Risk Assessment

Template-based Peptide Modeling for Celiac Risk Assessment of Newly Expressed Proteins in GM Crops


**Significance:** Care should be exercised when applying peptide modeling to celiac disease risks until methods are validated.

Newly expressed proteins in genetically modified (GM) crops are subject to celiac disease risk assessment according to EFSA guidelines. Amino acid identity matches between short peptides (9aa) and known celiac restricted epitopes are required to be further evaluated through peptide modeling; however, validated methods and criteria are not yet available. In this investigation, several structures of HLA-DQ2.5/peptide/TCR (T-cell receptor) complexes were analyzed and two template-based peptide modeling software packages were evaluated using various peptides including ones not associated with celiac disease. Structural characterization that residues at P(osition)1, P2, P5, P8, and P9 in the 9aa restricted epitopes also contribute to the binding of celiac peptides to the HLA-DQ2.5 antigen in addition to the presence of the motif Q/EX1PX2 starting at P4 or P6. The recognition of the HLA-DQ2.5/peptide complex by TCR is through specific interactions between the residues in the restricted epitopes and some loop structures in the TCR. The template-based software package GalaxyPepDock seems to be suitable for the application of peptide modeling when an estimated accuracy value of >0.95 combined with >160 interaction similarity score are used as a threshold for biologically meaningful in silico binding. Nevertheless, caution should be exercised when applying peptide modeling to celiac disease risk assessment until methods are rigorously validated and further evaluated to demonstrate its value in the risk assessment of newly expressed proteins in GM crops.

Foodborne Pathogens

Survival and Virulence of *Listeria monocytogenes* During Storage on Chocolate Liquor, Corn Flakes, and Dry-Roasted, Shelled Pistachios at 4°C and 23°C


**Significance:** These findings on *Listeria monocytogenes* virulence and survival on three low-moisture foods (LMFs) during storage have relevance for predictive modeling used in microbial health risk assessments and support the addition of LMFs to food safety questionnaires conducted during listeriosis outbreaks. *This work was supported by the ILSI North America Food Microbiology Committee.*

The survival and virulence of *Listeria monocytogenes* was assessed during storage on three low-moisture foods (LMFs), chocolate liquor, corn flakes and shelled, dry-roasted pistachios (a w 0.18, 0.27, 0.20). The LMFs were inoculated with a 4-strain cocktail of *L. monocytogenes* at 8 log CFU/g, dried, equilibrated and then stored at 4°C, 25-81% relative humidity (RH) and
23°C, 30-35% RH for at least 336 days. At 4°C, *L. monocytogenes* remained stable on the LMFs for at least 336 days. At 23°C, *L. monocytogenes* levels declined on the chocolate liquor, corn flakes and pistachios at initial rates of 0.84, 0.88 and 0.32 log CFU/g/month, respectively. After 8 months at 23°C, *L. monocytogenes* concentrations on the chocolate liquor and corn flakes decreased to below the limit of detection (i.e., 0.48 log CFU/g). Relative populations of each strain were assessed before (i.e., day 0) and after 6 and 12 months of storage at 23°C and 4°C, respectively. Generally, a decline in the relative abundance of the serotype 1/2a strain was observed during storage, coupled with the relative increase of other strains, depending on the LMF and storage temperature. The total viable populations of *L. monocytogenes* quantified by PMAxx-qPCR after 12-plus months of storage at 4°C were significantly higher than that obtained by plating on TSA-YE by 1.8 to 3.7 logs. Decreases in the culturable population of *L. monocytogenes* during storage on the LMFs were the result of both cellular inactivation and transition to a viable-but-non-culturable state. The surviving cells, specifically after long-term storage at 4°C on the chocolate liquor and pistachios, remained infectious and capable of intracellular replication in Caco-2 enterocytes. These results have great relevance for predictive modeling used in microbial health risk assessments and support the addition of LMFs to food safety questionnaires conducted during listeriosis outbreaks.

