ToxCast Data

Prioritizing Environmental Chemicals for Obesity and Diabetes Outcomes Research: A Screening Approach Using ToxCast™ High-Throughput Data

Environmental Health Perspectives, Vol. 124, No. 8; pp. 1141–1154
DOI: 10.1289/ehp.1510456
Link to full text: Click here

Significance: The spectrum of environmental chemicals to consider in research related to diabetes and obesity is much broader than indicated by research papers and reviews published in the peer-reviewed literature but more research is required to put these screening-level analyses into context.

This review is intended to help researchers generate hypotheses about chemicals that may contribute to diabetes and to obesity-related health outcomes by summarizing relevant findings from the U.S. Environmental Protection Agency (EPA) ToxCast™ high-throughput screening (HTS) program. The aim was to develop new hypotheses around environmental chemicals of potential interest for diabetes- or obesity-related outcomes using high-throughput screening data. Researchers identified ToxCast™ assay targets relevant to several biological processes related to diabetes and obesity (insulin sensitivity in peripheral tissue, pancreatic islet and β cell function, adipocyte differentiation, and feeding behavior) and presented chemical screening data against those assay targets to identify chemicals of potential interest. The results of this screening-level analysis suggest that the spectrum of environmental chemicals to consider in research related to diabetes and obesity is much broader than indicated by research papers and reviews published in the peer-reviewed literature. Testing hypotheses based on ToxCast™ data will also help assess the predictive utility of this HTS platform. More research is required to put these screening-level analyses into context, but the information presented in this review should facilitate the development of new hypotheses.

Ingredient Safety

Advances in Assessing Ingredient Safety

M.L. Dourson, R.G. York
Regulatory Toxicology and Pharmacology, Vol. 79, Suppl. 2; pp. S112–S118
DOI: 10.1016/j.yrtph.2016.07.008
Link to full text: Click here

The safety of food ingredients will be assessed in the 21st century by mixture of traditional methods, such as the “safe” dose concept, which is thought to be an accurate but imprecise estimation of dose below the population threshold for adverse effect, and contemporary methods, such as the Benchmark Dose (BMD), Chemical Specific Adjustment Factors (CSAF), physiologically-based
pharmacokinetic models, and biologically-informed dose response modeling. New research on the horizon related to toxicology may also improve these risk assessment methods, or suggest new ones. These traditional, contemporary and new methods and research will be briefly described.

**The Relevance of International Assessments to GRAS Determinations**

C. Kruger  
*Regulatory Toxicology and Pharmacology, Vol. 79, Suppl. 2; pp. S119–S123*  
DOI: 10.1016/j.yrtph.2016.06.010  
Link to full text: Click here

A discussion of the risk assessment process as applied to the Generally Recognized As Safe (GRAS) determination of safety for new ingredients can benefit from an international perspective. When we think about how risk assessments are performed around the world it is critical to assess what can be learned. What are the similarities? What are the differences? What are the takeaways? It is important to talk about the similarities in processes, because it validates the approach taken by risk assessors who are charged with protecting the food supply. It is also instructive to evaluate the differences in order to determine where improvements can be made to our process.

**Foodborne Pathogens**

**Inhibition of Listeria monocytogenes by Buffered Dry Vinegar in Reduced-Sodium Ready-to-Eat Uncured Turkey Stored at 4°C**

M.K. Badvela, J.S. Dickson, J.G. Sebranek, W.D. Schroeder  
*Journal of Food Protection, Vol. 79, No. 8; pp. 1396–1403*  
DOI: 10.4315/0362-028X.JFP-15-370  
Link to full text: Click here

*Significance:* The dry vinegar ingredients were effective in inhibiting Listeria monocytogenes obtained from multiple sources in reduced-sodium ready-to-eat uncured turkey stored at 4°C without adversely impacting the quality attributes.

