U.S. FOOD & DRUG ADMINISTRATION

2020-2021

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 formal partnerships 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 National Center for Natural Products Research (NCNPR) at the University of Mississippi, Oxford; 2) the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) at the University of Maryland, College Park; 3) the Institute for Food Safety and Health (IFSH)/National Center for Food Safety and Technology (NCFST) at the Illinois Institute of Technology; and 4) the Western Center for Food Safety (WCFS) at the University of California, Davis.

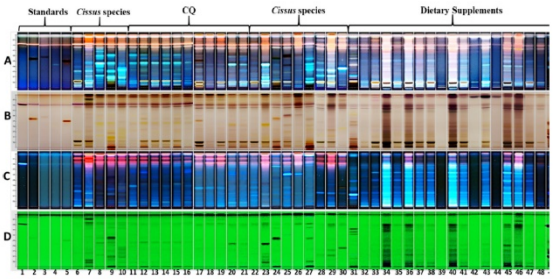
This report highlights selected research and capacity building efforts conducted by the COEs during the Cooperative Agreement budget period of September 1, 2020 to August 31, 2021.

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

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

Analytical Investigations to Assure the Overall Quality of BDS



The NCNPR has focused on developing several analytical methods to determine the botanical authenticity and assure the quality of various botanical-based dietary supplements in the past year. These studies include developing various analytical methods such as Ultra-Performance Liquid Chromatography (UPLC) (ultra violet (UV), evaporative light scattering detector (ELSD), and mass spectrometry (MS)), nuclear magnetic resonance (NMR), and gas chromatography (GC)-MS. For example, stem extract of the plant *Cissus quadrangularis* L. native to tropical Asia, Arabia, and much of Africa has been indigenously used to repair fractures and join bones. Cell- and animal-based studies increased the use of *Cissus*-based supplements to alleviate weight loss, diabetes, high cholesterol, and many other biological symptoms. However, chemical constituents responsible for the perceived health claims are largely unknown. A total of ten phenolic reference standards originated from aerial parts (stem and leaf) together with hyphenated analytical methods, ultra-high performance liquid chromatography photodiode array detection mass spectrometric method (UHPLC-PDA-MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF), were successfully implemented to assure the

overall quality and authenticity of *Cissus*-based botanical dietary supplement products. Moreover, five other *Cissus* species were acquired from our collaborating partners and are instrumental in probing the overall quality of raw materials (extracts) originated from closely related species and plant parts used in these finished products. Upon analysis of nineteen dietary supplements compared with botanical reference material, only one-third of products were identified to be originated from *C. quadrangularis* with total amounts ranging from 5 to 800 mg per daily dose and the rest either with inferior quality or not related to *Cissus* plants altogether. Moreover, these resources allowed us to develop an economically feasible orthogonal analytical high-performance thin layer chromatography (HPTLC) method as a visual, rapid analytical tool to probe the quality assessment of botanical raw materials used in *Cissus*-containing dietary supplements. The developed HPTLC analytical method is simple and transferable to other stakeholders, including CFSAN's Office of Regulatory Science, to assure the overall quality of *C. quadrangularis*-based dietary supplements.

In another project, to demonstrate the robustness of hyphenated chromatographic methods to assure the overall quality and safety of raw materials utilized in dietary supplements, an ultra-high-performance liquid chromatography method was developed to quantitatively determine nine phytochemicals of *Moringa oleifera* leaves. The inhibitory potential of *M. oleifera*'s extract together with a simple and robust analytical method allowed the NCNPR to investigate the overall quality of supplement products claimed to contain *M. oleifera,* aka miracle tree or drumstick tree. All tested dietary supplements contained phenolic compounds and the quantities in these products ranged from 0.42 to 2.57 mg/100 mg sample weight. However, the difference in the phenolic contents between the authentic plant material and the nutraceuticals stresses the significance of chemical fingerprint profiling and quantitative analysis for quality assurance. A similar approach was extended to probe the overall quality and toxicity concerns of three closely related species of *Tinospora* and supplement products containing such botanical raw materials. This research was funded through CFSAN's Cooperative Agreement with the NCNPR.

Safety of Botanical Ingredients in Cosmetics and other Personal Care Products

Botanical extracts and single compounds extracted from botanicals are an integral part of many natural and organic cosmetics, fragrances, and personal care products. To effectively address the safety concerns of such ingredients, integrated non-animal testing strategies and unique resources were assembled at the NCNPR to fulfill the research needs of CFSAN's Office of Cosmetics and Colors (OCAC), CFSAN, and the FDA. Under this directive, several projects concerning the safety and quality assessment of botanical and synthetic ingredients used in cosmetics and other personal care products have been undertaken. The general, long-term goals include i) the application of a combination of alternative (non-animal) methods to identify potential skin sensitizers, ii) the 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 methods. A total of five independent, non-animal-based methods are routinely investigated, and relevant data are compiled, shared with the OCAC team, and published in peer-reviewed journals. For example, two major natural components, umbelliferone and farnesene, were identified as potential skin sensitizers using an integrated testing strategy by complementing the unique resources available at OCAC and NCNPR to

assure the safety of chamomile-based finished cosmetic products. In another project under this program, the skin sensitization potential of oakmoss and atranol-like compounds in these fragrance raw materials have been investigated. Both atranol and chloroatranol, along with six additional oakmoss constituents, were identified as potential sensitizers using Organisation for Economic Co-operation and Development (OECD) and other agencies' accepted methods.

