Foodborne Pathogens

Thermal Resistance of *Listeria monocytogenes* in Natural Unsweetened Cocoa Powder Under Different Water Activity


*Significance:* The impact of water activity on thermal resistance of *L. monocytogenes* in cocoa powder is presented.

*L. monocytogenes* can survive in dry conditions for long periods. Despite an increasing research studying *Salmonella* inactivation in low-moisture foods, there is a general lack of knowledge related to *L. monocytogenes* inactivation in low-moisture foods during thermal processing and the factors impacting their survival in these products. Cocoa powder is an essential and widely incorporated ingredient in many desserts and drinks that do not need thermal processing. This study evaluated the thermal resistance of *L. monocytogenes* in cocoa powder and investigated the impact of water activity (aw) on its survival in cocoa powder. Natural unsweetened cocoa powder was inoculated with a 3-strain *L. monocytogenes* cocktail (~9.0 Log10 CFU/g), equilibrated to aw 0.30, 0.45 or 0.60 at 22 °C and subjected to isothermal treatments. Survivors were enumerated to obtain thermal-inactivation parameters. *L. monocytogenes* population was stable in cocoa powder (aw 0.30) over the first month of storage, then decreased gradually but remained detectable after 12-month storage at 22 °C. Thermal inactivation of *L. monocytogenes* in cocoa powder at target aw and different temperatures showed a log-linear trend. Heat resistance of *L. monocytogenes* is aw-dependent with the highest resistance at aw 0.30. The range of D-values (in min) at 70, 75 and 80 °C at aw 0.30 and 0.45, respectively, were: 21.9–5.0 and 7.3–1.8. The range of D-values (in min) at 65, 70 and 75 °C at aw 0.60 was 9.1–2.0. The z-value at aw 0.30, 0.45, and 0.60 was 15.5, 15.9, and 14.9 °C, respectively. In summary, *L. monocytogenes* can survive in cocoa powder stored at 22 °C for an extended time. Thermal resistance of *L. monocytogenes* adapted to low aw cocoa was conversely related to aw. This study provides valuable information for the food industry to develop thermal inactivation strategies to control *L. monocytogenes* in cocoa powder.

Effect of Food Structure, Water Activity, and Long-Term Storage on X-Ray Irradiation for Inactivating *Salmonella* Enteritidis PT30 in Low-Moisture Foods


*Significance:* The efficacy of X-ray irradiation for inactivating *Salmonella* in low-water-activity foods following a range of storage periods was assessed.

Recent outbreaks and recalls of low-moisture foods contaminated with *Salmonella* have been recognized as a major public health risk that demands the development of new *Salmonella* mitigation strategies and technologies. This study aimed to assess the efficacy of X-ray irradiation for inactivating *Salmonella* on or in almonds (kernels, meal, butter), dates (whole fruit, paste), and wheat (kernels, flour) at various water activities (aw) and storage periods. The raw materials were inoculated with *Salmonella* Enteritidis PT30, conditioned to 0.25, 0.45, and 0.65 aw in a humidity-controlled chamber, processed to various fabricated products, and reconditioned to the desired aw before treatment. In a storage study, inoculated almond kernels were stored in sealed tin cans for 7, 15, 27, and 103 weeks, irradiated with X-ray (0.5 to 11 kGy, targeting up to a ~2.5-log reduction) at the end of each storage period, and plated for *Salmonella* survivors to determine the efficacy of irradiation in terms of D10-value (dose required to reduce 90% of the population). *Salmonella* was least resistant (D10-value = 0.378 kGy) on the surface of almond kernels at 0.25 aw and most resistant (D10-value = 2.34 kGy) on the surface of dates at 0.45 aw. The *Salmonella* D10-value was 61% lower in date paste than on whole date fruit. Storage of almonds generally had no effect on the irradiation resistance of *Salmonella* over 103 weeks. Overall, these results indicate that product structure (whole, meals, powder, or paste), water activity (0.25 to 0.65 aw),
and storage period (0 to 103 weeks) should be considered when determining the efficacy of X-ray irradiation for inactivating Salmonella in various low-water-activity foods.

