



Fresh Ideas for

With the growing consumer demand around the world for fresh, minimally processed food, food manufacturers are looking for new ways of getting these items to shoppers safely. Separate groups of researchers in Australia are developing technologies that may help both address concerns about microorganisms that can cause food poisoning and be of general economic value in extending the length of time that food stays fresh, thus allowing transportation of fresh foods to distant markets.

Many of the microorganisms that cause food poisoning from fresh vegetables come from human feces (for instance, *Shigella*) or animal sources (for instance, *Cryptosporidium parvum*). Other potentially dangerous microorganisms, including *Bacillus cereus*, *Clostridium botulinum*, and *Listeria monocytogenes*, occur naturally in soil and decaying plant matter. Much of the microflora on fresh vegetables reflects the specific types present during growth and harvest, although contamination may also occur postharvest during storage, transport, and display.

L. monocytogenes has been a pathogen of particular public health concern in the United States. Each year, the bacterium causes a high death rate for the number of illnesses contracted, with an estimated 2,500 hospitalizations and 500 deaths. The bacterium is the primary trigger for the recall of foods such as hot dogs, lunch meats, unpasteurized dairy products, and softened cheeses. Of the 55

food product recalls logged in the United States in 1999, 25 resulted from *L. monocytogenes* contamination.

Listeriosis, the illness caused by eating food contaminated with *L. monocytogenes*, produces flu-like symptoms such as fever, chills, and upset stomach. If the infection spreads to the nervous system, symptoms such as headache, stiff neck, confusion, or convulsions can occur. A pregnant woman can transmit listeriosis through the placenta to the fetus, with severe or fatal effects. Under U.S. policy, if any *L. monocytogenes* is detected in ready-to-eat foods through standard testing, the manufacturer must remove the items from the marketplace.

For fresh-cut vegetables that are eaten raw, there is no one treatment that can be relied on to substantially reduce the number of contaminating microorganisms. Antimicrobial washes alone are not completely effective. The most effective means of reducing risk is a combination of control measures aimed at minimizing contamination in the first place and limiting the growth of microorganisms between the farm field and the plate, together with optimized washing procedures. Refrigeration alone is not a sufficient deterrent because *L. monocytogenes*, for example, reproduces well in refrigerated conditions. So, although they may not provide a single, all-encompassing solution, the developments in Australia may be helpful in making fresh foods safer for the consumer.



Battling Bacteria

In a cooperative research program at the University of Melbourne and Food Science Australia, a government/academic/industry cooperative in Werribee, Victoria, researchers have isolated a naturally occurring food preservative with a demonstrable effectiveness in killing *L. monocytogenes*. The preservative, a bacteriocin named piscicolin 126, is one of many small proteins, or peptides, produced by lactic acid bacteria.

Lactic acid bacteria have long played a role in food preservation because of their ability to produce these proteins, which attack bacteria closely related to the producing organism. Nisin, produced by *Lactococcus lactis*, is the most thoroughly characterized bacteriocin. It has been commercially available since the late 1950s, and many countries permit its use as a food preservative in canned products, cheeses, and dairy spreads. The U.S. Food and Drug Administration (FDA) permitted its use in 1988 as an antimicrobial agent to inhibit the growth of *C. botulinum* spores and toxin formation in certain pasteurized cheese products. The zero-tolerance policy of the U.S. government for *L. monocytogenes* has recently increased the interest in developing new variations of bacteriocins.

John Coventry, a researcher at Food Science Australia, says that he and colleagues



began screening food for bacteria with antimicrobial properties nine years ago. The researchers screened some 700,000 colonies, of which 300 were found to have potentially valuable traits. Five years ago, they identified and then cloned piscicolin 126, which proved to be a very rugged, heat-stable peptide. The research team is now part of the Australian Cooperative Research Centre (CRC) for International Food Manufacture and Packaging Science, also known as the CRC for Food and Packaging. CRC organizations are collaborative ventures between companies, government research institutions, and universities.

Piscicolin 126 is produced by the lactic acid bacterium *Carnobacterium piscicola* JG126, which was isolated from spoiled ham. In the initial experiments in the mid-1990s, applying piscicolin 126 to ham paste reduced the viable counts of *L. monocytogenes* to below the detection level (lower than 100 colony-forming units per gram) immediately after it was applied. This level was maintained throughout the 14 days of the test, surpassing the effectiveness of both a commercial antilisterial product and nisin. Piscicolin 126 was also tested successfully in Camembert cheese without inhibiting cheese starter bacteria. This means neither the quality nor the production process of the cheese is affected.

“Piscicolin 126 has many potential applications, especially in the area of minimally processed foods such as salad products,

smoked salmon, and fresh-cut vegetables,” Coventry says. “The real application may be as . . . a spray or coating for food. That way, if *Listeria* enters the product’s surface because of poor or suboptimal manufacturing processes, then an additional barrier of control exists.”

In situ approaches are also possible. Nisin is already produced in cheeses by starter culture fermentation, and piscicolin 126 could ultimately have a similar mode of application. Care needs to be taken, however, to prevent *Listeria* from developing resistance, for instance by adding the bacteriocin in an established way using typical measures of care so that the protein is not exposed to *Listeria* in an unplanned way.

