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In this report... There is a need to develop new antimicrobials that ensure food safety and extend product shelf life. It appears that nanoparticles of zinc oxide have strong antimicrobial activity. On another front, care must be taken to properly thermally treat peanut butter in order to kill large populations of *Salmonella*. Also, an irreversible polymer combined with barcodes on packages will help consumers determine whether a product is safe for consumption.

Zinc oxide nanoparticles have antimicrobial efficacy

Outbreaks caused by foodborne pathogens, such as *E. coli*, *Salmonella* and *Listeria*, continue to draw public attention to food safety issues. There is a need to develop new antimicrobials to ensure food safety and extend product shelf life.

Zinc oxide (ZnO) is one of five zinc compounds listed as generally recognized as safe by the FDA. Zinc salt has been used to treat zinc deficiency in diets. However, the antimicrobial function of ZnO in foods has not been extensively explored.

It appears that ZnO nanoparticles possess strong antimicrobial activity against *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 in culture media or liquid egg. With this in mind, USDA-ARS scientists prepared ZnO powder, ZnO film and polyvinylpyrrolidone-capped ZnO (ZnO-PVP) from ZnO nanoparticles. Then they prepared zinc oxide quantum dots (ZnO-QDs) in three forms: as a powder, as a ZnO-polystyrene (PS) film and as a ZnO-PVP gel. They investigated their antibacterial activity against various pathogens in culture media and liquid egg at 24 C or 4 C. The inhibitory effects of ZnO were concentration-dependent and also related to storage temperature and the type of application. After 48 hours of incubation at 24 C, the cell populations of *L. monocytogenes* and *E. coli* O157:H7 in growth media containing ZnO-PVP (3.2 mg of ZnO per ml) were 3.7 and 3.0 log units per ml, respectively. The controls had 9.0 log units.

In liquid egg stored for eight days at 24 C, *Listeria* and *Salmonella* cells in the controls increased from 3.8 to 7.2 and from 4.5 to 9.7 log units per ml, respectively. The cells in the samples treated with 1.12 mg of ZnO per ml were reduced to 1.4 log units per ml for *Listeria* and 3.4 log units per ml for *Salmonella*.

When stored at 4 C for 41 days, liquid egg samples containing ZnO had 3 log units per ml of *Salmonella* cells, which is one log less than the initial cell counts. The control had 7.4 log units per ml. Directly adding ZnO-PVP made it more effective than the ZnO powder and ZnO-PVP coating. The investigators did not see any antimicrobial activity from the ZnO-PS film.

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Thermal inactivation of *Salmonella* in peanut butter may be limited

In addition to the current peanut butter contamination issue in the United States, a large multi-state foodborne outbreak was caused by *S. Tennessee* in peanut butter in 2006.

With this in mind, scientists at the University of Georgia attempted to determine, in peanut butter, the rates of thermal inactivation at different temperatures of three strains of *S. Tennessee* associated with the 2006 outbreak. Their efforts indicate that the outbreak-associated *Salmonella* strains were more thermal tolerant than other serotypes tested. Thermally treating peanut butter at 71 C to 77 C for less than 20 minutes is not sufficient to kill large populations of *Salmonella* in highly contaminated peanut butter.

The researchers inoculated commercial peanut butter samples with the *Salmonella* strains and heated them at 71 C, 77 C, 83 C and 90 C. At least three independent trials were conducted at each temperature and for each group of *Salmonella*. The investigators found that the thermal inactivation curves for the bacteria were upwardly concave, indicating that the bacteria were experiencing rapid death at the beginning of the thermal treatment—within 20 minutes. This was followed by lower death rates for the remaining cells.

A nonlinear Weibull model was used to describe the thermal inactivation of *Salmonella*. The calculated minimum times needed to obtain a 7-log reduction at all temperatures for the composited three outbreak-associated strains were significantly higher, compared with those of a five-strain mixture of other *Salmonella* serotypes.

