaloupe, respectively, compared to time 0. Gene Set Enrichment Analysis (GSEA) determined that pathways (i.e., biological processes) related to carbohydrate metabolism, nutrient transport, cell signaling, and general stress response were upregulated. Data on the acid adaptation response of *Salmonella* on cut cantaloupe and will aid in evaluating any cross-protection mechanisms that the pathogen employs during this stress. In addition, this project provides important scientific basis for the food industry to develop more effective control measures to mitigate *Salmonella* contamination in fresh-cut melons and is essential to establish science-based standards for the FDA Preventive Controls Rule.

Growth kinetics of *Listeria monocytogenes* and *Salmonella enterica* during rehydration of dehydrated plant foods, storage of rehydrated plant foods, and storage of heat-treated plant foods

Joelle Salazar[2], Megan Fay[2], Pravalika Lingareddygari[1,2], Madhuri Mate[1], Supriya Sridhar[1], Girvin Liggans[2]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration

Dehydrated plant food products such as potatoes, onions, carrots, and peppers have low water activities and do not support the growth of pathogenic bacteria such as *S. enterica* or *L. monocytogenes*. Once rehydrated, the higher water activity (>0.92) and relatively neutral pH of these foods would require a product assessment to determine the extent to which they support the growth of pathogenic bacteria. Many of the dehydrated plant foods in retail and food service establishments are dehydrated at the manufacturer level using heat and are rehydrated onsite; however, once rehydrated, these products could be held for later use. The first objective of this study was to evaluate the growth kinetics of *S. enterica* and *L. monocytogenes* during rehydration of heat-dehydrated green bell pepper (utilizing 5 or 25°C water) and during subsequent storage (at 5, 10, or 25°C) for up to 14 days. After dehydration, green bell pepper was inoculated with a four-strain cocktail of either *L. monocytogenes* or *S. enterica* at 4 log CFU/g and dried at ambient for 24 h. Inoculated dehydrated green bell pepper was rehydrated with 5 or 25°C water, followed by storage at one of the three temperatures. The highest growth rate (1.46±0.49 log CFU/g per d) was observed by *L. monocytogenes* on 25°C-rehydrated green bell pepper during 25°C storage which resulted in a 1 log CFU/g increase in only 0.90 d (16.4 h). The second objective of this study was to determine the growth kinetics of *L. monocytogenes* and *S. enterica* in non-

dehydrated but heat-treated green bell pepper. Chopped green bell pepper was cooked in an oven at 177°C (350°F) for 25 min, followed by cooling at 5°C for 20 min. Cooked peppers were inoculated with either pathogen cocktail at 4 log CFU/g and stored at the three different temperatures. The highest growth rate (4.96±0.35 log CFU/g per d) was observed by *S. enterica* during storage at 25°C, leading to a 1 log CFU/g increase in only 0.20 d (4.80 h). This growth rate was significantly higher than any growth rate observed for either pathogen during storage of rehydrated green bell pepper. Understanding the growth kinetics of these pathogens in rehydrated plant foods and cooked plant foods can inform discussions surrounding whether specific time and temperature conditions for rehydrating and storing of these plant foods should be recommended.

Evaluating the effectiveness of antimicrobial chemicals for treatment of seeds for sprouting

Yikai Yang[1], Xinyue Ma[1], Mocang Deng[1], Tam Ngo[1] and Tong-Jen Fu[2]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration

The availability of safe and effective seed treatment methods is critical in reducing microbial hazards in sprouts and will allow sprout growers to meet the requirements set forth in the Produce Safety Rule. Treatment of seeds for sprouting with 6-10% hydrogen peroxide (H_2O_2) has been recommended by some government agencies. Published studies evaluating the efficacy of H_2O_2 focused primarily on alfalfa seeds. The efficacy of this treatment in reducing pathogens on other types of seeds has not been determined.

This study evaluated the efficacy of H_2O_2 in reducing *Salmonella* on artificially inoculated seeds, as affected by sanitizer concentration (6, 8, or 10% H_2O_2), treatment time (10 min and 1 h) and seed type (alfalfa, clover, broccoli, onion, radish, and mung beans). The impact of different treatment conditions on seed germination and sprout yield was also examined. Treatment of seeds with 6-10% of H_2O_2 for 10 min resulted in a $\leq$ 2-log reduction in *Salmonella* population. Extending the treatment time from 10 min to 1 h resulted in a slight increase in log kill. The treatment was more effective on alfalfa, clover and mung beans than on broccoli, radish or onion seeds. The treatment did not affect the germination percentage of any seed type under any concentration/time combinations. Treatment with 10% H_2O_2 for 1 h was the most effective on alfalfa seeds, achieving a 3.1-log reduction in *Salmonella* population, but it lowered the sprout yield by ~30%. Overall, treatment with 6-10% of hydrogen peroxide for up to an hour was only marginally effective in reducing *Salmonella* on seeds for sprouting.

Research findings will inform the development of standardized approach for evaluating seed treatment efficacy and will provide FDA with needed knowledge in reviewing seed treatment processes during sprout operation inspections.

