U.S. FOOD & DRUG ADMINISTRATION

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CENTER FOR FOOD SAFETY AND APPLIED NUTRITION

CENTERS OF EXCELLENCE ANNUAL REPORT

Western Center for Food Safety WCFS UCDAVIS

Joint Institute for Food Safety and Applied Nutrition UMD FDA

1848 THE UNIVERSITY OF MISSISSIPPI National Center for Natural Products Research

IFSH INSTITUTE FOR FOOD SAFETY AND HEALTH

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Introduction

The Center for Food Safety and Applied Nutrition, known as CFSAN, is one of six product-oriented centers, in addition to a nationwide field force, that carry out the mission of the Food and Drug Administration (FDA). FDA is a scientific regulatory agency responsible for the safety of the nation's domestically produced and imported foods, cosmetics, drugs, biologics, medical devices, and radiological products. CFSAN has the responsibility for ensuring that the United States food supply is safe, secure, sanitary, and properly labeled, as well as ensuring the safety and proper labeling of dietary supplements and cosmetic products. To help accomplish these goals, CFSAN recognizes the value of fostering collaborations with external partners to leverage research and regulatory resources in support of our science and capacity building activities. These partnerships assist the FDA in fulfilling its public health mission and in expanding the science base upon which future regulatory programs are developed.

CFSAN's Centers of Excellence (COE) program is one of several approaches CFSAN uses to collaborate with external partners to fulfill its public health mission. The COE program consists of cooperative agreements with four academic institutions and provides opportunities to build diversified channels for infusing innovative ideas and knowledge, encourages dialogue among government, academia and industry, and develops novel approaches to solve complex food safety issues. COEs also partner and collaborate with other domestic and international organizations to conduct food safety research and capacity building. This collaboration leverages CFSAN's resources and enhances our ability to ensure public health. It also allows CFSAN to reach a larger portion of the global food safety community. CFSAN currently supports four COEs: 1) the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) at the University of Maryland, College Park; 2) the Western Center for Food Safety (WCFS) at the University of California, Davis; 3) the National Center for Natural Products Research (NCNPR) at the University of Mississippi; and 4) the Institute for Food Safety and Health (IFSH)/National Center for Food Safety and Technology (NCFST) at the Illinois Institute of Technology.

This report highlights selected research and capacity building efforts conducted by the COEs during the cooperative agreement budget period of September 1, 2019 to August 31 2020. Unless otherwise noted, all activities in this document were funded by CFSAN through cooperative agreements with the COEs.

Joint Institute for Food Safety and Applied Nutrition (JIFSAN) - University of Maryland, College Park

The Joint Institute for Food Safety and Applied Nutrition (JIFSAN) was established in 1996 at the University of Maryland, College Park. The Institute is a jointly administered, multidisciplinary research, education and outreach program. The research program includes: genome sequencing and genomic analysis, bioinformatics, foodborne pathogens, development of training metrics, and risk assessment modeling. Additionally, JIFSAN's undergraduate internship program supports the science and research programs at CFSAN. JIFSAN's education and outreach programs serve the FDA internally, domestically, and internationally. The International Training Center is a train-the-trainer program and includes Food Safety Preventive Controls Alliance (FSPCA) and Produce Safety Alliance (PSA) training and coordinates the international implementation of the PSA training program through the Produce International Partnership for Education and Outreach (PIP). It also provides training on Good Agricultural Practices (GAP), Good Aquacultural Practices (GAqP), Good Fishery Vessel Practices (GFvP), Commercially Sterile Packaged Food (CSPF), Food Inspector Training Course (FIT), and Collaborative Food Safety Training Centers. The Food Safety Risk Analysis Professional Development Program provides courses that focus on risk assessment methods and analysis to address food safety issues worldwide, and hosts FoodRisk.org that offers online resources for food safety risk analysis.

JIFSAN Director – Dr. Jianghong Meng
CFSAN Project Officer – Dr. Chad P. Nelson

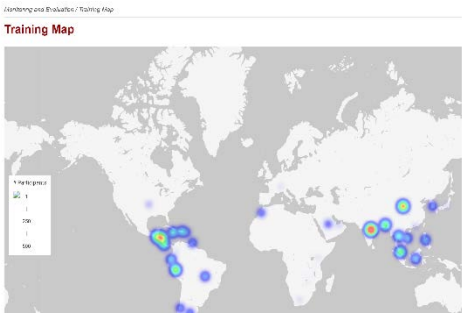
Research

Salmonella enterica and *Listeria* in Surface Water

Whole genome sequencing (WGS) has been broadly used to provide detailed characterization of foodborne pathogens. Applications in food safety using WGS approaches include outbreak detection and characterization, source tracking, determining the root cause of a contamination event, profiling of virulence and pathogenicity attributes, antimicrobial resistance monitoring, quality assurance for microbiology testing, and many others. FDA established the GenomeTrakr network in 2013 with the goal to build a global one health WGS database where human pathogens are rapidly characterized and linked to closely related food and environmental isolates for the rapid investigation of illnesses/outbreaks. A pilot program to engage international partners for isolating and sequencing

Salmonella from surface water was initiated with university collaborators from four Latin American countries (Argentina, Mexico, Brazil, and Chile) in 2018. The sampling collection began in March 2019. The two universities in Chile have partnered and obtained 480 samples in 16 visits to four rivers in Chile. The isolation rates of *Salmonella* ranged from 16% to 53%, with an average of 38%. A 300% increase in the isolation rate was detected in Maule rivers in warm months compared to cold months. The National Autonomous University of Mexico processed 324 surface water samples from 86 different sampling points (rivers, dams, ponds, lakes, and irrigation canals) from May of 2019 to October 2019. Out of these samples, 254 were *Salmonella* positive. Additional 58 samples were collected from January 2020 to February 2020 across 33 different sampling points. The overall prevalence of *Salmonella* was 78%. A total of 596 isolates of *Salmonella* enterica and 120 isolates of *Listeria* from water samples in Chile have been sequenced. Serotype and antimicrobial resistance (AMR) of each isolate were predicted based on the WGS data. There were 142 antimicrobial-resistant *S. enterica* isolates. Among 171 *Salmonella* isolates from water samples in Mexico, 137 of which were predicted to be antimicrobial-resistant. This study provided a comprehensive analysis of *Salmonella* in Latin American surface waters associated with produce production. The data will aid in the expansion of the global WGS database, further validate environmental sampling and analysis methods, assess the distribution and subtypes of *Salmonella* in these waters, and provide insight into the proficiencies and barriers faced by other nations in these efforts.