In addition to botanical-based research, the current program's mission was expanded to include cannabidiol (CBD) and cannabis due to recent legislative and cosmetic market developments. Under this task, the skin sensitization potential of hemp extracts, cannabinoids, and other terpenes, as well as dermal absorption of CBD and qualitative analysis of various cosmetic products, are ongoing, and results will be reported in due course. Additionally, with the direction of OCAC's leadership, two other analytical methods were developed to probe the presence/absence and quality of per- and polyfluoroalkyl substances (PFAS) and hexapeptides. These ingredients are added to several cosmetics to increase their durability and water resistance or alleviate age-related wrinkles. Like previous year's mandate, the NCNPR will continue to implement both in-house methods and validated methods that may serve as a proactive, orthogonal testing strategy for OCAC's mission to address the safety of botanical ingredients in cosmetics without animal-based testing. This research was funded through CFSAN's Cooperative Agreement with the NCNPR.

Adverse Effects of BDS – Modulation of Drug-Metabolizing Enzymes and Transporters (DMET) and Implications to Herb-Drug Interactions

There is a growing trend for herbal remedies in the US far exceeding the available information on their risk-benefit ratios. Considering that the escalating appeal of herbal remedies is likely to continue, some consumers' lack of awareness of herb-drug interactions is a potential risk. More recently, physicians and other health care providers have become familiar with the situation and are calling for more research that can provide scientifically valid information about the safety and efficacy of such remedies. Specifically, with either chronic or acute consumption of multiple botanical dietary supplement products, deleterious effects related to pharmacokinetic herb-drug or herb-herb interactions have been reported and mainly attributed to modulation of xenobiotic receptors and transporters. The main goal of this project was to accumulate scientifically valid experimental evidence to probe the herb-drug and herb-herb interactions of botanical ingredients by studying the modulation of xenobiotic enzymes and transporters. The NCNPR has established a battery of *in vitro* methods to assess the potential of adverse interactions, specifically upon chronic or acute consumption. In the past 12 months, we have optimized several *in vitro* methods to address the potential safety concerns of several extracts prepared from medicinal plants to probe their ability to modulate several isoforms of xenobiotic cytochrome P450 (CYP450) enzymes. For example, *Bulbine natalensis* is an African-folk medicinal plant used as a dietary supplement for enhancing sexual function and muscle strength by presumably boosting testosterone levels, but no scientific safety information is available when bulbine-containing supplements are concomitantly taken with prescription drugs. Based on the experimental data gathered, consumption of *B. natalensis* containing supplements appears to pose a potential herb-drug interactions risk if taken with conventional medications that are substrates of CYP1A2, 2B6, 2C9, and 3A4 isozymes and may contribute to unanticipated adverse reactions or

therapeutic failures. This research component is a demonstrated example of expertise along with alternative methods available at the NCNPR that may serve as a proactive testing strategy for CFSAN's mission to address the safety aspects of botanical dietary supplements. This research was funded through CFSAN's Cooperative Agreement with the NCNPR.

Capacity Building and Leveraging Research Efforts

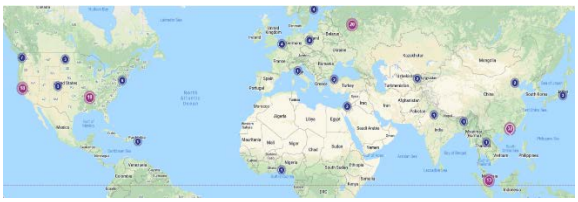
The establishment of the COE on botanical ingredients and unique resources at the NCNPR allowed the COE to pursue and establish several opportunities to impact and provide overall benefit to the ongoing cooperative agreement with CFSAN. Some of the leveraging research efforts with various entities are highlighted below.

The Consortium for Health and Military Performance, Department of Military and Emergency Medicine (CHAMP) at the Uniformed Services University of the Health Sciences, and the NCNPR collaborated to study the quality of supplements consumed by military personnel. The CHAMP is providing the NCNPR with the most popular dietary supplements taken by soldiers. Researchers at the NCNPR are implementing state-of-the-art analytical instrumentation for the (non)targeted analysis of numerous exogenous components in these products to ensure the overall quality of the botanical dietary supplements.

In association with the Duke Clinical Research Institute (DCRI) as a data coordinating Center, a collaborative five-year grant from National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) for the Drug-Induced Liver Injury Network has been awarded to the NCNPR. Under these collaborative efforts, scientists at the NCNPR are using different analytical approaches to identify potential hepatotoxic ingredients in herbal dietary supplements suspected of liver toxicity.

In association with the University of Mississippi Medical Center (UMMC) as a clinical center, a collaborative five-year grant from the Office of Dietary Supplements (ORS) of the NIH has recently been awarded to the NCNPR and identified as a component of the NIH Consortium for Advancing Research on Botanicals and Other Natural Products (CARBON) Program. Under this program, the Center's research is directed towards generating sufficient data to optimally design future clinical studies to evaluate the utility of *Spirulina*-derived oral supplements in promoting resilience against viral infections such as influenza. This research was funded by CHAMP, NIDDK/NIH, and ORS/NIH.

Global Impact of the Supplementary Role of Research Conducted by the COE



One of the metrics for evaluating the efficacy of any research program is the influence on projects being carried out by researchers not directly affiliated with the COE. As a part of the collaboration with the Drug-Induced Liver Injury Network (DILIN), the chemical analyses of various kratom-based supplements were instrumental in establishing eleven cases of liver injuries, mostly in males with a median age of 40 years. All these subjects were symptomatic and

developed jaundice with a median latency of 14 days. However, their liver injury pattern was variable, requiring hospitalization, and all eventually recovered. Nevertheless, the analytical personnel and instrumentation facilities of the NCNPR were used to establish the link between a detailed chemical analysis of botanical dietary supplements and clinical manifestation. These invaluable, collaborative efforts allowed us to contribute positively with scientifically valid analytical data to address the ongoing supplements-induced liver injury cases.