Decontamination of sprout seeds by dry heat treatment

Arlette Shazer and Tong-Jen Fu
Food and Drug Administration

Numerous studies have been performed in the search for effective methods to treat seeds for sprouting. Although chemical treatments are the most studied, physical treatments are gaining increasing interest. This research evaluated the efficacy of dry heat in reducing *Salmonella* population on artificially inoculated seeds, as affected by treatment time, temperature, relative humidity (RH), and seed type. Germination percentage and sprout yield of treated seeds were determined.

Heat treatment experiments were performed to determine the best conditions that can achieve the highest reduction in *Salmonella* while maintaining seed viability. Conditions evaluated for mung beans (10 g) included 60°C at 60% or 80% RH, 70°C at 40%, 60% or 80% RH, and 80°C at 20% or 40% RH for 6, 16, and 24 h. Overall, the best treatment conditions that were able to reduce *Salmonella* population on mung beans to below the detection limit (< 0 log cfu/g) while keeping seed germination percentage and sprout yield ratio at > 90% were 60°C, 80% RH or 70°C, 60% RH for 16 h. Heat treatment conditions evaluated for alfalfa seeds included 60°C at 40%, 60% or 80%RH and 70°C at 40% or 60%RH for 6, 16, or 24 h. Preliminary results showed that treatments at 70°C/60%RH, 70°C/40%RH, or 60°C/60% RH for 24 h or at 60°C/80%RH under all 3 time periods (6, 16, 24 h) were able to reduce the pathogen by > 3 log units. Experiments examining the impact of these treatment conditions on seed germination and sprout yield are ongoing.

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

Impact of temperature on pathogen proliferation during sprouting and postharvest storage

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

Sprouts pose a particular food safety concern as conditions that promote seed germination also promote pathogen growth. Developing ways to minimize proliferation of pathogens, if present, during sprouting is crucial in the overall approach to reduce public health risks of sprouts. This study investigated how germination temperature may affect pathogen growth during sprouting of artificially inoculated seeds and how this temperature effect is influenced by seed disinfection. Persistence of pathogens during postharvest storage of sprouts at different temperatures was also examined.

Sprouting at 4°C resulted in a decrease in *Salmonella* level by ~3.5 logs compared with an increase of 2-4 logs during sprouting at 10, 20, or 30°C. Treatment of inoculated seeds with 19,000 ppm NaClO or 20,000 ppm $Ca(OCl)_2$ reduced the *Salmonella* population by 2.6 or 2.4 logs, respectively. When the treated seeds were sprouted at 20 or 30°C, *Salmonella* proliferated rapidly and reached a similar level (8 - 9 log cfu/g) as those observed in sprouts grown from seeds treated with water only. The pathogen

either decreased by 2 logs or increased by 2 logs when the treated seeds were sprouted at 4°C or 10°C, respectively. For sprouts germinated at 4°C and then stored at 4°C, the *Salmonella* population decreased during the 21 days of storage. But when these sprouts were stored at 25°C (i.e., under temperature abuse conditions), the *Salmonella* level increased by > 5 logs after 2 days of storage. These results suggest that, although pathogen proliferation could be inhibited when sprouts are germinated at refrigeration temperature, maintaining the cold chain during storage is critical to prevent pathogen regrowth.

Research findings will inform risk assessment of cold grown sprouts. An understanding of pathogen survival and persistence in sprouts during postharvest storage will aid development of guidelines for proper postharvest storage and handling of sprouts.

Potential interruption of virus adhesion by modifying contact surfaces in nanoscale and by altering virus-surrounding environment

Ao Guo[1], Rong R. Wang[1], Ralu Divan[3], Carol Shieh[2]
[1]Illinois Institute of Technology, Department of Chemistry; [2]Food and Drug Administration; [3]Center for Nanoscale Materials, Argonne National Laboratory

Human enteric viruses can be indirectly transmitted via contact surfaces. One of the research objectives was to evaluate the interruption of virus transmission by studying possible inactivation of MS2 and hepatitis A virus (HAV) upon contacting metal surfaces. MS2 bacteriophage (surrogate) and HAV were deposited onto metal coupons including stainless steel (type 304) and circular-shape 99.9% copper (rough 11.74 cm^2, smooth 1.44 cm^2 and 1.46 cm^2 surfaces) for virus recovery. MS2 remained stable on the stainless-steel surface; but was inactivated ~1 log/min of contact across all three copper (Cu) surfaces from three manufactures (R^2 = 0.8 - 0.9). The interaction of MS2 and Cu was affected by both the virus-surrounding pH and medium complexity. Greater virus inactivation was observed (1) in a simple medium such as PBS when compared to complex medium such as 0.5% beef extract and (2) in acidic pH environments than in neutral and alkaline environments. This MS2 and Cu interaction phenomenon was upheld in foods examined. The greatest reduction of MS2 by Cu occurred in the 70 μm pore-filtered strawberry juice (acid and clear with fewer solids), followed by unfiltered acidic strawberry juice, and lastly food-grade neutral beef broth. HAV survived on a copper contact surface longer than MS2 at a rate of reduction of 0.15 log/min (R^2 = 0.9). When the virus suspension media (PBS with 1% TSB and with 1% MEM) were compared, there was no significant difference in MS2 survival upon Cu-contact. Thus, we conclude that HAV survives approximately 6 to 7 times greater than MS2 upon contacting Cu surfaces. Unlike stainless-steel, polymer, and glass, enteric viruses can be inactivated by Cu surfaces upon contact but is influenced by the virus-surrounding food components and pH values.