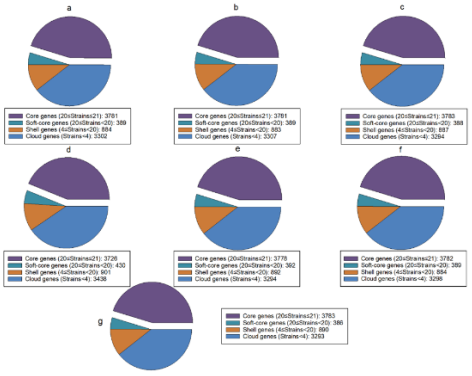
Monitoring and Impact Evaluation of JIFSAN's Programs



In order to measure the impact of our training programs, JIFSAN has developed monitoring and evaluation survey tools, implemented surveys, and a database for JIFSAN's training programs. The database of JIFSAN's historical trainings has been maintained and a heat map created showing all trainings since the beginning of the monitoring and impact program in 2013. In 2020, two online surveys were developed and conducted for JIFSAN's International Produce Safety Programs. The surveys for grower training in Latin America have been deployed for courses conducted via an online survey tool. Summary data have been shared with instructors to help identify knowledge gaps and participants' learning experience to improve the training courses. A second survey is under development and will be deployed to lead trainers who have previously conducted trainings. The goal is to understand the impacts of JIFSAN's trainings through analyzing teaching effectiveness and determining the number of growers who have been trained by these lead trainers. A metrics database and web application for all JIFSAN trainings have been developed, where the web application is used to add new information to the database and to edit/view existing information. The web application can be found at https://jifsan.umd.edu/metrics. Historical data are updated and made compatible with both the new database structure and the data upload feature of the web application, where users can upload a spreadsheet of training data for automated entry. A web-based heatmap showing locations of origin of training participants has been completed, and additional features are in

progress. This will allow JIFSAN and CFSAN to more easily determine geographic areas to focus future PSA trainings.

Whole Genome Sequencing (WGS) Analysis of Non-Top 7 Serogroups Shiga Toxin-producing *Escherichia coli* (STEC) Suggests Novel Serotypes and Relatedness to Human Clinical Isolates



Shiga toxin-producing *E. coli* (STEC) can cause severe foodborne disease. It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and fruits. Although *E. coli* O157:H7 is the most predominant STEC, as many as 400 serotypes of STEC have been identified. At least 130 serotypes have been implicated in human infections. Over the years, non-O157 STEC have emerged as rising human enteric pathogens responsible for outbreaks and sporadic cases of illnesses worldwide. The application of WGS techniques for pathogen detection, AMR identification, and public health surveillance has significantly increased. In this study, focusing on the less common non-top 7 STEC isolated from cattle feces, WGS was used to determine the serotypes and the presence of virulence genes and AMR determinants in their genomes. A total of 284 isolates of STEC from cattle feces were sequenced using Illumina MiSeq. These isolates were previously serotyped using PCR analysis and did not belong to the top 7 serogroups (O157, O26, O45, O103, O111, O121, and O145) that are most frequently associated STEC infections in humans. A single nucleotide polymorphism (SNP)-based phylogenetic tree was built to examine the relatedness between these isolates and human clinical isolates of STEC obtained from National Center for Biotechnology Information (NCBI), indicating that some cattle isolates were closely related to clinical isolates. The results suggest STEC from cattle, that are not part of the top 7 serogroups, have the potential to cause human illness. We were able to demonstrate the utility of WGS as a surveillance tool to characterize the novel serotypes, as well as AMR and virulence profiles of *E. coli* in cattle that could potentially cause human illness.

Capacity Building

Produce Safety Rule (PSR) Training in Guadalajara and Puebla, Mexico

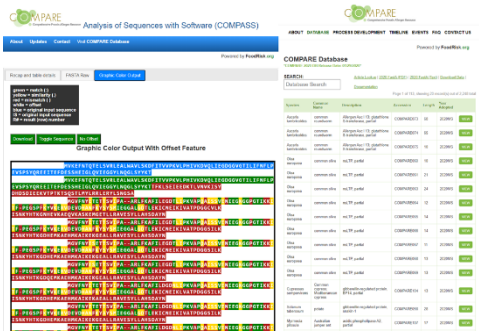
Foodborne illness continues to be a concern for the consumption of fresh fruit and vegetables. Exporters of produce to the U.S. need to be trained in the elements of the FDA Food Safety Modernization Act (FSMA)-Produce Safety Rule in the same manner that domestic audiences are trained. During the current reporting period, two PSR Train-the-Trainer training programs were offered in Mexico by the PIP. The PIP is a collaboration between JIFSAN, the PSA, and the FDA. An additional partner in the program was the Instituto Interamericano de Cooperación para la Agricultura (IICA), which has a formal Collaborative Training Initiative with JIFSAN for the advancement of food safety. A different university partner collaborated at each of the training locations. The first program was conducted in Guadalajara, December 9-13, 2019, in partnership with the University of Guadalajara, División de Ciencias Básicas for 61 participants. The second program was conducted in Puebla, February 24-28, 2020, in partnership with Benemérita Universidad Autónoma de Puebla for 60 participants. Prior to the training programs, the participants from industry, government and academia had been vetted by FDA's Latin America Office and IICA to ensure that they could become qualified to apply for PSA Lead Trainer (LT) status after completion of the course. Those participants who achieve the status of LT will be able to register their own grower training courses with PSA which helps to fulfill the FSMA requirement that at least one person from every farm must receive appropriate PSR training. IICA provided financial and logistical support for these trainings. The trainings were also funded through FDA's Cooperative Agreement with JIFSAN.

