ILSI North America

Food Safety Briefs

February 2018

Foodborne Illness

Natural Antimicrobial/Antioxidant Agents in Meat and Poultry Products as Well as Fruits and Vegetables: A Review

Aziz M, Karboune S. *Crit Rev Food Sci Nutr.* 2018 Feb 11;58(3):486-511. doi: 10.1080/10408398.2016.1194256.
Article Link

Significance: This review highlights the antimicrobial/antioxidant activities of natural preservatives, and reports on their proposed mechanisms of action.

Synthetic preservatives are widely used by the food industry to control the growth of spoilage and pathogenic microorganisms and to inhibit the process of lipid oxidation extending the shelf-life, quality and safety of food products. However, consumer's preference for natural food additives and concern regarding the safety of synthetic preservatives prompted the food industry to look for natural alternatives. Natural antimicrobials, including plant extracts and their essential oils, enzymes, peptides, bacteriocins, bacteriophages, and fermented ingredients have all been shown to have the potential for use as alternatives to chemical antimicrobials. Some spices, herbs and other plant extracts were also reported to be strong antioxidants. The antimicrobial/antioxidant activities of some plant extracts and/or their essential oils are mainly due to the presence of some major bioactive compounds, including phenolic acids, terpenes, aldehydes, and flavonoids. The proposed mechanisms of action of these natural preservatives are reported. An overview of the research done on the direct incorporation of natural preservatives agents into meat and poultry products as well as fruit and vegetables to extend their shelf-life is presented. The development of edible packaging materials containing natural preservatives is growing and their applications in selected food products are also presented in this review.

Exponentially Increased Thermal Resistance of *Salmonella* and *Enterococcus faecium* at Reduced Water Activity

Liu S, Tang J, Tadapaneni RK, Yang R, Zhu MJ. *Appl Environ Microbiol.* 2018 Feb 9. doi: 10.1128/AEM.02742-17.
Article Link

Significance: This paper demonstrates the relationship between pathogen thermal resistance and food moisture content.

Salmonella exhibited prolonged survivability and high tolerance to heat in low-moisture foods. Reported thermal resistance parameters of *Salmonella* spp. in low-moisture foods is unpredictable due to various factors. We report here that an external factor temperature-dependent water activity ($a_{w,\ treatment\ temperature}$) of bacterial cells plays an important role in the sharply increased thermal resistance of *Salmonella* Enteritidis PT 30 (*S* Enteritidis) and its potential surrogate *Enterococcus faecium* NRRL B-2354 (*E. faecium*). In our study, silicon dioxide granules, as inert carriers, were separately inoculated with these two microorganisms and heated at 80°C with controlled relative humidity between 18-72% (result in corresponding $a_{w,80°C}$ of bacteria between 0.18 and 0.72) in custom-designed test cells. Inactivation kinetics of both microorganisms fitted a log-linear model (R2=0.83-0.97). Reduced $a_{w,80°C}$ of bacterial cells increased the D80°C-values (the time needed to achieve 1 log reduction of a bacterial population at 80°C) exponentially for *S* Enteritidis and *E. faecium* on silicon dioxide. The log-linear relationship between $D_{80°C}$ of both strains in silicon dioxide and $a_{w,80°C}$ also were verified for organic wheat flour. *E. faecium* showed consistently higher D80°C values than *S.* Enteritidis over the tested $a_{w,80°C}$ range. The estimated zaw values (the $a_{w,80°C}$ needed to alter 1 log of D80°C) of *S* Enteritidis and *E. faecium* were 0.31 and 0.28, respectively. This study provides an insight into the interpretation of thermal resistances of *Salmonella* that could guide the development and validation of thermal processing of low-moisture foods.Importance In this paper, we established that thermal resistance of the pathogen *Salmonella* and its surrogate *Enterococcus faecium*, as reflected by D values at 80°C, increases sharply with reducing relative humidity in the environment. The log-linear relationship between $D_{80°C}$ of both strains in silicon dioxide and $a_{w,80°C}$ also were verified for organic wheat flour. The results provide a new quantitative insight into how thermal resistance of microorganism's change in low moisture systems, and should aid in the development of effective thermal treatment strategies for pathogen control in low-moisture foods.

