Foodborne Pathogens

Population Dynamics of *Listeria monocytogenes*, *Escherichia coli* O157:H7, and Native Microflora During Manufacture and Aging of Gouda Cheese Made With Unpasteurized Milk


**Significance:** Generally, lactic acid and mesophilic bacterial populations remained consistent at approximately 8 to 9 log CFU/g during aging, whereas yeast and mold populations steadily increased in gouda cheese production.

Cheeses made with unpasteurized milk are a safety concern due to possible contamination with foodborne pathogens. *Listeria monocytogenes* and *Escherichia coli* O157:H7 have been implicated in several outbreaks and recalls linked to Gouda cheese made with unpasteurized milk. The U.S. Food and Drug Administration Code of Federal Regulations requires cheeses made with unpasteurized milk to be aged at a minimum of 1.7°C for at least 60 days before entering interstate commerce. The goal of this study was (i) to assess the population dynamics of *L. monocytogenes* and *E. coli* O157:H7 during aging of Gouda cheese when the pathogens were inoculated into the unpasteurized milk used for manufacture and (ii) to compare the native microbial populations throughout manufacture and aging. Unpasteurized milk was inoculated with *L. monocytogenes* at 1 or 3 log CFU/mL or with *E. coli* O157:H7 at 1 log CFU/mL, and Gouda cheese was manufactured in laboratory-scale or pilot plant-scale settings. Cheeses were stored at 10°C for at least 90 days, and some cheeses were stored up to 163 days. Initial native microflora populations in unpasteurized milk did not differ significantly for laboratory-scale or pilot plant-scale trials, and population dynamics trended similarly throughout cheese manufacture and aging. During manufacture, approximately 81% of the total *L. monocytogenes* and *E. coli* O157:H7 populations was found in the curd samples. At an inoculation level of 1 log CFU/mL, *L. monocytogenes* survived in the cheese beyond 60 days in four of five trials. In contrast, *E. coli* O157:H7 was detected beyond 60 days in only one trial. At the higher 3-log inoculation level, the population of *L. monocytogenes* increased significantly from 3.96 ± 0.07 log CFU/g at the beginning of aging to 6.00 ± 0.73 log CFU/g after 150 days, corresponding to a growth rate of 0.04 ± 0.02 log CFU/g/day. The types of native microflora assessed included Enterobacteriaceae, lactic acid bacteria, mesophilic bacteria, and yeasts and molds. Generally, lactic acid and mesophilic bacterial populations remained consistent at approximately 8 to 9 log CFU/g during aging, whereas yeast and mold populations steadily increased. The data from this study will contribute to knowledge about survival of these pathogens during Gouda cheese production and will help researchers assess the risks of illness from consumption of Gouda cheese made with unpasteurized milk.

Thermal and Chemical Treatments to Reduce *Salmonella* on Alfalfa (*Medicago sativa*) and Broccoli (*Brassica oleracea* var. *italica*) Seeds Before and During the Sprouting Process: A Hurdle Approach


**Significance:** Thermal and chemical treatments used as a hurdle approach to control *Salmonella* on alfalfa and broccoli seeds significantly reduced the pathogen concentration on seeds but were ineffective to eliminate *Salmonella* and to control its growth during the sprouting process.

Sprouts are vehicles of foodborne diseases caused by pathogens such as *Salmonella*. The aim of this study was to evaluate thermal and chemical treatments applied as a hurdle approach to reduce *Salmonella* in alfalfa (*Medicago sativa*) and broccoli (*Brassica oleracea* var. *italica*) seeds before and during their germination. Seeds, inoculated and then dried at 55°C for 48 h, were subjected to a chemical treatment and a thermal shock with (i) 75 mM caprylic acid at 70°C for 5 s, (ii) 0.04% CaO at 70°C for 5 s, or (iii) 1% H₂O₂ at 70°C for 5 s. After each treatment, seeds were immersed in water at 3°C for 5 s. Next, the imbibition
process was carried out with 0.016% H$_2$O$_2$ at pH 3.0. Finally, the seeds were transferred to a rotary drum-type germinator and were sprayed with the same chemical solution that was applied before the imbibition process, for 20 s at intervals of 5 min for 40 min at 3 rpm. All chemical treatments reduced Salmonella at least 5 log CFU/g on both seeds. Germination rates between 90 and 93% were obtained after application of thermal and chemical treatments. Salmonella was not detected after the imbibition stage when caprylic acid and H$_2$O$_2$ treatments were applied. However, during the germination process of both seeds, Salmonella counts of >6 log CFU/g were obtained despite all treatments being applied at different stages of the sprouting process. These results demonstrated that thermal and chemical treatments used as a hurdle approach to control Salmonella on alfalfa and broccoli seeds significantly reduced the pathogen concentration on seeds >5 log but were ineffective to eliminate Salmonella and to control its growth during the sprouting process. The production of safe sprouts continues to be a major challenge for industry.