Global Water and Food Safety Summit

JIFSAN co-hosted with FDA the Global Water and Food Safety Summit at The Hotel at the University of Maryland, November 19-21, 2019. The goal of this meeting was, "to assemble a variety of international experts in the field to address the impact, importance, and challenges of microbiological sampling of water for food safety and public health." The summit incorporated detailed general sessions with multiple break-out meetings, which drew approximately 170 people to attend this 3-day conference. It provided an excellent forum for the participants to engage in the formation of new and important collaborations, promote global data sharing as part of a global open source WGS database, understand the root causes and potential environmental sources of produce

contamination, as well as contribute to our greater understanding of the risks of pathogen contamination of fresh and fresh cut produce farm systems across the U.S. and around the world.

COMprehensive Protein Allergen REsource (COMPARE) Allergen Database



COMPARE is a transparent resource for the identification of protein sequences that are known or putative allergens. More information is available at http://comparedatabase.org/. It was created via the development of an automated "rule-based" sorting algorithm tool, combined with a review of the literature associated with the identified sequences. Public sequence listings and peer-reviewed literature are informatically and programmatically combed for any indication of the discovery of new allergens. COMPARE was designed to make the broader scientific community and the public aware of putative allergens. JIFSAN provided regular feature updates according to user feedback and added a color-coded sequence-alignment visualization tool. This visualization tool facilitates the assessment on the degree of shared sequence similarity between an amino acid sequence of interest and allergen sequences within the COMPARE database. It is a repository that makes available a transparent process to find and review allergens. The tool is available at https://comparefasta.foodrisk.org. This work was funded by the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) and CFSAN's Cooperative Agreement with JIFSAN.

Western Center for Food Safety (WCFS) - University of California, Davis

The Western Center for Food Safety (WCFS) was established in 2008 at the University of California, Davis, to address the development of approaches and data that are critical to understanding the risks associated with the interface between production agriculture and food protection. This information is used to develop scientifically validated "best practices" for mitigating risks at the production, harvest and postharvest (versus processing) level. In addition to research, the Center provides education, outreach, and technical assistance to food safety stakeholders. The WCFS' research portfolio includes projects related to exploring the sources of microbial contamination on fresh produce and nuts, including agricultural water and soil, as well as collaborations with other academic institutions to increase our understanding of best agricultural practices across varying agro-ecological landscapes. The WCFS' research and outreach efforts assist CFSAN and the food safety community in the implementation of FSMA provisions and regulations.

WCFS Principle Investigators – Dr. Robert Atwill and Dr. Linda Harris
WCFS Program Manager – Dr. Michele Jay-Russell
CFSAN Project Officers - Dr. Samir Assar and Ms. Scarlett Salem

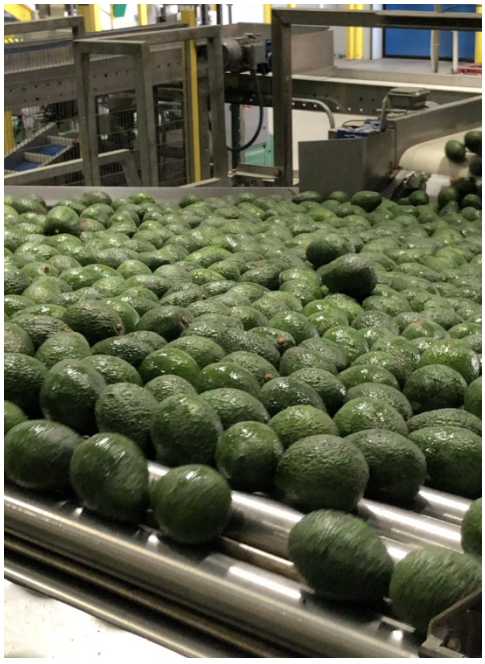
Research

Dispersal and Risk Mitigation of Airborne Bacterial Pathogens from Confined Animal Feeding Operations (CAFOs) in California

There is an urgent need to better quantify the risks and clarify the key mechanisms for produce contamination by airborne transmission of bacterial pathogens originating from nearby CAFO facilities (e.g., beef cattle feedlots, poultry facilities, dairy farms). Routes of airborne contamination of produce from CAFOs might occur by several mechanisms, including but not limited to direct deposition of contaminated fugitive dust from CAFO pen surfaces onto 1) nearby produce, 2) surface irrigation water canals, and 3) equipment surfaces that come into contact with raw produce prior to and during harvesting. WCFS initiated an epidemiological longitudinal study to better characterize the food safety risks from airborne dispersal of bacteria from California CAFOs, using Imperial Valley as the project location given its proximity to the Yuma growing region with its history of foodborne outbreaks and recalls associated with contaminated leafy greens. Two pilot sampling events were conducted in 2020, March 23rd to 26th and April 12th to 18th, at seven

cattle feedlots located in Imperial Valley, California. These sampling dates resemble the key dates of the 2018 outbreak from the Yuma growing region. Samples were taken at distances downwind (n=126) from the feedlots ranging from 2 feet to just over 1 mile, along with a set of samples taken upwind (n=42). Among these 168 air samples, one sample from an upwind site (2.4%) and ten from downwind sites (7.9%) tested positive for *E. coli*, with all positive samples within 100 feet of the closest feedlot cattle pen. The concentration of *E. coli* ranged from 1 to 2 colony-forming units (CFU) per 1000 L of sampled air. All samples that were located greater than 100 feet from the feedlot tested negative for *E. coli*. This longitudinal sampling will lead to the development of good agricultural practices to reduce bioaerosols from emanating from these livestock facilities, improved produce safety from the greater Yuma growing region, and strengthen relationships between the CAFO industry, academics, and regulatory agencies like FDA in terms of greater industry appreciation of food safety risks generated from having livestock facilities in proximity to produce farms.