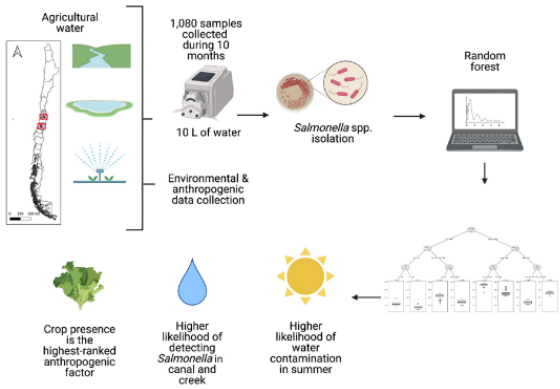
In another example to illustrate the global impact of ongoing research efforts, the NCNPR has initiated a program to allow global access to detailed analytical results that are not usually part of scientific publications. The University of Mississippi's library services digital initiatives were utilized to store globally accessible metadata to effectively carry out this program. The scientists at the NCNPR culminated in a digital campaign to disseminate the detailed analytical data of exogenous components, including ergogenic amines in dietary supplements. As a result, the COE collects data on readership, downloading history, and other metrics, including the type of global organizations accessing this information. The NCNPR will continue to utilize such digital initiatives to help the scientific community and further the transparency and scientific integrity of NCNPR's research activities. This research was funded by DILIN and NIH.

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. 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. Eric R. Olson

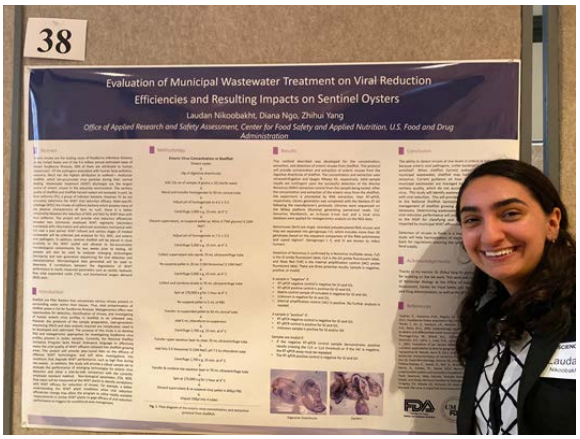
Salmonella enterica and *Listeria* in Surface Water



Surface water is one of the primary sources of irrigation water for produce production; therefore, their contamination by foodborne pathogens, such as *Salmonella* and *Listeria*, may substantially impact food safety and public health. Whole genome sequencing (WGS) provides a robust tool to determine detailed characterization of microbial pathogens. Its applications in food safety 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, as well as many others. Its increased use worldwide has allowed attaining significant progress in our understanding of the epidemiology of foodborne diseases. To engage international partners for better understanding of surface water as a potential source of *Salmonella* and other foodborne pathogens, we established a collaboration with university researchers in Mexico, Brazil, and Chile. Despite the Covid-19 pandemic, the project has made impressive progress. The two universities (Universidad Chile and Universidad Andres Bello) in Chile have partnered and obtained 1,140 samples in 38 visits

to four rivers in Chile. The isolation rates of *Salmonella* ranged from 8.3% to 45%, with an average of 28%. A 300% increase in isolation rate was detected in southern rivers in warm months compared to cold months. A total of 642 isolates of *Salmonella* enterica and 142 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 and 123 isolates of *S. enterica* were found to have antimicrobial-resistance. The National Autonomous University of Mexico processed 454 surface water samples from 160 different sampling points (rivers, dams, ponds, lakes, and irrigation canals). The overall prevalence of *Salmonella* was approximately 80%. Among 171 *Salmonella* isolates sequenced from Mexico, 137 were predicted to be antimicrobial-resistant. The two Brazilian universities (Federal University of Paraiba and Federal University of Rio de Janeiro) joined the project in August 2020. A total of 60 samples from in small rivers and irrigator canals near farms were collected in the State of Rio de Janeiro in 2020. Approximately 60% of the samples were positive for *Salmonella*. In Paraíba State, Northeastern Brazil, 42 water samples were collected in January 2021 and seven (16%) samples were positive for *Salmonella.* 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. This research was funded through CFSAN's Cooperative Agreement with JIFSAN.

Undergraduate Student Internship Program

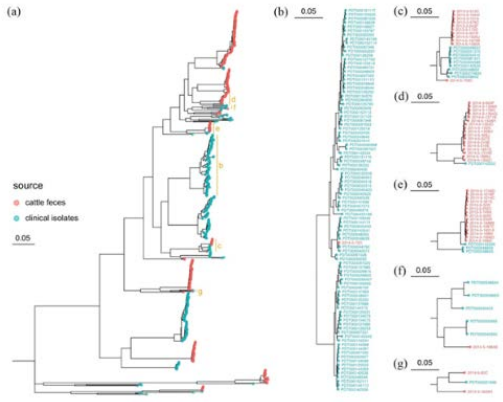


JIFSAN provides a unique internship program designed to offer University of Maryland (UMD) undergraduate students an opportunity to work with FDA scientists on specific projects related to food safety, applied nutrition and public health. These research- and programmatic-related training opportunities enhance the students' knowledge of and experience in science, particularly in a regulatory environment, and familiarize them with career opportunities in the regulatory sector of public service. JIFSAN recruits students, from across academic disciplines, who are full-time UMD undergraduates and have completed two college semesters. During the 2020-2021 funding period, fifteen undergraduate students participated in the JIFSAN Internship Program, logging in over 7,500 hours. Interns have produced posters, presentations and are co-authors in peer-reviewed publications. This research was funded through CFSAN's Cooperative Agreement with JIFSAN.