A little more than two hours was needed to reduce bacterial levels in the three-strain mixture of *S. Tennessee* by 7 logs. Almost 1.5 hours were needed for the five-strain mixture of other *Salmonella* serotypes.

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Smart packaging to alert consumers when refrigerated foods go bad

If you have ever wondered whether the milk in your grocer's refrigerator might have gone bad, then a partnership between the University of Rhode Island and a food safety company will soon put you at ease.

Barcodes created by researchers at SIRA Technologies for use on refrigerated products incorporate a nearly invisible ink. When conditions indicative of contamination exist, the ink will turn red, and the label's barcode is rendered incapable of transmitting data when scanned.

A decade ago, the university researchers began studying thermochromic pigments—those that change color at certain temperatures. The heat-sensitive polymer the scientists developed turned from red to yellow at 180 F, the temperature at which a person would suffer a burn. The material turned back to red when it cooled.

When the university scientists modified their discovery into an irreversible polymer, one that does not revert to its original color after changing, scientists at SIRA Technologies, Pasadena, CA, took notice. SIRA had developed a barcode that could sequester pathogens from animal blood and quantify the colony of pathogens with colored organic beads until the color emerges to activate the barcode and report the contamination. However, constant pathogenic mutations made it impossible to keep current with marketplace needs. The company's subsequent search for an irreversible thermochromic ink led them to partner with the University of Rhode Island in what is now trademarked and patented as The Food Sentinel System.

The Food Sentinel System's distribution date is late 2009, perhaps early 2010. First applications will relate to any food product in the cold chain, accounting for more than 50% of every dollar spent in food markets. A likely emphasis will be on muscle meats, dairy, seafood and poultry. Such emphasis would be provoked by buyer preference and the need from retailers and packagers.

A patent for the school's thermochromic polymer was received in 2004, and additional patent applications are being pursued. We're told that other thermochromic indicators are commercially available, but they are expensive and lack the archival feature required by regulatory agencies to track and trace products on a global scale. They also rely on human examination to judge whether the product has been rendered unsafe for consumption. The cost of the new barcode and polymer will be less than four cents each.

Patent. 6,706,218. Thermochromic polymers for rapid visual assessment of temperature. Issued March 16, 2004. The patent covers a thermochromic polymer-based temperature indicator composition comprised of a polythiophene and a carrier medium. The polythiophene is present in an amount of about 0.05% to about 5.0% by weight, based on the total weight of the composition. When the composition is placed in a heat-exchange relationship with an article, the composition will exhibit a color change when a design temperature or a temperature beyond the design temperature is reached in the article.

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Technique detects contamination in two hours

In the hours following an outbreak of *Salmonella*, there are many questions. And answers can be hard to find. To address some concerns, Iowa State University researchers have developed a technique for testing for the presence of *Salmonella* that may give investigators better, faster answers.

The process begins with testing a food, in most cases produce, using a strip of adhesive tape. The tape is applied to the produce, then carefully removed, taking a sample of whatever is on the skin of the produce. That sample is then put on a slide and soaked in a special warm, soapy mixture that contains a genetic marker that binds with *Salmonella* and gives off a fluorescent glow when viewed under an ultraviolet light. This genetic marker technique, Fluorescent In-Situ

Hybridization (FISH), can tell investigators if the produce is contaminated with *Salmonella* in about two hours. Current methods of detecting *Salmonella* take one to seven days.

The researchers indicate that FISH will be a good tool for outbreak investigations and routine surveillance, especially since all that is needed is tape, a heat block, a small centrifuge and a fluorescence microscope. It has the potential to be very portable.

The tape-FISH technique can be used to test large volumes of produce, and it could be very valuable as a basic research tool. Researchers would be able to determine how *Salmonella* and other types of organisms interact on produce surfaces.