Identification and use of novel disinfectants to disrupt regulation of desiccation and persistence in *Salmonella* and STEC and their sanitation efficacy

Joelle Salazar, Susanne Keller, Rachel Streufert, Megan Fay
Food and Drug Administration

Desiccation resistance and persistence of foodborne pathogens in low-moisture environments are problematic for the food industry as well as for regulators. Pathogens, like *Salmonella enterica*, typically develop greater resistance in a dried environment or during drying processes. However, exactly why this occurs is not well-understood. Both survival on drying and persistence in the dried environment may be related to biofilm formation or the production of extracellular polysaccharides, as well as changes to membrane lipids and/or proteins, and cell-to-cell communication related to quorum sensing. Consequently, compounds known to promote biofilm formation or play roles in quorum sensing were evaluated for their ability to improve the desiccation resistance of *Salmonella*. A variety of compounds were examined, including bovine serum albumin, sucrose, catalase, trehalose, inositol, lactone, peptidoglycan, and two components of peptidoglycan (N-acetylglucosamine and N-acetylmuramic acid). A six-strain cocktail of *Salmonella* was diluted 1:10,000 in buffered peptone water (BPW) or in BPW with the individual components and placed onto filter disks. Filters were dried at ambient in a biosafety cabinet with the blower on. The *Salmonella* population reduction on the filters was assessed after 24 h. The population reduction after 24 h when *Salmonella* was diluted with peptidoglycan was -0.97±0.18 log CFU, which was lower than but not significantly different compared to when diluted in BPW (-1.66±0.62 log CFU). None of the other tested components, including the two components of peptidoglycan, significantly improved *Salmonella* survival on drying. Future work will include the examination of the effect of peptidoglycan or other cell-membrane components on the survival of Shiga-toxin producing *E. coli* (STEC) on drying. Overall, results of this study will aid in understanding how *Salmonella* and STEC survive in low-moisture environments and food matrices.

Fate of *Listeria monocytogenes* on hard-cooked eggs treated with citric acid

Joelle Salazar[2], Diana Stewart[2], Megan Fay[2], Bashayer Khouja[1]
[1]Illinois Institute of Technology [2]Food and Drug Administration

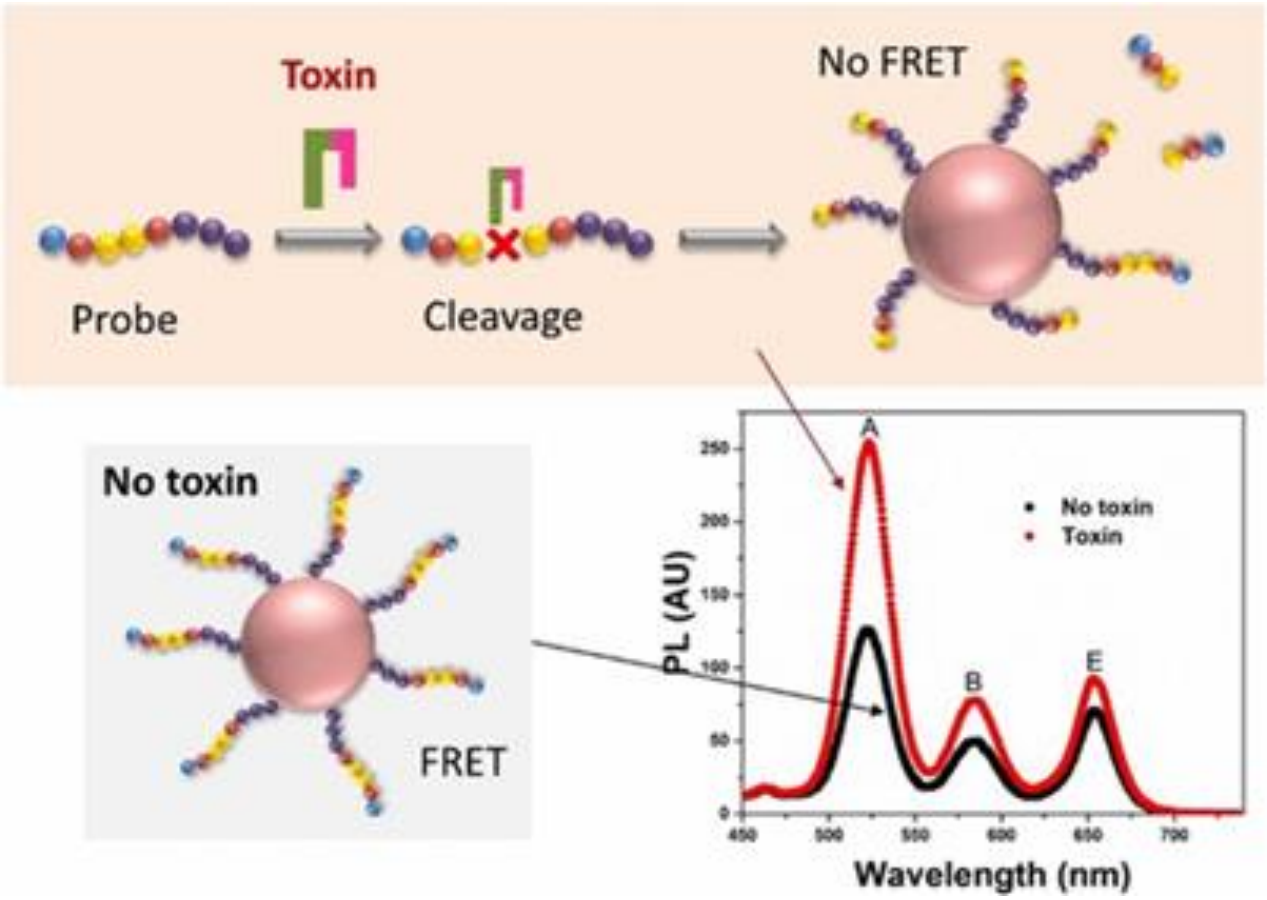
Commercially-prepared peeled hard-boiled eggs are available for food service and the public in various forms including refrigerated retail packs. Potential contamination with *Listeria monocytogenes* after the cooking and peeling steps is of concern since this pathogen can proliferate at refrigeration temperatures and there may be no additional hurdles to inhibit growth if the eggs are contaminated after peeling. In the past decade there have been recalls associated with *L. monocytogenes* contamination of hard-boiled eggs, presumably due to cross-contamination either after peeling or during packaging. In one such instance, eggs were treated by submersion in molar pH 2.5 citric acid for up to 24 h prior to packaging. However, the eggs were not always refrigerated during treatment and the efficacy of such a short acidification step was not properly validated. The objectives of this study, therefore, were to 1) determine the survival of *L. monocytogenes* on hard-boiled eggs during a 24-h citric acid treatment at 5 or 25°C and to 2) evaluate the effectiveness of a 5 or 25°C 24-h citric acid treatment at reducing *L. monocytogenes* during 28-d storage at 5 or 25°C when inoculated pre- or post-treatment. For