**Differential Modulation of Listeria monocytogenes Fitness, In Vitro Virulence and Transcription of Virulence-Associated Genes in Response to the Presence of Different Microorganisms**


**Significance**: This study found that *Listeria monocytogenes* differentially alters the expression of virulence-associated genes as a survival strategy when confronted with different bacterial genus and species.

Interactions between *Listeria monocytogenes* and food-associated or environmental bacteria are critical not only for the growth but also for a number of key biological processes of the microorganism. In this regard, limited information exists on the impact of other microorganisms on the virulence of *L. monocytogenes*. In this study, the growth of *L. monocytogenes* was evaluated as single culture or in co-culture with *L. innocua*, *Bacillus subtilis*, *Lactobacillus plantarum* and *Pseudomonas aeruginosa* in tryptic soy broth (10°C/10 days and 37°C/24 hours). Transcriptional levels of 9 key virulence genes (inIA, inIB, inIC, inIJ, sigB, prfA, hly, plcA, plcB) and invasion efficiency and intracellular growth in Caco-2 cells, were determined for *L. monocytogenes* following grown in monon- or in co-culture for 3 days at 10°C or 9 hours at 37°C. The growth of *L. monocytogenes* was negatively affected by the presence of *L. innocua* and *B. subtilis*, while the effect of cell-to-cell contact on *L. monocytogenes* growth was dependent on the competing microorganism. Co-cultivation affected the *in vitro* virulence properties of *L. monocytogenes* in a microorganism-specific manner, with *L. innocua* mainly enhancing and *B. subtilis* reducing the invasion of the pathogen in Caco-2 cells. Assessment of the mRNA levels of *L. monocytogenes* virulence genes in the presence of the four tested bacteria revealed a complex pattern in which the observed up- or down-regulation was only partially correlated with growth or *in vitro* virulence and mainly suggested that *L. monocytogenes* may display a microorganism-specific transcriptional response. Importance *Listeria monocytogenes* is the etiological agent of the severe foodborne disease listeriosis. Important insight regarding the physiology and the infection biology of this microorganism has been acquired the past twenty years. However, despite the fact that *L. monocytogenes* co-exists with various microorganisms throughout its lifecycle and transmission from the environment to foods and then to the host, there is still limited knowledge related to the impact of surrounding microorganisms on *L. monocytogenes* biological functions. In this study we showed that *L. monocytogenes* modulates specific biological activities (i.e., growth and virulence potential) as a response to co-existing microorganisms and differentially alters the expression of virulence-associated genes when confronted with different bacterial genus and species. Our work suggests that the interaction with different bacteria plays a key role in the survival strategies by *L. monocytogenes* and supports the need to incorporate biotic factors in the research conducted for the identification of mechanisms deployed by this organism to be established in different environments.

**Foodborne Illness**

**Detecting Foodborne Disease Outbreaks in Florida Through Consumer Complaints**


**Significance**: Complaints to the Florida Complaint and Outbreak Reporting System were the main source of identifying outbreaks of Norovirus, non-typhoidal *Salmonella* enterica and scombroid food poisoning, as well as rare outbreaks of *Clostridium perfringens*, *Cryptosporidium* spp., *Shigella* spp., and *Vibrio vulnificus*.

The Florida Complaint and Outbreak Reporting System (FL-CORS) database is used by the Florida Department of Health’s Food and Waterborne Disease Program (FWDP) as one of the tools to detect foodborne disease outbreaks. We present a descriptive
and spatial network analysis of the FL-CORS data collected during 2015-2018. We also quantified foodborne disease outbreaks (FBO) which were investigated and confirmed due to a filed complaint and the etiological agents involved in these outbreaks. An increasing number of unique complaints filed in FL-CORS was observed during 2015 to 2018, with a sharp increase during 2017-2018 and a different seasonal pattern in 2018. The preferred mechanism of reporting varied by age group with younger people more frequently filing complaints online and older persons preferring reporting in-person or by phone. Spatial network analysis revealed that 87% of complaints had the same county of residence and county of presumed exposure. Frequency of complaints was negatively associated with linear distance between place of residence and place of exposure at zip code level. Counties located in North and Central Florida, as well as some coastal areas in South Florida had higher incidence rates of complaints. Those counties tend to have a larger population density, and some are popular vacation destinations. On average, 96 FBO were reported in Florida annually, of which 60% were confirmed with successful identification of the causative agent. 56% of the confirmed FBO were triggered by a complaint. Throughout the years, 2.4 - 2.8 FBO and 1.4 confirmed FBO were identified per 100 complaints. Ciguatera toxin was cause of 40% of all FBO in Florida, and only 28% were detected through complaints. In contrast, complaints were the main source of identifying outbreaks of Norovirus, non-typhoidal Salmonella enterica and scombroid food poisoning, as well as rare outbreaks of Clostridium perfringens, Cryptosporidium spp., Shigella spp., and Vibrio vulnificus.