Evaluation of the Impact of Packinghouse and Postharvest Practices on the Growth/Survival of *Listeria monocytogenes* (LM) on Avocados



Avocados and products made from them (e.g., guacamole) have been subject to numerous recalls in the past few years, with LM being a particular concern. Some studies to address this concern have included evaluation of the growth of foodborne pathogens in avocado pulp, determination of the potential for organisms to internalize into whole fruit through the stem end, and evaluation of antimicrobial treatments for reduction of pathogens on the surface of avocados. This was a new area of research for WCFS scientists and key contacts with the California avocado industry were established to ensure the research design was relevant to commercial practice. The specific aims of this project were to 1) tour at least two California avocado packinghouse facilities through the California Avocado Commission, and 2) evaluate survival/growth of LM on the surface of avocados during commercially-relevant conditions of storage and distribution. WCFS consulted with Dr. Mary Lu Arpaia, University of California Riverside, who is a subtropical horticulturist with extensive experience with the California avocado industry. She organized a tour of a nursery, organic avocado farm, and three packinghouses that were a mix of organic and conventional, wet and dry packing. A detailed summary of this tour was prepared. Several connections were made with the food safety staff and plant managers who were interested in collaborating on the study. A review of the literature was undertaken and a table of outbreaks was prepared, as well as an annotated bibliography. Unfortunately, any laboratory-based work was prevented due to the COVID-19 pandemic. This project was intended to be a data gathering and methods development project to expand our expertise to avocados. A draft bibliography and

a summary of outbreaks, recalls, and existing data in the form of tables that the avocado industry and FDA can use as resources have been prepared. Packinghouse flow diagrams are being developed based on the tours with a goal of understanding the range of packinghouse practices common in the California avocado industry. The long-term goal of this research is to provide data that will support improved postharvest handling practices for avocados.

Growth of Foodborne Pathogens During Soaking of Tree Nuts

The Almond Board of California previously funded a project to look at the impact of soaking of raw almonds. The data showed that pathogens (*E. coli* O157:H7, LM, and *Salmonella*) grow at ambient temperatures if held for longer than ~8 h. Cooler temperatures were protective; no change in population levels were observed at lower temperatures. Subsequent low-temperature drying had no impact on microbial loads. This project has provided validated methods to inoculate and recover pathogens from almonds and similar systems. Almonds are the most commonly "soaked" tree nut, however many others are as well, such as Brazil nuts, cashews, hazelnuts, macadamia, pecans, pine nuts, and walnuts. There is a common belief that these nuts are somehow "activated" through possible initiation of germination but the data to support this is limited. The specific aims of this project were to 1) determine the ability of *E. coli* O157:H7, LM, and *Salmonella* to grow in a range of tree nuts other than almonds during soaking, and 2) investigate a standardized method to determine if "germination" is activated in tree nuts during common soaking procedures. Three tree nuts (cashews, pecans, walnuts) were selected for in-depth study based on an assessment of relative popularity of soaking these products, both at home and commercially. Chandler variety unpasteurized/treated walnut kernel halves (raw) received from a California walnut processor were inoculated with separate five-strain cocktails of *E. coli* O157:H7, LM, and *Salmonella*. Water was added at a 1:4 ratio (w/v) and walnuts were held at 15, 18, and 23°C. Populations of *E. coli* O157:H7, LM, and *Salmonella* significantly increased over 24 h at 23°C; no significant increases were seen at 15 and 18°C. Current work includes determining growth curves for these organisms at 23°C and evaluating survival/growth during soaking of cashews and pecans. The data generated for almonds and the preliminary data for walnuts suggest that soaking nuts at ambient temperature and for longer periods of time (>8 h) will lead to increases in populations of pathogens should they be present. Recommendations for soaking under cold or cool temperatures, over shorter time periods coupled with basic sanitation principles and possibly sourcing of treated raw materials should be included best practice recommendations for soaking tree nuts in the home or under commercial conditions that will minimize food safety risks.

Characterize Indicator *E. coli* Population Dynamics in Response to the Addition of Inoculated Treated BSAAO (commercial HTPP) During Two Leafy Green Growing Seasons in the Desert

Biological soil amendments of animal origin (BSAAO), including livestock manure, compost, and heat-treated amendments, are used frequently as organic fertilizers to improve physical and chemical properties and nutrient content of soils in crop production. Untreated BSAAOs are a known reservoir for zoonotic bacterial pathogens. Application of composted or treated BSAAOs to soils for production of fresh produce is expected to result in reduced risk of pathogen contamination. However, preliminary data suggest that heat-treated poultry pellets (HTPP) produced by a validated process may still pose an unexpected risk for microbial contamination with foodborne pathogens. To address research and extension questions about the safe use of BSAAOs in a southwestern desert climate, experimental field trials were conducted over two leafy green growing seasons using HTPP-amended soil to measure potential growth and persistence of indicator *E. coli* in soil, and its transfer to leafy greens grown in this soil. The field studies in combination with environmental sampling will help determine the extent that agricultural, temporal and biological factors influence survival of zoonotic foodborne pathogens in soil amended with HTPP, and provide data for comparison with other regions of the United States. Preliminary results indicate that the *E. coli* persists in all amended and unamended plots up to 70 days after inoculation, then begins to decrease. *E. coli* inoculum concentration in soil samples collected from Day 0 until just prior to harvest were highest among samples amended with raw poultry manure compared with soil amended with HTTP or steer manure compost. Notably, the inoculum was recovered from soil in unamended plots (controls), but with significantly more reduction compared with soil samples amended with any BSAAO. To date, there is no difference in *E. coli* recovery and persistence in soil from organic versus conventional fields. The research results and outreach efforts from this multi-year project will build-on previous studies related to the safe use of biological soil amendments. It is anticipated that through this work new strategies to reduce introduction of microbial hazards into leafy green fields during pre-harvest production will be discovered, which will benefit industry stakeholders and protect consumers.

Rural and Underserved Community Outreach in the Deep South and Mississippi Delta (DSMD)

To assist the farmers and growers of the DSMD regions to improve their food safety practices for produce, irrigation water quality courses were taught on September 17 and 18, 2019 in Thomaston, Alabama, at the Rural Heritage Center, and May 27, 2020 (virtually over Zoom). The topics covered included aseptic water sampling technique, basic water microbiology, calculation of FSMA water quality profiles, irrigation system inspection, and how to conduct an environmental assessment of their irrigation water supplies. The September 2019 workshops had 24 participants (12 first day/12 second day) from Alabama and Mississippi's Black Belt region; the May 2020 workshops had 45 participants from Mississippi rural farming communities. The Deep South Food Alliance staff were also trained in water collection techniques and irrigation system inspection to aid in their future workshops. Based on a pre/post workshop survey, participants showed a 40% improvement in general knowledge of water quality and sampling techniques after this training event. This outreach effort was in cooperation with the Deep South Food Alliance with a cooperative agreement with the Local Food Safety Collaborative and their partners at the National Farmers Union Foundation. It was also funded through FDA's Cooperative Agreement with the WCFS.

