Update

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Preventing Pathogens from Reaching Home Plate

According to estimates from the U.S. Centers for Disease Control and Prevention (CDC; www.cdc.gov), food-borne pathogens account for 76 million illnesses, 325,000 hospitalizations and 5,000 deaths in the U.S each year. Moreover, fresh produce is catching up with chicken as a major cause of *Salmonella* (Figure 1) infections in the U.S., primarily due to increased consumption of fruits and vegetables. Several outbreaks of food poisoning in recent years have been linked to cantaloupes, sprouts, lettuce, tomatoes and other fresh food.

"Fresh-cut produce is the fastest growing sector of the fresh produce industry," says Andrew von Eschenbach, acting commissioner of the Food and Drug Administration (FDA; www.fda.gov). In 2004, the agency moved forward on an action plan to minimize food-borne illness associated with fresh produce consumption, which targets the reduction of microbial food safety hazards such as *Salmonella*.

Processing produce into fresh-cut form increases the risk of bacterial contamination and growth because the natural exterior barrier of the produce is broken by peeling, slicing, coring, trimming or mashing. The danger exists, whether or not washing or other treatment is applied prior to packaging for consumption, according to the FDA.

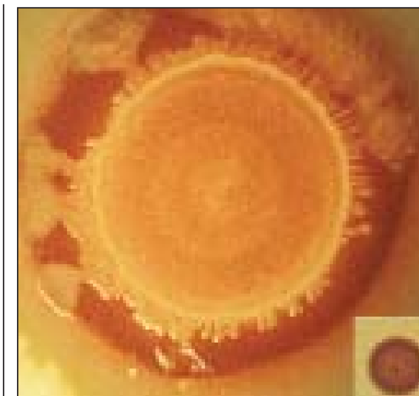
Against this backdrop, the FDA, together with the Food Safety and Inspection Service (FSIS; www.fsis.usda.gov), the public health agency in the U.S. Dept. of Agriculture, plans to develop sampling protocols to facilitate efficient and reliable detection of pathogens in or on fresh produce and in the fresh-produce production environment.

Last month, the Agency issued the "Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables" to provide clearer guidance on how to reduce health hazards that may be introduced during the production process. It also recommends that processors of fresh produce consider implementing a preventive control program, such as the Hazard Analysis and Critical Control Points (HACCP; www.cfsan.fda.gov) system, to build safety into the processing operations for fresh-cut fruits and vegetables in order to prevent, eliminate or reduce to acceptable levels the microbial, chemical and physical hazards associated with food production. FSIS is looking at machine vision as a way to help implement its HACCP program (box, p.10). "This would free up inspectors so they have time to take a close, careful look at the samples the machine-vision system judges suspect," says FSIS.

Irradiation and cold plasma

The CDC has reported that outbreaks of food-borne illnesses caused by *Campylobacter*, *Escherichia coli* O157:H7 (EHEC), *Listeria monocytogenes* (LM), *Salmonella enteritis*, and *Yersinia enterocolitica* have declined. Although this is good news, controlling these pathogens remains a primary goal for the meat industry. Consumer aversion to traditional chemical preservatives has left food processors with less flexibility in choosing preservation methods. Some of these approaches include pulsed electric fields, pulsed light, irradiation, heating and high hydrostatic pressure. However, they do not all satisfy both industry demands and consumer preferences.

Currently, irradiation is the only known method to completely eliminate *E. coli* O157:H7 bacteria in raw meat. Irradiation also significantly reduces levels of other pathogenic organisms, including *Cyclospora*, *Listeria*, *Salmonella*, *Campylobacter jejuni* and the protozoan parasite *Toxoplasma gondi* on raw products and frequently extends the shelf life of food. The short, high-energy waves of the irradiation process transfer into the molecules of



A colony of *Salmonella enteritis.* Courtesy of ARS.

the microbe, creating reactive chemicals that damage the cells' DNA without significant increases in the temperature of the food. This damage interferes with cell replication, and thus duplication and reproduction of the organism fail.