objective 1, hard-boiled eggs were inoculated with either 4 or 7 log CFU/egg *L. monocytogenes*, dried for 10 min, and treated by submersion in citric acid for 24 h. *L. monocytogenes* was enumerated throughout the treatment process. Regardless of temperature, *L. monocytogenes* was only reduced by 1-1.5 log CFU/egg over 24 h. For objective 2, eggs were either dip inoculated with 1 log CFU/egg of *L. monocytogenes*, followed by treatment as previously described (pre-process contamination) or first treated with citric acid, dried for 10 min, and spot inoculated with 1 log CFU/egg (post-process contamination). After drying 10 min, eggs were stored for 28 d at either 5 or 25°C. During storage, *L. monocytogenes* was enriched via the FDA BAM method. After 28-d storage, *L. monocytogenes* was detected on 2/6 eggs after 28-d storage at 25°C, but not detected at 5°C for pre-contamination experiments. For post-contamination, the pathogen was detected in 6/6 samples for both storage temperatures. Future experiments will examine other organic acids for hard-boiled egg treatment, including malic, lactic, and acetic acid.

Chemistry and Packaging Platform

Lauren Jackson, FDA

The Food Chemistry and Packaging Platform aims to investigate approaches to prevent, reduce or mitigate the formation of hazardous chemical contaminants during processing, and to prevent the cross-transfer of pre-formed natural toxins, allergens or man-made (environmental) contaminants in the food production environment. Another platform goal is to evaluate factors affecting migration of packaging constituents and contaminants into food.



Systematic approaches for sampling foods for allergens and gluten

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

Agricultural commingling and cross-contact often result in heterogeneous distribution of food allergens and gluten throughout a lot of food. The main goal of this project is to mimic practically relevant cross-contact scenarios for several food allergens and gluten and study their distribution in lots of high-risk foods and to evaluate sampling plans for measuring allergen and gluten content of these products. A secondary goal of this project is to assess the effectiveness of dry cleaning procedures for allergen removal from equipment in the manufacture of low water activity foods.

Over the past year, a study conducted in collaboration with scientists from several FDA/CFSAN offices (OFS, OARSA, OAO), IIT/IFSH, North Carolina State University and General Mills studied sampling plans for determining peanut concentrations in lots of wheat flour. This study was also designed to simulate the conditions that resulted in allergen recalls associated with peanut-contaminated wheat flour. Ten, 45-kg lots of wheat flour with varying peanut content (0-200 µg peanut protein/g) were produced in a pilot-scale hammer mill in the IFSH GMP pilot plant from wheat berries spiked with peanuts (blanched, raw). Thirty-two samples from each lot were collected during milling to study the effect of discrete and composite sampling methods on peanut protein content. A 5-g sample was taken from each of the 32 samples, extracted with extraction buffer, and peanut protein content was estimated using ELISA by analyzing duplicate aliquots of the sample extract. Regression analysis revealed a linear relationship between the log variance and the log of peanut protein concentration for samples obtained by the discrete sampling method. The total variance values (Vt) from peanut analyses were partitioned into variance between samples (Vs) and aliquots analyzed (Va). At all peanut concentrations, the Vs represented >95% of the Vt. Work is currently underway to measure the peanut content of composite samples and compare the variances for the two sampling methods. In addition, OAO is currently constructing operating characteristic (OC) curves to evaluate various sampling plans for peanut in wheat. The data generated in this project will be useful for the development of sampling plans for allergens in food products.