Monitoring and Impact Evaluation Research on JIFSAN and Inter-American Institute for Cooperation's (IICA) Produce Safety Training Programs

JIFSAN developed monitoring and evaluation survey tools, implemented surveys, and established databases for JIFSAN's training programs. In 2020, two online surveys were developed and conducted for JIFSAN and IICA's International Produce Safety Rule training 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 evaluate participants' learning experiences to improve the training courses. These surveys continued to be deployed for all JIFSAN and many of IICA's grower trainings in 2021. A second survey was developed and deployed to those who completed the "train-the-trainer" courses of the Produce Safety Alliance - Produce Safety Rule (PSA-PSR) and USDA-FAS Food Safety and Agricultural Sustainability Training (FAST) PSR. Data collection has been completed and the analysis will take place shortly. The goal is to understand the impacts of these trainings through analyzing teaching effectiveness and determining the number of growers who have been trained by these lead trainers. As part of this research work, JIFSAN is also partnering with IICA to conduct behavioral experiments to evaluate alternative supplemental training material being developed to better reach growers, their managers, and owners of produce farms. The experimental approach has been developed and evaluation tools will follow shortly. This research was funded through CFSAN's Cooperative Agreement with JIFSAN.

Genomic Analysis of Bacterial Pathogens Associated with Foodborne Outbreaks



The National Center for Biotechnology Information (NCBI) Pathogen Detection database contains over 600,000 genomes for foodborne pathogens, mostly contributed by US public health agencies (FDA, USDA, CDC, and local public health laboratories). The FDA contributes isolates collected from food and environmental sources through GenomeTrakr. JIFSAN is interested in exploring several mission relevant, big data challenges, including machine learning for source attribution, risk assessment of foodborne pathogens, and functional genomics, among others. We focused on performing rigorous, innovative, "big data" analyses across the public, genomic database for *Salmonella*, utilizing improved metadata to test various hypotheses surrounding source attribution, risk

assessment, functional genomics, or others specific to the candidates' research interest. For example, we benchmarked hybrid assembly approaches of the Maryland Super-Read Celera Assembler (MaSuRCA), St. Petersburg genome assembler (SPAdes), and Unicycler for bacterial pathogens using Illumina and Oxford Nanopore sequencing by determining genome completeness and accuracy, antimicrobial resistance (AMR), virulence potential, multilocus sequence typing (MLST), phylogeny, and pan genome. Ten bacterial species (10 strains) were tested for simulated reads of both mediocre- and low-quality, whereas 11 bacterial species (12 strains) were tested for real reads. Our research demonstrates the hybrid assembly pipeline of Unicycler as a superior approach for genomic analyses of bacterial pathogens using Illumina and Oxford Nanopore sequencing. This research was funded through CFSAN's Cooperative Agreement with JIFSAN.

International Training - Virtual Grower Training (VGT)

The PSA Grower Training program was developed to provide growers with training that would satisfy the requirements under §112.22 (c) of the FSMA Produce Safety Rule. In order to provide access to the course during COVID-19 restrictions, PSA temporarily amended their policy to allow remote delivery of the course. The *Virtual PSA Grower Training Initiative* was developed, which partnered FDA, JIFSAN and IICA, to offer virtual training in Latin American countries. The pilot program was completed in August 2020 and the virtual training program began in September 2020. A total of sixteen PSA Virtual Grower Training (VGT) programs were held from September 2020 through August 2021 with a total of 290 participants. Eleven training events were held in Mexico, 4 in Honduras, and 1 in Argentina. In order to increase the Lead Trainer cadre in each country, selected PSA trainers were coached through the PSA supplemental application process. To date, one instructor from each country for a total of 3 have submitted applications and 6 are at various stages in the process. PSA has extended the remote delivery option for VGT through March 30, 2022 and potentially longer. This training was funded through FDA's Cooperative Agreement with JIFSAN. IICA also provided financial and logistical support.

The United States Department of Agriculture (USDA) - FoodData Central

FoodData Central (FDC) is a modern website that grants users unprecedented access to food composition data (https://fdc.nal.usda.gov). Not only does FDC provide access to five different food composition datasets, but also its interface invites users to explore unique features of each dataset. For example, FDC allows users browsing branded foods to filter their search by market country, ingredients, and brand owner. FDC provides users browsing foundation foods with links and tabs showing measurements and metadata of individual samples contributing to each food that they select. In order to ensure that FDC received the features that USDA desires, JIFSAN was tasked with contracting the services of JBS International to develop FDC. In 2020 and 2021, search results were split into sections for each of the five datasets, so when a user runs a search, they can click different tabs to view search results from each dataset. The application's database was converted from a relational database to a graph database which enables data points to be connected in various ways. USDA has created ontologies for the graph database to leverage. The ontologies include categories such as types of food and transformations such as cooking/preparation methods of foods. The new graph database was also trimmed and optimized to improve search times. In addition, the presentation of foundation foods was changed to allow users to easily click various links or tabs to view information about individual samples. These changes were made in order to improve data presentation, user experience, and application performance of FDC. This work was funded through a Cooperative Agreement with USDA-ARS.