**Foodborne Illness**

*Can Aggregated Restaurant Inspection Data Help Us Understand Why Individual Foodborne Illness Outbreaks Occur?*


**Significance:** Food handling practices in outbreak restaurants may have increased contamination of foods through cross-contamination, which in turn increased transmission.

Restaurant inspections seek to identify and correct risk factors for foodborne illness, but restaurant inspection data are not typically used more broadly as a food safety surveillance tool. In 2015, there was an outbreak of Salmonella serotype Newport infections associated with multiple restaurants in a chain (Chain A), primarily in Minnesota. The outbreak was associated with tomatoes that were likely contaminated at the point of production. The objective of this study was to demonstrate the potential usefulness of aggregated restaurant inspection data in aiding individual outbreak investigations. Reports of the last inspection for all Chain A restaurants that preceded the first reported case meal date in the outbreak were obtained from local health departments and the Minnesota Department of Health. Ordinal logistic regression was used to assess differences in risk factor and good retail practices violation categories and specific violations in restaurants with zero cases (non-outbreak restaurants) (n=25), 1–2 cases (n=16) and >3 cases (n=13). For restaurants with a “protection from contamination” violation in the routine inspection that preceded the outbreak, the proportional odds ratio for outbreak level was 4.92 (95%CI: 1.57, 15.39; p-value=0.01). These findings suggest that food handling practices in the outbreak restaurants may have increased contamination of foods through cross-contamination, which in turn increased transmission at outbreak restaurants. These data suggest that aggregated data from routine inspection reports can provide useful information to aid in outbreak investigations and other foodborne illness surveillance and prevention activities.

**Food Packaging**

*Advances in Using Nanotechnology Structuring Approaches for Improving Food Packaging*


**Significance:** This review focuses on the functions and applications of widely studied nanostructures for developing novel food packaging materials.

Recent advances in food packaging materials largely rely on nanotechnology structuring. Owing to several unique properties of nanostructures that are lacking in their bulk forms, the incorporation of nanostructures into packaging materials has greatly improved the performance and enriched the functionalities of these materials. This review focuses on the functions and applications of widely studied nanostructures for developing novel food packaging materials. Nanostructures that offer antimicrobial activity, enhance mechanical and barrier properties, and monitor food product freshness are discussed and compared. Furthermore, the safety and potential toxicity of nanostructures in food products are evaluated by summarizing the migration activity of nanostructures to different food systems and discussing the metabolism of nanostructures at the cellular level and in animal models.

**Heavy Metals**

*A Review of the Evidence to Support Interim Reference Level for Dietary Lead Exposure in Adults*


**Significance:** Use of the 12.5 μg/day interim reference level as a benchmark for dietary lead intake is one way FDA will ensure that dietary lead intake in adults is reduced.
FDA developed the interim reference level (IRL) for lead of 3 μg/day in children and 12.5 μg/day in women of childbearing age (WOCBA) to better protect the fetus from lead toxicity. These IRLs correspond to a blood lead level (BLL) of 0.5 μg/dL in both populations. The current investigation was performed to determine if the IRL for WOCBA should apply to the general population of adults. A literature review of epidemiological studies was conducted to determine whether a BLL of 0.5 μg/dL is associated with adverse effects in adults. Some studies reported adverse effects over a wide range of BLLs that included 0.5 μg/dL adding uncertainty to conclusions about effects at 0.5 μg/dL; however, no studies clearly identified this BLL as an adverse effect level. Results also showed that the previously developed PTTDI for adults of 75 μg/day lead may not be health protective, supporting use of a lower reference value for lead toxicity in this population group. Use of the 12.5 μg/day IRL as a benchmark for dietary lead intake is one way FDA will ensure that dietary lead intake in adults is reduced.

**Caffeine**

**Effects of Acute Caffeine Consumption Following Sleep Loss on Cognitive, Physical, Occupational and Driving Performance: A Systematic Review and Meta-Analysis**


**Significance:** This systematic review and meta-analysis found that acute caffeine consumption improved measures of cognitive, physical, occupational, and driving performance in sleep-deprived/restricted individuals.