A second study evaluated the effectiveness of a push-through cleaning method using flour and corn meal to purge egg or milk powder from a continuous mixer/auger system. Nonfat dry milk (NFDM; 500 g) or whole egg powder (WEP; 500 g) was conveyed through a continuous lab-scale mixer/auger system comprised of a hopper, tee and screw conveyor. All-purpose flour (9000 g) was then added to the hopper to push-through residual allergen remaining in the system (2-10 g). Flour samples (~88 g) collected at one-minute increments as they exited the unit were homogenized and analyzed in triplicate for milk or egg concentrations by ELISA (Neogen Veratox for Total Milk or Egg; LOQ = 2.5 ppm milk or egg). Push-through trials were completed in triplicate for each allergen. Visual inspection and qualitative allergen specific lateral flow devices (LFDs; Neogen Reveal 3D tests for milk or egg) were also utilized for residual allergen determination. Studies were also completed in triplicate using coarsely ground

corn meal (9000-15,500 g) to purge residual WEP in a similar manner. Egg concentrations in flour or corn meal decreased as a function of the amount of push-through. The final flour samples obtained after 9000 g of push-through contained <LOQ, 11 ppm (18% CV) and 4.5 ppm (3% CV) egg in the triplicate trials. Trials with NFDM indicate that less than 9000 g of push-through flour was required to achieve flour samples having milk concentrations <LOQ. This suggests that NFDM was easier to flush from the system than WEP. However, when the system was disassembled, NFDM or WEP residue was visibly present or detectable by LFDs on the auger and other inner surfaces of the unit after the push-through trials were completed. Push-through with flour or coarsely ground corn meal was successful in reducing the amount of residual NFDM and WEP from the mixer/auger unit and aided in reducing allergen cross-contact. However, full unit disassembly was required to ensure complete allergen removal. Future studies will investigate an improved unit design (i.e., eliminating the dead-zone), conducting trials in a better controlled environment (humidity, temperature) and use of other push-through materials such as salt.

Current assessment of food-grade lubricant transfer into foods

Yoon S. Song[2], John L. Koontz[2], Louis B Edano[2], Pin-Chun Chao[1]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration

H1 (food-grade) lubricants are indirect additives used by machinery for manufacturing, processing, packaging, or transport of food. According to 21 CFR 178.3570, the maximum level of H1 lubricants that are permissible in foods is 10 ppm. Although modern equipment has been designed to minimize transfer of lubricants during processing and packaging, incidental food contact can still occur. There is, however, a lack of data for FDA to determine whether safety issues should be addressed concerning the use of food-grade lubricants in the production of foods.

In this project, the transfer of Petrol-Gel lubricant from a hydraulic piston filler into a semi-solid model food was determined at conventional operating conditions. Xanthan gum solutions with concentrations of 2.3% at 25°C and 1.9% at 50°C were used to simulate the viscosity of ketchup at 50°C (970 cP). Analytical methods to quantify aluminum (a tracer compound) in both Petrol-Gel and xanthan gum solutions were successfully developed and validated by using ICP-MS combined with a microwave-assisted acid digestion technique. A total of 1.35 g of Petrol-Gel was applied to four ring gaskets in the filler, and each 50 g sample of xanthan gum solution was collected into a 100-mL plastic tube during 500 filling cycles at 25 and 50°C. Results showed that the concentrations of Petrol-Gel transferred into xanthan gum solutions at each filling cycle ranged from 1.6 to 63.5 ppm at 25°C and from 1.6 to 35.0 ppm at 50°C. In general, the transfer of Petrol-Gel was significantly higher at 50°C than at 25°C. The concentrations of Petrol-Gel in 3 samples at 25°C and 12 samples at 50°C were higher than the current FDA's regulatory limit of 10 ppm. However, the average concentrations of Petrol-Gel in xanthan gum solutions at 25 and 50°C were calculated to be 2.84 and 4.1 μg/g, respectively. At the same operating conditions at 25°C, the total amount of Petrol-Gel transferred into 2.3% xanthan gum solution was higher than that into 50% ethanol (64.5 mg vs. 39.8 mg) reported from the previous study. It can be concluded that the transfer of Petrol-Gel was mainly attributed to physical action between mechanical components and ring gaskets. To obtain a more conservative estimate of dietary

exposure, the project has been extended to evaluate transfer of several low-viscosity H1 lubricants to foods during the operation of a chain-driven conveyor belt at different conditions including lubrication brush, sprayer, the belt speed, and/or the lubricant viscosity. Ultimately, this work will allow FDA to better estimate human exposure to food lubricants and enable updating of the maximum levels of these additives permissible in foods.

Development of a quantum dot-based microfluidic device for the rapid detection of biologically active botulinum neurotoxin in complex media

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

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

In the last year, we have been developing an analogous nanosensor for detection of BoNT serotype F. A biorecognition peptide that is specific for the F serotype has been ordered and conjugation chemistry with 800 nm emitting QDs has been optimized. We have verified that this peptide-QD complex is able to detect the F-type light chain in buffer solution in under 2 hours total detection time and have determined preliminary limits of detection and sensitivity benchmarks, which compare favorably to the mouse bioassay for this toxin serotype. Currently we are optimizing the sensor performance and evaluating critical limit of detection, sensitivity, and selectivity benchmarks, with plans to evaluate its performance on F-type holotoxin and multiplexed (simultaneous) detection of A, B, E, and F serotypes in the next few months.

