Food Safety Briefs
September 2016

Foodborne Pathogens

Evaluation of a Recirculating Dipper Well Combined With Ozone Sanitizer for Control of Foodborne Pathogens in Food Service Operations
G. Almeida, K.E. Gibson
DOI: 10.4315/0362-028X.JFP-16-055
Link to full text: Click here

Significance: The integration of ozone into a dipper well could be a potential critical control point to reduce the incidence of microbial contamination during retail food service.

In this study, a recirculating dipper well ozone sanitation system (DWOSS) was evaluated for the control and inactivation of Escherichia coli, Listeria innocua, PRD1 bacteriophage, and Staphylococcus aureus present on a stainless steel (SS) disher. In a low ozone (O3) demand medium, the DWOSS achieved over a 5-log reduction for E. coli, L. innocua, and PRD1 at 30 s when exposed to 0.45 to 0.55 ppm of residual O3. A >5-log total CFU reduction was achieved for S. aureus at a 600-s exposure time and 0.50 ppm of residual O3. When evaluated in the presence of high O3 demand medium (10% skim milk), the DWOSS performed significantly better for all microbe–exposure time combinations compared with a conventional dipper well with respect to the reduction of microbes on the SS disher. In addition, the DWOSS was evaluated under two neglect scenarios to determine its ability to control microbes in 10% skim milk medium on the SS disher and within the dipper well basin itself over an extended period of use (2 h of use per day over 5 days).

Effect of Power Levels on Inactivation of Escherichia coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes in Tomato Paste Using 915-Megahertz Microwave and Ohmic Heating
DOI: 10.4315/0362-028X.JFP-16-044
Link to full text: Click here

Significance: Increasing power levels of microwave heating ensures heating uniformity and microbiological safety and preserves quality aspects of tomato paste.

The effect of power levels on inactivation of Escherichia coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes in tomato paste was investigated using 915-MHz microwave heating (MW) and ohmic heating (OH). Heating uniformity, pathogen inactivation, and quality aspects were determined with 1.8-, 2.1-, 2.4-, and 3.0-kW MW and corresponding OH. GInaFit was used to analyze pathogen inactivation. The heating uniformity of MW-treated samples was inferior...
to that of OH-treated samples at low power levels of 1.8 to 2.4 kW but improved as the power level increased. Pathogen inactivation of MW-treated samples was significantly higher than that of OH-treated samples at low power levels of 1.8 to 2.4 kW but was not significantly different at the highest power level of 3.0 kW. Quality aspects (color, pH, and lycopene content), except for L*, of MW-treated samples were not significantly degraded by increased power levels.

**Commercially Available Rapid Methods for Detection of Selected Food-borne Pathogens**

*W.B. Valderrama, E.G. Dudley, S. Doores, C.N. Cutter*

*Critical Reviews in Food Science and Nutrition, Vol. 56, No. 9; pp.1519–1531*

DOI: 10.1080/10408398.2013.775567

Link to full text: Click here

**Significance:** This review describes the basic mechanism and capabilities of commercially available methods for rapid detection of food borne pathogens and discusses the difficulties of choosing the most convenient method.

Generally, the enumeration and isolation of food-borne pathogens is performed using culture-dependent methods. These methods are sensitive, inexpensive, and provide both qualitative and quantitative assessment of the microorganisms present in a sample, but these are time-consuming. For this reason, researchers are developing new techniques that allow detection of food pathogens in shorter period of time. This review identifies commercially available methods for rapid detection and quantification of *Listeria monocytogenes*, *Salmonella spp.*, *Staphylococcus aureus*, and *Shiga toxin-producing Escherichia coli* in food samples. Three categories are discussed: immunologically based methods, nucleic acid-based assays, and biosensors.

**Most Common Foodborne Pathogens and Mycotoxins on Fresh Produce: A Review of Recent Outbreaks**

*F. Yeni, S. Yavaş, H. Alpas, Y. Soyer*

*Critical Reviews in Food Science and Nutrition, Vol. 56, No. 9; 2016; pp. 1532–1544*

DOI: 10.1080/10408398.2013.777021

Link to full text: Click here

**Significance:** This paper reviewed the most common foodborne pathogens on fresh produce, traced back the investigations of the outbreaks caused by these pathogens, and reviewed an international early warning and notification system aimed at detecting foodborne outbreaks.

