2022
Annual Report of Collaborative Research Program

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

2022 IIT IFSH NCFST Annual Report of Collaborative 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, 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.

Enhancing the safety of high pressure processed (HPP) juices

Alvin Lee[1], Catherine Rolfe[1,2], Nathan Anderson[2], Glenn Black[2]
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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.

The purpose was to develop validation guidelines for HPP inactivation and post-HPP recovery of pressure resistant and matrix-adapted *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* in HPP-treated juices. Main objectives included: screen bacterial isolates for barotolerance and select isolates for validation studies, assess the effects of juice matrices and define HPP parameters, determine impact of recovery methods on enrichment procedures, define protocol for shelf-life analysis, and provide framework for guidance document related to HPP-treated juices. Ten strains of each microorganism were prepared in three growth conditions (neutral, cold-adapted, or acid-adapted) and assessed for barotolerance or sensitivity. Cold-adapted cells were prepared at 17°C. Acid-adapted cells were prepared in intermediate pH 5.0 broth. Approximately 6 log CFU/mL of bacterial strains were inoculated into buffer (pH 3.5). Pressure treatment applied at sublethal levels for the initial bacterial screening. Analyses were conducted 0h, 24h and 48h (4°C storage) post-HPP. Pressure resistant and sensitive strains from each (6 strains with neutral, cold-adapted, or acid-adapted growth conditions) were used to evaluate HPP inactivation with increasing pressure levels (200 – 600 MPa) in juice matrices (apple and orange). A 75-day shelf-life analysis was conducted on HPP-treated juices inoculated with acid-adapted resistant strains for each pathogen and examined for inactivation and recovery.

Individual strains of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* demonstrated significant ($p < 0.05$) differences in reduction levels in response to pressure treatment in high acid environments. It was found that *E.* coli O157:H7 was the most barotolerant of the three microorganism in multiple matrices. Bacterial screening resulted in identification of pressure resistant strains *E. coli* O157:H7 TW14359, *Salmonella* Cubana, and *L. monocytogenes* MAD328, and pressure sensitive strains *E. coli* O15:H7 SEA13B88, *S.* Anatum, and *L. monocytogenes* CDC. HPP inactivation in juice matrices (apple and orange, single strength) confirmed acid adaptation as the most conservative preparation compared to neutrally grown and cold-adapted. Shelf-life studies conducted for 75 days in cold storage with mild temperature abuse reached a 5-log reduction in HPP-treated juices immediately following pressure treatment for *L. monocytogenes* MAD328, after 24 h in cold storage for *S.* Cubana, and after 4 days of cold storage for *E. coli* O157:H7 TW14359. Recovery of injured cells was observed for *L. monocytogenes* in orange juice but still maintained a 5-log reduction. These results suggest the preferred inoculum preparation for HPP validation studies is the use of acid-adapted, pressure resistant strains. At 586 - 600 MPa, critical inactivation (5-log reduction) was achieved during

post-HPP cold storage, suggesting sufficient HPP lethality is reached at elevated pressure levels with a subsequent cold holding duration. The shelf-life studies concluded with unexpected results. The microorganism reoccurring during shelf-life studies for orange juice was *L. monocytogenes* which was previously thought to be the most sensitive to pressure application compared to *E. coli* O157:H7 and *Salmonella* spp. Further, modifications to the *E. coli* O157:H7 recovery and enrichment procedures in both apple and orange juice did not prove substantial for sub-lethally injured cells. *Salmonella* results were as predicted with continued loss in viability following pressure treatment and lack of recovery following refrigerated storage.

Limited research has been conducted on the effects of pressure application on membrane permeability and injured cell recovery. The disruption of the cell membrane during HPP treatment may lead to altered response mechanisms against selective agents included for enrichment procedures. Pressure induced cell damage may allow for better recovery of injured cells that have undergone stress adaptations. A thorough understanding of the effects of high pressure on *E. coli* O157:H7 membrane structure and function is necessary to develop proper recovery mediums and procedure. In addition, exploration of how *L. monocytogenes* stress adaptation influences recovery during cold storage conditions would provide beneficial guidance for shelf-life applications. Further genetic studies would be advantageous to conceptualize the gene expression following acid adaptation, pressure treatment, and cold storage, especially in a complex juice matrix.

In relation to guidance development, the information generated from the project is being synthesized into manuscript and guidance document are also being developed with FDA collaborators. Consumer interest in functional beverages and minimal processed juices has led to increasing research of juice products treated with minimal processing. HPP has been shown to provide effective inactivation of pertinent microorganisms in high acid juice products to comply with the FDA Juice HACCP rule in various studies. The current study demonstrated the variability associated with individual strains of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* when treated in high acid food environments.

The results of this research propose barotolerant and acid-adapted strains of juice-associated pathogens should be used in cocktail inoculations for validation studies of HPP-treated juice. A pressure level of 586 MPa achieved a 5-log reduction of *E. coli* O157:H7 after 4 days in cold storage for apple and orange juice, however, data suggests increasing pressure and increasing hold times may provide greater inactivation. Further research on target pressure and hold time combinations in relation to different juice types would be beneficial. Additional assessment of acid-adapted *E. coli* O157:H7 enrichment methodology from high acid juices would provide reassurance of complete inactivation.

The findings of the project was originally scheduled to be tested in a High Pressure Processing and validation workshop at the Institute for Food Safety and Health, IIT in April 2020. This workshop was to give training to industry users and laboratory scientists who conduct studies using HPP, and provide an overview of how challenge studies should be conducted for HPP treated juices. However, the workshop was cancelled due to COVID-19 and will be rescheduled. A presentation session in partnership with the Cold Pressure Council was conducted in September 2021 in Las Vegas. The findings of the project were presented to industry members

so that they understand how to conduct and interpret appropriate challenge studies for their juice products.

Although former research for the project has concluded and manuscripts in preparation for publication, future directions and research activities will be discussed at the annual IFSH Juice and Beverage Task Force meeting and ideas put towards the next research grant.