In addition to the solution-based work, we have begun translating the technology to a microfluidic chip platform. This work is being done in collaboration with FDA's Center for Biologics Evaluation and Research (CBER) and San Jose State University. Polymer substrate has successfully been functionalized with BoNT-selective peptides, and cleavage in the presence of BoNT light chain has been demonstrated. This work was significantly disrupted due to the COVID19 pandemic but work was resumed in the last month.

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

project will be a facile, hand-held technology that can quickly and accurately detect BoNT or other proteolytic food toxins in the field.

Factors affecting the decomposition kinetics of opiate alkaloids in poppy seeds

Shalaka Shetge[1], Daria Kleinmeier[2], Benjamin Redan[2]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration

Some types of poppy seeds that enter the food markets may contain elevated concentrations of alkaloid opiate compounds (also referred to as opium alkaloids). Poppy seeds with such elevated alkaloid levels have been implicated in multiple reports of adverse reactions. To ensure that food-grade poppy seeds are low in opiate alkaloid compounds, heating and other treatments have been recommended to degrade or remove these compounds. However, there are limited data on the effectiveness of potential treatments to reduce levels of alkaloid compounds from poppy seeds. In our previous report, we subjected poppy seeds containing morphine at relatively lower concentrations (<20 mg/kg) to dry heat and steam treatments, water washing, and baking. This project accomplished milestones on this project to assess the effect of these treatments on poppy seeds with relatively higher levels of alkaloid compounds. Poppy seed sample (5 g) containing 210 mg/kg morphine underwent heat treatments at 120, 160, 180 and 200°C over 120 min. Similar to our previous results, dry heat treatments at 160 °C and higher were able to reduce levels of morphine, codeine, and thebaine. Additionally, the half-lives of codeine and morphine at 200°C were approximately 30-40 min. Water washing treatments significantly reduced all alkaloid levels. However, only limited effectiveness was observed with steam application. Baking muffins with incorporated poppy seeds did not reduce concentrations of any alkaloid compound. Information from this research will be useful to both industry and FDA field investigators to evaluate control conditions that spice suppliers use to reduce opiate alkaloids in poppy seeds.

Influence of the environment, polymer structure, and nanoparticle capping agent on the quantity and form of metal ion transport from products manufactured with nanostructured materials

Tianxi Yang[2], Teena Paulose[1], Saloni Shah[1], Benjamin W. Redan[2], James C. Mabon[3], Timothy V. Duncan[2]
[1]Illinois Institute of Technology, IFSH; [2]Food and Drug Administration; [3]University of Illinois UC

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 manufactured and characterized AgNPs with different surface capping agents and incorporated them into AgNP/LDPE test materials. Release of silver from these materials into food simulants under conditions relevant to potential use scenarios (long term room temperature and refrigerated storage) was then evaluated. The impact of different food components on released Ag content and form was also examined. This work resulted in a peer reviewed publication during the last year (Yang et al. *ACS Applied Materials and Interfaces*, **2021**, 13, 1398–1412). An additional two manuscripts are planned in the next 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.

Predictive migration model parameter determination for EVOH copolymers of high and low ethylene content

John L. Koontz, Yoon S. Song, Louis B. Edano
Food and Drug Administration

FDA's safety evaluation of food contact materials is driven by probable exposure assessments based on combining migration data with consumer usage information. Migration models used as tools in calculating potential additive and contaminant migration from food contact materials are designed conservatively for regulatory compliance testing to provide a safety margin for consumers. The generally recognized Piringer diffusion model requires a polymer-specific parameter, A_P, to describe the basic diffusion behavior of a polymer in relation to its migrants. Ethylene vinyl alcohol (EVOH) is a random copolymer of ethylene and vinyl alcohol used as a high-oxygen barrier within multilayer flexible packaging. However, A_P values for EVOH films of any ethylene content are not currently available. The goal of this project is to determine the permeability of small molecular mass surrogate compounds through EVOH using an isostatic permeation method for determination of diffusion coefficients and subsequent calculation of model A_P values.

EVOH monolayer films of two ethylene contents, 44 mol% and 32 mol%, were studied using a homologous series of *n*-alkyl acetates (C1-C4) as surrogate migrants – methyl acetate (74.1 g/mol), ethyl acetate (88.1 g/mol), *n*-propyl acetate (102.1 g/mol), and *n*-butyl acetate (116.2 g/mol). Diffusion coefficients (D_P), permeability coefficients, and solubility coefficients of surrogates were determined by an isostatic permeation method at 90 °C, 100 °C, 110 °C, and 121°C. Diffusivity of *n*-alkyl acetates increased with temperature and was greater in EVOH 44 mol% ethylene than EVOH 32 mol%. Migrant size had a greater effect on diffusion behavior at

90°C than at 121°C for both EVOH grades. Solubility coefficients of migrants were greater in high ethylene 44 mol% EVOH likely due to the copolymer's lower crystallinity.

The higher ethylene content of EVOH 44 mol% allowed increased migrant solubility and mobility within the polymer matrix due to its lower crystallinity, lower T_g, and closer proximity to its T_m relative to EVOH 32 mol%. Polymer-specific modeling constants for EVOH copolymers of high and low ethylene content used in food contact applications have been defined. Mean A'_P values for EVOH 44 mol% and EVOH 32 mol% were defined using four *n*-alkyl acetates as -0.1 and -1.5, respectively. The outcome of this research provided A_P polymer parameters for EVOH copolymers used in migration models to aid consumer exposure assessment in multilayer flexible packaging materials.