Every year millions of people are affected and thousands of them die due to infections and intoxication as a result of foodborne outbreaks, which also cause billions of dollars’ worth of damage, public health problems, and agricultural product loss. A considerable portion of these outbreaks is related to fresh produce and caused by foodborne pathogens on fresh produce and mycotoxins. *Escherichia coli O104:H4* outbreak, occurred in Germany in 2011, has attracted a great attention on foodborne outbreaks caused by contaminated fresh produce, and especially the vulnerability and gaps in the early warning and notification networks in the surveillance systems in all around the world.
Molecular Detection of Foodborne Pathogens: A Rapid and Accurate Answer to Food Safety
Critical Reviews in Food Science and Nutrition, Vol. 56, No. 9; pp. 1568–1584
DOI: 10.1080/10408398.2013.782483
Link to full text: Click here

Significance: This paper describes the conventional methods, as well as recent developments in food pathogen detection, identification, and quantification, with a major emphasis on molecular detection methods.

Food safety is a global health concern. For the prevention and recognition of problems related to health and safety, detection of foodborne pathogen is of utmost importance at all levels of food production chain. For several decades, a lot of research has been targeted at the development of rapid methodology as reducing the time needed to complete pathogen detection tests has been the primary goal of food microbiologists. With the result, food microbiology laboratories now have a wide array of detection methods and automated technologies such as enzyme immunoassay, polymerase chain reaction, and microarrays, which can cut test times considerably. Nucleic acid amplification strategies and advances in amplicon detection methodologies have been the key factors in the progress of molecular microbiology.

Salmonella

Hydrosol of Thymbra capitata Is a Highly Efficient Biocide against Salmonella enterica Serovar Typhimurium Biofilms
F. Karampoula, E. Giaouris, J. Deschamps, A.I. Doulgeraki, G-J.E. Nychas, F. Dubois-Brissonnet
Applied and Environmental Microbiology, Vol. 82, No. 17; pp. 5309–5319
DOI: 10.1128/AEM.01351-16
Link to full text: Click here

Significance: Hydrosol of Thymbra capitata demonstrated significant antimicrobial action against both planktonic and biofilm cells of Salmonella enterica serovar Typhimurium.

This study aimed to evaluate the antimicrobial activity of hydrosol of the Mediterranean spice Thymbra capitata against both planktonic and biofilm cells of Salmonella enterica serovar Typhimurium and to compare its action with that of benzalkonium chloride (BC), a commonly used industrial biocide. The disinfectant activity following 6-min treatments was comparatively evaluated for both disinfectants by calculating the concentrations needed to achieve the same log reductions against both types of cells. Their bactericidal effect against biofilm cells was also comparatively determined by in situ and real-time visualization of cell inactivation through the use of time-lapse confocal laser scanning microscopy (CLSM). Results revealed that hydrosol was almost equally effective against biofilms and planktonic cells, whereas a 200-times-higher concentration of BC was needed to achieve the same effect against biofilm compared to planktonic cells. Time-lapse CLSM revealed the significant advantage of the hydrosol to easily penetrate within the biofilm structure and quickly kill the cells, despite the three-dimensional (3D) structure of Salmonella biofilm.
E. coli

Shiga Toxin-Producing Escherichia coli Strains Isolated From Dairy Products — Genetic Diversity and Virulence Gene Profiles


DOI: 10.1016/j.ijfoodmicro.2016.04.032
Link to full text: Click here

Significance: Differences between human and dairy strains of STEC were identified but these differences were not sufficient to associate PFGE and virulence gene profiles to a putative lower pathogenicity of dairy strains based on their lower incidence in disease.

The genetic diversity of 197 strains, mainly Escherichia coli (STEC), from serotypes O157:H7, O26:H11, O103:H2, O111:H8 and O145:28 were compared against strains recovered in dairy products against strains from human, meat and environment cases. A set of reference-collection STEC isolates from dairy products were characterized by PFGE DNA fingerprinting and a subset of these by virulence-gene profiling. PFGE profiles of restricted STEC total DNA showed high genomic variability, enabling all dairy isolates to be differentiated. High-throughput real-time PCR screening of STEC virulence genes were applied on the O157:H7 and O26:H11 STEC isolates from dairy products and human cases. The virulence gene profiles of dairy and human STEC strains were similar. Frequency-wise, stx1 was more prevalent among dairy O26:H11 isolates than in human cases ones (87% vs. 44%) while stx2 was more prevalent among O26:H11 human isolates (23% vs. 81%). For O157:H7 isolates, stx1 (0% vs. 39%), nleF (40% vs 94%) and Z6065 (40% vs 100%) were more prevalent among human than dairy strains.