A reduced-sodium ready-to-eat (RTE) uncured turkey was manufactured with buffered dry vinegar treatments to validate the inhibition of Listeria monocytogenes and spoilage microflora and to determine the effects on sensory and quality attributes. Two different five-strain inocula of *L. monocytogenes* obtained from different sources were used for evaluating the efficacy of the buffered dry vinegar treatments. The results showed that 0.6 and 0.8% buffered dry vinegar with a sodium base (BDV-SB) and buffered dry vinegar with a potassium base (BDV-PB) at 0.7 and 0.9% controlled *L. monocytogenes* for 12 weeks. The untreated control product containing no buffered dry vinegar showed >1 log increase in *L. monocytogenes* populations counts at the end of 2 weeks. Statistical analysis confirmed that the dry vinegar treatments inhibited the growth of *L. monocytogenes* compared with the untreated control. No significant differences were seen in the inhibition of *L. monocytogenes* between the two different five-strain inocula. Instrumental color results showed no significant differences between the treatments. Purge loss results showed no significant differences between the dry vinegar treatments, but significant differences were seen between the untreated control and dry vinegar treatments at a few testing intervals.
Internalization of Listeria monocytogenes in Whole Avocado
Y. Chen, P. Evans, T.S. Hammack, E.W. Brown, D. Macarisin, Dumitru
Journal of Food Protection, Vol. 79, No. 8; pp. 1440–1445
DOI: 10.4315/0362-028X.JFP-16-075
Link to full text: Click here

Significance: Dye mixed with inoculum was useful for guiding subsequent sampling, but dye penetration patterns were not always consistent with bacterial penetration.

This study evaluated the potential internalization of Listeria monocytogenes from the surface of avocados into the edible portions of the fruit during certain post-harvest practices simulated in a laboratory setting. One set of intact avocados was spot inoculated with L. monocytogenes on the stem scar, and the second set was hydrocooled in water contaminated with L. monocytogenes. Under these experimental conditions, L. monocytogenes internalized into the avocado pulp through the stem or stem scar after both spot inoculation and hydrocooling. In avocados spot inoculated with 50, 130, 500, and 1,300 CFU per fruit, bacteria were detected in the edible portion adjacent to the stem scar within 15 days postinoculation during storage at 4°C. In avocados hydrocooled in water containing L. monocytogenes at 106 and 108 CFU/ml, bacteria reached the bottom end of the fruit, and the populations in the edible portion adjacent to the stem scar reached up to 5.90 to 7.19 log CFU/g within 10 to 15 days during storage at 4°C.

Loss of SigB in Listeria monocytogenes Strains EGD-e and 10403S Confers Hyperresistance to Hydrogen Peroxide in Stationary Phase under Aerobic Conditions
M. Boura, C. Keating, K. Royet, R. Paudyal, B. O’Donoghue, C.P. O’Byrne, et al.
Applied and Environmental Microbiology, Vol. 82, No. 15; pp. 4584–4591
DOI: 10.1128/AEM.00709-16
Link to full text: Click here

Significance: SigB is the most important stress gene regulator in L. monocytogenes and other Gram-positive bacteria.

This study showed conclusively that unlike all other stresses, loss of sigB results in hyperresistance to H2O2 (more than 8 log CFU ml−1 compared to the wild type) in aerobically grown stationary-phase cultures of L. monocytogenes strains 10403S and EGD-e. Furthermore, growth at 30°C resulted in higher resistance to oxidative stress than that at 37°C. Oxidative stress resistance seemed to be higher with higher levels of oxygen. Under anaerobic conditions, the loss of SigB in 10403S did not affect survival against H2O2, while in EGD-e, it resulted in a sensitive phenotype. During exponential phase, minor differences occurred, and this result was expected due to the absence of sigB transcription. Catalase tests were performed under all conditions, and stronger catalase results corresponded well with a higher survival rate, underpinning the important role of catalase in this phenotype. This study also assessed the catalase activity in protein lysates, which corresponded with the catalase tests and survival. In addition, reverse transcription-PCR (RT-PCR) showed no differences in transcription between the wild type and the ΔsigB mutant in various oxidative stress genes.
Effect of Hydrogen Peroxide in Combination with Minimal Thermal Treatment for Reducing Bacterial Populations on Cantaloupe Rind Surfaces and Transfer to Fresh-Cut Pieces