**Mycotoxins**

**Cytotoxic Effects of Individual and Combined Sterigmatocystin and Nivalenol on Liver Hepatocellular Carcinoma Cells**


**Significance:** Predictive models could help to better understand the interaction between mycotoxins and their implications in food safety assessment.

Since humans are exposed to different mycotoxins through daily intake, there is increasing concern about the adverse effects of the interactions between them. Cytotoxicity of sterigmatocystin (STE) and nivalenol (NIV) alone and in combination in human hepatocarcinoma (HepG2) cells was evaluated by MTT assay. Furthermore, ROS production and alteration of \( \Delta \Psi_m \) as mechanisms of action were assessed. Cells were treated with concentrations ranging from 0.15 to 5 \( \mu M \) for NIV and from 0.78 to 50 \( \mu M \) for STE individually and in binary combinations. The combination ratio between the mixture STE + NIV was 10:1. The IC\(_{50}\) values of NIV ranged from 0.96 to 0.66 \( \mu M \), whereas no IC\(_{50}\) values were obtained for STE at any time tested. For the combinations studied, synergistic, antagonistic and additive effects were obtained with the two type of analyses performed, the isobologram analysis and the Combenefit method. No relevant effects on ROS and \( \Delta \Psi_m \) were observed. In conclusion, predictive models based on combination data could help to better understand the interaction between mycotoxins and their implications in food safety assessment. However, a further analysis of the molecular mechanism underlying these interactive effects is required.

**Food Packaging**

**A Review on Techniques Utilized for Design of Controlled Release Food Active Packaging**


**Significance:** This review gathers and presents the strategies utilized for release controlling of active compounds from food active packaging systems.

Active packaging (AP) is a new class of innovative food packaging, containing bioactive compounds, is able to maintain the quality of food and extend its shelf life by releasing active agent during storage. The main challenge in designing the AP system is slowing the release rate of active compounds for its prolonged activity. Controlled-release active packaging (CRP) is an innovative technology that provides control in the release of active compounds during storage. Various approaches have been proposed to design CRP. The purpose of this review was to gather and present the strategies utilized for release controlling of active compounds from food AP systems. The chemical modification of polymers, the preparation of multilayer films and the use of cross-linking agents are some methods tried in the last decades. Other approaches use molecular complexes and irradiation treatments. Micro- or nano-encapsulation of active compounds and using nano-structured materials in the AP film matrix are the newest techniques used for the preparation of CRP systems. The action mechanism for each technique was described and an effort was made to highlight representative published papers about each release controlling approach. This review will benefit future prospects of exploring other innovative release controlling methods in food CRP.
Migration Regularity of Phthalates in Polyethylene Wrap Film of Food Packaging

Significance: This paper presents a model to evaluate the migration behaviors and hazardous properties of phthalic acid esters in food packaging materials.