This research was funded through a USDA grant to IIT IFSH, and is in alignment with CFSAN's 2020-25 Science and Research Strategic Plan by addressing Strategic Goal 1.

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 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 examined the effect of stand-time on temperature distribution across the meat thickness for the standard shapes of slabs, cylinders and spheres. The results showed that adequate equilibration could occur if the thicknesses were small enough. Furthermore, spheres more easily reached equilibration than cylinders, which more easily reached equilibration than slabs. Experimentally, gellan gum gel was used as a meat substitute and cast into slab and cylinder shapes for microwave heating and stand time equilibration observation. Dual infrared cameras were trained on opposite sides of the microwave-heated gels. To prevent evaporative cooling during the stand time, the shapes were wrapped with pieces of common 2mil black trash bag material, cut to fit the shapes exactly with complete surface contact. These bags are only semitransparent to infrared radiation. Nevertheless they allow surface temperatures to be monitored through calibrating the radiation attenuation of the material against a black body heat source of known temperature. It was found that across the surface, temperatures did not sufficiently approach equilibrium in the two minute stand time. Furthermore, longer times recommended elsewhere did not help. The results from the modeling and experimental approaches can be explained by the wavelength of microwaves in the oven and in meat, but holds for food in general. The energy distribution across the surface of the food is determined by the microwave wavelength in the oven, while that in the food by its wavelength there. The difference is inversely proportional to $\sqrt{\varepsilon'}$, where ε' is the dielectric constant of the food. For meat it is approximately 52. With a free space wavelength of 2450MHz microwaves of ~12cm, in the food it is reduced to ~1.7cm. The separation between hot and cold points in the food is proportional to these values. Therefore, it is much more

difficult to bridge the separation in hot and cold points along the length of the food than it is through the thickness of it. Taken together, these results indicate that it is not advisable to use a microwave oven to cook meat from raw.

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 represented by different heat transfer coefficients in the model. Previous results showed that the model, when 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. Experimental studies meant to examine a non-smooth food surface such as that of broccoli had been impeded by COVID-19 restrictions on lab access but are resuming.

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.

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

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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 on *Salmonella* reduction and impact on germination, as affected by H_2O_2 concentration (6, 8, 10%), treatment time (10 min, 1 h) and seed type (alfalfa, clover, broccoli, radish, onion, mung beans). The impact of different treatment conditions on seed germination and sprout yield was also examined. Treatment with 6% H_2O_2 for 10 min reduced *Salmonella* on the six types of seeds by 0.6 - 1.9 log CFU/g. Increasing H_2O_2 concentration from 6% to10% resulted in a similar log kill (0.6 - 2.3 log CFU/g). Extending treatment time to 1 h (at 10% H_2O_2) led to a slight increase in log kill (1.0 - 3.3 CFU/g). H_2O_2 was the most effective on alfalfa seeds, reducing *Salmonella* by 2.5 or 3.3 logs when seeds were treated with 6% or 10% H_2O_2, respectively, for 1 h. Seed germination or sprout yield was not affected under most conditions except treatment with 10% H_2O_2 for 1 h, where germination was reduced for all but mung beans and the yield was lowered by ~ 40% for clover seeds. Overall, except for alfalfa, treatment with H_2O_2 was not able to achieve a > 3-log reduction in *Salmonella* on seeds (the minimum level the Environmental Protection Agency (EPA) will consider registering an antimicrobial).

Research findings will provide the FDA with needed knowledge regarding factors that may affect the efficacy of seed treatment and in reviewing seed treatment processes during sprout operation inspections.

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

Decontamination of sprout seeds by dry heat treatment

Arlette Shazer and Tong-Jen Fu
Food and Drug Administration

The Produce Safety Rule requires sprout growers to treat seeds prior to sprouting. Industrial adoption of chemical treatments by sprout growers has been hindered by the lack of EPA approved seed treatment chemicals. CFSAN is interested in exploring physical methods which do not need an EPA approval. This research evaluated the efficacy of dry heat in reducing *Salmonella* population in artificially inoculated seeds, as affected by treatment time (6, 16, and 24 h), temperature (60, 70 and 80°C), relative humidity (20, 40, 60, and 80%RH), seed type

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

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

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

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

Impact of temperature on pathogen proliferation during sprouting and postharvest storage

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Sprouts pose a particular food safety concern as conditions that promote seed germination also promote pathogen growth. Developing ways to minimize proliferation of pathogens, if present, during sprouting is crucial in the overall approach to reduce public health risks of sprouts. This study examined how sprouting temperature (4, 10, 20, 30°C) may affect pathogen proliferation and how this temperature effect is influenced by pathogen type (*Salmonella*, *E. coli* O157:H7) and seed disinfection (water, 19,000 ppm sodium hypochlorite (NaClO), 2,000 or 20,000 ppm calcium hypochlorite ($Ca(OCl)_2$). Persistence of pathogens during postharvest storage of sprouts as affected by storage temperature (4, 10, 25°C) was also examined. Experiment conducted this year included those for alfalfa seeds inoculated with a high level of *Salmonella* (~ 4 log CFU/g) and treated with water or 2000 ppm $Ca(OCl)_2$ and those for seeds inoculated with a low level of *Salmonella* (~ 1 log CFU/g) and treated with water or 20,000 ppm $Ca(OCl)_2$.

Results obtained from seeds inoculated at the high level were similar to that observed previously, i.e., sprouting at 4°C resulted in a decrease in *Salmonella* population compared with an increase when sprouting was conducted at 10, 20, or 30°C. Treatment with 2,000 ppm $Ca(OCl)_2$ resulted in a reduction in *Salmonella* count in alfalfa seeds by about 1 log. But the pathogen re-grew during sprouting and reached levels similar to those observed in sprouts grown from seeds treated with water. For sprouts germinated at 4°C and then stored at 4°C, the level of *Salmonella* decreased during the 21 days of storage. But when the 4°C sprouts were stored at 25°C, the level

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

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

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.

