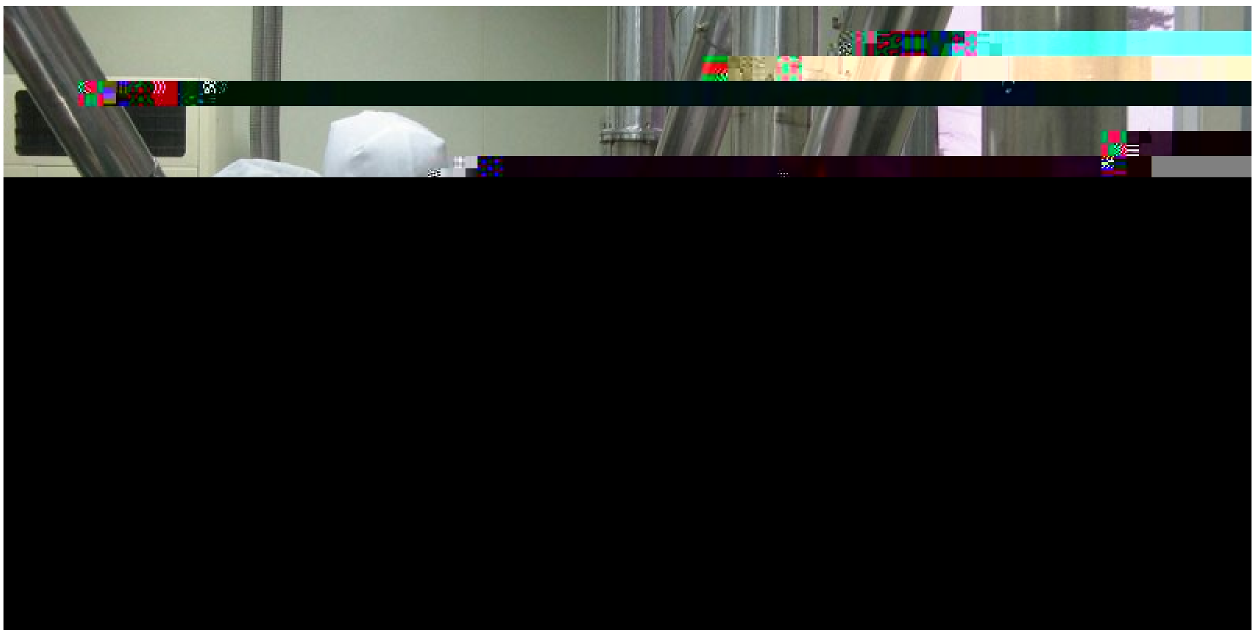
# September 2024

## Annual Report of Research

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



Heat Transfer Challenges in Blanching for Microorganism Reduction: Complex Geometries and Heat Transfer Coefficients

Greg

Linear ($R^2$ >0.86) relationships between *Salmonella* reduction and apple cube $a_w$ were observed for all conditions (n=13). The lowest and highest *Salmonella* reduction when reaching the same apple aw was estimated for LTMBLA and HTLBMA. On $a_w$ 0.60 apple cubes, estimated *Salmonella* reduction was 4.37 ± 0.18 and 8.88 ± 0.51 log CFU/sample for LTMBLA and HTMBLA, respectively. On $a_w$ 0.30 apple cubes, estimated *Salmonella* reduction was 4.18 ± 0.21 and 8.93 ± 0.36 log CFU/sample for LTMBLA and MTLBHA, respectively. For apple cubes dried to the same aw, higher *Salmonella* reduction was estimated for LTMBHA than LTMBLA, and for HTMBLA than LTMBLA ($p<0.05$), respectively. The bed depth and airflow had a significant ($p<0.05$) interactive effect on *Salmonella* reduction.

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

This work was supported by the Agriculture and Food Research Initiative, Sustainable Agricultural Systems Program Grant No. 2020-68012-31822 from the USDA National Institute of Food and Agriculture and in part through CFSAN’s Cooperative Agreement with IFSH and the DFPST operating budget.