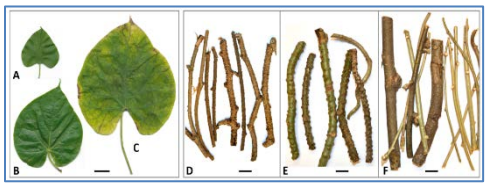
National Center for Natural Products Research (NCNPR) - University of Mississippi

The National Center for Natural Products Research (NCNPR) was established in 2001 at the University of Mississippi, Oxford, to assist the FDA with implementation of the Dietary Supplement Health & Education Act of 1994 (DSHEA). The cooperative research, education, and outreach programs developed by the NCNPR address scientific issues related to the safety of botanical dietary supplements (BDS) and botanical ingredients and complement the diverse activities of both the public and private sectors. Specifically, the NCNPR 1) assists in the identification and development of a list of BDS and botanical ingredients, based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize further research; 2) acquires, validates, and characterizes authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to the FDA, for evaluation of their safety; 3) exchanges technical and scientific information, analytical methods, and reference material with the FDA scientists and other stakeholders; 4) collaborates with the FDA scientists in research areas of mutual interest; and, 5) coordinates scientific workshops and conferences on BDS-related topics of public health relevance to address high priority science and research needs.

NCNPR Director - Dr. Ikhlas A. Khan
NCNPR Assistant Director - Dr. Amar G. Chittiboyina
CFSAN Project Officer - Dr. Gregory O. Noonan

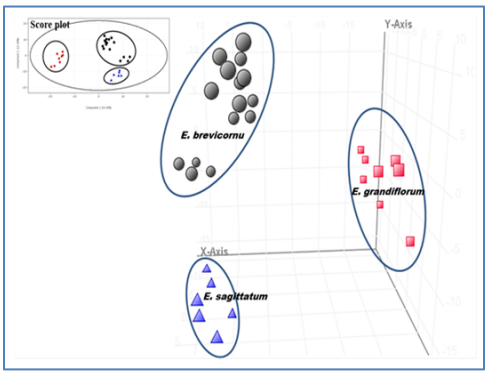
Research

Authentication of Botanicals and the Development of Chemical Fingerprints



Authentication of botanicals - Classical taxonomy
Macroscopic- and microscopic-techniques were performed under this cooperative agreement to assure authenticity or detect possible adulteration in powdered, commercial plant samples. Several species of *Tinospora,* and *Salvia* were sourced from various collaborating partners. Morphological resemblances, overlapping geographical occurrence, history of traditional use, or confusion in species identification often result in some ambiguity with closely related species of botanical raw materials in various dietary supplements. This is particularly important for *Tinospora*, and *Salvia* because certain species have been reported to be causative agents with suspected liver toxicity. Morphological studies together with high-performance thin-layer chromatography were successfully applied for the unambiguous identification of three *Tinospora* species. Additionally, to assess the overall quality of *Tinospora* species specific raw materials in commerce, compounds, such as borapetoside B and C for *T. crispa*, and tinosineside A for *T. sinensis* were established as quality markers.

Avocado oil is traditionally extracted from the mature fruit pulp and is prized for its high nutrient value due to the substantial amounts of triglycerides (TGL) and unsaturated fatty acids (FA). Recently the popularity of avocado oil has been promoted with the newly introduced avocado seed oil. However, there is insufficient evidence to support either the claimed health benefits or the safe use of oils derived from an avocado seed. A high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC/ESI-MS) method was developed for the quantification of 13 TGL in authenticated and commercial avocado oils. The precision and accuracy of the HPLC/ESI-MS method were verified by FA analysis using a gas chromatography–mass spectrometry (GC/MS) method, and the results from these two methods were in good agreement. The statistical analysis based on TGL and FA compositional data revealed that among the 19 commercial oils analyzed, seven samples were adulterated with soybean/sesame oil or mislabeled. None of the oils claimed as avocado seed oil were derived from avocado seeds. The compilation of such triglyceride and fatty acid compositional data as chemical signatures might serve as an important tool to assess the overall quality of avocado oils found in commerce.



In another project, to delineate the robustness of hyphenated chromatographic methods in quality assessment of dietary supplements, closely related species of *Epimedium*, commonly known as horny goat weed, were studied with the help of an ultra-high performance liquid chromatography (UHPLC) method to determine the relative concentrations of thoroughly characterized 15 prenylflavonoids. Even though, the total content of 15 prenylflavonoids was found to be similar in all three studied species (*E. grandiflorum*, *E. brevicornum*, and *E. sagittatum*), chemometrics together with relative presence/absence and concentrations of prenylflavonoids, the majority of the commercial supplement products were derived from *E. grandiflorum* as the botanical source. In addition to the liquid chromatographic method, a high-performance thin-layer chromatography (HPTLC) method was also developed as a more convenient, less-expensive and faster visual aid (based on chemical fingerprints) to qualitatively differentiate the closely related species. A similar approach was also applied to the investigation of supplements purported to contain *Vangueria agrestis* as a botanical ingredient.