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

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

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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). Only the addition of peptidoglycan increased the desiccation resistance of *Salmonella*. Therefore, desiccation resistance may be influenced by cell membrane components. To further examine any influence that cell membrane structure may have on desiccation resistance, various Gram-positive and Gram-negative strains were assessed including *Enterococcus*, *L. monocytogenes*, *Bacillus*, *Staphylococcus*, and *Micrococcus* (Gram-positive) and *Salmonella*, *Shigella*, *E. coli*, *Enterobacter*, *Klebsiella*, and *Proteus* (Gram-negative). Results suggest that the Gram-positive

strains are more desiccation resistant than their Gram-negative counterparts. Specifically, log CFU reductions after a 24 h desiccation on membrane filters were 1.17, 0.80, and 0.61 for *Staphylococcus*, *Micrococcus*, and *Bacillus*, respectively, whereas reductions were 4.73, 3.99, and 5.21 for *Shigella*, *Enterobacter*, and *Proteus*, respectively. 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

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Peeled hard-boiled eggs are available in small retail packages and in larger-scale foodservice containers. In the last decade, several recalls associated with *Listeria monocytogenes* have occurred, presumably due to cross-contamination either after peeling or during packaging. Commercially prepared hard-boiled are generally treated in a pH 2.5 citric acid solution (~0.03%) right after peeling for up to 24 h, however the efficacy of this step for reduction of *Listeria* on the eggs or the ability of this acidification to allow *Listeria* survival after cross-contamination is not understood. Initially, the objectives of this study were to 1) determine the reduction 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 when contaminated pre- or post-acid 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 detection on 2/6 eggs treated at 25°C and 0/6 eggs treated at 5°C for pre-contamination experiments. In post-contamination trials, 6/6 eggs were positive after 28-d storage, regardless of treatment temperature.

Due to the inefficacy of pH 2.5 (0.03%) citric acid treatment for reduction of *L. monocytogenes* populations on hard-boiled eggs stored either pre- or post-contamination, additional milestones were added to the project to examine the effect of 1) other organic acids including acetic, malic, and lactic and 2) an increased concentration of citric acid. For these trials, the previously listed objectives for pH 2.5 citric acid were completed using 2% solutions of acids without pH adjustment. *L. monocytogenes* populations inoculated onto hard-boiled eggs at ~8 log CFU/egg and treated for 24 h in 2% organic acids were reduced by 3.15, 3.46, 4.78 and 2.88 log CFU/egg after 5°C treatment and 5.17, 4.17, 4.76, and 2.37 log CFU/egg after 25°C treatment for acetic, lactic, malic, and citric acids, respectively. The 2% acetic, lactic, and malic acid treatments all resulted in significant reductions in *L. monocytogenes* populations on the hard boiled eggs regardless of treatment temperature, however 25°C treatment resulted in faster reductions overall and higher reductions after 24 h for 2% acetic and lactic acid-treated eggs. Storage trials were

completed on eggs which were contaminated either pre-treatment or post-treatment, with the acid treatments done at either 5°C or 25°C prior to refrigeration. Reduction of *L. monocytogenes* presence over the 28-d storage period was markedly better when the eggs were contaminated prior to acid treatment, regardless of the acid used or the temperature of the treatment, however 5°C treatment did result in fewer positive samples than those treated at 25°C. Acetic acid treatment eliminated the *L. monocytogenes* population after the first day of storage at 5°C but eggs treated at 25°C allowed sporadic positives over the storage period. Overall, for egg contamination followed by 5°C treatment, the efficacy of the acids relative to each other followed acetic/malic > citric > lactic. For eggs contaminated prior to 25°C acid treatment, the acid efficacy followed malic > citric > acetic > lactic. *L. monocytogenes* on eggs inoculated acid treatment was best controlled with either 2% citric or acetic acid regardless of treatment temperature, but only citric acid allowed a complete elimination of *L. monocytogenes* detection after 7-d storage.

This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget and supports CFSAN's 2020-2025 Science and Research Strategic Plan by addressing Strategic Goal 1.

Evaluation of viral and bacterial microbiomes of leafy greens and herbs

Joelle Salazar[2], Wei Zhang[1]
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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 understanding of the bacterial and viral communities in these food products. The goal of this study therefore was to assess the microbiomes of various types of leafy greens and herbs. Leafy greens and herbs were acquired from two sources: 1) collected at local farmers markets and 2) acquired from local retail grocers at the point of sale to consumers. Methodology was determined to identify both the surface microbiomes (phyllosphere) and the microbiomes within these plant tissues (endophytes). Thus far, bacterial microbiomes of Romaine lettuce, green leaf lettuce, kale, spinach, cabbage, cilantro, basil, and parsley have been determined. Although differences were observed between farmers market and retail samples, the overall core microbiomes appear similar. The core microbiomes of both leafy greens and herbs were dominated by *Acinetobacter*, *Bacillus*, *Bradyrhizobium*, *Pseudomonas*, and *Staphylococcus*. 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.

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

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Fresh produce is considered ready-to-eat (RTE) foods and are minimally processed prior to retail distribution and consumer consumption. Due to the minimal processing, fresh produce are commonly associated with outbreaks due to foodborne pathogen contamination. In response to the prevalence of outbreaks associated with fresh produce, novel methodologies for the reduction or elimination of bacterial pathogens on these food matrices are needed. Ultrasonication technology is a re-emerging method which can be utilized in the processing of fresh produce. "Power ultrasound" uses frequencies between 20 and 100 kHz and is a non-thermal cost-effective method. Recently, power ultrasound coupled with either chlorine or peroxyacetic acid (PAA) has been shown to reduce *Salmonella* Newport on lettuce, spinach, and grape tomatoes by 1.3-3.6 log CFU/g. Since only a moderate reduction in pathogen population was observed with these two antimicrobials, the aim of the current study is to evaluate the efficacy of power ultrasound technology coupled with different organic acids to reduce *Listeria monocytogenes* and *S. enterica* on select outbreak-associated matrices including romaine lettuce, peaches, and cucumbers. For preliminary studies, power ultrasound was evaluated to reduce both pathogens when dried onto membrane filters. Results suggest that either 20 or 40 kHz treatment for 1 min was capable to reducing both pathogens by 1-2 log CFU/g. Currently, power ultrasound coupled with different organic acids, including malic, citric, lactic, and acetic, are being examined. This project will provide novel information and fills a data gap on a non-thermal processing technology for the reduction of foodborne pathogens on RTE vegetables and fruits.