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, the Center for Nutrition Research, and the 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.

Interim IFSH Director – Dr. Jason Wan
CFSAN Project Officer - Dr. Les Smoot

Enhancing the Safety of High Pressure Processed (HPP) Juices

HPP juice manufacturers are required to demonstrate a 5-log reduction of the pertinent microorganism to comply with FDA Juice HACCP. However, there is currently no consensus on validation approaches for shelf-life studies in terms of storage conditions, sampling times, or temperature abuse. The HPP inactivation and post-HPP survival of acid-adapted *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* in apple and orange juice were evaluated during a 75-day shelf-life using quantitative and qualitative assessments. *E. coli* O157:H7 TW14359, *Salmonella cubana* and *L. monocytogenes* MAD328, previously screened to be barotolerant, were acid-adapted in intermediate pH 5.0 TSBYE and inoculated into apple juice (pH 3.50±0.20) and orange juice (pH 3.88±0.10) at approximately 6.00 log CFU/mL, pressure treated at 586 MPa (180s, 4°C initial) and stored at 4°C for 75 days with temperature abuse conducted on days 3 (30°C, 2h) and 12 (30°C, 2h and room temperature, 1h). Significantly ($p<0.05$) greater log reduction occurred for *S.* Cubana (approximately 3 logs) and *L.*

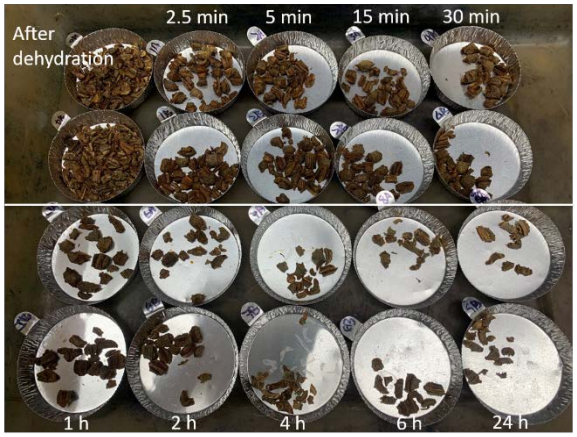
monocytogenes MAD328 (>5 logs) compared to *E. coli* O157:H7 TW14359 (<1 log) following initial post-HPP analyses. While complete inactivation was not observed, bacterial inactivation exceeded 5-log after immediate 586 MPa treatment for *L. monocytogenes* MAD328, after 24 h in cold storage for *S.* Cubana, and after 4 days of cold storage for *E. coli* O157:H7 TW14359. Contradictory qualitative analyses results were obtained for *E. coli* O157:H7 TW14359 with typical *E. coli* O157:H7 colonies observed on microbiological agar plates but not in qualitative enrichments. Similarly, qualitative enrichments of *L. monocytogenes* MAD328 in orange juice were observed during cold storage time on days 35 and 75 but >5 log reduction was maintained. These results suggest certain juice types may require a cold holding time following HPP to achieve a 5-log reduction and certain juice matrices may interfere with qualitative enrichments of bacterial targets. This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

Survival of *Salmonella enterica* on Cut Melons and Transcriptomic Response of the Pathogen on Melon Treated with Organic Acid

Foodborne outbreaks of *Salmonella enterica* have been associated with fresh-cut melons in the U.S. including cantaloupe, watermelon, and honeydew. Research has shown that the antimicrobial activity of organic acids, such as malic acid and citric acid, may reduce pathogen populations on fresh produce, including melons. This study was divided into two objectives: 1) examine the survival of *Salmonella* on melon flesh treated with organic acids, and 2) determine the differential gene regulation of the pathogen on the treated flesh. For objective 1, different serotypes of *Salmonella* (Typhimurium, Enteritidis, and Newport) were evaluated for their abilities to survive on cut cantaloupe and honeydew flesh treated with or without malic or citric acid. Cut melon flesh was treated by submersion in either 2% malic or citric acid for 1 min or left untreated. Flesh was then inoculated with 4 or 7 log CFU/g of one of the *Salmonella* serotypes and stored at 4°C for 7 days. All tested *Salmonella* strains survived on untreated and citric acid-treated cantaloupe and honeydew flesh with no significant decrease in population during 7-day storage at 4°C, regardless of the initial inoculation level. On malic acid-treated melon flesh, the average *Salmonella* population decreased <1 log CFU/g over 7-day storage for both initial inoculation levels (4 and 7 log CFU/g). For objective 2, the differential gene regulation of serotypes Newport and Typhimurium on malic acid-treated cantaloupe flesh was examined using RNA-seq. RNA was extracted at time 0 (after inoculation and prior to storage) and after 1 day storage of cantaloupe flesh at 4°C. Samples for sequencing were prepared using the TruSeq Stranded mRNA Kit and run on a MiSeq. After 1-day storage, a total of 169 and 492 genes were differentially regulated in Newport and Typhimurium on malic acid-treated cantaloupe, respectively, compared to time 0. Gene Set Enrichment Analysis (GSEA) determined that pathways (i.e., biological processes) related to carbohydrate metabolism, nutrient transport, cell signaling, and general stress response were upregulated. Data on the acid adaptation response of

Salmonella on cut cantaloupe will aid in evaluating any cross-protection mechanisms that the pathogen employs during this stress. In addition, this project provides important scientific basis for the food industry to develop more effective control measures to mitigate *Salmonella* contamination in fresh-cut melons and is essential to establish science-based standards for the FDA Preventive Controls Rule. This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