D.O. Ukuku, S. Mukhopadhyay, D. Geveke, M. Olanya, B. Niemira

Journal of Food Protection, Vol. 79, No. 8; pp. 1316-1324

DOI: 10.4315/0362-028X.JFP-16-046

Link to full text: Click here

Significance: The microbial safety of fresh-cut pieces from treated cantaloupes was improved at day 6 of storage at 5°C and day 3 of storage at 10°C.

Whole cantaloupe rind surfaces were inoculated with Salmonella, Escherichia coli O157:H7, and Listeria monocytogenes at 107 CFU/ml. Intervention treatments used were (i) water (H2O) at 22°C, (ii) H2O at 80°C, (iii) 3% hydrogen peroxide (H2O2) at 22°C, and (iv) a combination of 3% H2O2 and H2O at 80°C for 300 s. The strength of pathogen attachment (SR value) at days 0, 3, and 7 of storage was determined, and then the efficacy of the intervention treatments to detach, kill, and reduce transfer of bacteria to fresh-cut pieces during fresh-cut preparation was investigated. Populations of E. coli O157:H7 attached to the rind surface at significantly higher levels than Salmonella and L. monocytogenes, but Salmonella exhibited the strongest attachment (SR value) at all days tested. Washing with 3% H2O2 alone led to significant reduction of bacteria and caused some changes in bacterial cell morphology. A combination treatment with H2O and 3% H2O2 at 8°C led to an average 4-log reduction of bacterial pathogens, and no bacterial pathogens were detected in fresh-cut pieces prepared from this combination treatment, including enriched fresh-cut samples.

Microbial Safety and Overall Quality of Cantaloupe Fresh-Cut Pieces Prepared from Whole Fruit after Wet Steam Treatment

D.O. Ukuku, D.J. Geveke, L. Chau, B.A. Niemira

International Journal of Food Microbiology, Vol. 231; pp. 86–92

DOI: 10.1016/j.ijfoodmicro.2016.05.019

Link to full text: Click here

Significance: Minimal wet steam treatment of cantaloupe rind surfaces designated for fresh-cut preparation will enhance the microbial safety of fresh-cut pieces, by reducing total bacterial populations.

The objectives of this study were to use a wet steam process to 1) reduce indigenous spoilage microflora and inoculated populations of Salmonella, Escherichia coli O157:H7 and Listeria monocytogenes on the surface of cantaloupes, and 2) reduce the populations counts in cantaloupe fresh-cut pieces after rind removal and cutting. The average inocula of Salmonella, E. coli O157:H7 and Listeria monocytogenes was 107 CFU/ml and the populations recovered on the cantaloupe rind surfaces after inoculation averaged 4.5, 4.8 and 4.1 log CFU/cm2, respectively. Presence and growth of aerobic mesophilic bacteria and Salmonella, E. coli O157:H7 and L. monocytogenes were determined in fresh-cut cantaloupe samples. There were no visual signs of physical damage on all treated cantaloupe surfaces immediately after treatments and during storage. All fresh-cut pieces from treated cantaloupes rind surfaces were negative for bacterial pathogens even after an enrichment process. Steam treatment significantly changed the color of the fresh-cut pieces.
Prevalence and Amounts of Salmonella Found on Raw California Inshell Pistachios

Journal of Food Protection, Vol. 79, No. 8; pp. 1304–1315
DOI: 10.4315/0362-028X.JFP-16-054

Significance: The prevalence and levels of Salmonella in pistachios are within those observed for other tree nuts, but the limited number of serovars isolated suggests a narrow and persistent contamination source.