Evaluation of foodborne pathogen survival on dehydrated and rehydrated enoki and wood ear mushrooms

Joelle Salazar
Food and Drug Administration

Only sporadic foodborne outbreaks associated with mushrooms (fresh, dried, or unspecified) due to contamination with bacterial pathogens have occurred in the U.S. However, in 2020, a multistate outbreak due to *Listeria monocytogenes* contamination of enoki mushrooms occurred, which resulted in a total of 36 illnesses, 31 hospitalizations, and four deaths. The outbreak prompted an Import Alert and subsequent sampling in 2021 of imported mushrooms from South Korea and China. This was the first known outbreak due to *L. monocytogenes* contamination of a fungus. The lack of listeriosis outbreaks is interesting as studies have identified persistent strains of *L. monocytogenes* in mushroom production and processing facilities. Due to this fact and the recent enoki mushroom outbreak, this study aims to understand the survival of *L. monocytogenes* on different mushroom varieties. In addition, *Salmonella enterica* will also be evaluated due to the recent dried wood ear mushroom outbreak. Both foodborne pathogens will be examined on enoki and wood ear mushrooms. Different preparations will also be evaluated including fresh, fresh-cut, dehydrated, and rehydrated. To date, the survival and growth of both pathogens have been

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

Clostridium botulinum challenge study in commercially prepared cold brew coffee

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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. Unlike traditional hot brew, cold brew is prepared by brewing the grounds at ≤25°C for approx. 8 to 36 hrs. Since temp greatly affects the aqueous solubility of compounds, the chemical composition and antimicrobial activity of cold brew extracts likely differs from that of hot brew. Although several studies have been conducted on the antimicrobial activity of hot brew, to our knowledge there are no reports on the inhibitory effect of cold brew on the growth of *Clostridium botulinum*. FDA needs the data to evaluate the filings submitted by the producers.

Five type A (69-A, CAM2-A, Clovis-A, Giorgio-A, CDC-CR1-A) and 5 proteolytic type B (Mush3-B, 6891-B, TJ-980B, NCA1-B, 7273-B) strains of *Clostridium botulinum* were selected and enumerated for use in the 10- strain spore cocktail in the challenge study of cold brew coffee. A survey of 24 commercially available cold brew coffee products (black coffee) was conducted. These 24 products were evaluated for pH, water activity, Brix, titratable acidity, and total dissolved solids. Five products were chosen for a challenge study based on high pH or low Brix. A 5-month challenge study of 5 different commercially available cold brew coffee products (Five additional products were made by diluting the 5 products with water, total dissolved solids between 0.48-0.69%) with a 10-strain spore cocktail of *C. botulinum*. Coffee samples were inoculated with 3 log spores/ml with the 10-strain cocktail. The coffee products were sampled at time 0, 1-month, 3-months, and 5-months. Water activity, pH, Brix, spore enumeration (MPN), aerobic plate count, anaerobic plate count, lactic acid bacteria, yeast and mold, and presence of botulinum toxin (Endopep-MS) were monitored for each sample time-point.

The initial challenge study was designed to last 9-months, however, the anaerobic jars for 7 and 9-months leaked, and a compound formed in the coffee that inactivated all *C. botulinum* spores before they were able to grow and produce toxin. One of the coffee products tested (pH 6.58, Brix 2.3, TDS 1.89%) produced *C. botulinum* toxin Type A and Type B at the 3 and 5-month sampling points. The diluted version of this coffee (pH 6.71, Brix 0.8, TDS 0.64%) also produced Type A and Type B toxin at 3 and 5-month sampling points. All other coffee tested did

not produce toxin during the duration of the challenge study. The coffee that produced toxin is a shelf stable product that has been thermally processed. This coffee contained added potassium phosphates. The *C. botulinum* spore population increased from 3.2 log MPN/ml at Time 0 to 4.2, and 4.13 log MPN/ml for 1-month and 3-months, respectively, before dropping to 2.81 log MPN/ml at 5-months. This suggests limited *C. botulinum* growth produced toxin at 3 and 5-months. Three additional lots of this coffee were purchased and inoculated with the 10-strain spore cocktail to confirm the initial results. All 3 lots tested produced botulinum toxin Type A and Type B at 5 and 9-months with no observed increase in the MPN/ml concentration of *C. botulinum*.

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

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

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

Peanut protein concentrations measured in discrete flour samples were overestimated compared to the amount of peanut added into wheat lots. In contrast, measured total soy protein levels were underestimated compared to the amount of soy added into wheat kernel lots. The measured protein values by ELISA may be over/under-estimated due to a variety of factors including differences in ELISA kit standards/calibrants, protein extraction method, assay antibodies, food matrix, form and state of the allergen of interest (e.g., raw, blanched, roasted).

The Vt, Vs, and Va increased with an increase in P and S concentration, and their regression analyses showed a linear best fit on a log-log scale. For both peanut and soy, Vs>Va and over 95% of Vt resulted from Vs. The Vt=Vs+Va was estimated from the equations:

$$\text{Peanut: } Vs=(5/Ns)2.4235\ P^{1.5926} \text{ and } Va=(1/Na)0.0653\ P^{1.6033}$$

$$\text{Soy: } Vs=(1/Ns)1.1596\ S^{1.6204} \text{ and } Va=(1/Na)0.0046\ S^{1.8594}$$

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

This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget and supports CFSAN's 2020-25 Science and Research Strategic Plan by addressing Strategic Goal 1. The project was also funded by the Office of the Chief Scientist Challenge Grant Program.