Depending on the size of the organism, the amount of DNA, and how quickly the organism can repair itself, the organism's sensitivity to the irradiation varies. There may be some nutritional content changes as a result of this treatment, but no more than with other processing methods, such as cooking, canning, or heat pasteurization, says the FDA, which has officially stated that food conditioned with this technology is not hazardous to human health.

Like irradiation, cold plasma treatment invasively causes an upset in the cell that leads to cell death. A cold plasma is a gas with free electrons that are accelerated by an electric or electromagnetic field. The electrons collide with gas molecules, fragmenting them to create reactive agents, such as ions, free radicals and other atomic particles, all of which increase the entropy of the system.

At the Univ. of Wisconsin (UW; www.wisc.edu), a team of scientists led by Frank Denes, professor of biosystems engineering in the College of Agricultural and Life Science, is using cold plasma as the basis for two pathogen-killing devices. "The plasma treatments primarily disturb the microorganisms' homeostasis, reducing some

bacteria populations by a factor of 1,000 to 100,000," Denes says. In a practical sense, the devices could help processors meet stepped-up regulatory standards for food safety.

The first device is a sandwich-sized block of white ceramic, one side of which features a grid of over 200 individually housed electrodes. When the reactor is "on," the electrodes work together to produce a constant and uniform flow of plasma. "The reactor can be suspended, electrodes pointing downward, above any surface in need of disinfection, such as food moving on a conveyer belt," explains Denes.

The second device decontaminates water and other fluids. It is physically similar to a glass jug that holds about one liter of liquid, and is fitted with caps that house the electrical components used for plasma production. As liquids swirl inside the reactor, the plasma disrupts the contaminant cells' metabolism. Within 20 s, the reactor can kill up to 100,000 colony-forming units (CFU) per mL of liquid, according to Denes. Although cold plasma has been used for various manufacturing processes, Denes says his invention is one of the first that works at atmospheric pressure.

Too much pressure proves fatal

High-pressure processing (HPP), a technology more than a century old, was resurrected in the past decade under the joint leadership of Patrick Dunne, a senior research chemist at the U.S. Army Soldier Systems Center (www.natick.army.mil) and Edmund Ting of Avure Technologies, Inc. (www.avure.com). The process is the only FDA- and USDA-approved technology that can kill *E. coli, Salmonell*a and *Listeria* pathogens inside packaged, moist food products (*e.g.*, vegetables, sauces and ready-to-eat meats) without the use of additives or additional heat processing, says Dunne. But there's one deadly pathogen for which the efficacy of this process must still be tested — *Clostridium botulinum.*

"*Clostridium* has been a stumbling block in the commercialization of low-acid, shelf-stable foods," Dunne says. HPP must be demonstrated with a prototype unit before R&D begins at a greater production rate.

The process subjects liquid and solid foods (solids must be suspended in water) contained in a steel vessel to pressures of up to 78,000 psi, which fatally damages proteins and other key cellular structures. Vessels are uniquely designed to safely withstand these pressures over many cycles. Process temperature can be specified from below 0°C (to minimize any effects of adiabatic heat) to above 100°C, according to the FDA. "In contrast to thermal processing, economic requirements for throughput may limit practical exposure times to less than 20 min," says the organization. Dunne's pressure cycle lasts no more than 6 min vs. one hour or longer for a typical high-temperature cycle.

Liquid from a low-pressure pump is compressed to generate high-pressure process fluid. "The pressures appear to have little effect on covalent bonds; thus, processed foods will not undergo significant chemical transformations due to the pressure treatment itself," says Dunne. Chemical changes in the food generally will be a function of the process temperature and time selected for pressure treatment. Food companies are testing the technology on products ranging from orange juice, to guacamole, to deli meats that are now being sold across the U.S.

The Army is planning to develop a high-pressure, thermal sterilization process for low-acid foods, which is expected to be approved by regulatory agencies this year. Mashed potatoes processed with a new high-pressure sterilization unit might be ready for field testing by the end of the year.