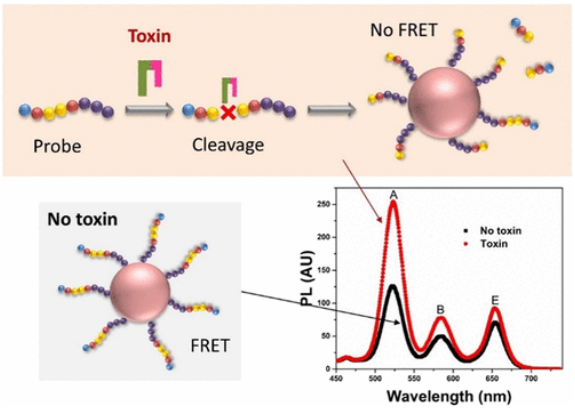
Growth Kinetics Of *Listeria monocytogenes* And *Salmonella enterica* During Rehydration Of Dehydrated Plant Foods, Storage Of Rehydrated Plant Foods, And Storage Of Heat-Treated Plant Foods



Dehydrated plant food products such as potatoes, onions, carrots, and peppers have low water activities and do not support the growth of pathogenic bacteria such as *S. enterica* or *L. monocytogenes*. Once rehydrated, the higher water activity (>0.92) and relatively neutral pH of these foods would require a product assessment to determine the extent to which they support the growth of pathogenic bacteria. Many of the dehydrated plant foods in retail and food service establishments are dehydrated at the manufacturer level using heat and are rehydrated onsite; however, once rehydrated, these products could be held for later use. The first objective of this study was to evaluate the growth kinetics of *S. enterica* and *L. monocytogenes* during rehydration of heat-dehydrated green bell pepper (utilizing 5 or 25°C water) and during subsequent storage (at 5, 10, or 25°C) for up to 14 days. After dehydration, green bell pepper was inoculated with a four-strain cocktail of either *L. monocytogenes* or *S. enterica* at 4 log CFU/g and dried at ambient temperature for 24 h. Inoculated dehydrated green bell pepper was rehydrated with 5 or 25°C water, followed by storage at one of the three temperatures. The highest growth rate (1.46±0.49 log CFU/g per d) was observed by *L. monocytogenes* on 25°C-rehydrated green bell pepper during 25°C storage which resulted in a 1 log CFU/g increase in only 0.90 d (16.4 h). The second objective of this study was to determine the growth kinetics of *L. monocytogenes* and *S. enterica* in non-dehydrated but heat-treated green bell pepper. Chopped green bell pepper was cooked in an oven at 177°C (350°F) for 25 min, followed by cooling at 5°C for 20 min. Cooked peppers were inoculated with either pathogen cocktail at 4 log CFU/g and stored at the three different temperatures. The highest growth rate (4.96±0.35 log CFU/g per d) was observed by *S. enterica* during storage at 25°C, leading to a 1 log CFU/g increase in only 0.20 d (4.80 h). This growth rate was significantly higher than any growth rate observed for either pathogen during storage of rehydrated green bell pepper. Understanding the growth kinetics of these pathogens in rehydrated plant foods and cooked plant foods can inform discussions surrounding whether specific time and temperature conditions for rehydrating and storing of these plant foods should

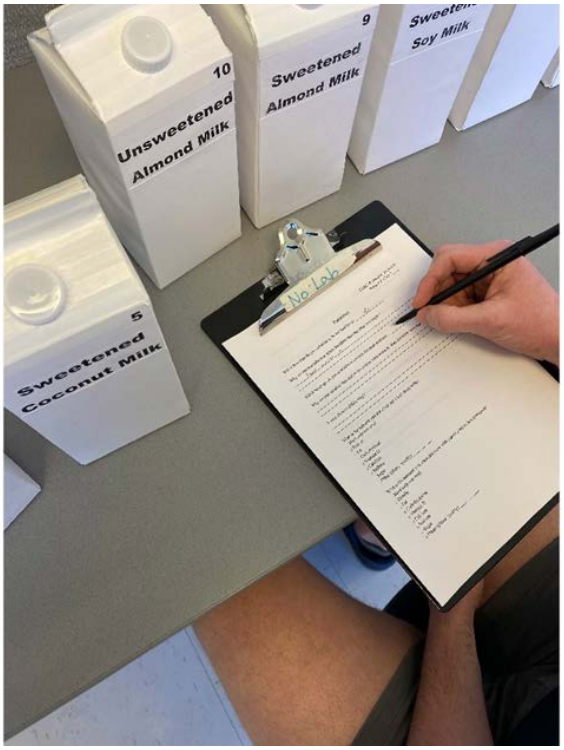
be recommended. This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

Development of a Quantum Dot-Based Microfluidic Device for the Rapid Detection of Biologically Active Botulinum Neurotoxin in Complex Media



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 active toxin that is able to harm human beings if ingested. The current project extends this nanosensor to detect additional BoNT serotypes of relevance to food safety, and it aims to translate the technology to a microfluidic chip-based platform for rapid field-based detection. In the last year, we have been developing an analogous nanosensor for detection of BoNT serotype F. A biorecognition peptide that is specific for the F serotype has been ordered and conjugation chemistry with 800 nm emitting QDs has been optimized. We have verified that this peptide-QD complex is able to detect the F-type light chain in buffer solution in under 2 hours total detection time and have determined preliminary limits of detection and sensitivity benchmarks, which compare favorably to the mouse bioassay for this toxin serotype. Currently we are optimizing the sensor performance and evaluating critical limit of detection, sensitivity, and selectivity benchmarks, with plans to evaluate its performance on F-type holotoxin and multiplexed (simultaneous) detection of A, B, E, and F serotypes. In addition to the solution-based work, we have begun translating the technology to a microfluidic chip platform. This work is being done in collaboration with FDA's Center for Biologics Evaluation and Research (CBER) and San Jose State University. Polymer substrate has successfully been functionalized with BoNT-selective peptides, and cleavage in the presence of BoNT light chain has been demonstrated. The most important deliverable of this project is a reliable method that can detect 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.