Current assessment of food-grade lubricant transfer into foods

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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 the last year, we have determined the transfer of Petrol-Gel lubricant from a hydraulic piston filler into a semi-solid model food at conventional operating conditions. 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. 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, which were lower than the current FDA's regulatory limit of 10 ppm. We have concluded that the transfer of Petrol-Gel was mainly attributed to physical action between mechanical components and ring gaskets.

In addition to a hydraulic piston filler, we have evaluated transfer of low-viscosity H1 lubricants from chain sprayers during the operation of a chain-driven conveyor belt, which often occurs in the baking industry. We have successfully determined the operation parameters at worst-case scenario conditions: conveyor belt speed (15ft/ min. at low), lubricant (polyalphaolefin; viscosity grade 32), and sprayer (0.5s on-time; 2.0s off-time; 0.95-cm nozzle height from the chain; 10 psi. pressure). The image analysis using Matlab™ showed that over 90% of the lubricant droplets splashed to the surface of the conveyor belt were located within 0.5 cm from the edge of the chain. The greatest distance from the chain at which lubricant droplets were observed was 2.0 cm. The current study extends to evaluate how the brush-type chain lubricator can affect the splash pattern on the conveyor belt system. Ultimately, this work will allow FDA to better estimate human exposure to food lubricants and enable updating 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

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

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

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

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

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

Factors affecting the decomposition kinetics of opiate alkaloids in poppy seeds

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Some poppy seeds that enter the food markets may be from poppy cultivars with elevated concentrations of opiate alkaloids (for example, morphine and codeine), perhaps intended for pharmaceutical purposes. There is a need to determine the effect of thermal and water washing treatments on reducing the levels of opiate alkaloids from poppy seeds, especially if the seeds are from sources typically used for pharmaceutical purposes. A second goal of this project was to determine the fate of opiate alkaloids from poppy seeds during baking. This study examined how the major opiate alkaloids present in poppy seeds (morphine, codeine, and thebaine) respond to thermal treatments. After characterizing the opiate alkaloids in a sample of poppy seeds (n=15), two types of seeds were selected for further experiments. Opiate alkaloids from poppy seeds were extracted using a procedure adapted from a previously reported method. After an initial screening, poppy seeds containing relatively lower and higher concentrations of morphine were selected for further experiments. Poppy seeds were treated up to 120 min at 120-200 °C. For steam treatments, poppy seeds were placed on a stainless-steel wire fine mesh screen that was positioned over a glass beaker containing boiling water for 30 min. For the washing experiments, poppy seeds were mixed with room temperature water for 5 min. In order to determine the effect of baking on opiate alkaloids, a muffin was selected as a model baked product. Batter for one muffin was prepared and then poppy seeds were either incorporated into the batter or applied to the surface of the batter. The muffins were baked for 16 min at 200 °C. An ultraperformance liquid chromatography−tandem mass spectrometry (UPLC−MS/MS) method was used to quantify the opiate alkaloids in the samples.

Morphine in poppy seed samples ranged from 3.6-261 ppm, codeine from 1.9-378 ppm, and thebaine from 8.1-217 ppm. Two poppy seed samples were selected to undergo further experiments to model opiate alkaloid degradation in samples with relatively low (14.7 ± 4.2 ppm; S-6)) and high (210.0 ± 19.6 ppm; S-13) morphine concentrations. At 200 °C, thebaine exhibited the shortest half-life of approximately 3 min, while that of codeine and morphine was 32−39 min across both samples. The steam treatment significantly ($P < 0.05$) decreased morphine concentrations in one sample type by 21%. A 5 min water-washing treatment resulted in a significant ($P < 0.05$) reduction in opiate alkaloid levels in both poppy seed sample types. For sample S-6, morphine, codeine, and thebaine were reduced by approximately 80, 75, and 60%, respectively, after the wash treatment; for S-13, morphine, codeine, and thebaine were reduced by 79, 69, and 46%, respectively. Opiate alkaloids were found to be stable after undergoing thermal treatment in a baked product. After 16 min of baking at 200 °C, there were no significant ($P > 0.05$) differences in opiate alkaloid concentrations when either sample S-6 or S-13 was incorporated into the model baked product. Similarly, no reduction in opiate alkaloid concentrations was observed for poppy seeds applied to the surface of the muffin.

The results from the heat treatment experiments indicated that the three major opiate alkaloids are relatively stable. At 200 °C, the half-life of morphine and codeine was found to be approximately 32−39 min. Use of elevated heat treatments may not be useful for industry because it can affect the flavor of the poppy seeds. The experiments evaluating the impact of

baking on opiate alkaloid concentrations in poppy seed muffins showed no significant effects, which indicates the importance of monitoring opiate alkaloids in seeds provided by spice suppliers. Future work may have to take these limitations into account to develop mitigation techniques relevant to industry. Because limited degradation was found after baking, this suggests that mitigation of opiate alkaloids by spice suppliers may be important for reduction of these compounds in the food supply. Information obtained from this research will be useful to industry and governmental bodies to help evaluate control conditions that spice suppliers use to reduce opiate alkaloids in poppy seeds supplied to the food industry.

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

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

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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 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 last year (Yang et al. ACS Applied Materials and Interfaces, 2021, 13, 1398–1412), and two additional manuscripts currently under review.

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.