## Isothermal Inactivation Kinetics of Salmonella Montevideo on Partially Dried Apple Cubes

Xiyang Liu[1], Alvin Lee[1], Elizabeth Grasso-Kelley[2], Suresh Vakkalagadda[3], and Nathan Anderson[2]

*[1]Illinois Tech IFSH; [2]Food and Drug Administration; [3]Illinois Tech FdSN*

Thermal resistance of *Salmonella* is known to increase as the water activity decreases. The dynamic nature of apple drying from high to low water activity poses challenges in predicting microbial lethality. Additional data on the thermal inactivation kinetics of *Salmonella* on apple cubes can assist in predicting microbial inactivation during drying. In addition, a preliminary study has shown that *Salmonella* Montevideo appeared to be the most thermally resistant compared to five other *Salmonella* strains under isothermal treatments. The purpose of this study is to investigate *Salmonella* Montevideo inactivation on partially dried apple cubes with different $a_w$ during isothermal treatment at various temperatures.

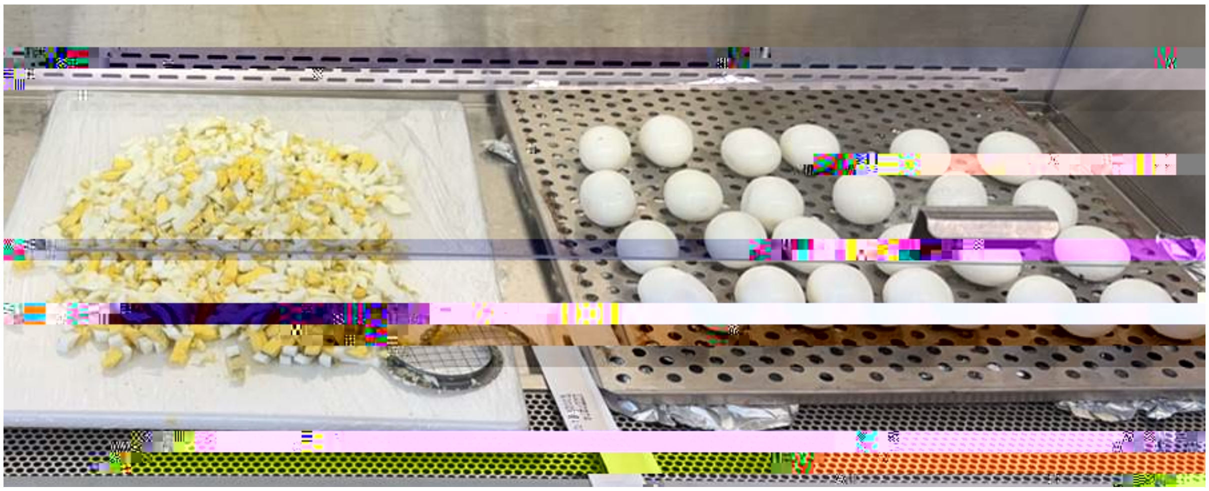
Gala apple cubes (6.40mm) were pre-dried to $a_w$ 0.45, 0.60 or 0.75. *Salmonella* Montevideo 488275 was harvested from lawn culture grown on tryptic soy agar with yeast extract (TSAYE) and inoculated onto the pre-dried apple cubes (3% v/w) to achieve ~8 log CFU/g population. After ~96h reequilibration (45, 60, or 75% RH), inoculated apple cubes were packed into aluminum test cells under controlled RH and isothermally treated in a water bath. At various time points (n=6), triplicate samples were collected and cooled in an ice-water bath, and *Salmonella* was enumerated on TSAYE with ammonium iron citrate and sodium thiosulfate.