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

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

This project completed several milestones, even with the COVID-19 restrictions in place. We worked with the FDA's statistical team at OAO to design our sampling plan for plant-based beverages. In all, we procured over 100 unique samples at local retail markets.

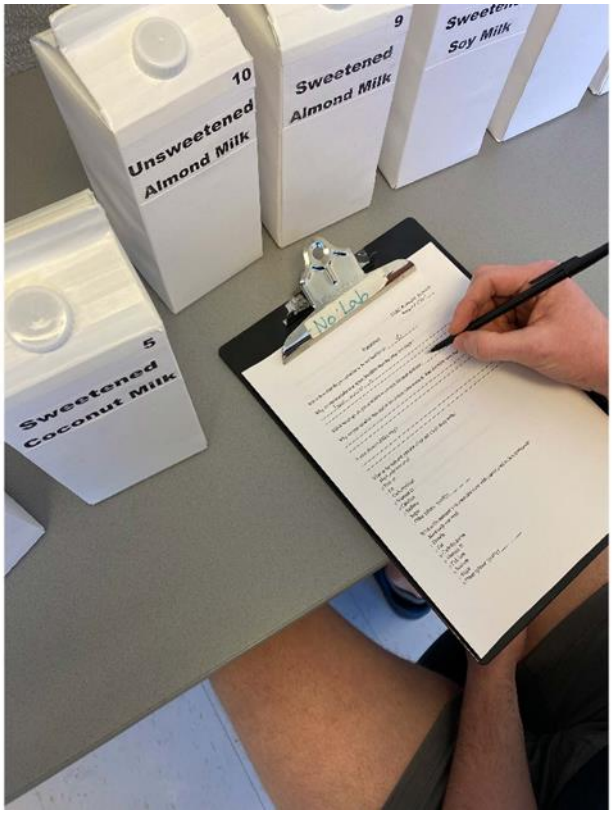
We have completed analysis of the beverage samples for free and total choline using the LC-MS/MS, based off AOAC method 2015.10. These results are being used to produce a draft manuscript. We are currently about mid-way through analysis of the mineral/trace metal analysis for calcium, magnesium, potassium, phosphorous, selenium, and zinc. The ICP-MS method is based off the FDA's elemental analysis manual. NIST milk samples are being used as a reference material to ensure that our system produces results within 10-20% of the certified values. We are validating the vitamin A method on our systems (using AOAC Method 992.06). Additionally, we are in the process of setting up the LC-MS method for quantitation of Vitamins D2 and D3, based off AOAC Method 2016.05. We have identified AOAC Method 2015.14 to use for analysis of B vitamin complex, and will likely adopt this method to additionally include B12 (as cyanocobalamin).

Key processing equipment purchased for this project included a 25 gallon direct drive Likwifier and Atomo 3.0 Homogenizer. Both pieces of equipment have been installed in the GMP pilot plant space and start up training on the homogenizer has been completed. Practice runs using the Bantam 1S HTST with the Atomo 3.0 homogenizer will be conducted to fine tune flow rate, holding time, and homogenizer pressure. Pre-Trial batching will be conducted in the Likwifier to determine the best method for ingredient addition and the sensitivity of temperature control during heating.

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).



Plant-based milk alternative – consumer perspectives

Britt Burton-Freeman, Indika Edirisinghe, Olivia O'Neill, August Neyrey
Illinois Institute of Technology, IFSH

Nutrition labelling aims to provide consumers with the information they need to make informed and healthy choices about the foods they eat. Food labelling is intended to be factual and not misleading. Use of the word "milk" on non-dairy, plant-based milk alternatives, such as soy milk, almond milk, coconut milk, among many more has raised concern that broad associations with the term "milk" may suggest nutritional equivalency between dairy-based milks and plant-based milk alternatives.

Previous research indicates that consumers understand that plant-based milk alternatives are not cow's milk (personal communication, FDA); however, it is not clear if consumers understand the nutritional differences between plant-based milks and dairy milk when choosing to drink them. In a pilot study conducted in our lab previously in a diverse community in on the south side of Chicago, consumers indicated that "nutritional value" was an important consideration when purchasing both dairy milk and plant-based milks, but price and taste were also important considerations when selecting to purchase these beverages. In this same study, consumers indicated protein and calcium were the two nutrients they associated most with plant-based and dairy milks. They also associated fat and Vitamin D with dairy milk and Vitamin D, sugar and sodium with plant-based milks. When asked about the healthiest milk, 49% chose almond milk as the healthiest milk option. The results of the pilot study suggested some confusion in consumer perception and understanding of nutrients in plant-based vs dairy milks, as well as inconsistencies in how consumers are defining "healthy" relative to milk beverages. It is also unclear to what degree environmental factors play a role in consumer's decision to choose plant-based over dairy milks, and if consumers understand the nutritional trade-offs. Therefore, in follow up to the pilot study we have designed a series of experiments to better understand consumer choices when selecting a "milk" beverage to purchase and to consume focusing specifically on nutritional attributes and trade-offs mediating ingestion choices. The study had 100 completers. The main findings are summarized: Taste and nutrients were the top two factors that participants considered when purchasing Dairy milk (DM) or Plant-based milk alternatives (PBMA), which aligned with the results of the pilot study. DM was most associated with Vitamin D and calcium before providing nutritional labels and after label exposure. PBMA was most associated with protein before and after nutrition labels exposure. People generally understand nutrient content differences between PBMA and DM, but confusion remains on the protein content of almond, rice, and coconut milk beverages. Those 35 or older (mainly Gen X, but also Baby Boomers) and people with higher BMIs were more confused about protein content than younger and leaner participants, in that they reported almond, rice and coconut as having high protein content. Confusion regarding sugar content was also apparent, suggesting a misunderstanding between inherent and added sugar. The analysis is mostly complete and a manuscript is being prepared.