To determine the prevalence of Salmonella in pistachios, a total of 3,966 samples (1,032 floaters and 2,934 sinkers) were collected within 4 months of the 2010, 2011, and 2012 harvests from storage silos and were stored at 4°C; 100-g subsamples were enriched for the presence of Salmonella. Twenty-one of the floater samples and 11 of the sinker samples were positive for Salmonella: 2.0% prevalence (95% CI, 1.3 to 3.1%) and 0.37% prevalence (95% CI, 0.21 to 0.67%), respectively, for a weighted average prevalence of 0.61%. Levels of Salmonella were determined for positive samples using a most-probable-number (MPN) method with multiple 50-g, three 5.6-g, and three 0.56-g subsamples. Geometric mean levels of Salmonella in floaters and sinkers were 0.66 MPN/100 g (0.14 to 5.3 MPN/100 g) and 0.18 MPN/100 g (0.10 to 0.62 MPN/100 g), respectively. Seven different serovars were identified among the isolates, with nine pulsed-field gel electrophoresis fingerprints; as many as four serovars were isolated from some samples. Salmonella serovars Montevideo (44%), Enteritidis (19%), Senftenberg (16%), Worthington (12%), and Liverpool (9.4%) were most commonly isolated from the initial 100-g samples.

Enzymatic Digestion for Improved Bacteria Separation from Leafy Green Vegetables

D. Wang, Z. Wang, F. He, A.J. Kinchla, S.R. Nugen
Journal of Food Protection, Vol. 79, No. 8; pp. 1378–1386
DOI: 10.4315/0362-028X.JFP-15-581

Significance: Enzyme digestion prior to separation can improve the efficiency of bacteria separation and increase the likelihood of detecting pathogens in the final detection assay.

The benefits of using enzyme digestion followed by immunomagnetic separation to isolate Salmonella from spinach and lettuce were investigated. Enzymatic digestion using pectinase and cellulase was able to break down the structure of the leafy green vegetables, resulting in the detachment and release of Salmonella from the leaves. Immunomagnetic separation of Salmonella from the liquefied sample allowed an additional separation step to achieve a more pure sample without leaf debris that may benefit additional downstream applications. We have investigated the optimal combination of pectinase and cellulase for the digestion of spinach and lettuce to improve sample detection yields. The concentrations of enzymes used to digest the leaves were confirmed to have no significant effect on the viability of the inoculated Salmonella. Results reported that the recovery of the Salmonella from the produce after enzyme digestion of the leaves was significantly higher than traditional sample preparation methods to separate bacteria (stomaching and manually shaking).
Inactivation of Salmonella, Listeria monocytogenes and Enterococcus faecium NRRL B-2354 in a Selection of Low Moisture Foods

G. Rachon, W. Peñaloza, P.A. Gibbs


DOI: 10.1016/j.ijfoodmicro.2016.04.022

**Significance:** Results showed that the pathogens *Salmonella* and *L. monocytogenes* and the surrogate *E. faecium* NRRL B-2354, can survive well (maximum reduction < 0.8 log) in low moisture foods maintained at 16 °C.