Following post-inoculation equilibration at 45, 60, and 75% RH, the $a_w$ of apple cubes was 0.44 ± 0.01, 0.61 ± 0.02, and 0.76 ± 0.01, respectively. *Salmonella* populations post-equilibration were significantly greater ($p<0.05$) on apple cubes at $a_w$ 0.45 and 0.60 (8.46 ± 0.20 and 8.48 ±

# Microbiology Platform

Elizabeth Grasso-Kelley, FDA and Alvin Lee, Illinois Tech IFSH

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



# Evaluation of the Risk for Clostridium botulinum and Toxin Production in Commercial Plant-Based Meat Alternative Products

Catherine Rolfe[2], Travis Morrissey[2], Viviana Aguilar[1], Guy Skinner[2]
*[1]Illinois Tech IFSH; [2]Food and Drug Administration*

Plant-based meat alternative (PBMA) products contain a mixture of plant-based ingredients with common protein sources of wheat gluten, soybean, and pea and are intended to replace traditional animal-based meats. These products appeal to a variety of consumers, from those who are strictly vegan or vegetarian to those who aim to reduce their red meat intake or have allergies such as alpha-gal syndrome. PBMAs are produced from plant-derived ingredients, with a variety of

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

## Efficacy of Dry-heat Treatment in Reducing Salmonella and E. coli O157:H7 Populations on Sprout Seeds

Arlette Shazer, Tong-Jen Fu
*Food and Drug Administration*

The Produce Safety Rule requires that seeds used to grow sprouts be treated to reduce pathogens. Treatments may be applied at sprout operations or by seed suppliers. Numerous studies have been performed in search of effective seed treatments. Most of these studies focused on treatments applicable to sprout growers. Very few have examined treatments that may be applied by seed suppliers. Although chemical treatments are the most studied, their industrial use are limited due to the lack of EPA approved chemicals. Physical methods, such as dry heat, have increasingly been evaluated. Dry-heat treatments have the added advantages in that they are scalable and can avoid the need for a post-treatment drying step typically required for chemical treatments. These advantages make dry heat treatment a potential option for seed suppliers. The efficacy of dry heat for decontamination of seeds differed among published studies. For dry heat to be recommended as a seed treatment option, research is needed to better understand factors that may affect treatment efficacy and to identify conditions that can effectively decontaminate seeds while preserving their germination capability. In the previous study, we have evaluated the impact of dry heat in reducing *Salmonella* on inoculated mung beans. Factors such as treatment temperature, treatment time, relative humidity and treatment scale on pathogen reduction, seed germination and sprout yield were examined. In this study, experiments will be performed to examine the impact of various treatment conditions on inactivation of *Salmonella* and *E. coli* O157:H7 on other types of seeds (e.g., alfalfa) to understaesentie( t)-2 (ype)4 (a)3.9

Evaluation of Strain-Specific Phenotypic and Genomic Differences on the Survival of Listeria monocytogenes on Selected Vegetables During Frozen and Thawed Storage associated with hard-cooked eggs and previous research suggesting that citric acid at pH 2.5 may not be effective at reducing *L. monocytogenes* on hard-cooked eggs, it is important to understand how the addition of treated or untreated contaminated hard-cooked eggs into deli salads affects the survival and/or growth of *L. monocytogenes*.

Hard-boiled eggs (HBEs) were submerged in 2% citric acid or water at 5°C for 24 h as a pre-treatment. The HBEs were then inoculated with a high inoculation level of *L. monocytogenes* for modeling studies (4 log CFU/HBE) or at a low level for survival studies (1 log CFU/HBE). The inoculum was allowed to dry and the eggs were chopped. Chopped HBEs were stored at 5, 10 or 25ºC for up to 14 days. It was determined that there was no significant difference between the growth rates of *L. monocytogenes* on citric acid treated and untreated chopped HBEs when stored at 5ºC. However, there was a significant difference between growth rates at 10 and 15ºC betwee the HBE treatments; longer lag phases were observed in the citric acid treated HBEs, along with