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Fruit Flies as Potential Vectors of Foodborne Illness

Black EP, Hinrichs GJ, Barcay SJ, Gardner DB. *J Food Prot.* 2018 Feb 23:509-514. doi: 10.4315/0362-028X.JFP-17-255. Article Link

Significance: This study documents the ability of fruit flies to transfer pathogenic bacteria between contaminated sources and ready-to-eat foods.

Fruit flies are a familiar sight in many food service facilities. Although they have been long considered as "nuisance pests," some of their typical daily activities suggest they may pose a potential public health threat. The aim of this study was to provide evidence of the ability of small flies to transfer bacteria from a contaminated source, food, or waste to surfaces or ready-to-eat food. Laboratory experiments were conducted by using purpose-built fly enclosures to assess the bacterial transfer capability of fruit flies. *Drosophila repleta* were capable of transferring *Escherichia coli* O157:H7, *Salmonella* Saint Paul, and *Listeria innocua* from an inoculated food source to the surface of laboratory enclosures. In addition, using an inoculated doughnut and noncontaminated lettuce and doughnut surfaces, fly-mediated cross-contamination of ready-to-eat food was demonstrated. Fruit flies were shown to be capable of accumulating approximately 2.9×10^3 log CFU of *E. coli* per fly within 2 h of exposure to a contaminated food source. These levels of bacteria did not decrease over an observation period of 48 h. Scanning electron micrographs were taken of bacteria associated with fly food and contact body parts and hairs during a selection of these experiments. These data, coupled with the feeding and breeding behavior of fruit flies in unsanitary areas of the kitchen and their propensity to land and rest on food preparation surfaces and equipment, indicate a possible role for fruit flies in the spread of foodborne pathogens.

Efficacy of Peracetic Acid in Inactivating Foodborne Pathogens on Fresh Produce Surface

Singh P, Hung YC, Qi H. *J Food Sci.* 2018 Feb;83(2):432-439. doi: 10.1111/1750-3841.14028. Article Link

Significance: This paper evaluates the efficacy of several sanitizers to remove foodborne pathogens from fresh produce.

Washing treatment with effective sanitizer is one of the critical steps in ensuring fresh produce safety. This study was to evaluate the efficacy of peracetic acid (PAA; VigorOx® 15 F&V), chlorine-based sanitizers (acidic electrolyzed water [AEO], near neutral electrolyzed water and bleach), lactic acid, and deionized (DI) water to reduce *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium DT104 from fresh produce surfaces. A 5-strain cocktail of *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium DT104 was separately prepared and used for surface inoculation on produce samples (*E. coli* O157:H7 on romaine lettuce, lemons, tomatoes, and blueberries; *L. monocytogenes* on romaine lettuce and cantaloupe; *S.* Typhimurium DT104 on lemons, tomatoes, cantaloupe, and blueberries). PAA at 45, 85, and 100 mg/L; AEO, NNEO, and bleach at 100 mg/L of free chlorine; lactic acid at 2%; and DI water were used for washing inoculated produce in an automated produce washer for 5 min. In general, PAA at 100 mg/L achieved the highest microbial inactivation of *E. coli* O157:H7 (lettuce, lemon, tomato, and blueberry at 2.2, 5.7, 5.5, and 6.7 log CFU/g, respectively), *S.* Typhimurium DT104 (lemon, tomato, cantaloupe, blueberry at 5.4, 6.8, 4.5, and 5.9 log CFU/g, respectively), and *L. monocytogenes* (lettuce and cantaloupe at 2.4 and 4.4 log CFU/g, respectively). Efficacy of sanitizers on produce with coarse surface (for example, lettuce and cantaloupe) was lower than produce with smooth texture (lemon, tomato, and blueberry). Cross-contamination of *E. coli* O157:H7 among romaine lettuce heads during simulated retail crisping process was greatly reduced by the application of PAA and NNEO. PRACTICAL APPLICATION: NNEO and PAA showed high efficacy in foodborne pathogen removal from fresh produce. Produce surface texture plays an important role in pathogen removal. NNEO and PAA effectively prevented cross-contamination during the crisping process.