The aims of this study were to obtain data on survival and heat resistance of cocktails of Salmonella, Listeria monocytogenes and the surrogate Enterococcus faecium (NRRL B-2354) in four low moisture foods (confectionery formulation, chicken meat powder, pet food and savoury seasoning) during storage before processing. Inoculated samples were stored at 16 °C and cell viability examined at day 0, 3, 7 and 21. At each time point, the heat resistance at 80 °C was determined. The purpose was to determine a suitable storage time of inoculated foods that can be applied in heat resistance studies or process validations with similar cell viability and heat resistance characteristics. The main inactivation study was carried out within 7 days after inoculation, the heat resistance of each bacterial cocktail was evaluated in each low moisture food heated in thermal cells exposed to temperatures between 70 and 140 °C. The Weibull model and the first order kinetics (D-value) were used to express inactivation data and calculate the heating time to achieve 5 log reduction at each temperature. Results showed that the pathogens *Salmonella* and *L. monocytogenes* and the surrogate *E. faecium* NRRL B-2354, can survive well (maximum reduction < 0.8 log) in low moisture foods maintained at 16 °C, as simulation of warehouse raw material storage in winter and before processing. The D_{50} value of the pathogens and surrogate did not significantly change during the 21 day storage (p > 0.05). The inactivation kinetics of the pathogens and surrogate at temperatures between 70 and 140 °C, were different between each organism and product. *E. faecium* NRRL B-2354 was a suitable *Salmonella* surrogate for three of the low moisture foods studied, but not for the sugar-containing confectionery formulation. Heating low moisture food in moisture-tight environments (thermal cells) to 111.2, 105.3 or 111.8 °C can inactivate 5 log of *Salmonella*, *L. monocytogenes* or *E. faecium* NRRL B-2354 respectively.

**E. coli**

Survival and Reduction of Shiga Toxin-Producing Escherichia coli in a Fresh Cold-Pressed Juice Treated with Antimicrobial Plant Extracts

S. Hatab, R. Athanasio, R. Holley, A. Rodas-Gonzalez, C. Narvaez-Bravo

*Journal of Food Science*, Vol. 81, No. 8; M1987–M1995

DOI: 10.1111/1750-3841.13382

**Significance:** This paper provides new data on the growth and survival of Shiga-toxigenic *Escherichia coli* in raw cold-pressed juice and essential oils with potential as natural antimicrobials.

This study was conducted to evaluate the survival of 7 Shiga-toxigenic *Escherichia coli* (STEC) in fresh cold-pressed juice and the antimicrobial efficacy of 4 essential oils (EO: achillea, rosemary, sage, and thyme). The minimum inhibitory
concentration (MIC) and minimum bactericidal concentration (MBC) of each EO was determined using microdilution assays evaluated at pH levels 4 and 7; as well as at 4 and 25 °C; daily for up to 5 d. Results indicated that 5 of 7 serotypes survived well in cold-pressed raw juice for at least 4 d at 4 °C and pH 3.5 with no significant reduction in viability. The EO showed varying degrees of antimicrobial activity against the 7 STEC. The MIC and MBCs were lowest for thyme (2 μg/L) and highest for sage (15 to 25 μg/L). The antimicrobial activity was enhanced at low pH and temperature. Data showed that although the top 7 STEC could survive low pH and temperature in vitro and in cold-pressed juices, EO, especially from thyme and rosemary, reduced STEC to an undetectable level at 4 °C, suggesting that they could be used as natural antimicrobials in juice.

A Rapid and Highly Specific Immunofluorescence Method to Detect Escherichia coli O157:H7 in Infected Meat Samples

B. Balakrishnan, S. Barizuddin, T. Wuliji, M. El-Dweik

International Journal of Food Microbiology, Vol. 231; pp. 54–62
DOI: 10.1016/j.ijfoodmicro.2016.05.017

Link to full text: Click here

Significance: Protein-A magnetic beads may be used as a platform to detect other bacterial pathogens.

The present study attempts to develop an immunofluorescence technique that uses Protein-A-coated, magnetic beads as the platform. The immunofluorescence technique described here is a direct detection method in which E. coli O157:H7 cells are labeled with tetramethylrhodamine (TRITC) fluorescent dye. TRITC-labeled bacteria are captured by the desired antibody (Ab), which is immobilized on the Protein-A magnetic beads. The Ab immobilization procedure is also evidenced by microscopy using fluorescein isothiocyanate (FITC)-labeled Ab. The minimum bacterial concentration detected by this method is $1.2 \pm 0.06 \times 10^3$ CFU ml$^{-1}$. The high specificity of this method was proved by using the specific monoclonal Ab (MAB) in the test. The proposed protocol was successfully validated with E. coli O157:H7-infected meat samples.

