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2009 PROJECT PROPOSALS

CHEMICAL CONSTITUENTS
AND ALLERGENS PLATFORM

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Chemical Contaminants and Allergens

Title: Cleaning and Validation to Prevent Allergen Cross-Contact

Project Leader(s): Lauren Jackson

New or Continuing: Continuing **Start: October 1, 2008** **End: September 2010**

Background and Justification:
Undeclared allergens can be inadvertently introduced into a food due to cross-contact during manufacture. Cleaning is considered a first line of defence against cross-contact on shared processing lines. The challenges to removing allergenic foods from processing equipment in a dry manufacturing environment are substantial. Currently little information exists on the effectiveness of dry cleaning methods for allergen removal and methods that could be used to validate/verify cleaning procedures in a dry environment. In addition, although cleaning solutions are often reused, little is known about the safety of this practice. Research is needed to determine the effectiveness dry cleaning procedures (compressed air, vacuum, dry steam, brushing, cloths/wipes) for removal of allergenic foods from different food-contact surfaces and to determine the best practices for validating/verifying dry cleaning effectiveness. Research is also needed to evaluate the safety of cleaning solution reuse.

Project Objectives (and Milestones, with timeline, if a continuing project):

Objectives:
The main objective is to determine the effectiveness of a variety of dry cleaning procedures (compressed air, vacuum, brushing, CO₂ or grit blasting, cloths/wipes) for removing allergenic foods (milk, egg and peanut) from different food-contact surfaces. A second objective is to study methods for validating/verifying dry cleaning effectiveness. The final objective is to study the effectiveness of cleaning solution reuse on removal of milk residues from a pilot-scale dairy processing system.

Milestones:

1. Complete studies on determining the effectiveness of different dry cleaning procedures (vacuum, compressed air, brushing, grit/CO₂ blasting, water- and sanitizer solution moistened cloths) on removal of various allergenic foods (peanut, egg, milk) from a variety of food-contact surfaces (stainless steel, Teflon, etc) (Complete by September 2009).
2. Example the effects of use of a dry steam/vacuum system for removing allergenic food residues from belting material and study methods for validating the effectiveness of allergen removal (Complete by February 2010).
3. Conduct an in-depth study of analytical methods (ELISA, LFD, ATP, total protein, visual methods, etc) for validating/verifying the effectiveness of dry cleaning procedures (Complete by April 2010).
4. Study milk/juice cross-contact in a model dairy processing operation (at NCFST) and determine the safety of cleaning solution reuse. This study will investigate the efficacy of different cleaning procedures (a simple water rinse, an intermediate cleaning procedure, and a complete sanitation cycle) on removal of milk proteins from a pilot-scale HTST system (Complete by September 2010).

Benefits to Stakeholders:
The information generated in this project will assist the food industry and FDA in the establishment of best practices for allergen cleaning and validation/verification in a dry manufacturing environment. In addition, this work will assist the food industry and FDA by determining the safety of cleaning solution reuse.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Chemical Constituents and Allergens

Title: Impact of processing on food allergens

Project Leader(s): Tong-Jen Fu

New or Continuing: Continuing

Start: October 1, 2008

End: June 30, 2011

Background and Justification:

Information relating to the impact of processing on food allergenicity is critical for allergen risk assessment and for the development of effective allergen control measures. Components derived from the eight major allergenic foods are used in a wide range of food applications. Research is needed to determine the allergenic potential of these ingredients and how it may be affected by processing conditions. Research is also needed to identify processing approaches that can supplement cleaning to eliminate allergenic residues from food contact surfaces. Commercial ELISA test kits are increasingly used to ensure the absence of undeclared allergens in food and to validate allergen control measures. Whether the allergenic potential of thermally processed foods is accurately indicated by commercial test kits remains to be determined. Allergen cross contact can occur through the use of common processing media (e.g., frying oil or Clean-In-Place cleaning solutions). Detection of allergens in these media can be challenging either due to extreme heat, the non-aqueous nature of frying oils or the high acidity (or alkalinity) of the cleaning solutions. Research is needed to determine whether commercial ELISA kits are effective in quantifying allergenic residues in re-used processing media.