The FISH concept came to ISU researchers after they learned about an Italian group that was using a similar approach to look for bacteria on ancient catacombs. Those researchers were hoping to identify and remove bacteria that were slowly eating away at the relics.

Investigators decided that using the FISH on produce could be useful. They made several improvements in speed and sensitivity over the existing tape-FISH approach. They hope that the tape-FISH approach can help speed investigations of produce contamination, such as last summer's outbreak of *Salmonella St. Paul*, which was eventually traced to imported jalapeno and Serrano peppers. *S. St. Paul* is the sixth most common serovar infecting humans in the United States and has emerged in other countries, such as Japan.

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High CO₂ and O₂ atmospheres extend the shelf life of fresh-cut orange and grapefruit

Fresh-cut orange and grapefruit have high respiration rates and are very susceptible to microbial spoilage. For this reason, they have a short shelf life. Generally, high respiration rates are associated with shorter post-harvest product life.

Scientists at Texas A&M University set out to extend the shelf life of fresh-cut orange and grapefruit stored at 5 C by reducing the leakage of juice from the product and preventing microbial growth. Their efforts indicate that fresh-cut citrus can be commercialized using a combination of high concentrations of CO₂ and O₂.

In their work, the researchers cut sanitized orange and grapefruit into cubes—some segments with membrane and sliced segments with skin. The extent of juice leakage was determined by weighing any juice released from the product every seven days.

Fresh cuts of citrus were dipped in a 0.5% citric acid solution as chemical treatment. The researchers evaluated several combinations of CO₂ and O₂ gas concentrations in continuous and closed systems for controlled and modified atmospheres.

The researchers monitored plate counts of total aerobic microorganisms, yeast and molds at 0, 7, 14 and 21 days. For modified atmosphere storage, about 250 g of cubed citrus were placed in 490, 950 and 3780 mL glass jars. These were flushed with pure oxygen until concentrations of 100% O₂ were reached. The CO₂ samples were tracked for 21 days. Microbial counts were evaluated at 0 and 21 days. The investigators detected little juice leakage in the cut citrus.

It appears that the chemical treatment did not preclude microbial growth. Microbial growth in orange fresh-cut pieces was limited to some extent in the controlled atmosphere storage

environment using 21% O₂ and 7% CO₂. A high oxygen atmosphere of 54% by itself did not extend shelf life. However, bacteria, yeasts and mold were effectively controlled when that high oxygen atmosphere was combined with 46% CO₂. Modified atmosphere storage in a 490-mL jar also controlled the growth of microorganisms because of the rapid build up of CO₂ that was created by reduced headspace.

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Acid tolerance response induces resistance to nisin in *L. monocytogenes*

L. monocytogenes is a ubiquitous foodborne pathogen responsible for many outbreaks of foodborne illness associated with ready-to-eat (RTE) foods. The use of acid and nisin to control *L. monocytogenes* in RTE foods as part of multiple-hurdle technology is a promising approach, but may become ineffective if cross-resistance emerges.

Nisin is a peptide made by *L. lactis*. It is a small molecule that kills Gram positive bacteria by binding to their membrane and by disrupting the proton motive force. When food is processed, it is heated to kill bacteria, but some bacteria still survive. Adding nisin to the food provides a second barrier for the growth of the bacteria.

Scientists at Rutgers University used fluorescent anisotropy to examine the effect of the acid tolerance response (ATR) on the membrane fluidity of *L. monocytogenes*. Membrane fluidity involves the viscosity of the lipid bilayer of a cell membrane. Changes in membrane-dependent functions are believed to depend upon cell-membrane fluidity. The effect of the ATR on the bacteria's resistance to acid and nisin was also investigated. The ATR is a complex defense system that can minimize the lethal effects of an extremely low pH environment. It sometimes enables pathogens to survive harsh acidity.

It appears that alterations in the membrane fluidity of the acid-tolerant strains might confer cross-resistance to other stresses. This cross-resistance could decrease the efficacy of a multiple hurdle approach to food safety.