Food Packaging

Human Exposure Assessment of Silver and Copper Migrating From an Antimicrobial Nanocoated Packaging Material Into an Acidic Food Simulant

J.C. Hannon, J.P. Kerry, M. Cruz-Romero, S. Azlin-Hasim, M. Morris, E. Cummins

Food and Chemical Toxicology, Vol. 95, No. 9; pp. 128–136
DOI: 10.1016/j.fct.2016.07.004
Link to full text: Click here

Significance: The calculated margin of exposure suggests current migration limits may be conservative for specific nano-packaging applications.

To examine the human exposure to a novel silver and copper nanoparticle (AgNP and CuNP)/polystyrene-polyethylene oxide block copolymer (PS-b-PEO) food packaging coating, the migration of Ag and Cu into 3% acetic acid (3% HAc) food simulant was assessed at 60 °C for 10 days. Significantly lower migration was observed for Ag (0.46 mg/kg food) compared to Cu (0.82 mg/kg food) measured by inductively coupled plasma – atomic emission spectrometry (ICP-AES). In addition, no distinct population of AgNPs or CuNPs were observed in 3% HAc by nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). The predicted human exposure to Ag and Cu was used to calculate a margin of exposure (MOE) for ionic species of Ag and Cu, which indicated the safe use of the food packaging in a hypothetical scenario (e.g. as fruit juice packaging).
Norovirus

**Effect of Process Temperature on Virus Inactivation during High Hydrostatic Pressure Processing of Contaminated Fruit Puree and Juice**

H. Pan, M. Buenconsejo, K.F. Reineke, Y.C. Shieh

*Journal of Food Protection*, Vol. 79, No. 9; pp. 1517–1526

DOI: 10.4315/0362-028X.JFP-16-004

Link to full text: Click here

**Significance:** This research illustrates use of predictive inactivation and a feasible means for manipulating high pressure processing parameters for effective virus inactivation in fruit juices and puree.

This study evaluated the effectiveness of high pressure processing (HPP) holding temperatures (<40°C) and pressures for inactivating surrogates (murine norovirus [MNV] and MS2 coliphage) in pomegranate and strawberry juices and strawberry puree using a 24-liter HPP system. MNV inactivation in juices was conducted at 300 MPa for 3 min with various holding temperatures (10 to 30°C). A regression equation was used to predict a 2.6-log reduction in juices at 0°C holding temperature and indicated that MNV inactivation was inversely proportional to temperature increase. MNV survival during HPP did not differ significantly in pomegranate and strawberry juices. However, MS2 coliphage inactivation was greater as the holding temperature increased (from 15 to 38°C) at 600 MPa for 3 min. The increased inactivation trend is presumably similar to that for hepatitis A virus, but the holding temperature was not correlated with the reduction of HPP resistant MS2 in strawberry puree. When the HPP holding pressure was evaluated independently in strawberry puree, a 5-log reduction of MNV was predicted through regression analysis at the holding pressure of 424 MPa for 3 min at 20°C. These parameters should inactivate >5 log PFU of MNV in juices, based upon a greater inactivation in berry juice than in puree (1.16-versus 0.74-log reduction at 300 MPa).

**A State-by-State Assessment of Food Service Regulations for Prevention of Norovirus Outbreaks**


DOI: 10.4315/0362-028X.JFP-16-088

Link to full text: Click here

**Significance:** Adoption and compliance with federal recommended food service regulations may decrease the incidence of foodborne norovirus outbreaks.

This study analyzed the food service regulations of all 50 states and the District of Columbia to describe differences in adoption of norovirus-related Food Code provisions into state food service regulations. In addition, potential correlations between adoption of these regulations and characteristics of foodborne norovirus outbreaks reported to the National Outbreak Reporting System from 2009 through 2014 were assessed. Of the 51 states assessed, all required food workers to wash their hands, and 39 (76%) prohibited bare-hand contact with ready-to-eat food. Thirty states (59%) required exclusion of staff with vomiting and diarrhea until 24 h after cessation of symptoms. Provisions requiring a certified food protection manager (CFPM) and a response plan for contamination events (i.e., vomiting)
were least commonly adopted; 26 states (51%) required a CFPM, and 8 (16%) required a response plan. States that adopted the provisions prohibiting bare-hand contact (0.45 versus 0.74, \( P = 0.07 \)), requiring a CFPM (0.38 versus 0.75, \( P = 0.09 \)), and excluding ill staff for \( \geq 24 \) h after symptom resolution (0.44 versus 0.73, \( P = 0.24 \)) each reported fewer foodborne norovirus outbreaks per million person-years than did those states without these provisions.