Project Objectives (and Milestones, with timeline, if a continuing project):

Objectives:

To determine the impact of processing on food allergen detection. To determine the impact of processing on the allergenicity of food and food ingredients, and to identify processing approaches to reduce / eliminate allergenic residues from food contact surfaces

Milestones:

1. Characterize the impact of thermal processing on the structural and immunological properties of food allergens. Analytical tools including circular dichroism, differential scanning calorimetry and inhibition ELISA will be used (June 2010).
2. Determine the effect of heat on the allergenicity of egg, milk and peanut. Standard reference materials will be used as model foods (June 2010).
3. Determine the allergenicity of ingredients from egg, milk or peanut and determine whether their allergenicity is affected by heat treatment (Dec. 2010).
4. Determine whether the allergenic potential of thermally processed foods / food ingredients is accurately indicated by commercial ELISA kits (Dec. 2010).
5. Determine the efficacy of elevated dry heat treatment for reduction of the allergenicity of foods deposited on stainless steel surfaces (June 2011).
6. Evaluate commercial ELISA test kits for detection of allergen residues in re-used cooking oils and cleaning solutions and determine whether allergen quantitation as determined by the test kits reflects the allergenic nature of the re-used media (June 2011).

Benefits to Stakeholders:

Data generated will help the FDA and the food industry to better assess the allergenic potential of processed foods and will provide baseline information with respect to FALCPA implementation and compliance. The study will help to develop effective allergen control measures by providing 1) alternative approaches to reduce allergenic residues from food contact surfaces, 2) a better understanding of the efficacy of commercial test kits for detection of allergens in processed foods and shared cooking/cleaning media, and 3) information relating to the safety of cleaning solution / cooking media reuse.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Chemical Constituents and Allergens

Title: Structural characterization of food allergens

Project Leader(s): Yuzhu Zhang, Tong-Jen Fu

New or Continuing: New

Start: July 1, 2009

End: June 30, 2011

Background and Justification:

It is not clear what makes a protein an allergen. Research has increasingly been focused on the structural characterization of proteins in order to understand the molecular basis of protein allergenicity. Although linear IgE-binding epitopes for a number of food allergens have been determined, information relating to the conformational epitopes of allergens is limited. The identification and characterization of conformational epitopes require an understanding of the 3D structure of the proteins. Only very few food allergens of which the 3D structures have been determined. Previously, we have successfully elucidated the crystal structures of a peanut and an almond allergen and determination of a number of other allergens are in the pipeline. In this project, work will continue to elucidate the structures of additional allergens at high resolutions. Research will also be conducted to identify and characterize the linear and conformational epitopes of food allergens and to assess the effect of food processing on the IgE binding characteristics and thus the allergenicity of proteins. Protein engineering techniques will also be used to identify the amino acid sequences that are critical to protein allergenicity. Manipulations of the IgE binding epitopes through molecular biology or food processing techniques will allow the development of foods with lower allergenicity as well as reagents for immunotherapy in the near future.

Project Objectives (and Milestones, with timeline, if a continuing project):

Objectives:

To determine the crystal structure of food allergens. To determine conformational epitopes on milk and peanut allergens and the allergenicity of food proteins before and after the elimination of conformational

Milestones:

1. Determine the crystal structures of the Brazil nut allergen Ber e 2 and the hazelnut allergen Cor a 9 (using X-ray crystallography tool).
2. Develop a novel method for determining conformational epitopes of food allergens using milk allergens (alpha-lactalbumin and beta-lactoglobulin) as model systems. (12 months).
3. Determine the changes in the IgE binding epitopes and overall protein allergenicity as affected by different food processing conditions or protein mutagenesis.
4. Design recombinant proteins with reduced allergenicity but maintaining original biochemical properties using alpha-lactalbumin and beta-lactoglobulin as model systems. (12 months).

Benefits to Stakeholders:

This study will help the FDA and food industry with a better understanding of the molecular basis of protein allergenicity which will lead to a better risk assessment of food allergens. The research will also provide a novel approach for the development of foods with reduced allergenicity as well as reagents that are useful for the treatment of food allergy.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Chemical Contaminants and Allergens / Microbiology

Title: Best Practices for Cleaning/Sanitation of Nut Butter Processing Equipment

Project Leader(s): Lauren Jackson; Lou Tortorello

New or Continuing: New

Start: July 1, 2009 End: June 30, 2011

Background and Justification:

In February 2007, an outbreak of *Salmonella* serotype Tennessee associated with peanut butter consumption was reported in the U.S. In 2004, raw almonds distributed throughout the United States and internationally were implicated as the source of the *Salmonella enterica* serotype Enteritidis infections. Currently, the FDA and the California Department of Public Health are investigating *Salmonella* contamination in pistachios and pistachio products. Peanuts, almonds and other types of nuts can become contaminated with *Salmonella* strains during growth, harvest, transportation, storage. Consequently, pathogens can be introduced into processed nut products, such as nut butters, manufactured from contaminated nuts. In addition to microbial hazards that can be present in nut butters, allergenic proteins from one nut product (e.g. peanut butter) can be introduced into another nut-containing product (e.g. almond butter) if they are produced on the same processing lines.