Methodology

Effect of Immunomagnetic Bead Size on Recovery of Foodborne Pathogenic Bacteria

Chen J, Park B. *Int J Food Microbiol.* 2018 Feb 21;267:1-8. doi: 10.1016/j.ijfoodmicro.2017.11.022. Article Link

Significance: This article assesses the effect of immunomagnetic bead size on efficiency of foodborne pathogen detection.

Immunomagnetic separation (IMS) as a culture-free enrichment sample preparation technique has gained increasing popularity in the development of rapid detection methods for foodborne pathogens. While the use of magnetic nanoparticles in IMS is on the rise due to substantially larger surface area compared to conventional magnetic microparticles, the effects of immunomagnetic bead (IMB) size on pathogen cell recovery are not fully understood. In this study we used IMBs of different

sizes (100, 500, and 1000nm diameters) to capture *Salmonella* Enteritidis, a common foodborne pathogen, from buffer solutions as well as food matrices (chicken carcass rinse and liquid egg white). The IMS recovery and non-specific binding rate were compared. The recoveries of *Salmonella* cells in buffers was highest using the 100nm IMBs (88-96%), followed by the 500nm (31-89%) and 1000nm (4.1-61%) IMBs, demonstrating a significant size effect. The non-specific binding rates of *E. coli* also increased as IMB size decreased. A 2-72% reduction in *Salmonella* recovery was observed in chicken carcass rinse and liquid egg white samples compared to in buffers, and this reduction was more significant using 500 and 1000nm IMBs. However, lower IMS recoveries (10-56%) was found in 100nm IMBs two months after preparation. Overall, magnetic nanoparticles yielded superior IMS efficiency to micrometer size IMBs and were less subjective to interference from food matrices. Nevertheless, their long term stability remains an obstacle towards successful use in IMS.

Comparison Between Digital PCR and Real-Time PCR in Detection of *Salmonella typhimurium* in Milk

Wang M, Yang J, Gai Z, Huo S, Zhu J, Li J, et al. *Int J Food Microbiol.* 2018 Feb 2;266:251-256. doi: 10.1016/j.ijfoodmicro.2017.12.011. Article Link

Significance: This article compares the efficiency of two commonly used PCR methods for rapid detection of foodborne *Salmonella typhimurium*.

As a kind of zero-tolerance foodborne pathogens, *Salmonella typhimurium* poses a great threat to quality of food products and public health. Hence, rapid and efficient approaches to identify *Salmonella typhimurium* are urgently needed. Combined with PCR and fluorescence technique, real-time PCR (qPCR) and digital PCR (ddPCR) are regarded as suitable tools for detecting foodborne pathogens. To compare the effect between qPCR and ddPCR in detecting *Salmonella typhimurium*, a series of nucleic acid, pure strain culture and spiking milk samples were applied and the resistance to inhibitors referred in this article as well. Compared with qPCR, ddPCR exhibited more sensitive (10^{-4}ng/μl or 10^{2}cfu/ml) and less pre-culturing time (saving 2h). Moreover, ddPCR had stronger resistance to inhibitors than qPCR, yet absolute quantification hardly performed when target's concentration over 1ng/μl or 10^{6}cfu/ml. This study provides an alternative strategy in detecting foodborne *Salmonella typhimurium*.

LAMP-Based Group Specific Detection of Aflatoxin Producers Within *Aspergillus* Section *Flavi* in Food Raw Materials, Spices, and Dried Fruit Using Neutral Red for Visible-Light Signal Detection

Niessen L, Bechtner J, Fodil S, Taniwaki MH, Vogel RF. *Int J Food Microbiol.* 2018 Feb 2;266:241-250. doi: 10.1016/j.ijfoodmicro.2017.12.013. Article Link

Significance: This paper demonstrates a novel LAMP method for rapid detection of several aflatoxin producing species in a single analysis.