Not your ordinary water

Meanwhile, scientists from the ARS and the Univ. de Navarra are studying how to improve the washing techniques used in the produce packing and processing industries. Conventional commercial washing and sanitization methods used to remove microbial contaminants from produce surfaces *(e.g.,* soaking produce in water mixed with a small amount of a fruit and vegetable wash, followed by a water rinse) have been rated "adequate, at best," says Gary Rodrick, a professor with the Univ. of Florida's Institute of Food and Agricultural Sciences (UF; www.ufl.edu). "But the effectiveness of such techniques varies, due to human error or not following protocol," he adds. UF scientists are conducting experiments to learn how these organisms survive on produce surfaces, even after the food has been exposed to sanitizing solutions, like chlorine.

Cantaloupe and other produce with coarse surfaces are breeding grounds for bacteria. *Salmonella* cells, for instance, produce hairlike structures and cellulose that help them attach, colonize and maintain a fairly stable, sheltered life on a melon's surface. "The polymers form a biofilm that shields the bacteria from sanitizing solutions," explains Bassam Annous, a microbiologist with ARS. "The *Salmonella* cells on the rind can be transferred to the melon's internal tissues during cutting," he says.

Together with Joseph Sites, a mechanical engineer, Annous recently developed a commercial-scale surface pasteurization treatment that resulted in a 99.999% reduction in the population of *Salmonella* cells on the surface of artificially contaminated cantaloupe. "The process involves immersing the melons in water at 169°F for three minutes to kill the pathogens, then sealing each melon in a plastic bag to prevent the fruit from potential recontamination upon its subsequent immersion in an ice-water bath," says Annous. The treatment also extends the fruit's shelf life by reducing native microflora that may cause spoilage. Best of all, it does not compromise melon quality, say the scientists.

A company called EAU, short for Electric Aquagenics Unlimited (www.eau-x.com), has made inroads in bringing its electrolyzed water technology to the front lines as a replacement for chemical cleaners. The firm's

Empowered Water generators use a unique combination of cell technology, salt and electricity to modify the behavior of water molecules, thereby creating a non-toxic, oxidized antimicrobial solution capable of killing many pathogens in less than a minute. "The electrolyzed water is stable, inexpensive to produce, and broadly applicable, due to its various inherent sanitizing characteristics," says James Thatcher, senior vice president of food safety.

The high oxidation-reduction potential (ORP) of Empowered Water first damages the cell walls, allowing infiltration by water. The microbe reaches capacity, causing an osmotic, or hydration, overload. The acidic fluid and water continue to flood the cell faster than the cell can expel it, literally causing the cell to burst. "This "physical kill"quality uniquely differentiates Empowered Water from chemical and pharmaceutical cleaners and disinfectants, because the pathogens cannot build up resistance to it.

The "Primacide A" brand disinfecting fluid is capable of replacing chlorinated water that is currently used at capacities of up to 50,000 gal/h in poultry processing plants. "It is up to 80 times more effective at killing pathogens than chlorinated water, without the toxicity," the company claims.

Over $30 million in R&D and at least a dozen university and research institutions have been part of the effort to validate and improve this water production technology — and field tests have been conducted at Tyson Foods and other facilities. "At the acidity levels that EAU employs, testing results demonstrate zero toxicity throughout every application," says Thatcher. The firm is now petitioning the FDA for approval to use its Primacide products along the entire food chain process to disinfect fruits and vegetables.

Ozone, the gas that protects the Earth from ultraviolet radiation, is another very promising and versatile non-chemical sanitization technology. Its usefulness as a sanitizing agent comes from its unstable molecular structure. "When you expose an apple to ozone, bacteria on the fruit's surface will begin absorbing ozone molecules immediately," says Rodrick. "Those molecules break apart within seconds, and when they do, they release enough energy to cause the bacteria to explode."

The FDA recently gave the go-ahead to use ozone-enriched water commercially in U.S. supermarkets and food-processing facilities. Rodrick has tested a commercial ozone sanitizing system developed by Fresh Food Technology in Burley, ID, that washes fruits and vegetables in ozone-enriched water. Based on numerous tests in supermarkets, ozone killed almost 100% of the bacteria on produce received

MACHINES WITH AN "EYE" FOR CONTAMINATED SURFACES

Machine-vision systems enable food-safety investigators to detect contamination the human eye often can't see. Scientists at the Scientists at the Agricultural Research Service's Instrumentation and Sensing Laboratory in Beltsville, MD, have spearheaded the development of this technology for the identification of fecal contamination. Yud-Ren Chen, an agricultural engineer, Moon Kim, a biophysicist, and others, had built a multispectral imaging apple-inspection system that uses reflectance from apples illuminated by halogen lamps in the invisible near-infrared and visible-color light bands, as well as fluorescence techniques, for detection. It takes pictures at different wavelengths simultaneously, creating multiple images that are used to find the wavelengths best suited to spotting fecal contamination or bacteria.