Food Processing Safety

Recent Developments in Applications of Radio Frequency Heating for Improving Safety and Quality of Food Grains and Their Products: A Review


Significance: This review highlights the potential applications and challenges of using radio frequency technology for reducing post-harvest losses of food grains.

Food grains constitute a vital part of the daily diet of the population worldwide, and are generally considered as safe products with high storage stability due to their low moisture contents. However, post-harvest losses (PHL) caused by insects, fungi, food-borne pathogens, and undesirable enzymes remain a major concern for the grain industry. Thermal treatments are commonly used to reduce the PHL of grains and their products without any chemical residues. Among which, radio frequency (RF) technology has been regarded as a promising alternative to traditional heating methods for improving safety and quality of food grains due to its fast, volumetric, and deep penetration heating characteristics. This review provided comprehensive information about principles of RF technology and its main applications including disinfection, pasteurization, enzyme inactivation, drying, and roasting for processing food grains and their products. The methods to improve the RF heating uniformity and effects of RF heating on product quality were also reviewed. Finally, the current problems and recommendations for future work related to RF processing of grains and their products were discussed. This review would improve the understanding of RF heating for food grains and their products and promote the application of RF technology in the food grain industry.

Mycotoxins

Biotin-Streptavidin System-Mediated Ratiometric Multiplex Immunochromatographic Assay for Simultaneous and Accurate Quantification of Three Mycotoxins


Significance: A novel radiometric multiplex immunochromatographic assay is presented for the simultaneous quantitative detection of aflatoxin B1, fumonisin B1, and ochratoxin A.

The quantitative multiplex immunochromatographic assay (mICA) has received an increasing amount of attention in multitarget detection. However, the quantitative results in the reported mICAs were obtained by recording the signals on the test lines that with which various analyte-independent factors readily interfere, resulting in inaccurate quantitation. The ratiometric strategy using the T/C value (ratios of signals on the test line to those of the control line) for signal correction can effectively circumvent these issues to enable more accurate detection. Herein, we present for the first time a novel ratiometric mICA strip with multiple T lines for the simultaneous quantitative detection of aflatoxin B1, fumonisin B1, fumonisin B1, and ochratoxin A (OTA) using highly luminescent quantum dot nanobeads (QBs) as enhanced signal reporters. To achieve reliable ratiometric signal output, a biotin–streptavidin system was introduced to replace the conventional anti-mouse IgG antibody for reliable reference signals on the control line that are completely independent of the signal probe and analyte. By using stable T/C values as quantitative signals, our proposed QB–mICA method can successfully detect three mycotoxins with concentrations as low as 1.65 pg/mL for AFB1, 1.58 ng/mL for FB1, and 0.059 ng/mL for OTA. The detection performance of the developed QB–mICA strip, including precision, specificity, and reliability, was further evaluated using artificially contaminated cereal samples. The results demonstrate the improved accuracy and reliability of quantitative determination by comparison with the anti-mouse IgG antibody. Thus, this work provides a promising strategy for developing a ratiometric mICA method for accurately quantifying multiple analytes using the biotin–SA system, opening up a new direction in quantitative mICAs.

Food Packaging

Natural Biodegradable Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Nanocomposites With Multifunctional Cellulose Nanocrystals/Graphene Oxide Hybrids for High-Performance Food Packaging