leli salad

recipes including potato, chicken, macaroni, tuna and egg salad. Deli salads were then stored for up to 1 month at 5, 10, or 15ºC. Minimal differences in survival in deli salads was observed at 5 and 10°C regardless of if

treatment. Understanding the microbiome of these non-sterile products is essential and will aid in understanding pathogen interaction and survival and dynamics during enrichment. A few published studies have identified the microbiomes of PIF production facilities or the PIF manufacturing process in Asian countries, however, no study has identified the microbiomes of commercially-available PIF in the U.S. Furthermore, *C. sakazakii* has a high tolerance to desiccation and other stressors. On abiotic surfaces, *C. sakazakii*, especially in its sessile form, displays a high tolerance to sanitizers. However, the molecular mechanisms by which *C. sakazakii* responds to these harsh conditions are not well understood. Understanding how *C. sakazakii* overcomes the stress associated with PIF manufacturing plants and the sanitizers used in these environments will aid in creating science-based preventive controls. The main aims of this study are therefore to 1.) identify the microbiome of PIF and assess the population dynamics of *C. sakazakii* and the native microbiota in PIF during standard enrichment, and 2.) assess the inactivation and molecular response of *C. sakazakii* to desiccation stress in PIF and on food-contact surfaces with and without sanitizer treatment.

This research will fill data gaps on the microbiomes of PIF products and the population dynamics of *C. sakazakii* and native microbiota during enrichment. The study will also evaluate the survival mechanisms of *C. sakazakii* in response to PIF production environmental conditions.

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

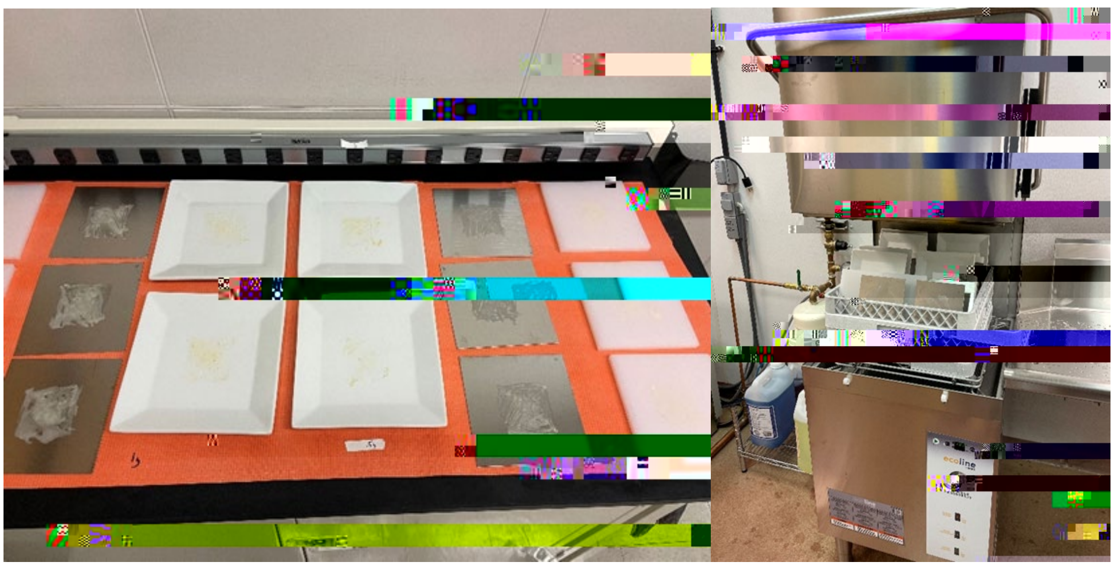
Assessment of Population Dynamics of Cronobacter sakazakii and Salmonella enterica in Powdered and Reconstituted Infant Formula During Storage