**Inactivation of Human Norovirus and Tulane Virus in Simple Media and Fresh Whole Strawberries by Ionizing Radiation**


DOI: 10.1016/j.ijfoodmicro.2016.05.013

Link to full text: Click here

**Significance:** Human norovirus and Tulane virus are highly resistant to ionizing radiation and, therefore, the technology may not be suitable to eliminate viruses in fresh produce at the currently approved levels.

Electron beam (E-beam) and gamma radiation were evaluated for efficacy against a human norovirus (NoV) GII.4 strain and Tulane virus (TV). Virus survival following ionizing radiation treatments was determined using direct quantitative reverse transcriptase PCR (RT-qPCR), the porcine gastric mucin magnetic bead (PGM-MB) binding assay followed by RT-qPCR, and plaque assay. In simple media, a high dose of E-beam treatment was required to completely abolish the receptor binding ability of human NoV (35.3 kGy) and TV (19.5–24.1 kGy), as assessed using the PGM-MB binding assay. Both human NoV and TV were more susceptible to gamma irradiation than E-beam, requiring 22.4 kGy to achieve complete inactivation. In whole strawberries, no human NoV or TV RNA was detected following 28.7 kGy of E-beam treatment using the PGM-MB binding assay. The PGM-MB binding assay is an improved method to detect viral infectivity compared to direct RT-qPCR.

**Viability of Murine Norovirus in Salads and Dressings and Its Inactivation Using Heat-Denatured Lysozyme**


DOI: 10.1016/j.ijfoodmicro.2016.06.006

Link to full text: Click here

**Significance:** Norovirus can survive for 5 days in contaminated salads.

The viability of norovirus in various types of salads and dressings was examined using murine norovirus strain 1 (MNV-1) as a surrogate for the closely related human norovirus. In addition, the inactivation of MNV-1 in salads was examined using heat-denatured lysozyme, which had been reported to inactivate norovirus. MNV-1 was inoculated in 4 types of salads (coleslaw, thousand island salad, vinaigrette salad, egg salad) and 3 types of dressings (mayonnaise, thousand island dressing, vinaigrette dressing), stored at 4 °C for 5 days. The results revealed that in the vinaigrette dressing, the infectivity of MNV-1 decreased by 2.6 log PFU/mL in 5 days, whereas in the other dressings and salads, the infectivity of MNV-1 did
not show any significant decrease. Next, 1% heat-denatured lysozyme was added to the 4 types of salads, and subsequently it was found that in 2 types of salads (thousand island salad, vinaigrette salad), the infectivity of MNV-1 decreased by >4.0 log PFU/g, whereas in coleslaw salad, a decrease of 3.0 log PFU/g was shown. However, in egg salads, the infectivity of MNV-1 did not show such decrease.

**Food Allergy**

**A Novel Immunoassay Test System for Detection of Modified Allergen Residues Present in Almond-, Cashew-, Coconut-, Hazelnut-, and Soy-Based Nondairy Beverages**

J. Masiri, L. Benoit, M. Meshgi, J. Day, C. Nadala, M. Samadpour


DOI: 10.4315/0362-028X.JFP-15-493

Link to full text: [Click here](#)

**Significance:** The development of a highly sensitive and rapid test specifically designed to detect trace quantities of highly modified allergen residues in plant-based, dairy-free beverages will aid food manufacturers and regulatory agencies in monitoring products for these modified allergens when testing environmental and food samples.

A growing number of plant-based milk substitutes have become commercially available, providing an array of options for consumers with dietary restrictions. However, beverages prepared with soy and tree nuts can be a significant concern to consumers because of potential contamination with food allergens. Allergen residues from plant-based beverages are modified during manufacturing, thereby decreasing the sensitivity of antibody-based detection methods. Many commercially available allergen detection kits are less effective for allergens derived from nondairy milk substitutes. A panel of polyclonal antibodies directed against the modified proteins present in almond, cashew, coconut, hazelnut, and soy milks were developed and incorporated into rapid lateral flow immunoassay tests configured in both sandwich and competitive format. The tests had robust detection capabilities when used with a panel of various brand-name products, with a sensitivity of 1 ppm and selectivity values of 3 to 5 ppm in nondairy beverages. Minimal cross-reactivity to extracts prepared from common commodities was observed.

**Egg and Milk Proteins as Hidden Allergens in Food: 5-Year (2010 to 2014) Results of Food Allergen Monitoring in Piedmont, Italy**

D.M. Bianchi, D. Adriano, S. Astegiano, S, Gallina, M. Caramelli, L. De castelli


DOI: 10.4315/0362-028X.JFP-16-013

Link to full text: [Click here](#)

**Significance:** Food manufacturers need to improve their allergen control programs to reduce allergen exposure and risk.