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

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

AOAC method 2012.10 was used to analyze PBMA samples for vitamin A (as retinyl palmitate) using HPLC-DAD. The results revealed that the highest mean vitamin A amounts were in coconut beverages (93.2 µg vitamin A equivalents/100 g portion), while the lowest amounts were in cashew beverages (27.2 µg vitamin A equivalents/100 g portion). AOAC method 2016.05 was used for the analysis of vitamins D2 and D3 in PBMA samples using LC-MS/MS. Mean vitamin D levels were highest in rice beverages (4.33 µg vitamin D2/100 g portion) and lowest in cashew beverages (0.73 µg vitamin D2/100 g portion). FDA EAM 4.7 was adapted for analysis of key elements in the PBMA samples, including calcium, potassium, phosphorous, magnesium, and zinc. Results for calcium showed that the highest mean value was in coconut beverages (180 mg Ca/100 g portion), while the lowest levels of calcium was found in rice beverages (42.9 mg Ca/100 g portion). For comparison, amounts of vitamin A, vitamin D3, and calcium in fluid bovine milk were 33.7 µg/100 g, 2.08 µg/100 g, and 105.8 mg/100 g, respectively. There was variation in analyzed micronutrients across type and brand. AOAC method 2015.14 is currently being used to analyze samples for B vitamin complex.

Processing equipment has been purchased and installed in the IFSH GMP pilot plant space. Practice runs using the Micro Thermics Bantam 1S HTST with the Atomo 3.0 homogenizer were conducted to fine tune flow rate, holding time, and homogenizer pressure. Pre-trial batching was conducted in the Likwifier to determine the best method for ingredient addition and the sensitivity of temperature control during heating. We have obtained a vitamin/mineral premix from an ingredient supplier for use in our trials. We are currently conducting experimental trials to process a model almond beverage with added micronutrient premix. The model almond beverage will be analyzed for various micronutrients using the above-described methods.

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

Assessment of undeclared allergens in dark chocolate products

Lauren Jackson
Food and Drug Administration

Undeclared milk is responsible for more than one third of all food product recalls in the U.S. over the past decade due to allergen-related causes. Milk is also a leading cause of consumer adverse reactions to foods recalled due to undeclared allergens, and a high percentage of adverse reactions have been associated with dark chocolate products. A limited survey conducted by FDA in 2013-2014 found that 75% of dark chocolate products with a milk advisory statement had milk concentrations above 2.5 µg/g, with the majority containing greater than 1,000 µg/g milk. Since many chocolate products formulated without milk are manufactured on shared equipment with products containing milk, cross-contact is a likely explanation for the presence of milk residues in the products evaluated in this survey. The Food Safety Modernization Act (FSMA) requires that food facilities implement controls to prevent allergen cross-contact when allergen hazards are likely to occur. As the original FDA survey was conducted prior to FSMA implementation, current information is needed on the prevalence undeclared of undeclared milk in dark chocolate products and whether these products contain potentially hazardous levels of milk. The main goals of this project are to 1) survey dark chocolate products without milk or milk-based ingredients in the ingredient list for the presence and concentrations of milk, 2) evaluate lot-to-lot differences in the milk concentrations for the surveyed dark chocolate products, and 3) compare the levels of milk in the products as measured with ELISA kits to the xMAP Food Allergen Detection Assay (FADA), and 4) use the xMAP FADA to determine the presence and concentrations of other undeclared allergens in the dark chocolate products.

In the past year, 198 dark chocolate samples (bars, chips, nuggets) without milk or milk-based ingredients listed in the ingredient list or in a "contains" statement were obtained from retail stores in the Chicago area or Washington, DC area or on-line. These included chocolates with an advisory statement for milk, a milk-free or dairy-free statement, or no statement regarding milk on the label. These samples represented 42 unique dark chocolate products with four to five different lots obtained for each product. Each sample (100 – 150 g) was homogenized using a food processor. Preliminary qualitative screening of chocolate samples for milk and peanut was accomplished using the Neogen Veratox® for Total Milk ELISA kit. Chocolate samples testing positive for milk (i.e. ≥ 2.5 µg/g) with the qualitative method were evaluated further for milk concentrations using the Neogen Veratox® for Total Milk ELISA. The Morinaga Milk Protein (Casein) ELISA assay was used as a confirmatory test for samples having a positive response with the Neogen Veratox® Milk ELISA kit. Each sample was analyzed in triplicate, and Pacari dark chocolate samples spiked with nonfat dry milk (NFDM) at 5 µg/g and 25 µg/g, and 50 µg/g were used as reference samples to estimate milk recoveries for each test kit.

Of the 198 chocolate samples analyzed for milk, 46% contained levels > 2.5 µg/g milk (as NFDM), and

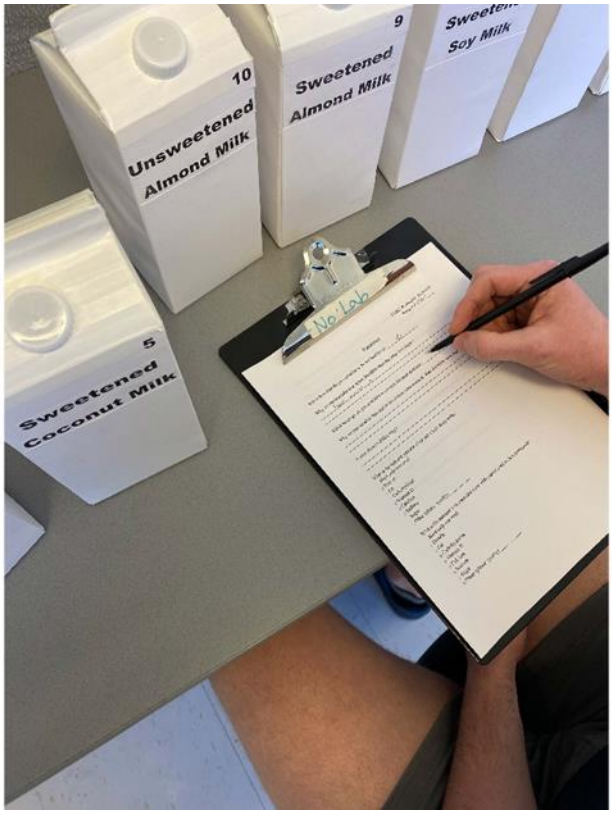
all of positive samples had an advisory statement for the presence of milk on the label. Of the samples that had an advisory statement for milk, 56% tested positive for milk. Twenty percent of all samples tested contained milk at concentrations >1000 µg/g. Concentrations of milk were generally inconsistent across lots of the same product. The type or verbiage used in the advisory statement (i.e. "may contains milk", "may contain traces of milk", "made in a shared facility", etc.) did not correlate with the concentrations of milk found in the chocolate samples. None of the samples that a milk-free, dairy-free or no statement regarding milk contained detectible concentrations of milk. Work is currently underway to quantify milk and other allergens in a select number of products using the xMAP FADA.