Plant-Based Milk Alternative – Consumer Perspectives



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 on the south side of Chicago, consumers indicated that "nutritional value" was an important consideration when purchasing both dairy milk and plant-based milks; and further, they 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 sugar, sodium and Vitamin D with plant-based milks. Almond milk was chosen as the healthiest milk option. To follow up on these findings we have designed a series of experiments focusing specifically on nutritional attributes and trade-offs mediating ingestion choices. The study had 100 people complete the follow up study. The main findings are summarized as follows. Taste and nutrients were the top two factors that participants considered when purchasing Dairy milk (DM) or Plant-based milk alternatives (PBMA), which aligned with the results of the pilot study. DM was most associated with Vitamin D and calcium before providing nutritional labels and after label exposure. PBMA was most associated with protein before and after nutrition label exposure. People generally understand nutrient content differences between PBMA and DM, but confusion remains on the protein content of almond, rice, and coconut milk beverages. Those 35 or older (mainly Gen X, but also Baby Boomers) and people with higher BMIs were more confused about protein content than younger and leaner participants, in that they reported almond, rice and coconut as having high protein content. Confusion regarding sugar content was also apparent, suggesting a misunderstanding between inherent and added sugar. The analysis is mostly complete and a manuscript is being prepared. This research was funded through CFSAN's Cooperative Agreement with IFSH and the DFPST operating budget.

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. Rachael Kozolup

Longitudinal Study to Investigate the Ecology and Epidemiology of Human Foodborne Pathogens in the California Central Coast

Between 2009 and 2018, FDA and CDC identified 40 foodborne outbreaks of Shiga toxin-producing *E. coli* (STEC) infections in the U.S. with a confirmed or suspected link to leafy greens. In November 2020, FDA launched a multi-year study to improve food safety through enhanced understanding of the ecology of human pathogens in the environment that may cause foodborne illness outbreaks associated with leafy greens grown in the Central Coast of California. The California Longitudinal Study (CALS) is a partnership between FDA, WCFS, the California Department of Food and Agriculture (CDFA), and agricultural stakeholders. The overall goal of CALS is to provide information on how pathogens survive in various reservoirs (e.g., prevalence in water, air, soil, animals) and how they move throughout the environment, leading to produce contamination prior to harvest. During the first year of the study, the CALS team addressed four aims:

1) design and initiate a sampling plan on private ranches and adjacent lands (e.g., composting operations, vineyards, cattle ranches); 2) develop and implement pathogen sampling for publicly accessible locations in Salinas Valley; 3) optimize and standardize field and laboratory methods across WCFS and FDA CFSAN laboratories; and 4) communicate study goals, regulatory compliance, and research progress to stakeholders including the CALS Industry Advisory Group and affiliated groups. A large part of the first year of the study was aimed at recruitment efforts to enroll a variety of agricultural operations, and standardization of laboratory methods. To date, approximately 50 sampling events have occurred resulting in over 1,200 individual samples analyzed. Approximately 90 STEC and *Salmonella* isolates were submitted to FDA's GenomeTrakr for whole genome sequencing. In the upcoming year culture-dependent and independent metagenomics will be expanded, including samples from air, soil, water and animals. This research was funded through CFSAN's Cooperative Agreement with WCFS.

Evaluation of Microbiological Risks Associated with Application of Crop Protection Sprays on Developing Walnuts

Crop protection sprays are part of an integrated pest management plan and can be employed through all stages of treenut production, including the period immediately before harvest. Crop protection sprays have varying application intervals, the minimum allowable time between application and harvest; some are as low as 0 days. In general, a concentrated pest control product will be mixed with 300 to 600 L of water per acre application, which is generally made with an air blast sprayer, but other methods such as with a handgun sprayer or aerial application are also employed. These crop protection sprays will introduce agricultural water directly to growing produce which is subject to the Produce Safety Rule. The specific aim of this project builds on our previous work to evaluate the survival of inoculated generic (non-pathogenic) *E. coli* on maturing walnuts within 1 month of anticipated harvest. Trees in an on-campus walnut research orchard were sprayed in September 2020 (husks were split and had begun to dehydrate) with a low-level cocktail of generic *E. coli* consisting of TVS 353, TVS 354, and TVS 355 strains. Walnut maturity impacted the survival of inoculated *E. coli* and should be considered when assessing the quality of water used for agricultural chemical application. The ability of pathogens to survive or grow in application-strength agricultural chemicals, especially those that may be applied closer to harvest, should also be considered. Studies are ongoing to determine the rate of decline in some insecticides, and to determine the impact of other variables such as presence of organic material. The microbiological data collected in this study will provide information on the likelihood for crop protection sprays applied to tree nuts to serve as a contamination source. The information generated will fill a needed data gap to assist FDA and state regulators on assessing agricultural-water based risks associated with near-harvest application of crop protection sprays applied to

tree nuts. Information generated here will help growers of tree crops to characterize the risks associated with direct application of agricultural water. While focused on tree nuts the work is relevant to other tree fruit. This information will also fill data gaps for the FDA produce risk assessment team. This research was funded through CFSAN's Cooperative Agreement with WCFS.