As a kind of polymer material additive, phthalic acid esters (PAEs) are widely used in food industry. However, PAEs are environmental endocrine disruptors with reproductive toxicity and teratogenic carcinogenicity, which are difficult to be degraded in the natural environment. In this paper, gas chromatography-mass spectrometer (GC-MS) methods for PAEs in polyethylene wrap film were optimized. For diisobutyl phthalate (DIBP) and dibutyl phthalate (DBP) that were mainly detected, the method had a good linearity in 1 to 500 ng/g. Then, we confirmed that the migration of DIBP and DBP from polyethylene wrap film increased with time and temperature. It is found that the migration law in different food simulations well followed the migration dynamics first-level model. The rate constant $K_1$ and initial release rate $V_0$ are inversely proportional to the polarity of the simulated liquid. We hope that this study can serve as a valuable reference for further research on the migration of food packing materials. Practical Application: In this paper, we present a simple example of applying migration model to evaluate the migration behaviors of PAEs in food packaging materials along with their hazardous properties. It can serve as a valuable reference for further research on the migration of food packing materials.

Biodegradable Films Based on Fruit Puree: A Brief Review

Significance: This review synthesizes the general aspects of fruit puree films and highlights their characterization for use as food packaging.

The production of fruit-film packaging has attracted increasing attention in scientific research due to the packaging’s environmentally friendly, nontoxic, and edible characteristics. The development of alternative packaging contributes to both minimizing the environmental impacts caused by the large consumption of non-biodegradable plastics and favoring the reduction of postharvest loss/waste of fruit. In addition, these fruit films have the potential to be functional packages due the presence of antioxidant and antimicrobial compounds that can migrate to the food matrix, acting as natural additives. The use of fruit puree to develop biodegradable films can be simpler and more practical than the developed of films from fruit flour or extracts, reducing the time, energy, and resources necessary to prepare the film-forming solution. A better understanding of the mechanical properties, bioactive compounds, and potential applications is interesting in terms of prospecting new specific ways to produce and use these films. In this study, we briefly review the general aspects of fruit puree films, highlighting their characterization for use as food packaging.

Chemical Contaminants
How the 62-year Old Delaney Clause Continues to Thwart Science: Case Study of the Flavor Substance β-myrcene

Significance: A case study is presented to question the application of the Delaney Clause based on classification alone, without consideration of the exposure potential or the human health relevance of effects observed in animals.

The Delaney Clause is a provision of the 1958 Food Additive Amendment to the Food, Drug and Cosmetic Act of 1938 which stipulates that if a substance is found by the Food and Drug Administration to be carcinogenic in any species of animal or in humans, then it cannot be used as a food additive. This paper presents a case study of β-myrcene, one of seven synthetic substances that was challenged under the Delaney Clause, ultimately resulting in revocation of its regulatory approval as a food additive despite a lack of safety concern. While it is listed as a synthetic flavor in 21 CFR 172.515, β-myrcene is also a substance naturally occurring in a number of dietary plants. The exposure level to naturally-occurring β-myrcene is orders of magnitude higher (estimated to be 16,500 times greater) than the exposure via β-myrcene added to food as a flavoring substance. The National Toxicology Program conducted genotoxicity testing (negative), a 13-week range-finding study, and a two-year cancer
bioassay in B6C3F1 mice and F344/N rats. An increase in liver tumors was seen in male mice and kidney tumors in male rats, ultimately resulting in β-myrcene being classified by IARC as a Class 2B carcinogen and being listed on California Proposition 65; in contrast, β-myrcene is not classified as a carcinogen by any other regulatory authority. The doses administered in the NTP bioassay were five-six orders of magnitude higher than human exposures, and the FDA concluded after a thorough evaluation that there was no safety concern associated with the use of β-myrcene as a flavor substance at the current use level. The Delaney Clause, however, does not consider the exposure potential or the human health relevance of effects observed in animals. The lack of options available to the US FDA led to the 2018 decision to remove β-myrcene from the list of approved food additives. This revocation has contributed to the ongoing erosion of trust in regulatory agencies (and industry), which has both economic implications for food manufacturers and consumers alike, and implications for consumer perception of safety of the US food supply. It is time for us to reconsider the rationale behind any legislation that relies on classification alone, and whether there is, in fact, a reason to still classify nongenotoxic carcinogens at all.