Effective cleaning and sanitation of nut butter lines are essential for preventing cross-contamination of microbial hazards such as *Salmonella* and cross-contact of allergens. However, little information exists on the effectiveness of sanitation procedures for controlling microbial hazards and allergens in nut processing operations. Research is needed to develop guidelines to clean and sanitize nut processing equipment to inactivate microbial hazards and remove allergenic food residues.

Project Objectives (and Milestones, with timeline, if a continuing project):

Objectives:

The objective of this project is to determine the effectiveness of a variety cleaning and sanitizing protocols for inactivating microbial hazards and removing allergenic food residues in a laboratory setting and in pilot-scale equipment. Ultimately, this research will be used to develop guidelines for effective cleaning and sanitizing of nut processing equipment.

Milestones:

1. Conduct a survey of sanitation procedures used by nut processors to clean and sanitize processing equipment (Complete by December 2009).
2. Determine the effectiveness of a variety of cleaning/sanitizing treatments on removing peanut and almond residues from food-contact surfaces in a laboratory setting (Complete by June 2010).
3. Evaluate the effectiveness of a variety of cleaning/sanitizing treatments on inactivation of several strains of *Salmonella* on a variety of food-contact surfaces in the presence of peanut and almond butters. (Complete by September 2010).
4. Study the effectiveness of cleaning/sanitizing treatments on inactivating *Salmonella* strains and allergen removal in pilot-scale experiments (Complete by September 2010).

Benefits to Stakeholders:

The information generated in this project will assist the food industry and FDA in the establishment of best practices for cleaning/sanitizing nut processing equipment to prevent microbial cross-contamination and allergen cross-contact.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Chemical Constituents and Allergens

Title: Chemical Inactivation of Protein Toxins on Food-Contact Surfaces

Project Leader(s): Lauren Jackson

New or Continuing: Continuing **Start: October 1, 2008** **End: June 30, 2010**

Background and Justification:
Ricin and abrin are potent plant toxins found in seeds of the castor bean plant and rosary pea plant, respectively. Both toxins have potential for being used as biological weapons since they are relatively easy to isolate and purify and can be disseminated as food contaminants. In the case of a deliberate contamination event with ricin or abrin in a food processing facility, remediation of the food-contact surfaces must be done safely and effectively. Little information has been published on chemical treatments that could be used to inactivate protein toxins such as ricin and abrin on food-contact surfaces especially in the presence of food residues. The results of this project will provide guidance for effective inactivation of ricin and abrin in the presence of presence of different classes of foods. Another important outcome of this work will be identification of surrogate(s) that could be used to validate inactivation procedures in a food production facility. This project will determine if rapid methods (ELISA and activity-based assay) can be used to estimate the biological activity of treated ricin and abrin. Overall, this project will enhance food defence by enhancing our abilities to recover from an intentional contamination event with protein toxins such as ricin and abrin.

Project Objectives (and Milestones, with timeline, if a continuing project):

Objectives:
Identify cleaning/sanitizing treatments that result in inactivation of protein toxins (abrin and ricin) on food-contact surfaces in the absence and presence of different classes of food matrices (high fat, high protein, high starch). Identify surrogate(s) that can be used to study chemical inactivation of abrin or ricin. Compare ELISA detection to cytotoxicity assay and an activity-based assay for measuring loss of ricin/abrin activity in the presence of cleaning/sanitizing solutions.