Caffeine is widely used to counteract the effects of sleep loss. This systematic review and meta-analysis examined the impact of acute caffeine consumption on cognitive, physical, occupational and driving performance in sleep-deprived/restricted individuals. 45 publications providing 327 effect estimates (EEs) were included in the review. Caffeine improved response time (44 EEs; g = 0.86; 95 % CI: 0.53-0.83) and accuracy (27 EEs; g = 0.68; 95 % CI: 0.48-0.88) on attention tests, improved executive function (38 EEs; g = 0.35; 95 % CI: 0.15-0.55), improved reaction time (12 EEs; g = 1.11; 95 % CI: 0.75-1.47), improved response time (20 EEs; g = 1.95; 95 % CI: 1.39-2.52) and accuracy (34 EEs; g = 0.43; 95 % CI: 0.30-0.55) on information processing tasks, and enhanced lateral (29 EEs; g = 1.67; 95 % CI: 1.32-2.02) and longitudinal (12 EEs; g = 1.60; 95 % CI: 1.16-2.03) measures of vehicular control on driving tests. Studies also typically indicated benefit of caffeine on memory (25 EEs), crystallized intelligence (11 EEs), physical (39 EEs) and occupational (36 EEs) performance. Ingestion of caffeine is an effective counter-measure to the cognitive and physical impairments associated with sleep loss.

**Food Allergens**

**Prevalence and Characteristics of Shellfish Allergy in the Pediatric Population of the United States**


**Significance:** Shellfish allergy (SA) is one of the most common food allergies causing anaphylaxis in adults and children. There are limited data showing the prevalence of SA in US children. A cross-sectional food allergy prevalence survey was administered via phone and the Web by the National Opinion Research Center at the University of Chicago from 2015 to 2016. Point prevalence SA estimates, complex survey weighted proportions, and 95% CIs were determined. Relative proportions of demographic characteristics were compared using weighted Pearson χ² statistics. The prevalence of SA was 1.3% (95% CI, 1.1–1.5), with more children allergic to crustaceans (1.2%; 95% CI, 1.0–1.3) than to mollusks (0.5%; 95% CI, 0.4–0.6). Mean ages of shellfish, crustacean, and mollusk allergy diagnoses were 5.0 (95% CI, 4.4–5.6), 5.1 (95% CI, 4.6–5.6), and 7.7 (95% CI, 5.7–9.7) years, respectively. More than half (54.9%; 95% CI, 48.1–61.4) of pediatric patients with SA had more than 1 lifetime food allergy-related emergency room visit, but only 45.7% (95% CI, 39.2–52.4) carried an epinephrine autoinjector. Children with SA were more likely to be black/Hispanic/Latino and have comorbid asthma, allergic rhinitis, or a parental history of asthma, environmental, or other food allergies (P < .001). The epidemiology of SA in the US pediatric population shows that crustacean allergy is more common than mollusk allergy. A disparity in SA children and epinephrine autoinjector carriage exists. Results from this study will lead to increased awareness of the need for detailed histories, specific diagnostic tests, and rescue epinephrine for anaphylaxis in US children with SA.
Extension of xMAP Food Allergen Detection Assay to Include Sesame


**Significance:** The modified xMAP FADA successfully detected sesame incurred or spiked in baked muffins, spice mix, canola oil, and in both raw and toasted sesame oils with limit of quantitation values ≤ 1.3 ppm of sesame. Canola oil, sesame oil, toasted sesame oil, and olive oil inhibited sesame detection, as did the detection of sesame incurred in foods containing oil.

An estimated 0.1 to 0.2% of the North American population is allergic to sesame, and deaths due to anaphylactic shock have been reported. Detecting and quantifying sesame in various food samples is critical to safeguard the allergic population by ensuring accurate ingredient labeling. Because of the modular nature of the xMAP Food Allergen Detection Assay (FADA), it was possible through method extension to add sesame as a validated additional analyte. Because raw and toasted sesame are both commonly used and the two display significantly different antigenicity, three antibodies, one monoclonal and two polyclonal, were conjugated to bead sets to ensure reliable detection. The modified xMAP FADA successfully detected sesame incurred or spiked in baked muffins, spice mix, canola oil, and in both raw and toasted sesame oils with limit of quantitation values ≤ 1.3 ppm of sesame. Canola oil, sesame oil, toasted sesame oil, and olive oil inhibited sesame detection, as did the detection of sesame incurred in foods containing oil (e.g., hummus). Despite this inhibition, the xMAP FADA was still able to reliably detect sesame at levels throughout the dynamic range of the assay (22 to 750 ng of protein per mL) in all the foods examined. Further, the high signal-to-noise ratio of the lowest calibration standard and preliminary studies conjugating the antibodies at higher concentrations indicate an ability to increase the sensitivity of the assay should the need arise.