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

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 (39.5±5.7 years, 58% > $40K household income, no allergies or intolerances to Dairy milk, DM) and aimed to better understand consumer preferences and motivation for milk choice, perceptions of nutritional differences between milks, whether perceptions change after viewing food labels, and if real-time consumption influences choice. Results of this research indicated Taste and nutrients were the primary and secondary factors for choosing DM and Plant-based milk alternative (PBMA), followed by price and ingredients, respectively. Calcium and protein were most associated with DM and PBMA, respectively. Pre- and post- review of carton Nutrition Facts labels revealed >3-fold increase in consumers associating calcium with almond milk. Added sugar was most associated with coconut milk pre-test; however, more participants associated added sugar with DM post-test (29% compared to 14%) after NF labels, despite zero added sugar. Routine was the primary reason for choosing DM whereas sensory and curiosity were the primary reasons for choosing PBMA. Environmental and ethical concerns were not primary drivers of choice. Overall, exposure to Nutrition Facts panels had limited influence in correcting misconceptions of protein content and revealed confusion about sugar/added sugar related to DM. Consumers also did not differentiate content of vitamin D or calcium in DM and PBMA but seemed to understand higher and lower calorie content of the milks. Consumers are not well informed about the nutritional differences of DM and PBMA.

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

Proficiency Testing Programs

Ravinder Reddy, FDA and Jason Wan, IIT IFSH

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

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

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 Peer-Reviewed Publications Calendar Year 2022

1. Ahmad, N.H., Hildebrandt, I.M., Pickens, S.R., Vasquez, S., Jin, Y., Liu, S., Halik, L.A., Tsai, H-C., Lau, S.K., D'Souza, R.C., Kumar, S., Subbiah, J., Thippareddi, H., Zhu, M-J., Tang, J., Anderson, N.M., Grasso-Kelley, E.M., Ryser, E.T., Marks, B.P. **2022**. Interlaboratory evaluation of *Enterococcus faecium* NRRL B-2354 as a *Salmonella* surrogate for validating thermal treatment of multiple low-moisture foods. *J Food Prot*. 85(11):1538-1552. https://doi.org/10.4315/JFP-22-054 CARTS 1344

2. Andrés-Hernández, L., Blumberg, K., Walls, R.L., Dooley, D., Mauleon, R., Lange, M., Weber, M., Chan, L., Malik, A., Møller, A., Ireland, J., Segovia, L., Zhang, X., Burton-Freeman, B., Magelli, P., Schriever, A., Forester, S.M., Liu, L., King, G.J. 2022. Establishing a common nutritional vocabulary - from food production to diet. *Front Nutr*. 21(9):928837. https://doi:10.3389/fnut.2022.928837. eCollection 2022.PMID: 35811979 Free PMC article.

3. Berry, S.C., Triplett, O.A., Yu, L.-R., Hart, M.E., Jackson, L.S., Tolleson, W.H. **2022**. Microcalorimetric investigations of reversible staphylococcal enterotoxin unfolding. *Toxins*. 2022, 14(8):554. https://doi.org/10.3390/toxins14080554

4. Carstens, C.K., Salazar, J.K., Sharma, S.V., Chan, W., Darkhoh, C. **2022**. Food safety attitudes, behaviors, and hygiene measures among predominantly low-income parents in houston, texas. *J. Food Prot*. 85(12):1745-1755. https://doi.org/10.4315/JFP-22-179

5. Carstens, C.K., Salazar, J.K., Sharma, S.V., Chan, W., Darkhoh, C. **2022**. Evaluation of the kitchen microbiome and food safety behaviors of predominantly low-income families. *Front. Microbiol*. 13:987925. https://doi.org/10.3389/fmicb.2022.987925 CARTS N/A

6. Davies, C.P., Thomas J., Julie H., Elizabeth R., Anderson, N.M., Grasso-Kelley, E., Hoffmann, M., Zheng, J. **2022**. Changes in the genomes and methylomes of three *Salmonella enterica* serovars after long-term storage in ground black pepper. *Front Microbiol*. 13:970135. https://doi:10.3389/fmicb.2022.970135 CARTS 1151

7. Duncan, T.V., Bajaj, A., Sharma, A., Gray, P.J., Weiner, R.G., Pillai, K.V. **2022**. Sulfides mediate the migration of nanoparticle mass out of nanocomposite plastics and into aqueous environments. *Nano. Impact*. 28:100426.

8. Duncan, T.V., Bajaj, A., Gray, P. **2022.** Surface defects and particle size determine transport of CdSe quantum dots out of plastics and into the environment. *J. Hazard. Mater.* 439:129687.

9. Fleischman, G. J., Yang, R., Shazer, A., Li, H. **2022.** Experimentally implementing the linear non-isothermal equation for simultaneously obtaining D and z values of *Salmonella* Senftenberg in skim milk using a differential scanning calorimeter. *J. Food Prot.* 85(10), pp.1410-1417. https://doi.org/10.4315/JFP-22-009

10. Fu, T.-J., Maks, N., Shazer, A.G., Chrysogelos, C. **2022**. Comparison of commercial test kits for detection of *Salmonella* and *E. coli* O157 in alfalfa spent sprout irrigation water. *J. AOAC Int*. 105(4):L1092. https://doi.org/10.1093/jaoacint/qsac008 CARTS 1482

11. Ho, K.K., Redan, B.W. **2022**. Impact of thermal processing on the nutrients, phytochemicals, and metal contaminants in edible algae. *Crit. Rev. Food Sci. Nutr*. 62:508. https://doi.org/10.1080/10408398.2020.1821598

12. Huang, Y., Tsai, M.F., Thorat, R.S., Xiao, D., Zhang, X., Sandhu, A.K., Edirisinghe, I., Burton-Freeman, B.M. **2022**. Endothelial function and postprandial glucose control in response to test-meals containing herbs and spices in adults with overweight/obesity. *Front. Nutr*. 9:811433. http://doi:10.3389/fnut.2022.811433. PMID: 35273988; PMCID: PMC8902252.