Milestones:

1. Evaluate the effect of several sanitizers (e.g. sodium hypochlorite, iodophors, quaternary ammonium compounds, peroxyacid-based) on the stability of ricin and abrin and several enzymes (surrogates) as measured by ELISA (ricin/abrin) and enzyme activity assays (surrogates). (Complete by August 2009).
2. Evaluate the effect of several sanitizers (e.g. sodium hypochlorite, iodophors, quaternary ammonium compounds, peroxyacid-based) on the stability of ricin and abrin as measured with a cytotoxicity and a RTQ-PCR assay. (Complete by December 2009)
3. Complete calculations needed to determine the kinetics for inactivating ricin, abrin and potential surrogates for each cleaning solution, concentration and temperature. (Complete by March 2010)
4. Write and submit several manuscripts describing the effects of cleaning compounds on inactivation of protein toxins for publication in peer-reviewed journals. (Complete by June 30, 2010)

Benefits to Stakeholders:
The results of this project will provide guidance for effective inactivation of ricin and abrin in the presence of presence of different classes of foods. Another important outcome of this work will be identification of surrogate(s) that could be used to validate inactivation procedures in a food production facility. This project will determine if rapid methods (ELISA and activity-based assay) can be used to estimate the biological activity of treated ricin and abrin. Overall, this project will enhance food defence by enhancing our abilities to recover from an intentional contamination event with protein toxins such as ricin and abrin.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Chemical Constituents and Contaminants

Title: Washing methods for reducing the levels of chemical hazards associated with fresh produce

Project Leader(s): Jack Cappozzo

New or Continuing: New **Start: July 1, 2009** **End: June 30, 2010**

Background and Justification:

Because of the expansion of world-wide trade, more commodity items are being exported into the United States. There are concerns as to the safety of these food commodities because of recent reports of the presence of banned antimicrobial agents and pesticides in imported farm-raised seafood and produce, respectively. While economic factors drive the use of imported commodities, the safety of these foods needs to be a priority. Sampling and inspection programs cannot keep up with the pace of imports, and there are gaps in enforcement in instances where firms fail to adhere to procedures that prevent intentional and inadvertent contamination of food.

This project will examine the use of ultrasound in combination with washing treatments as a means for removing pesticide residues from vegetables and fruits. This technology is currently being assessed at the NCFST for pathogen removal/inactivation in fresh produce. The objectives of the research pursued here are to: (1) measure the stability of a variety of pesticides on several varieties of vegetables, (2) study the effectiveness of washing treatments alone for removing pesticide residues in vegetables, and (3) determine the efficacy of the use of ultrasound in combination with washing/sanitizing treatments for removing pesticide residues from vegetables.

Project Objectives (and Milestones, with timeline, if a continuing project):

Milestones:

1. Determine the scope of pesticide use in the regional and international areas and evaluate data base for pesticides of interest.
2. Validate analytical methods for volatile and semi-volatile pesticides by GC-MS and LC-MS-MS and determine residue levels in control matrices.
3. Establish protocols for decontamination protocols to washing systems
4. Establish protocols for matrix contamination (spiking) and stability
5. System application of contaminated matrix, analytical testing and data collection.

Benefits to Stakeholders:

This research will enable industry and government stakeholders to identify risk mitigation strategies that may be influential in ensuring the safety of produce.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Chemical Constituents and Allergens

Title: Use of Ultrasound Processing to Reduce Acrylamide Formation in Food Matrices

Project Leader(s): Jack Cappozzo

New or Continuing: New **Start: July 1, 2009** **End: June 30, 2010**

Background and Justification:

High temperature processing has been shown to result in acrylamide formation in carbohydrate-rich foods containing asparagine and glucose. Reducing processing time and/or temperature are effective at reducing acrylamide formation in foods, but often at the expense of product flavor, texture, color, and possibly food safety. Other methods that do not compromise food quality and safety are needed for acrylamide mitigation. The enzyme, asparaginase, has been shown to have varying effectiveness in preventing acrylamide formation in different food matrices. The efficacy of the enzyme treatment is influenced by the ability of the enzyme to come in contact with the substrate (asparagine) in the food matrix. Other methods for acrylamide mitigation include use of techniques that reduce processing time.

Two new applications of ultrasound processing will be assessed for its ability to reduce acrylamide formation in a model system (French fries). The first will involve the use of ultrasound to aid in the pretreatment of potato slices with asparaginase. The second will involve the use of ultrasound to aid in frying of French fries. A final study will evaluate a combination of the two applications for acrylamide reduction in fried potatoes.