An ATR induced by lactic acid at pH 5.5 conferred 4 logs of protection when cells were exposed to pH 3.5. Hydrochloric acid (HCl) at pH 5.5 was unable to induce any similar protection. The ATR cells were also cross-resistant to 1000 IU per ml of nisin. These acid-tolerant cells manifested about 10% higher membrane fluidity compared to the non-acid-adapted controls. In contrast, the rigidity of nisin-resistant cells grown in the presence of 25 UI per ml and 250 UI ml of nisin did not change.

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Dielectric barrier discharge eliminates *E. coli* on almonds

Scientists at the University of Minnesota wanted to investigate the effectiveness of using nonthermal plasma (NTP) to disinfect almonds. They also looked for any quality changes in almonds after NTP treatment.

An NTP is any plasma which is not in thermodynamic equilibrium. In the context of food processing, an NTP is a type of antimicrobial treatment being investigated for application to fruits, vegetables and other foods with fragile surfaces. These foods are either not adequately sanitized or are otherwise unsuitable for treatment with chemicals, heat or other conventional food processing tools.

In this research, investigators developed a dielectric barrier discharge NTP reactor. The system contained two electrodes covered by epoxy resin plates that served as the dielectric barrier material. The scientists applied 20 kV to 30 kV high voltage power to the electrodes. Almonds were spiked with various levels of *E. coli* by dipping them in an *E. coli* culture broth and drying them.

For each batch treatment, 15 grams of almonds were placed in a single layer in the discharge chamber. The researchers found NTP to be effective in reducing *E. coli* levels on almonds, as evidenced by an almost 5-log reduction after 30-second treatments at 30 kV and 2000 Hz. The bactericidal effect of NTP on the *E. coli* that had been inoculated on almonds increased with the applied voltage and the frequency. The reduction rate varied with the types and grades of almonds used. The *E. coli* cells at the logarithmic phase were more sensitive to the NTP than those at the stationary and declining phases.

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Low-pressure cold plasma treatment removes *Aspergillus* and aflatoxins from nuts, legumes

Contamination of nuts by the fungus *Aspergillus* and by aflatoxins, produced from infections as secondary metabolites, cause serious economic and health-related problems. It is estimated that nearly one quarter of fruits and vegetables worldwide each year are contaminated with *Aspergillus* spp. Aflatoxins are known to cause a number of acute or chronic diseases, such as cancer, liver cirrhosis, skin disorders, hormonal defects and cardiovascular problems.

Considering the magnitude of potential health issues, eliminating fungus before the toxin levels reach the international limits is of great interest. With this in mind, scientists in Turkey explored the potential of applying low-pressure cold plasma using air gases or sulfur hexafluoride (SF₆) to inactivate *Aspergillus* on ready-to-eat nuts and legumes. They found that using a plasma treatment with the appropriately chosen gases may offer a non-thermal novel decontamination technique for sensitive dry food surfaces.

Plasma is a highly energized fourth state of matter. Depending on the system and on the types of gases used, plasma can contain certain excited atoms and molecules, ionized gases, radicals and sometimes free electrons. A low-pressure cold plasma prototype system for processing small samples was constructed by an interdisciplinary team at Suleyman Demirel University in Turkey. Using this prototype system, plasma was generated in less than 30 seconds. The plasma treatment used on various samples lasted from 30 seconds to 30 minutes. When the fungal

inactivation kinetics of plasma gases of air and SF₆ were compared, an air gas plasma treatment for 10 minutes resulted in a 2-log reduction of *Aspergillus* and continued with a lag phase up to 20 minutes in duration.

When comparing the mold viable counts between the control and SF₆-treated samples, the researchers found a nearly a 6-log decrease in the treated samples. Depending on the duration-gas combinations, a 20% to 40% decrease in aflatoxin levels was also observed.