13. Huang, Y., Edirisinghe, I., Burton-Freeman, B., Sandhu, A.K. **2022**. Pharmacokinetic evaluation of phytochemicals from selected herbs and spices in human plasma samples. American Society for Nutrition (ASN) meeting, Live-Online. *Curr. Dev. Nutr.*, (Suppl 1):288. Published online 2022 http://doi:10.1093/cdn/nzac053.029

14. Imanian, B., Donaghy, J., Jackson, T., Gummalla, S., Ganesan, B., Baker, R.C., Henderson, M., Butler, E.K., Hong, Y., Ring, B., Thorp, C., Khaksar, R., Samadpour, M., Lawless, K.A., MacLaren-Lee, I., Carleton, H.A., Tian, R., Zhang, W., Wan, J. **2022**. The power, potential, benefits, and challenges of implementing high-throughput sequencing in food safety systems. *NPJ Sci. Food*. 6(1):35.

15. Jackson, L.S., Al-Taher, F. **2022**. Chapter 13 - Processing issues: acrylamide, furan, and trans fatty acids. Ensuring Global Food Safety: Exploring Global Harmonization, 2nd Edition. A. Martinovic, S. Oh, and H. Lelieveld (Eds), Academic Press, London.

16. Jiao, H., Xu, W., Hu, Y., Tian, R., Wang, Z. **2022**. Citric Acid in Rice Root Exudates Enhanced the colonization and plant growth-promoting ability of *Bacillus altitudinis* LZP02. *Microbiol. Spectr*. 10(6):e01002.

17. Jiao, H., Xu, W., Hu, Y., Tian, R., Wang, Z. **2022**. Complete genome sequence data of *Bacillus altitudinis* LZPO2, a bacterium from the rice rhizosphere, for studying the promotion of plant growth. *Mol. Plant Microbe Interact*. 35(5):428.

18. Low, M, Robert S., Tang, J., Grasso-Kelley, E., Feng, Y. **2022**. Food handling practices for apple drying in home kitchens in the United States: a survey. *J. Food Prot*. 85(10):1418. https://doi.org/10.4315/JFP-22-106

19. Luo, S., Wang, H., Wang, Z., Xu, W., Xu, Tian, R., Zhou, J. **2022**. Internalization of myriocin involved in energy and affected expression of genes and proteins in the endocytosis pathway in *Fusarium oxysporum* f. sp. Niveum. *Biotechnol. Biotechnol. Equip*. 36(1):520.

20. Redan, B.W., Morrissey, T.R., Rolfe, C.A., Aguilar, V.L., Skinner, G.E., Reddy, N.R. **2022**. Rapid detection and quantitation of dipicolonic acid release from *Clostridium botulinum* spores using mixed-mode liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem*. 414(8):2767. https://doi.org/10.1007/s00216-022-03926-7

21. Salazar, J.K., Fay, M.L., Qi, Y., Liggans, G. **2022**. Growth kinetics *of Listeria monocytogenes* on cut red cabbage. *J. Food Prot*. 85(8):1128. https://doi.org/10.4315/JFP-22-072

22. Salazar, J.K., Tesfaldet, B., Zamperlini, M., Streufert, R., Fay, M.L., Keller, S.E. **2022**. Desiccation survival of *Salmonella enterica*, *Escherichia coli*, and *Enterococcus faecium* related to initial cell level and cellular components. *J. Food Prot*. 85(3):398. https://doi.org/10.4315/JFP-21-320

23. Shetge, S.A., Redan, B.W. **2022**. Effect of dry heat thermal treatments and baking on concentrations of the opium alkaloid noscapine in poppy seeds. *ACS Food Sci. Technol*. 2:541. https://doi.org/10.1021/acsfoodscitech.1c00428

24. Tian, R., Imanian, B. **2022**. ASAP 2: a pipeline and web server to analyze marker gene amplicon sequencing data automatically and consistently. *BMC Bioinformatics*. 23(1):27.

25. Tian, R., Widel, M., Imanian, B. **2022**. The light chain domain and especially the c-terminus of receptor-binding domain of the botulinum neurotoxin (BoNT) are the hotspots for amino acid variability and toxin type diversity. *Genes (Basel)*. 13(10):1915.

26. Yang, R., Fleischman, G., Shazer, A., Li, H. **2022**. Experimentally implementing the linear non-isothermal equation for simultaneously obtaining d and z values of *Salmonella* Senftenberg in skim milk using differential scanning calorimeter. *J. Food Prot*. 85(10):1410. https://doi.org/10.4315/JFP-22-009

27. Zhang, X., Zhao, A., Sandhu, A.K., Edirisinghe, I., Burton-Freeman, B.M. **2022**. Red raspberry and fructo-oligosaccharide supplementation, metabolic biomarkers, and the gut

microbiota in adults with prediabetes: a randomized crossover clinical trial, *The Journal of Nutrition*. 152(6):1438. https://doi.org/10.1093/jn/nxac037. PMID: 35421233.

28. Zhang, X., Xiao, D., Guzman, G., Edirisinghe, I., Burton-Freeman, B. **2022**. Avocado consumption for 12 weeks and cardiometabolic risk factors: a randomized controlled trial in adults with overweight or obesity and insulin resistance. *J. Nutr*. 152(8):1851. https://doi.org/10.1093/jn/nxac126. PMID: 35700149; PMCID: PMC9486596.