Project Objectives (and Milestones, with timeline, if a continuing project):

Milestones:

1. Establish and assess Acrylamide formation from matrix (potato) for time and temperature parameters. [Acrylamide Control: Concentration]
2. Determine intervention protocols with enzyme pre-treatment and conduct experimental trials according to parameters established in Milestone 1. [Enzyme Treated Results]
3. Determine intervention protocols with Ultra-sound and conduct experimental trials according to parameters established in Milestone 1. [Ultra-sound Treated Results]
4. Determine the feasibility of combining Enzyme Pre-treatment and Ultrasound as an added effective means of reducing acrylamide formation and devise test protocols from milestone 1, 2 and 3
5. Evaluate Data for Report and Publication

Benefits to Stakeholders:

This research will enable industry and government stakeholders to identify risk mitigation strategies that may decrease the acrylamide formation in cooked potato products.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Chemical Constituents and Allergens

Title: Detection of Clostridium Botulinum Toxin in Food Matrices by Liquid Chromatography Mass Spectrometry

Project Leader(s): Jack Cappozzo

New or Continuing: New

Start: June 1, 2009

End: May 31, 2010

Background and Justification:

Clostridium botulinum toxin stands out as one of the most lethal poisons known, having an LD₅₀ of 0.8g for 70 kg human. The toxin consists of a 100 kDa heavy chain linked to a 50 kDa light chain by disulfide bonds. The light chain is responsible for the neurotoxic properties of the toxin and acts by cleaving a protein receptor that is responsible for releasing acetylcholine, thus resulting in flaccid muscle paralysis and potential death. Today, *C. bot* toxin is used for cosmetic treatments and treatment of muscle spastic disorders. Foodborne botulism is a rare but serious illness caused by consuming foods that are contaminated with *C. bot* toxin. Outbreaks of botulism can occur in foods that are not properly processed or in the case of intentional contamination of food. Several methods are currently available for detecting the presence of *C. bot* in food including the DIG-ELISA and the mouse bioassay. The mouse bio-assay is considered the more accurate of the two methods, but it can take days to complete the assay. There is a need to develop a method for confirming the presence of *C. bot* in foods that is reliable and can provide results in < 1 day.

The purpose of this project is to develop an LC-MS-MS method for detecting the presence of *C. bot* toxin in a variety of food matrices. This project will use LC-MS-MS to characterize the profile of peptides resulting from enzymatically hydrolysing *C. bot* toxin. This method will likely be able to detect the toxin with high specificity and at a low level of detection.

Project Objectives (and Milestones, with timeline, if a continuing project):

Milestones:

1. Establish Safety Protocol for the Project.
2. Implement activity assays to study digested **C. bot** toxin and determine the inactivity of the preparation
3. Chromatograph digested preparations of digested **C. bot** toxin.
4. Implement spiking assay to food matrices and apply digestion preparation and measure inactivity and chromatographic assay for detection. Evaluate data for report and publication.

Benefits to Stakeholders:

This research will enable industry and government stakeholders to quickly identify a potential dangerous issue from an emerging technology.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Chemical Constituents and Allergens		
Title: Furan Levels in Home-Prepared Foods		
Project Leader(s): Lauren Jackson		
New or Continuing: Continuing	Start: October, 2008	End: June 30, 2010
Background and Justification: <p>Furan is a volatile cyclic ether found in a number of foods that receive a heat treatment, such as canned and jarred foods. Its presence is a potential concern since it is both carcinogenic and cytotoxic to rodents. Although information exists on the occurrence of furan in food, there is a need for data on the effects of thermal processing conditions and home food preparation conditions on furan levels in foods. The information may lead to methods for reducing furan levels during home food preparation and will allow a better estimate of human exposure from home-prepared foods.</p>		
Project Objectives (and Milestones, with timeline, if a continuing project): <i>Objectives:</i> Determine the effects of home food preparation conditions on furan levels in foods. <i>Milestones:</i> <ol style="list-style-type: none">1. Compare a SPME/GC method to a direct head space/GC method for determining furan levels in a variety of canned/jarred foods and validate SPME/GC method. (Complete by December 2008)2. Measure furan levels in several food products including jarred infant and toddler foods such as sweet potatoes, carrots, apple sauce, green beans, meals containing pasta), canned vegetables, fruits and beans, fruit juices, potato chips and/or crackers. (Complete by March 2009).3. Determine the effects of cooking/preparation conditions on furan levels in selected foods. These studies will determine the effects of coffee strength, brewing method and brand on furan levels in brewed coffee. Other studies will determine the levels of furan in canned soups and stews as affected by cooking method (microwaved vs. stove-top heating), furan levels in jarred toddler foods as affected by heating method, and furan formation in surface-browned foods such as bread (Complete by June 2010).		
Benefits to Stakeholders: <p>The information generated in this project may lead to methods for reducing furan levels during home food preparation and will allow a better estimate of human exposure from home-prepared foods.</p>		