The system was upgraded to a hyperspectral unit, which can take pictures at up to 256 different wavelengths simultaneously. A multispectral system generally uses only 2–4 wavelengths, scanning a whole object in a fraction of a second, which lends the technology for realtime use in processing plants. "The data are used to find common patterns in whole agricultural objects, so that any anomalies stand out," says Chen.

Machine-vision technology can be applied to detect tumors, bacteria, fungus or fecal matter in livestock, as well. To this end, the lab formed a cooperative R&D agreement (CRADA) with Stork Gamco, Inc., of Gainesville, GA — one of the largest manufacturers of chicken-processing plant equipment in the world — to commercialize the system and move it into use among the nation's 300-plus poultry processing plants.

This hyperspectral imaging system takes pictures at different wavelengths simultaneously, enabling the creation of images that reveal contamination.

The ARS Poultry Processing and Meat Quality Research Unit in Athens, Georgia, has signed a CRADA with Stork Gamco to use machine vision to spot contamination from "ingesta," partially digested food from the ruptured crops of chicken carcasses, and from fecal matter. The chicken plant of the future will likely have one system in place for the automated diagnosis of wholesomeness and one for fecal and contamination.

Meanwhile, eMerge Interactive, Inc. (eMerge; www.emergeinteractive.com) launched its VerifEYE food safety technology in 2002. Based on ARS's technology, it detects microscopic levels of organic contamination on meat and other surfaces. eMerge formed an agreement Cargill subsidiary Excel Corp., a meat packing company, to commercialize the VerifEYE Carcass Inspection System (CIS), which identifies organic contamination in realtime on full carcass (beef) sides on the rail within a slaughter plant. A portable version of the technology, also born of the ARS laboratories, is now available.

from suppliers. In slightly higher concentrations, it also killed yeasts and molds. Rodrick says ozone sanitization also increases the shelf life of fresh produce by up to two weeks.

Breeding natural pathogen killers

Bacteriophages — viruses that target bacteria, rather than human, plant or animal cells — have the potential to destroy pathogens on the surfaces of food, equipment, packaging and other contact materials. EBI, Inc. (www.ebi.com), which was formed to commercialize research begun at the National Institutes of Health (www.nih.gov), is awaiting regulatory approval to launch its first product based on this technology — a liquid of phages that eat *Listeria* monocytogenes. “For every bacteria, there is a phage that likes to latch on to them, take over their life processes and multiply, thereby killing the host cell,” says Mark Offerhaus, chief executive officer. With this in mind, the company has set a goal to mass produce phages for every pathogen found in and on food plants.

Tougher regulatory standards and the increased reporting of food contamination has pushed companies to put a higher priority on food safety. The trend has fueled the demand for more stringent and rapid testing and tracing systems for food products along the supply chain to the consumer (box below).

This month, Warnex Inc. (www.warnex.com) is launching the industry’s first polymerase chain reaction (PCR)-based quantitative test for *Campylobacter*. It detects and quantifies the pathogen in poultry rinses within 3 h. A test that uses PCR to detect *Salmonella* in ready-to-eat meats has been developed at ARS. Like current methods, the ARS technique detects contamination in meat products at 2–4 cells/25 g, yielding results within 8 h. Researchers note that the laboratory test is less expensive than commercial kits.

For information on other pathogen detection systems:

3M Microbiology	www.3m.com/microbiology
AES Chemunex	www.aeschemunex.com
Deibel Labs	www.deibellabs.com
Food Safety Net Services	www.food-safetynet.com
Microbac	www.microbac.com
Neogen	www.neogen.com
Q Labs	www.qlaboratories.com
Weber Scientific	www.weberscientific.com