Aflatoxins can be produced by 21 species within sections *Flavi* (16 species), *Ochraceorosei* (2), and *Nidulantes* (3) of the fungal genus *Aspergillus*. They pose risks to human and animal health due to high toxicity and carcinogenicity. Detecting aflatoxin producers can help to assess toxicological risks associated with contaminated commodities. Species specific molecular assays (PCR and LAMP) are available for detection of major producers, but fail to detect species of minor importance. To enable rapid and sensitive detection of several aflatoxin producing species in a single analysis, a *nor1* gene-specific LAMP assay was developed.

Specificity testing showed that among 128 fungal species from 28 genera, 15 aflatoxigenic species in section Flavi were detected, including synonyms of *A. flavus* and *A. parasiticus*. No cross reactions were found with other tested species. The detection limit of the assay was 9.03pg of *A. parasiticus* genomic DNA per reaction. Visual detection of positive LAMP reactions under daylight conditions was facilitated using neutral red to allow unambiguous distinction between positive and negative assay results. Application of the assay to the detection of *A. parasiticus* conidia revealed a detection limit of 211 conidia per reaction after minimal sample preparation. The usefulness of the assay was demonstrated in the analysis of aflatoxinogenic species in samples of rice, nuts, raisins, dried figs, as well as powdered spices. Comparison of LAMP results with presence/absence of aflatoxins and aflatoxin producing fungi in 50 rice samples showed good correlation between these parameters. Our study suggests that the developed LAMP assay is a rapid, sensitive and user-friendly tool for surveillance and quality control in our food industry.

Pathogen Detection

Probing and Quantifying the Food-Borne Pathogens and Toxins: From *In Vitro* to *In Vivo*

Liu JM, Wang ZH, Ma H, Wang S. *J Agric Food Chem.* 2018 Feb 7;66(5):1061–1066. doi: 10.1021/acs.jafc.7b05225. Article Link

Significance: This review summarizes the current state of nanomaterial-assisted *in vitro* pathogen detection methods, and provides perspective on non-invasive *in vivo* quantification of target pathogens.

Development of real-time and *in situ* analytical methods for determination of food-borne pathogens and toxins ingested into the human body would be a promising research direction in the food-safety area. The present perspective starts with summarization of the up-to-date progress of the nanomaterial-assisted *in vitro* detection methods for pathogens and toxins and finally focuses on application of animal bioimaging to *in vivo* study, including prospective strategies for *in vivo* quantification of target pathogens or toxins and in vivo investigation of their behaviors inside the living body, with the assistance of real-time and non-invasive optical bioimaging. This perspective provides the advisory direction for food-safety research, from *in vitro* to *in vivo*, along with a prospective discussion of the further development roadmap of the food-safety detection techniques, especially the bioimaging-guided methods for investigation and mediation of the food contamination effect to human health.

Microbial Diversity

Microbial Diversity of Consumption Milk During Processing and Storage

Porcellato D, Aspholm M, Skeie SB, Monshaugen M, Brendehaug J, Mellegård H. *Int J Food Microbiol.* 2018 Feb 2;266:21-30. doi: 10.1016/j.ijfoodmicro.2017.11.004. Article Link

Significance: This article identifies several factors that influence microbial composition of bovine milk during production and storage.