Joelle Salazar[2], Diana Stewart[2], Ravinder Reddy[2], Megan Fay[2], Wei Zhang[1]
[1]*Illinois Tech IFSH; 350.k89f (noi).f 8.04 0 0 8-0.004 Tc 0.004 Tw 12 0 0 12 308.88 363.4 Tc 0.004 -0.01 Tw 12*

# Chemistry and Packaging Platform

Lauren Jackson, FDA and Brian Schaneberg, Illinois Tech IFSH

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



# Assessment of Allergen Cross-Contact Risk Associated with Production of Oil-Roasted Nut and Peanut Products

Robert Beverly, Lauren Jackson
*Food and Drug Administration*

Oil roasting is a common method for roasting peanuts and tree nuts. During the roasting process, peanut particulates can contaminate the roasting oil. When the oil is reused for roasting or frying other products with different allergen profiles, there is a potential for allergen cross-contact. It is not well understood how the oil roasting process and oil regeneration treatments such as filtering affect peanut detection and peanut levels in roasting oil. Immunochemical methods such as ELISA have reduced efficacy when the target protein is denatured, as occurs during heating, and can underestimate the amount of allergen present. Quantitative proteomics methods utilizing mass spectrometry may be able to overcome the deficiencies of ELISA, as both unheated and heated peanut proteins can be digested into peptides for detection.

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

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

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

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

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

## Transfer of Seafood Allergens to Frying Oil and Subsequent Fried Products

Xingyi Jiang, Lauren Jackson
*Food and Drug Administration*

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

For shrimp proteins and gluten extraction from oil, the selection of defatting reagents, extraction buffer ingredients, extraction time and temperature were optimized. The developed method included the following three key steps: (1) centrifugation to separate oil phase and particulates; (2) defatting using an acetone:hexane mixture to reduce oil interference; and (3) protein extraction using 10 mM phosphate buffered saline (PBS, pH 7.4) containing 6 0 X U H D D Q G -mercaptoethanol (PBS-urea- 0 ( 7 K L V V W U D W H J \ Z D V G H Y H O R S H G E D V H G proteins existed as micro-particulates in oils. Compared to previous reported methodologies, this strategy requires only small amounts of defatting and extraction reagents, significantly simplifying the extraction procedure.

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

For quantification purpose, gluten-spike oil underwent a frying process at 180 °C for 3 min. Subsequently, the oil was utilized to prepare 12 g of fries (180 °C for 5 min). The results showed that around 250 ppm of gluten were presented in oil after frying, and about 4%, i.e., 109 ppm, was transferred to fries. In a real-world scenario, around 75 g/batch of breaded shrimp were fried in 750 g oil at 180 °C for 2.5 min. In total, ten batches were prepared. The oil was collected for protein extraction after the 1st, 3rd, 6th, and 10th batch. Subsequently, the oil from after completing 10 batches was utilized to prepare fries, chicken nuggets, and tarter tots following the instructions provided on their labels. Our results showed that the oil residual gluten level increased as a function of frying batch. The amount of gluten transferred was dependent on the undeclared peanut, almond, and cashew in various PBB. The higher frequency of allergen related recalls in recent years suggests that more information is needed about unintended allergen presence (UAP) in PPB and the effectiveness of cleaning and other allergen control practices used by firms manufacturing these products. This project is assessing peanut, tree nut and seed butters (peanut, almond, hazelnut, cashew, soy, sunflower, sesame, etc.) for the presence and concentrations of unintended allergens (especially various tree nuts, peanuts, soy, wheat/gluten, and sesame) and determine how these results relate to allergen advisory statements found on each product. A variety of major seed and nut butters will be purchased, and different lots of each product will be assessed to determine whether allergen concentrations vary between batches/lots. The xMAP food allergen detection assay (FADA) is being used to quantify the allergens present in the products. The second part of this study aims to evaluate cleaning procedures at a pilot-plant scale to determine their effectiveness at reducing cross-contact when manufacturing different PBB products on shared equipment.