Significance: The barrier properties, antibacterial activity, and migration level of ternary nanocomposites with potential for bioactive food packaging materials are presented.
High-performance and useful graphene oxide (GO) and cellulose nanocrystals (CNCs) are easily extracted from natural graphite and cellulose raw materials, and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is produced by bacterial fermentation from natural plant corn stalks, etc. In this study, novel ternary nanocomposites consisting of PHBV/cellulose nanocrystal-graphe oxide nanohybrids were prepared via a simple solution casting method. The synergistic effect of CNC with GO nanohybrids obtained by chemical grafting (CNC-GO, covalent bonds) and physical blending (CNC/GO, noncovalent bonds) on the physicochemical properties of PHBV nanocomposites was evaluated and the results compared with a single component nanofiller (CNC or GO) in binary nanocomposites. More interestingly, ternary nanocomposites displayed the highest thermal stability and mechanical properties. Compared to neat PHBV, the tensile strength and elongation to break increased by 170.2 and 52.1%, respectively, and maximum degradation temperature ($T_{\text{max}}$) increment by 26.3 °C, were observed for the ternary nanocomposite with 1 wt % covalent bonded CNC-GO. Compared to neat PHBV, binary, and 1:0.5 wt % noncovalent CNC/GO based nanocomposites, the ternary nanocomposites with 1 wt % covalent bonded CNC-GO exhibited excellent barrier properties, good antibacterial activity (antibacterial ratio of 100.0%), reduced barrier properties, and lower migration level for both food simulants. Such a synergistic effect yielded high-performance ternary nanocomposites with great potential for bioactive food packaging materials.

**Heavy Metals**

**Dietary Compounds to Reduce In Vivo Inorganic Arsenic Bioavailability**


**Significance:** This study found that Fe(III) and glutathione are effective in reducing arsenic bioavailability and tissue accumulation in BALB/c mice.

It is estimated that approximately 200 million people are exposed to arsenic levels above the World Health Organization provisional guideline value, and various agencies have indicated the need to reduce this exposure. In view of the difficulty of removing arsenic from water and food, one alternative is to reduce its bioavailability (the amount that reaches the systemic circulation after ingestion). In this study, dietary components [glutathione, tannic acid, and Fe(III)] were used to achieve this goal. As(III) or As(V) (1 mg/kg body weight) was administered daily to BALB/c mice, along with the dietary components, for 15 days. The results confirm the efficacy of Fe(III) and glutathione as reducers of arsenic bioavailability and tissue accumulation. Also, these treatments did not result in reductions of Ca, K, P, and Fe contents in the liver. These data suggest that use of these two compounds could be part of valid strategies for reducing inorganic arsenic exposure in chronically exposed populations.

**Caffeine**

**Coffee Consumption and Risk of Atrial Fibrillation in the Physicians’ Health Study**


**Significance:** Among men who participated in the Physicians’ Health Study, consumption of 1-3 cups of coffee per day was associated with a reduced risk of atrial fibrillation.

**Background:** Although coffee consumption is often reported as a trigger for atrial fibrillation (AF) among patients with paroxysmal AF, prospective studies on the relation of coffee consumption with AF risk have been inconsistent. Hence, we sought to assess the association between coffee consumption and risk of AF in men. **Methods and Results:** We prospectively studied men who participated in the Physicians’ Health Study (N=18 960). Coffee consumption was assessed through self-reported food frequency questionnaires. The incidence of AF was assessed through annual questionnaires and validated through review of medical records in a subsample. Cox proportional hazard models were used to calculate hazard ratios and 95% CIs of AF. The average age was 66.1 years. A total of 2098 new cases of AF occurred during a mean follow-up of 9 years. Hazard ratios (95% CI) of AF were 1.0 (reference), 0.85 (0.71-1.02), 1.07 (0.88-1.30), 0.93 (0.74-1.17), 0.85 (0.74-0.98), 0.86 (0.76-0.97), and 0.96 (0.80-1.14) for coffee consumption of rarely/never, ≤1 cup/week, 2 to 4 cups/week, 5 to 6 cups/week, 1 cup/day, 2 to 3 cups/day, and 4+ cups/day, respectively; adjusting for age, smoking, alcohol intake, and exercise (P for nonlinear trend=0.01). In a secondary analysis the multivariable adjusted hazard ratio (95% CI) of AF per standard deviation (149-mg) change in caffeine intake was 0.97 (0.92-1.02). **Conclusions:** Our data suggest a lower risk of AF among men who reported coffee consumption of 1 to 3 cups/day.
Food Allergens

Deriving Individual Threshold Doses From Clinical Food Challenge Data for Population Risk Assessment of Food Allergens


Significance: This report provides in-depth insights into the methodology applied by TNO and FARRP to derive individual maximum tolerable doses and minimum eliciting doses for objective symptoms from clinical food challenge data, with the aim of stimulating harmonization and transparency in quantitative food allergen risk assessment.