Heavy Metals

Locus-Specific Differential DNA Methylation and Urinary Arsenic: An Epigenome-Wide Association Study in Blood Among Adults With Low-to-Moderate Arsenic Exposure


Significance: This epigenome-wide association study examined the relationship between arsenic exposure and locus-specific DNA methylation, finding DNA methylation signatures that warrant further exploration as biomarkers of disease development.

Background: Chronic exposure to arsenic (As), a human toxicant and carcinogen, remains a global public health problem. Health risks persist as As exposure has ended, suggesting epigenetic dysregulation as a mechanistic link between exposure and health outcomes. Objectives: We investigated the association between total urinary As and locus-specific DNA methylation in the Strong Heart Study, a cohort of American Indian adults with low-to-moderate As exposure [total urinary As, mean(±SD) μg/g mean(±SD)μg/g creatinine: 11.7 (10.6)]. Methods: DNA methylation was measured in 2,325 participants using the Illumina MethylationEPIC array. We implemented linear models to test differentially methylated positions (DMPs) and the DMRcate method to identify regions (DMRs) and conducted gene ontology enrichment analysis. Models were adjusted for estimated cell type proportions, age, sex, body mass index, smoking, education, estimated glomerular filtration rate, and study center. Arsenic was measured in urine as the sum of inorganic and methylated species. Results: In adjusted models, methylation at 20 CpGs was associated with urinary As after false discovery rate (FDR) correction (FDR<0.05). After Bonferroni correction, 5 CpGs remained associated with total urinary As (pBonferroni<0.05), located in SLC7A11, ANKS3, LINGO3, CSNK1D, ADAMTSL4. We identified one DMR on chromosome 11 (chr11:2,322,050-2,323,247), annotated to C11orf2; TSPAN32 genes. Discussion: This is one of the first epigenome-wide association studies to investigate As exposure and locus-specific DNA methylation using the Illumina MethylationEPIC array and the largest epigenome-wide study of As exposure. The top DMP was located in SLC7A11A, a gene involved in cystine/glutamate transport and the biosynthesis of glutathione, an antioxidant that may protect against As-induced oxidative stress. Additional DMPs were located in genes associated with tumor development and glucose metabolism. Further research is needed, including research in more diverse populations, to investigate whether As-related DNA methylation signatures are associated with gene expression or may serve as biomarkers of disease development.

Caffeine

Effects of Caffeine Supplementation on Muscle Endurance, Maximum Strength, and Perceived Exertion in Adults Submitted to Strength Training: A Systematic Review and Meta-Analyses


Significance: Caffeine supplementation showed a significant ergogenic effect on muscle endurance and maximum strength in the bench press exercise, but more studies to examine its effects on lower-body strength are needed.

This study aimed to determine the effects of caffeine supplementation on muscle endurance, maximum strength, and ratings of perceived exertion (RPE) in individuals undergoing strength training with external resistance exercises. A search of three databases (PubMed, LiLACS, and CENTRAL) and gray literature was carried out to find randomized controlled trials, with a double-blind design, which investigated the effects of caffeine supplementation in healthy adults. Meta-analyses of weighted mean differences (WMD) and standardized mean differences (SMD) between caffeine and placebo groups from individual studies were performed using a random-effects model. Nineteen studies were included in the quantitative synthesis. Only the bench press and the leg press exercises were assessed in a sufficient number of studies to be included in meta-analyses. In the bench press exercise, caffeine supplementation improved strength resistance (WMD 0.87 (95% confidence interval (CI): 0.33, 1.41))
Association of Maternal Caffeine Intake During Pregnancy With Low Birth Weight, Childhood Overweight, and Obesity: A Meta-Analysis of Cohort Studies


Significance: This meta-analysis of cohort studies found that maternal caffeine consumption during pregnancy is associated with a higher risk of low birth weight and childhood overweight and obesity.