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

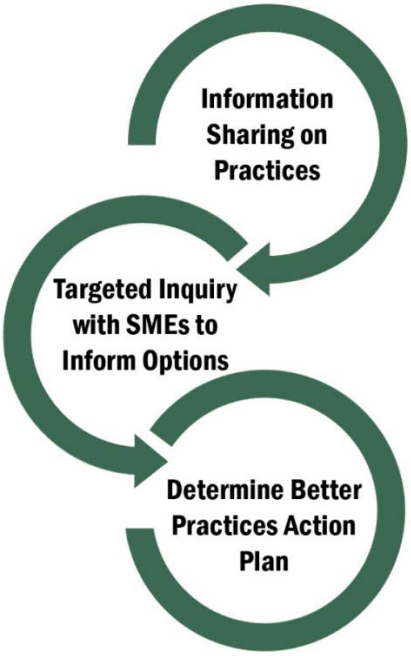
There is a need to quantify the risks and clarify the mechanisms for produce contamination by airborne transmission of bacterial pathogens originating from nearby CAFO facilities (e.g., beef cattle feedlots, poultry facilities, dairy farms). Routes of airborne contamination of produce from CAFOs might 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. In order to better quantify these risks and to develop targeted mitigation strategies if such risks are found to be unacceptably high, numerous knowledge gaps need to be resolved. A 6-month prospective cohort study was conducted 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. A total of 300 air samples were collected during monthly trips to Imperial Valley from November 2020 to April 2021. No *E. coli* O157 or other STEC were detected, but one air sample that was >1000 feet downwind was positive for *Salmonella* (1/300). A small number of samples were positive by PCR for the presence of Shiga-toxin I or II genes but were culture negative for STEC; these PCR-positives were found at sporadic distances from the feedlot suggesting that the bacterial source may be from the immediate vicinity of the air sampler and not fugitive dust from the nearby feedlot. A statistical model is being developed to analyze the effect of wind speed, wind direction, and other local factors on the prevalence and concentration of bacterial indicators in the proximity to feedlots in the Imperial Valley. In order to inform the livestock industry of these results and preliminary recommendations for minimizing fugitive dust and improving produce food safety, the executive director of the California Cattle Council has been briefed on the project's preliminary results. This project will clarify the food safety risks from fugitive dust and airborne bacteria from CAFOs and help lead to the development of good agricultural practices to reduce bioaerosols emanating from these livestock facilities, thereby improving produce safety from the greater Yuma growing region. This research was funded through CFSAN's Cooperative Agreement with WCFS.

Growth of Foodborne Pathogens During Soaking of Tree Nuts

Almonds are the most commonly "soaked" treenut. Brazil nuts, cashews, hazelnuts, macadamia, pecans, pine nuts, and walnuts can also be soaked prior to consumption. There is a common belief that these nuts are somehow "activated" through possible initiation of germination but the data to support this benefit is limited. The specific aim of this project was to determine the ability

of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* to grow in a range of tree nuts other than almonds during soaking. Walnuts were selected for in-depth study based on an assessment of relative popularity of soaking these products both at home and commercially and because they are an important U.S. crop. 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 by 2 to 3 log CFU/g over 24 h at 23°C; no significant increases were observed in 24 h at 15 and 18°C. Growth curves for the three pathogens were determined during soaking at 23°C and lag times of approximately 8 h were observed. *Salmonella* on walnuts that were soaked at ambient conditions for 24 h and then dried at 66 °C for 24 h decreased by ~1 log CFU/g at ≥12 h. The data generated for walnuts (and previously for almonds) 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. Soaking under cold or cool temperatures over shorter time periods coupled with basic sanitation principles, and possibly sourcing and use of treated rather than raw materials, should be included best practice recommendations for soaking tree nuts in the home or under commercial conditions. This research was funded through CFSAN's Cooperative Agreement with WCFS.

California Agriculture Neighbors



Since January 2021, the agricultural community in the Salinas Valley has come together in an effort known as California Agricultural Neighbors (CAN). CAN provides a roundtable opportunity to foster collaboration and discuss enhanced neighborly food safety practices when agriculture operations are adjacent to one another. The work of CAN stemmed from a 2019 initiative, the California Good Agriculture Neighbors Workshops, which was led by WCFS in partnership with the Western Institute for Food Safety and Security, UC Cooperative Extension and supported by funding from the California Department of Food and Agriculture (CDFA). WCFS scientists are representing CALS on the CAN Dialogue Group, organized by CDFA and the Monterey County Farm Bureau. The effort brings together industry (leafy greens, cattle ranching, viticulture, compost), academia, and government to foster a deeper understanding among Salinas Valley agriculture neighbors of the practices that commonly take place throughout the year. An Interim Report was published, "Neighbor-to-neighbor best practices to help enhance localized food safety efforts," in June 2021. As described in the report, WCFS presented at the first webinar in the series entitled: "Reservoirs of *E. coli* O157:H7 and other STEC." In the future, CAN will continue to develop the stepwise effort that includes information sharing, options generation, refinement, and the development of a near-term action plan for enhanced practices. Additionally, the engagement of an ever-broadening roster of stakeholders, starting with neighbors, expanding to subject matter experts (SMEs), and then including the broader industry and supply chain are planned per the Interim Report. WCFS will remain a key partner in this regional effort. This work was funded by the California Department of Food and Agriculture (CDFA).

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