Implications of Botanicals in Skin Sensitization

Botanical extracts and single compounds extracted from botanicals are increasingly used in cosmetics, fragrances, topical ointments and personal care products to enhance the products' "natural" appeal. In an attempt to effectively address the safety concerns of botanical ingredients in cosmetics, integrated testing strategies, as well as relevant, unique expertise, were assembled at the NCNPR to fulfill the research needs of CFSAN's Office of Cosmetics and Colors (OCAC) at the US FDA. Under the current program, several projects concerning the safety assessment of botanicals have been undertaken. The general, long-term goals include i) application of a

combination of non-animal alternative methods to identify potential skin sensitizers, ii) application of analytical methods for the authentication, characterization, and quantitation of candidate compounds of concern, and iii) evaluation of risk by complementing hazard studies with dermal absorption studies. Two in-house methods and three regulated methods, direct peptide reactivity assay (DPRA), KeratinoSens, and human cell line activation test (hCLAT), as an integrated nonanimal testing strategy, have been established at the NCNPR Center of Excellence. For this reporting period, comprehensive analysis of chamomile (*Matricaria recutita*) was completed and investigation of two additional, frequently used botanicals, aloe (*Aloe vera*), and rosemary (*Rosmarinus officinalis*) are ongoing. In addition to these three botanicals, our research mission was expanded to include cannabidiol (CBD) and cannabis, due to recent legislative developments and the related burgeoning of the CBD's cosmetic market. The combination of innovative in-house methods along with validated methods may serve as a proactive, orthogonal testing strategy for OCAC's mission to address the safety assurance of botanical ingredients in cosmetics without animal-based testing.

In another project under the OCAC program, the skin sensitization of oakmoss and atranol-like compounds in these fragrance raw materials have also been investigated. Along with two major allergens, atranol, chloroatranol, several other atranol-like compounds have been suspected to be a potential source of allergens. A chemical fingerprinting of a typical oakmoss sample has been developed with both GC-MS and HPLC-MS. Ten pure, fully characterized compounds were utilized in developing a chemical fingerprint of oakmoss extract and investigated further using non-animal methods (HTS-DCYA, DPRA, KS, and hCLAT). Both atranol and chloroatranol, along with six additional oakmoss constituents were identified to be potential sensitizers using the three alternative methods accepted by regulatory agencies.

Adverse Effects of Botanical Dietary Supplements – Drug-metabolizing Enzymes and Transporters (DMET): Herb-drug and herb-herb Interactions with Botanicals

Tens of thousands of botanical dietary supplements are currently in the global marketplace; however, very few have been evaluated for their potential herb-drug interactions. With the increasing use of herbal supplements for a variety of health benefits, the potential for adverse effects is also on the rise. With either chronic or acute consumption of multiple products, deleterious effects related to pharmacokinetic herb-drug or herb-herb interactions have been reported and attributed to modulation of DMET. The goal of this project was to evaluate the potential of herb-drug and herb-herb interactions. NCNPR has established a battery of *in vitro* methods to assess the potential of herbs to cause adverse effects in situations such as chronic or acute use. In the past 12 months, the team optimized several *in vitro* methods and screened a number of extracts prepared from a wide variety of medicinal plants to probe their ability to modulate several isoforms of cytochrome P450 enzymes. For example, dietary supplements are the most common forms of alternative therapy used by the HIV-positive population for various purposes. Some of these products have been reported to be causative agents of significant herb-drug interactions with antiretrovirals; leading to either treatment failure or readjustment of therapeutic doses. To gauge the implication of dietary supplement products in combination with antiretrovirals, scientists at the NCNPR conducted several experiments including inductive potentials of DMET. Ethanolic extracts of selected supplements were prepared and their effect

on the hepatic metabolism of five antiviral drugs (elvitegravir, rilpivirine, tenofovir, dolutegravir, and cobicistat) was evaluated in human liver cells. Of the five drugs tested, the metabolism of two drugs, rilpivirine, and dolutegravir, was enhanced at least three fold compared to control, suggesting faster metabolic clearance in the presence of these supplement products. Further experimental results indicated that isozyme CYP3A4 is the probable causative agent for the superior metabolic clearance and the final data agree for these drugs being substrates of 3A4 isoform. This is a demonstrated example of expertise along with alternative methods available at the COE that may serve as a proactive, testing strategy for CFSAN's mission to address the safety aspects of botanicals in various finished products.

Capacity Building

Public Awareness of Emerging Problems Associated with Botanicals

The Center hosted the 19th Oxford International Conference on the Science of Botanicals (ICSB) that was held on April 8 - 11, 2019 at The University of Mississippi. The agenda, program and other activities were organized to reflect the celebration of 25 years passage of DSHEA by reviewing the history, confronting ongoing issues and assessing future prospects with botanical dietary supplements. Due to the ongoing crisis with COVID-19, health, and safety implications, the ICSB organizing committee made the decision to cancel this year's annual meeting scheduled in Oxford, MS, for August 16 - 20, 2020. Unfortunately, due to risk and uncertainty with the ongoing pandemic situation, the committee reached this decision after concluding that it would be impossible to hold the conference as planned. Like many other professional meetings, the organizers also seriously considered conducting this year's meeting *via* virtual platforms; however, the uniqueness, international flavor and personal contacts integral to the annual gathering would be lost. Nevertheless, the organizers wish to thank our sponsors, including CFSAN, for their unwavering support. We also wish to recognize the outstanding work and ingenuity of our national and international colleagues, and the countless other volunteers who contributed to our past ICSB annual meetings.

Botanical Adulterants Prevention Program (BAPP)

The NCNPR, in association with two other nonprofit organizations, American Botanical Council (ABC) and American Herbal Pharmacopoeia (AHP), have initiated the 'botanical adulterants program' to educate members of the herbal and dietary supplement industry about ingredient and product adulteration so they will be able to produce safe, high quality, and economically successful products. The ABC-AHP-NCNPR BAPP is a long-term, multi-party coalition of herb quality and identity experts in university research groups, third-party analytical laboratories, government agencies, trade associations, and industry companies that examine the extent of suspected adulteration of herbal materials, particularly adulteration that is economically motivated. To date, the program has published more than 50 extensively peer-reviewed

documents, 20 Botanical Adulterant Bulletins, 10 Laboratory Guidance Documents, and Botanical Adulterants Monitor e-newsletters. All program publications are freely available on the program's website (http://cms.herbalgram.org/BAP/index.html). For this year, bulletins were published on several ongoing adulteration issues with oils belonging to oregano herb, olive and English lavender. In addition to these, a laboratory guidance document was published to authenticate saw palmetto berries and their extracts and detect adulteration with vegetable oils and fatty acids from animals. Specifically, with this platform, two publicly accessible press releases were issued on the BAPP's role in the COVID-19 pandemic and substantial toxicity associated with all parts of the oleander (*Nerium oleander*) plant. This effort is partially supported by the botanical industry partners, ABC and AHP.