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.

APPENDIX

IFSH Publications Calendar Year

Peer-reviewed Publications 2020

1. Bedford, B., Liggans, G., Williams, L., and Jackson, L.S. 2020. Allergen removal and transfer with wiping and cleaning methods used in retail and food service establishments. Journal of Food Protection. 83(7): 1248-1260. doi: https://doi.org/10.4315/JFP-20-025.

2. Daryaei, H., Sui, Q., Liu, H., Rehkopf, A., Peñaloza, W., Rytz, A., Luo, Y., and Wan, J. 2020. Heat resistance of Shiga toxin-producing *Escherichia coli* and potential surrogates in wheat flour at two moisture levels. Food Control. 108. doi: https://doi.org/10.1016/j.foodcont.2019.106788.

3. Durigan, M., Murphy, H., Deng, K., Kmet, M., Lindemann, S., Newkirk, R., Patel, V., Ulaszek, J., Warren, J., Ewing, L., Reddy, R., and Da Silva, A. 2020. Dead-end ultrafiltration for the detection of cyclospora cayetanensis from agricultural water. FDA Bacterial Analytical Manual (BAM), Chapter 19: https://www.fda.gov/media/140309/download.

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6. Hildebrandt, I.M., Marks, B.P., Anderson, N.M., and Grasso-Kelley, E.M. 2020. Reproducibility of *Salmonella* thermal resistance measurements via multi-laboratory isothermal inactivation experiments. Journal of Food Protection. 83(4): 609-614.

7. Koontz, J. L., Liggans, G. L., Redan, B.W. 2020. Temperature and pH affect copper release kinetics from copper metal foil and commercial copperware to food simulants. Food Additives & Contaminants: Part A, 2020. 37(3), 465–477. doi: 10.1080/19440049.2019.1704447.

8. Li, Y., Salazar J.K., He, Y., Desai, P., Porwollik, S., Chu, W., Paola, P.S., Tortorello, M.L., Juarez, O., Feng, H., McClelland, M., and Zhang, W. 2020. Mechanisms of Salmonella attachment and survival on in-shell black peppercorns, almonds, and hazelnuts. Frontiers in Microbiology. 11: 582202. doi: 10.3389/fmicb.2020.582202.

9. Lu, Y., Fu, T.J. 2020. Performance of commercial colorimetric assays for quantitation of total soluble protein in thermally treated milk samples. Food Analytical Methods. 13(6): 1337-1345.

10. Nguyen, A.V., Lee, D., Williams, K.M., Jackson, L.S., Bedford, B., Kwon, J., Scholl, P.F., Khuda, S.E. 2020. Effectiveness of antibody specific for heat-processed milk proteins and incurred calibrants for ELISA-based quantification of milk in dark chocolate matrices. Food Control, 2020:107760. https://www.sciencedirect.com/science/article/pii/S0956713520306769/pdf.

11. Qi Y., He Y., Beuchat L.R., Zhang W., Deng X., 2020. Glove-mediated transfer of Listeria monocytogenes on fresh-cut cantaloupe. *Food Microbiol*. 88:103396, doi: 10.1016/j.fm.2019.103396.

12. Rahman, S., Zasadzinski, L., Zhu, L., Edirisinghe, I., and Burton-Freeman, B. 2020. Assessing consumers' understanding of the term "Natural" on food labeling. Journal of Food Science: 85(6):1891-1896. doi: 10.1111/1750-3841.15128.

13. Redan, B.W., and Jackson, L.S. 2020. Overview of the American Chemical Society symposium on metals and trace elements in food safety, health, and food quality. Journal of Agricultural and Food Chemistry. 68, 46, 12773–12775. doi: https://doi.org/10.1021/acs.jafc.0c01763.

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20. Suehr, Q.J., Chen, F., Anderson, N.A., and Keller, S.E. 2020. Effect of pH on survival of *E. coli* O157, O121 and *Salmonella enterica* during desiccation and short-term storage. Journal of Food Protection. 83: 211-220.

Peer-reviewed Publications 2021 (To date)

1. Fay, M., Salazar, J.K., Ramachandran, P., and Stewart, D. 2021. Microbiomes of commercially-available pine nuts and sesame seeds. *PLoS ONE*. 16(6): e0252605.

2. Fleischman, G.J., Kleinmeier, D., Lunzer, J., and Redan, B.W. 2021. Differences in experimental outcomes from thermal processing: The case of poppy seeds and opium alkaloids. *Journal of Agricultural and Food Chemistry*. 69(27): 7499-7500.

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