Caffeine

Trends and Patterns of Caffeine Consumption among US Teenagers and Young Adults, NHANES 2003–2012

N.L. Tran, L.M. Barraj, X. Bi, M.M. Jack

Food and Chemical Toxicology, Vol. 94; pp. 227–242
DOI: 10.1016/j.fct.2016.06.007

Link to full text: Click here

Significance: Consumption of caffeine and caffeinated beverages has remained constant over the past 10 years despite new caffeine sources.

Caffeine consumption among US teenagers (13–17y), young adults (18–24y) and adults (25–29y) for a 10-year period was examined using NHANES 2003–12. Of the 85% who consume caffeine, 84% consume caffeinated beverages. Less than 7.1% of the population consume energy drinks. While mean caffeine intake among teenage caffeine consumers decreased from 62 to 55 mg/day ($p=0.018$) over the 10-year period, no discernable trend was observed for other age groups. Caffeine intake from energy drinks increased, and was only statistically significant
for age 18–24y accounting for <9% of total caffeine intake. Mean caffeine intake per consumption occasion was equivalent between coffee and energy drinks for teenagers and young adults. During a 30-min period mean caffeine consumption was similar when an energy drink was the only consumption event or when it occurred with other caffeinated beverage products suggestive of a substitution effect. Linear regression models of caffeine intake from energy drinks against caffeine from coffee, tea and soda among energy drink consumers in the upper 50th percentile shows a statistically significant inverse relationship ($R^2 = 28\%$, coffee: $\beta = -0.35$, $p < 0.001$; tea: $\beta = -0.44$, $p < 0.001$; soda: $\beta = -0.22$, $p = 0.036$) and further supports the substitution concept.

**Food Packaging**

**Antimicrobial Packaging for Extending the Shelf Life of Bread—A Review**

V.A. Jideani, K. Vogt

*Critical Reviews in Food Science and Nutrition,* Vol. 56, No. 8; pp. 1313–1324

DOI: 10.1080/10408398.2013.768198

Link to full text: Click here

**Significance:** This paper reviews active packaging systems used in food products and proposes systems that may have potential for extending the shelf life of bread.

Antimicrobial packaging is an important form of active packaging that can release antimicrobial substances for enhancing the quality and safety of food during extended storage. It is in response to consumers’ demand for preservative-free food as well as more natural, disposable, biodegradable, and recyclable food-packaging materials. The potential of a combination of allyl isothiocyanate and potassium sorbate incorporated into polymers in providing the needed natural antimicrobial protection for bread products is discussed. The role of double extrusion process as a means for obtaining a homogeneous mix of the sorbate into the polymer (polyethylene or ethylenevinylalcohol), is highlighted.

**Scientific Excellence**

**A Framework for Improving the Quality of Research in the Biological Sciences**

A. Casadevall, L.M. Ellis, E.W. Davies, M. McFall-Ngai, F.C. Fang

*mBio*, Vol. 7, No. 4; pp. e01256-16

DOI: 10.1128/mBio.01256-16

Link to full text: Click here

The American Academy of Microbiology convened a colloquium to discuss problems in the biological sciences, with emphasis on identifying mechanisms to improve the quality of research. Participants from various disciplines made six recommendations: (i) design rigorous and comprehensive evaluation criteria to recognize and reward high-quality scientific research; (ii) require universal training in good scientific practices, appropriate statistical usage, and responsible research practices for scientists at all levels, with training content regularly updated and presented by qualified scientists; (iii) establish open data at the timing of publication as the standard operating procedure throughout the scientific enterprise; (iv) encourage scientific journals to publish negative data that meet methodologic standards of quality; (v) agree upon common criteria among scientific journals for retraction of published papers, to provide consistency and transparency; and
(vi) strengthen research integrity oversight and training. These recommendations constitute an actionable framework that, in combination, could improve the quality of biological research.