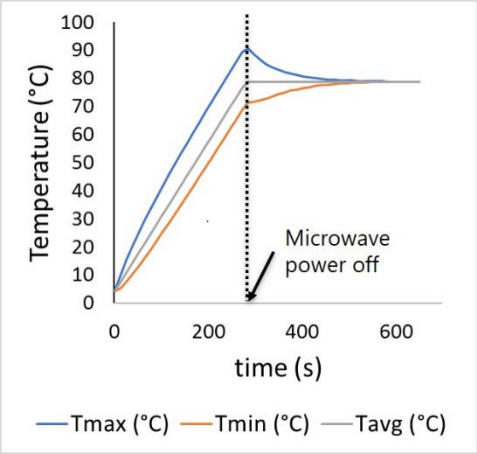
Institute for Food Safety and Health (IFSH)/National Center for Food Safety and Technology (NCFST) - Illinois Institute of Technology

The National Center for Food Safety and Technology (NCFST) was established in 1988 at the Illinois Institute of Technology's (IIT) Moffett Campus in Bedford Park, IL, to bring together scientists from the FDA, academia, and industry to work collaboratively on food safety issues. The NCFST is a part of IIT's Institute for Food Safety and Health (IFSH) and is a unique food research consortium of CFSAN's Division of Food Processing Science and Technology (DFPST), IIT faculty and students, and food and food-related industries. NCFST's research addresses the safety of processed foods; food safety implications of emerging technologies in food processing and packaging; and laboratory method performance. In addition to the NCFST, other Centers within the IFSH structure include the Center for Processing Innovation, Center for Nutrition Research, and Center for Specialty Programs. IFSH also coordinates FSMA training programs through the IFSH-led Food Safety Preventive Controls Alliance (FSPCA) and Sprout Safety Alliance, including Preventive Controls for Human Food, Preventive Controls for Animal Food, Foreign Supplier Verification Programs (FSVP), Intentional Adulteration, and Sprout Safety. The FSPCA also provides a Technical Assistance Network (TAN) to industry on inquiries which are not related to FSMA rule interpretation.

IFSH Director – Dr. Robert E. Brackett
IFSH Associate Director – Dr. Jason Wan
CFSAN Project Officer - Dr. Les Smoot

Research

Temperature Redistribution in Food During the Post-microwave Stand-time



A two- or three-minute time to allow solid food to stand covered after microwaving has become a common recommendation by the FDA Food Code, the USDA Food Safety and Inspection Service, various university extension services and others to assure complete heating. 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 of the ovens have dramatically decreased and the foods prepared in them have evolved to smaller single-serving portions. Since 1992, various foodborne illness outbreaks have been linked to microwave undercooking, including insufficient stand-times. Yet no studies on stand-time effectiveness have appeared to address

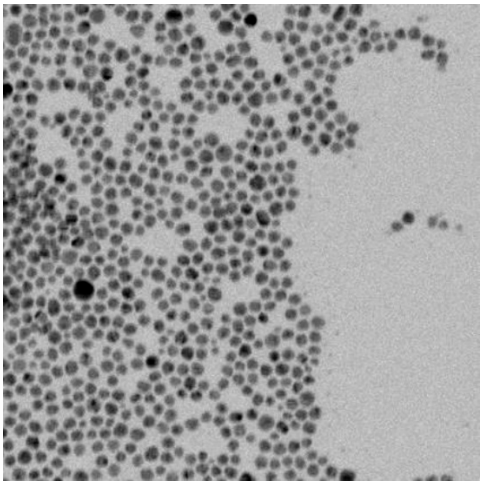
modern microwave heating. This project aims to fill that gap by examining the stand-time in microwave-cooked meat. Meat has been chosen because of the critical need for its complete cooking. The approach taken examines the microwave power distribution into a meat portion by mathematical modeling. The model includes a transition between microwave power on and power off (standing) by using an event that triggers it during a simulation. That event is the average temperature in the meat reaching 74°C, which is the minimum temperature set in the FDA Food Code as part of complete microwave cooking. This roughly simulates a consumer testing a microwaved meat portion several times and seeing 74°C each time. The simulation then continues for 120 seconds, after which the minimum temperature is noted. Complete cooking is achieved if that minimum temperature has been at 63°C for at least 15 seconds, which is the minimum adequate cook temperature and time in conventional cooking. In slab and cylindrical shapes of meat, results indicate that the two-minute stand-time doesn't consistently achieve complete cooking, though it performs better in cylindrical shapes than in slab shapes. Future work will involve experimentally measuring the power distribution across the meat surface and combining it with the present model to obtain a full three-dimensional investigation of stand-time. It is expected that the inconsistency in this case will be more pronounced.

Factors Affecting the Decomposition Kinetics of Opiate Alkaloids in Poppy Seeds

Food-grade poppy seeds are obtained mainly from poppy (*Papaver somniferum* L.) cultivars bred to accumulate lower amounts of opiate alkaloid compounds. However, some poppy seeds that enter the food markets may be from poppy cultivars with elevated concentrations of opiate compounds intended for pharmaceutical or other purposes. 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 can be used to degrade or remove these compounds. However, limited data exist on the effectiveness of potential treatments to reduce levels of opium alkaloids that may be present in seeds from poppy. Poppy seeds containing morphine at relatively lower and higher target concentrations were subjected to dry heat and steam treatments, water washing, and baking. Sample extracts were analyzed using liquid chromatography−tandem mass spectrometry (LC-MS/MS) for the opium alkaloids morphine, codeine, and thebaine. After screening 15 poppy seed samples, one sample was selected based on quantity available and level of morphine (14.7±4.2 mg/kg). Poppy seed sample (5 g) was subjected to heat treatments at 120, 160, 180 and 200°C over 120 min. The results overall from the dry heat treatments indicated that the alkaloid degradation of morphine, codeine, and thebaine was greater at higher temperatures and could be described using first-order degradation kinetics. These data were used to calculate the half-life of the alkaloid compounds at the tested temperatures. At 200°C, thebaine exhibited the shortest half-life of approximately 3 min, while codeine and morphine half-lives were approximately 30-40 min. Water washing treatments

significantly reduced all alkaloid levels, while steam application had limited effectiveness. Baking did not reduce concentrations of any alkaloid compound. Information obtained 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 supplied to the food industry.