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

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

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

Jeremiah Kidd[2], Lauren Jackson[2], Tina Gettis[1], Amandeep Sandhu[1]
*[1]Illinois Tech IFSH; [2]Food and Drug Administration*

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

Over the past year, two separate studies were completed. The first study evaluated the use of wet and dry wipes on their ability to remove allergens from food-contact surface, while the study determined the effectiveness of manual and mechanical washing methods at removing allergen-containing foods from food-contact surfaces. For the wiping study, three stainless steel (SS) and three textured white polyethylene (PE) coupons were contaminated with 0.5 g or 1 g of egg-based (powdered whole egg; reconstituted whole egg powder), gluten-containing (wheat flour; wheat-containing batter), or sesame-based (sesame flour; tahini) foods. After drying for 30 min at ambient temperatures (23 ± 2°C), coupons were wiped with either a dry paper wipe, a dry terry cloth, a wet terry cloth soaked in 200 ppm quat solution, or one, two, or three sanitizing wipes for 5 sec. A second type of sanitizing wipe was also evaluated for tahini-contaminated surfaces. After wiping treatments, coupons were tested for residual allergens with lateral flow devices (LFDs). Three independent trials were conducted for each experimental variable. For the washing study, coupons made of PE (n=3), SS (n=3), and ceramic (CE, n=3) were contaminated with 0.5 g or 1 g of egg-based (egg powder; reconstituted egg powder), gluten-containing (wheat flour; batter), or sesame-based (sesame flour; tahini) foods. After drying for 30 min at 23±2°C, coupons were subjected to a manual or mechanical washing treatment. The manual treatment used 3, 18.9 L buckets containing wash solutions at 43°C. Coupons were washed (10 sec), rinsed (10 sec), and then sanitized (60 sec), in neutral detergent solution, water, and 200 ppm quat solution, respectively. Mechanical treatments involved using contaminated coupons in commercial warewashing machines. Residual allergens on coupons were detected with LFDs. Washing trials were done in triplicate.

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

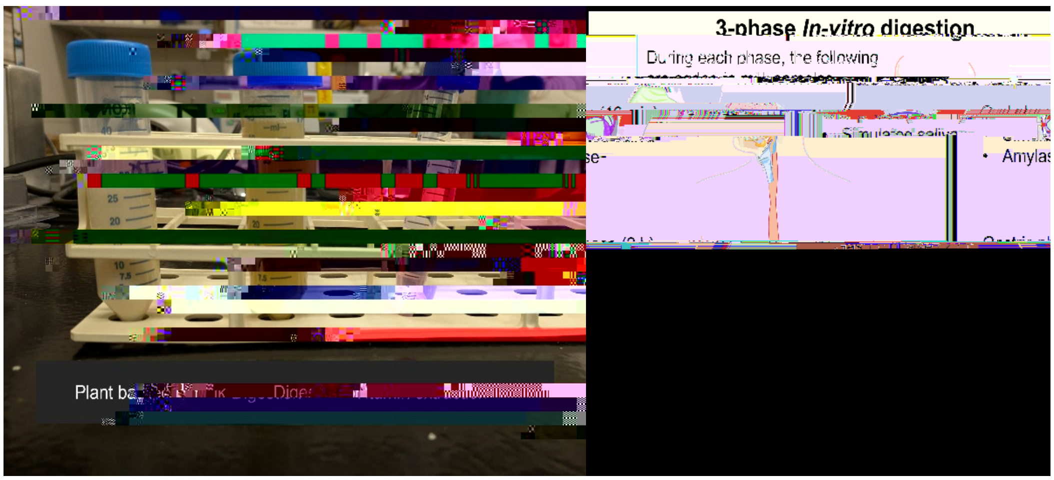
Overall, the results indicate that wet wipes are more effective for removal of allergens than dry wipes. Using multiple wet wipes facilitates removal of allergenic foods. The results from the washing study indicate that the nature of the food soil and surface impacted the effectiveness of washing treatments.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

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

# Nutrition Platform

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

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



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