Background: Epidemiological studies reported inconsistent results on the associations between maternal caffeine intake during pregnancy and risk of low birth weight (LBW) and childhood overweight and obesity in their offspring. Methods: We conducted a meta-analysis of cohort studies to quantitatively assess these associations. Pertinent studies were identified by searching PubMed and Embase through June 2019. Study-specifics risk estimates were combined using fixed effects models, or random-effects models when significant heterogeneity was detected. Dose-response analysis was modeled by using restricted cubic splines. Results: A total of 15 cohort studies, with 102,347 pregnant women, was included in the meta-analysis. The pooled relative risk (RR) for LBW was 1.33 (95% CI: 1.12, 1.57) for mothers with the highest compared with the lowest level of caffeine intake during pregnancy, with significant heterogeneity across studies (P = 49.3%, I^2 = 0.032). The pooled RR was 1.07 (95% CI: 1.02, 1.11) for each 100 mg/day increase of caffeine intake. Conclusions: Maternal caffeine intake during pregnancy is associated with higher risk of LBW and childhood overweight and obesity. Further studies may focus on investigating the potential mechanisms before the recommendation of complete avoidance of caffeine intake during pregnancy.

Food Allergens

Consensus Report From the Food Allergy Research & Education (FARE) 2019 Oral Immunotherapy for Food Allergy Summit


Significance: This article summarizes a discussion between health care providers, patient representatives, researchers, regulators and food allergy advocates on current knowledge and research gaps related to oral immunotherapy as a therapeutic option for food allergy.

Food allergy is a major health problem affecting 5% to 10% of the population in developed nations, including an estimated 32 million Americans. Despite the large number of patients suffering from food allergies, up until the end of January 2020, no treatment for food allergies had been approved by the US Food and Drug Administration. The only options were avoidance of food allergen triggers and acute management of allergic reactions. A considerable body of data exists supporting oral immunotherapy (OIT) as a promising, novel treatment option, including that for the now Food and Drug Administration-approved peanut OIT product Palforzia (Aimmune Therapeutics, Brisbane, Calif). However, data for long-term quality-of-life improvement with OIT varies, depending on the measures used for analysis. Like many therapies, OIT is not without potential harms, and burdens, and the evaluation of patient-specific risk-benefit ratio of food OIT produces challenges for clinicians and patients alike, with many unanswered questions. Food Allergy Research & Education organized the Oral Immunotherapy for Food Allergy Summit on November 6, 2019, modeled after the PRACTALL sessions between the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology to address these critical issues. Health care providers, patient representatives, researchers, regulators, and food allergy advocates came together to discuss OIT and identify areas of common ground as well as gaps in existing research and areas of uncertainty and disagreement. The purpose of this article was to summarize that discussion and facilitate collaboration among clinicians and patients to help them make better-informed decisions about offering and accepting OIT, respectively, as a therapeutic option.
Robustness Testing of the xMAP Food Allergen Detection Assay: A Multiplex Assay for the Simultaneous Detection of Food Allergens


**Significance:** This study characterizes the effects of incubation temperature, amounts of the antibody bead cocktail and concentrations of detection antibody and β-mercaptoethanol in the reduced-denatured extraction buffer on xMAP food allergen detection assay performance for simultaneous detection of allergens in food.

The xMAP food allergen detection assay (xMAP FADA) can simultaneously detect 15 analytes (14 food allergens plus gluten) in one analysis. The xMAP FADA typically employs two antibody bead sets per analyte, providing built-in confirmation that is not available with other antibody-based assays. Before an analytical method can be used, its reliability must be assessed when conditions of the assay procedure are altered. This study was conducted to determine the effects on assay performance associated with changes in incubation temperature, amounts of the antibody bead cocktail, and concentrations of detection antibody and β-mercaptoethanol in the reduced-denatured extraction buffer. The analysis of buffered-detergent extracts revealed lower responses at 22°C than at 37°C, but temperature had no effect on the analysis of reduced-denatured extracts. Changes in β-mercaptoethanol and detection antibody concentrations had an effect on the detection of only milk in the reduced-denatured extracts. A slight change in the measured bead count was observed when one-fourth of the bead cocktail was used, and a large decrease in the bead count was noted when one-eighth of the recommended amount was used, but this number (≥25) was still sufficient to provide reliable results. Overall, the xMAP FADA was very robust to changes in the assay procedure, which may inadvertently occur.