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Irradiation-induced furan formation limited in freshly cut fruits and vegetables

Furan, a potential carcinogen, has been found most often in thermally processed foods. Research in the past has shown that ionizing radiation can induce the formation of furan in solutions of simple sugars and ascorbic acid.

Many fruits and vegetables are rich in sugars and ascorbic acid. However, whether furan is induced by irradiation specifically in fresh produce is not known.

USDA-ARS scientists set out to determine whether furan can be induced by irradiation in common freshly cut fruits and vegetables which vary widely in their sugar and ascorbic acid content. They also wanted to determine that, if produced, what levels of furan might be found in irradiated produce. It appears that a high amount of sugars and a low pH are needed for the formation of furan in freshly cut produce.

In experiments, 19 freshly cut fruits and vegetables were irradiated to 5 kGy at 4 C. The scientists analyzed furan from the irradiated samples using solid phase microextraction and gas chromatography-mass selective detection techniques.

The formation of furan was correlated with the pH, sugar content, titratable acidity and ascorbic acid content of the produce. Results showed that almost all of the tested fruits and vegetables, when irradiated, produced non-detectable levels, or less than 1 nanogram (ng) per g, of furan. Actually, irradiation induced low ng-per-g levels of furan only in grape and pineapple.

The ARS researchers also wanted to see if the antibrowning agent calcium ascorbate would cause furan to develop. Dipping apple slices into calcium ascorbate before they were irradiated did not increase the potential for furan. The pH and the amount of simple sugars in fresh fruits and vegetables did play a role in furan formation. Low levels of furan were induced by irradiation only in those fruits that had a high amount of simple sugars and a low pH.

Considering the low levels of furan detected in irradiated fresh produce, irradiation-induced furan is unlikely to be a concern in these products.

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In brief. . .

A system of **high-voltage coils** attached to a small transformer, which generates a room-temperature plasma field inside a package, can ionize the gases inside. The process kills harmful bacteria, such as *E. coli* and *Salmonella*.

The technique, for which a patent application has been filed, can eliminate bacteria in packaged foods such as spinach and tomatoes. When placing two high-voltage, low-watt coils on the outside of a sealed food package, a plasma field forms. In the plasma field, which is a charged cloud of gas, oxygen ionizes and turns into ozone. Treatment times range from 30 seconds to about five minutes. Ozone kills bacteria, and the longer the gas in the package remains ionized, the more bacteria are killed. Eventually, the ionized gas will revert back to its original composition.

The process uses only 30 watts to 40 watts of electricity, less than most incandescent light bulbs. The outside of the container only increases a few degrees in temperature, so its contents are not cooked or otherwise altered.

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Traditional techniques for identifying **foodborne pathogens** take several days. Rapid, cost-effective detection techniques are needed to successfully monitor pathogens in the food supply. Scientists have developed a rapid method for detecting and identifying *Salmonella* serovars. They use immunomagnetic separation (IMS) technology, Fourier-transform infrared (FTIR) spectroscopy and multivariate analysis.

The researchers grew selected *Salmonella* serovars in selective broth overnight at 34 C. Anti-*Salmonella* magnetic beads were added to the culture, which was continuously shaken for 30 minutes at 20 rpm and collected to specifically isolate and concentrate *Salmonella*. The supernatant was removed, and the bacteria-bead complex was resuspended in water (5 μ L). Samples were dried under vacuum onto zinc selenide crystals and analyzed using FTIR. The *Salmonella* cells bound to immunomagnetic beads had distinctive, reproducible infrared spectra and higher absorbance than immunomagnetic beads or *Salmonella* alone. Infrared spectra analysis of antibody-bound microorganisms separated *Salmonella* into well-defined clusters with differentiation among serovars, presumably due to differences in cell envelope lipopolysaccharides.

So, it appears that combining IMS and FTIR makes it possible to quickly isolate and detect *Salmonella* serovars.

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