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



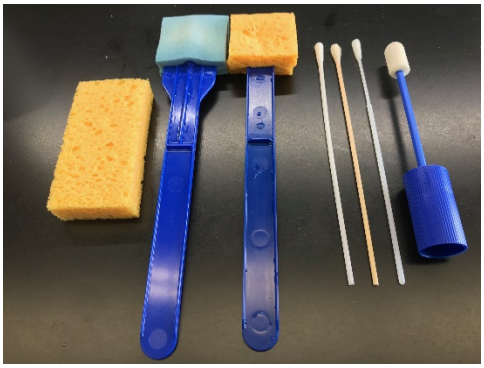
Polymer nanocomposites (PNCs) may be used in FDA regulated products like food packaging and medical devices. There is a need for FDA to better understand the safety of FDA-regulated products manufactured with PNCs. One critical aspect is being able to predict exposure to PNC components during product lifecycles. In the past year, we have developed a model system to study the extent to which food chemistry and nanoparticle surface treatment impacts the amount and form of nanoparticles released from PNC packages. The model system is based on silver nanoparticles (AgNPs) incorporated into low density polyethylene (LDPE), a common food contact polymer. After manufacturing and characterizing these materials, we assessed their release into food simulants under conditions relevant to potential use scenarios (long term room temperature and refrigerated storage). PNCs containing AgNP with diameter of approximately 10 nm were capped with different capping agents (alkyl sulfides and polyethylene glycol chains) to assess the impact of nanoparticle surface chemistry and dispersion characteristics. Release into different foods and aqueous solutions of food ingredients has also been explored, and most recently AgNPs have also been incorporated into other food contact polymers to study how polymer structure impacts release mechanisms. Analysis of food simulants by single-particle inductively coupled plasma mass spectrometry (ICP-MS) to discriminate between dissolved and particulate fractions has been completed and the data are currently being analyzed. 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. Such information will address knowledge gaps that hinder effective risk characterization decisions, which FDA's leadership has established as a research priority. Outcome 2 will be an assessment of whether published FDA chemistry guidance is accurate for PNCs. For instance, if food ingredients 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.

Impact of Temperature on Pathogen Proliferation During Sprouting and Postharvest Storage

Contaminated sprouts have been linked to many illness outbreaks. Seeds are often the source of contamination, but sprouts pose a particular 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 inoculated seeds and how this temperature effect is influenced by pathogen type, pathogen load, seed type, and seed disinfection. Persistence of pathogens on sprouts during postharvest storage as affected by storage temperature was also examined. Alfalfa seeds inoculated with *Salmonella* were pre-treated with sterile tap water, 19,000 ppm sodium hypochlorite (NaClO) or 20,000 ppm of calcium hypochlorite ($Ca(OCl)_2$) and were allowed to germinate at 4, 10, 20 or 30°C. After harvest, sprouts were divided and were stored at 4, 10 or 25 for up to 21 days. Sprout samples were taken at defined time intervals during sprouting and postharvest storage and analysed for levels of *Salmonella* by plate count. Sprouting at 4°C resulted in a decrease in *Salmonella* level (by 3 logs) compared with an increase of 3-5 logs during sprouting at 10, 20, or 30°C. Treatment of the inoculated seeds with 19,000 ppm NaClO or 20,000 ppm $Ca(OCl)_2$ reduced the *Salmonella* level by 2.8 or 2.9 log units, respectively. When the treated seeds were sprouted at 20 or 30°C, *Salmonella* population increased and reached a similar level (8 - 9 log CFU/g) as those observed in sprouts grown from untreated seeds. 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 cold-grown sprouts were stored at 25°C (i.e., under temperature abuse conditions), the level of *Salmonella* increased by as much as 7 log units after 2 days of storage. Although *Salmonella* 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 provide the FDA with needed knowledge regarding the growth behavior of pathogens and public health risk associated with sprouts grown at a low temperature. An understanding of pathogen survival in sprouts during postharvest storage will aid development of guidelines for proper storage and handling of sprouts.

Qualitative Comparison of Devices for Environmental Sampling of *Listeria monocytogenes* from Various Food Contact Surfaces

The FDA's 2015 Compliance document and FDA Bacteriological Analytical Manual (BAM) do not explicitly recommend types of devices for LM environmental sampling. Sponges and swabs of various materials are the most common, however wipes and other styles of devices are used by industry. Understanding the benefits and shortcomings of various devices for sampling allows FDA inspectors and food industry personnel to make informed choices about the devices used in environmental monitoring programs. For these comparisons, LM was inoculated into buffer, cheese



whey, or lettuce wash and dot-inoculated onto 64 sq.^{2} stainless steel sheets or high-density polyethylene (HDPE) cutting boards at various levels. In addition, process-contaminated ice cream, containing ~ 10 MPN/g of LM, was melted and inoculated similarly onto both surfaces. Surfaces were dried overnight and swabbed with the various devices followed by LM detection using the FDA Compliance Document's enrichment method. Devices tested included cellulose and polyurethane sponges, microfiber and cloth wipes, a large polyurethane swab, and a flocked nylon swab. Overall, there was little statistical difference between most devices, though the sponges and wipes performed better than the swabs. The flocked swab was statistically the worst performing device when a food matrix (whey, lettuce wash and ice cream) was used for inoculation. There was no discernible difference between sponge material or device type (handled or non-handled). In addition to comparing devices for each matrix/surface combination, results from multiple inoculation levels were used to calculate the limits of detection for LM with 95% confidence (LOD_{95}) in order to compare between the stainless steel and HDPE surfaces. LODs for ice cream, lettuce wash and cheese whey were all ~0.5 log higher for HDPE surfaces than stainless steel. Recovery between surfaces inoculated with LM in buffer differed substantially with HDPE surfaces having a 2 log CFU/surface higher LOD than for stainless steel. These results indicate that studies done using buffer as a matrix may not accurately represent results when using a complex food-based matrix. Overall, this information should be useful for food safety personnel evaluating or implementing a LM environmental sampling program.

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