Background: Food allergies are a significant public health issue and the only effective management option currently available is strict avoidance of all foods containing the allergen. In view of the practical impossibility to limit risks to zero, quantitative allergen risk assessment and management strategies are needed. Objective: To develop appropriate methods for informing population-based risk assessments and risk management programs to benefit all stakeholders, but particularly food allergic individuals. Methods: Individual thresholds for food allergens (maximum tolerable doses and minimum eliciting doses) can ideally be established through double-blind, placebo-controlled food challenges (DBPCFCs). If DBPCFC data is not available, data from widely used “open” food challenges (OFCs) using pre-defined objective criteria can also provide useful data regarding minimum eliciting doses. For more than 20 years, the Netherlands Organisation for Applied Scientific Research (TNO) and The Food Allergy Research and Resource Program (FARRP) at the University of Nebraska-Lincoln have been collecting individual maximum tolerable doses and minimum eliciting doses that produce objective symptoms from published and unpublished clinical data to better refine knowledge regarding the sensitivity of the population to food allergens. Results: In this paper we provide in depth insights into the methodology applied by TNO and FARRP to derive individual maximum tolerable doses and minimum eliciting doses for objective symptoms from clinical food challenge data. Over 90 examples for determining the individual allergic thresholds are presented. Conclusion: With the methodology presented in this paper, we aim to stimulate harmonization and transparency in quantitative food allergen risk assessment and risk management programs, encouraging their wider adoption.

Extraction Conditions Affect the Immunoreactivity of Peanut Allergens


Significance: The impact of preincubation temperature and probe sonication on peanut protein solubility and immunoreactivity are presented.

Peanut allergic consumers rely on food package labels to avoid foods containing peanut. The inadvertent presence of peanut in foods due to cross-contact can be fatal if ingestion of such food leads to an allergic reaction. Analytical methods are available to detect undeclared peanut in foods. However, depending on the type of food matrix and food processing parameters, method performance can be adversely affected due to reduction in the extraction efficiency of peanut proteins. Temperature and probe sonication were used as a preincubation treatment for peanut flour slurries to assess their effect on the total peanut protein solubility from raw, light-roasted, and dark-roasted peanut flours. The effect of these treatments on the immunoreactivity of peanut allergens (Ara h 1, Ara h 2, Ara h 3, and Ara h 6) was determined by an indirect enzyme-linked immunosorbent assay using antibodies raised against these individual peanut proteins. Preincubation at 50 °C did not significantly improve the peanut protein solubility, whereas an increase in protein solubility was observed when light- and dark-roasted peanut flour slurries were preincubated at 90 °C or sonicated. The immunoreactivity of peanut allergens varied depending on the degree of peanut flour roasting and type of preincubation treatment. Overall, the immunoreactivity of peanut allergens from most peanut flour slurries was unaffected when preincubated at 50 °C for up to 60 min or sonicated with a probe for up to 5 min, whereas preincubation at 90 °C resulted in a time-dependent reduction in immunoreactivity of peanut allergens. Sonication treatment may improve peanut protein extraction without markedly affecting their immunoreactivity. Practical Application: Extraction of peanut proteins is vital for developed analytical methods to estimate peanut allergens in foods. The manuscript describes the effect of two different temperatures (50 and 90 °C) and probe-type sonication on peanut protein solubility. The findings suggest sonication can improve peanut protein solubility without markedly affecting their immunoreactivity.