Bovine milk contains a complex microbial community that affects the quality and safety of the product. Detailed knowledge of this microbiota is, therefore, of importance for the dairy industry. In this study, the bacterial composition of consumption milk was assessed during different stages in the production line and throughout the storage in cartons by using culturing techniques and 16S rRNA marker gene sequencing. Monthly samples from two dairies were analyzed to capture the seasonal variations in the milk microbiota. Although there was a core microbiota present in milk samples from both dairies, the composition of the bacterial communities were significantly influenced by sampling month, processing stage and storage temperature. Overall, a higher abundance of operational taxonomic units (OTUs) within the order *Bacillales* was detected in samples of raw and pasteurized milk from the spring and summer months, while *Pseudomonadales* and *Lactobacillales* OTUs were predominant in the winter months. OTUs belonging to the order *Lactobacillales*, *Pseudomonadales*, *Clostridiales* and *Bacillales* were significantly more abundant in milk samples taken immediately after pasteurization compared to raw milk samples. During storage of milk in cartons at 4°C, the bacterial composition remained stable throughout the product shelf life, while storage at 8°C significantly increased the abundance of OTUs belonging to the genus *Bacillus* and the plate count levels of presumptive *Bacillus cereus*. The knowledge obtained in this work will be useful to the dairy industry during their quality assurance work and risk assessment practices.

Food Packaging

Processing and Characteristics of Canola Protein-Based Biodegradable Packaging: A Review

Zhang Y, Liu Q, Rempel C. *Crit Rev Food Sci Nutr.* 2018 Feb 11;58(3):475-485. doi: 10.1080/10408398.2016.1193463. Article Link

Significance: This article characterizes the physical properties of canola protein for use in food packaging applications.

Interest increased recently in manufacturing food packaging, such as films and coatings, from protein-based biopolymers. Among various protein sources, canola protein is a novel source for manufacturing polymer films. It can be concentrated or isolated by aqueous extraction technology followed by protein precipitation. Using this procedure, it was claimed that more than 99% of protein was extracted from the defatted canola meal, and protein recovery was 87.5%. Canola protein exhibits thermoplastic properties when plasticizers are present, including water, glycerol, polyethylene glycol, and sorbitol. Addition of these plasticizers allows the canola protein to undergo glass transition and facilitates deformation and processability. Normally, canola protein-based bioplastics showed low mechanical properties, which had tensile strength (TS) of 1.19 to 4.31 MPa. So,

various factors were explored to improve it, including blending with synthetic polymers, modifying protein functionality through controlled denaturation, and adding cross-linking agents. Canola protein-based bioplastics were reported to have glass transition temperature, T_g, below -50°C but it highly depends on the plasticizer content. Canola protein-based bioplastics have demonstrated comparable mechanical and moisture barrier properties compared with other plant protein-based bioplastics. They have great potential in food packaging applications, including their use as wraps, sacks, sachets, or pouches.

Photo-Curable Metal-Chelating Coatings Offer a Scalable Approach to Production of Antioxidant Active Packaging

Lin Z, Goddard J. *J Food Sci.* 2018 Feb;83(2):367-376. doi: 10.1111/1750-3841.14051. Article Link

Significance: This paper introduces a novel process to introduce metal chelating functionality onto common polymeric packaging materials, to enable removal of synthetic additive EDTA.

Interest increased recently in manufacturing food packaging, such as films and coatings, from protein-based biopolymers. Among various protein sources, canola protein is a novel source for manufacturing polymer films. It can be concentrated or isolated by aqueous extraction technology followed by protein precipitation. Using this procedure, it was claimed that more than 99% of protein was extracted from the defatted canola meal, and protein recovery was 87.5%. Canola protein exhibits thermoplastic properties when plasticizers are present, including water, glycerol, polyethylene glycol, and sorbitol. Addition of these plasticizers allows the canola protein to undergo glass transition and facilitates deformation and processability. Normally, canola protein-based bioplastics showed low mechanical properties, which had tensile strength (TS) of 1.19 to 4.31 MPa. So, various factors were explored to improve it, including blending with synthetic polymers, modifying protein functionality through controlled denaturation, and adding cross-linking agents. Canola protein-based bioplastics were reported to have glass transition temperature, T_g, below -50°C but it highly depends on the plasticizer content. Canola protein-based bioplastics have demonstrated comparable mechanical and moisture barrier properties compared with other plant protein-based bioplastics. They have great potential in food packaging applications, including their use as wraps, sacks, sachets, or pouches.