Cow’s milk and egg allergies are two of the most common food allergies. Manufacturers of food products containing milk or eggs or their derivatives as an ingredient are required by European Union regulations to list their presence on the ingredient label. Under European Union legislation, member states are mandated to carry out food safety monitoring programs to verify compliance with food labeling requirements. Through the Regional Integrated Plan for Food Safety, the Piedmont (Italy) regional authority carries out an annual program to
determine the presence of undeclared allergens in foods. In the 5-year period from 2010 to 2014, a total of 1,566 food samples were analyzed for the presence of hidden egg and milk proteins. The average positive percentage was 2.8% (3.6% egg and 2% milk proteins). Comparison between the allergen concentration and the published eliciting dose (ED) for egg proteins (0.03 mg) and for total milk proteins (0.1 mg) indicated a high risk of allergen exposure for sensitized consumers. The calculated exposure was up to 135× (for milk) the ED01 reported in the literature.

**Timing of Allergenic Food Introduction to the Infant Diet and Risk of Allergic or Autoimmune Disease: A Systematic Review and Meta-Analysis**


*Journal of the American Medical Association, Vol. 316, No. 11; pp. 1181–1192*

DOI: 10.1001/jama.2016.12623

Link to full text: [Click here](#)

**Significance:** Timing of egg or peanut introduction was not associated with risk of allergy to other foods and timing of gluten introduction was not associated with celiac disease risk.

The purpose of this systematic review and meta-analysis was to determine whether timing of allergenic food introduction during infancy influences risk of allergic or autoimmune disease. Databases were searched between January 1946 and March 2016. Intervention trials and observational studies that evaluated timing of allergenic food introduction during the first year of life and reported allergic or autoimmune disease or allergic sensitization were included. Of 16,289 original titles screened, data were extracted from 204 titles reporting 146 studies. There was moderate-certainty evidence from 5 trials (1915 participants) that early egg introduction at 4 to 6 months was associated with reduced egg allergy (RR=0.56; 95% CI, 0.36-0.87; I² = 36%). Absolute risk reduction for a population with 5.4% incidence of egg allergy was 24 cases (95% CI, 7-35 cases) per 1000 population. There was moderate-certainty evidence from 2 trials (1550 participants) that early peanut introduction at 4 to 11 months was associated with reduced peanut allergy (RR=0.29; 95% CI, 0.11-0.74; I² = 66%). Absolute risk reduction for a population with 2.5% incidence of peanut allergy was 18 cases (95% CI, 6-22 cases) per 1000 population. There was low-to very low-certainty evidence that early fish introduction was associated with reduced allergic sensitization and rhinitis. Timing of allergenic food introduction was not associated with other outcomes.

**Aflatoxins**

**Aflatoxin B1 Degradation by Liquid Cultures and Lysates of Three Bacterial Strains**

*O.A. Adebo, P.B. Njobeh, S. Sidu, M.G. Tlou, V. Mavumengwana*

*International Journal of Food Microbiology, Vol. 233, 16 September 2016; pp. 11–19*

DOI: 10.1016/j.ijfoodmicro.2016.06.007

Link to full text: [Click here](#)

**Significance:** This study showed the efficacy of crude bacterial lysates for detoxifying aflatoxin B1 indicating potential for application in the food and feed industry.
This study investigated the biodegradation of aflatoxin B1 (AFB1), using lysates of three bacterial strains (Pseudomonas anguilliseptica VGF1, Pseudomonas fluorescens and Staphylococcus sp. VGF2) isolated from a gold mine aquifer. The bacterial cells were intermittently lysed in the presence and absence of protease inhibitors to obtain protease free lysates, subsequently incubated with AFB1 for 3, 6, 12, 24, and 48 h to investigate whether any possible AFB1 degradation occurred using high performance liquid chromatography (HPLC) for detection. Results obtained revealed that after 6 h of incubation, protease inhibited lysates of Staphylococcus sp. VGF2 demonstrated the highest degradation capacity of 100%, whereas P. anguilliseptica VGF1 and P. fluorescens lysates degraded AFB1 by 66.5 and 63%, respectively. After further incubation to 12 h, no residual AFB1 was detected for all the lysates. Lower degrading ability was however observed for liquid cultures and uninhibited lysates. Data on cytotoxicity studies against human lymphocytes showed that the degraded products were less toxic than the parent AFB1. The mechanism of degradation by these bacterial lysates is enzymatic.