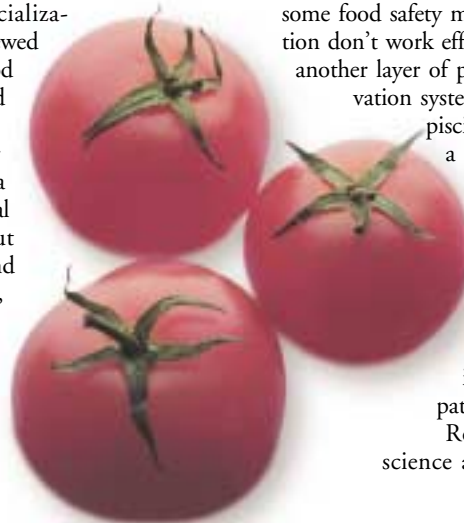
According to Coventry, routes to commercialization have been reviewed by the CRC for Food and Packaging, and piscicolin 126 is currently under evaluation by a potential commercial partner. “We’re out of the discovery and development phase, and now any further developmental work will depend on the commercial evaluation,” he says.

Other experts in food science agree that piscicolin 126 may be a valuable addition to the food preservation armory. “I think it’s a very important step to improving the preservative properties of our foods and limiting the growth of foodborne pathogens,” says Todd Klaenhammer, the William Neal Reynolds professor of food sciences and microbiology at North Carolina State University in Raleigh. “This sort of development is more important today than 20 years ago, as we need more preservation hurdles to protect our minimally processed and refrigerated foods from pathogens like *L. monocytogenes*.”

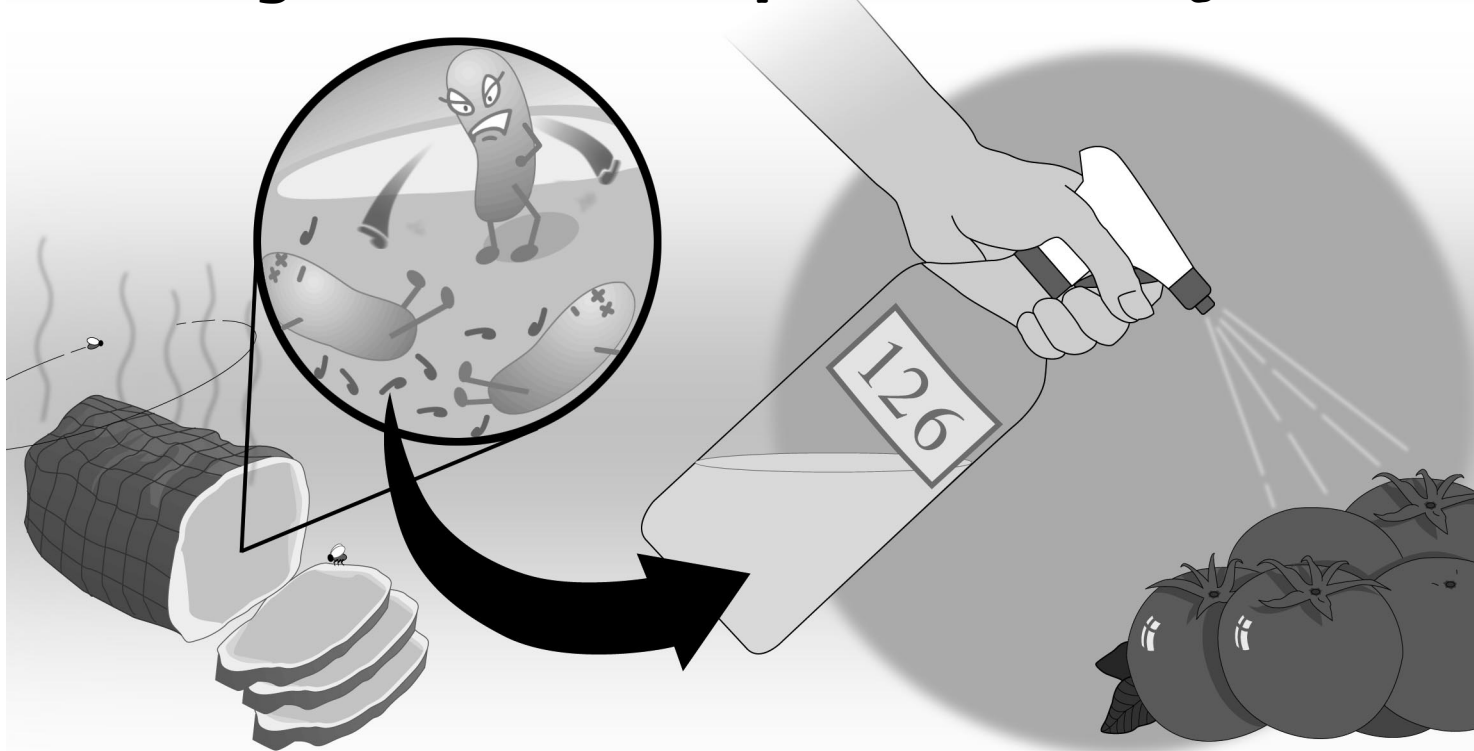
Klaenhammer adds, “Some of our new emerging pathogens are more adapted to food preservation techniques and, as a result, some food safety measures such as refrigeration don’t work effectively. Bacteriocins add another layer of protection to food preservation systems.” He emphasizes that

piscicolin 126 is only one of a variety of bacterium-like molecules that attack *L. monocytogenes*, and so it represents another potentially effective tool to add to the arsenal of bacteriocins that are currently available to inhibit growth of the pathogen.

Robert Buchanan, senior science advisor and head of the



Food Fight: The Battle to Keep Foods Fresher Longer



First isolated from spoiled ham, the bacterium *Carnobacterium piscicola* JG126 naturally produces an antimicrobial agent to kill nearby competing bacteria.

This antimicrobial agent, known as piscicolin 126, has been found to kill some bacteria that are dangerous to humans. Researchers at the University of Melbourne and Food Science Australia are developing ways to introduce piscicolin 126 into food products to control harmful bacteria.

Office of Science at the FDA's Center for Food Safety and Applied Nutrition, agrees with Klaenhammer's observation that bacteriocins, especially nisin, have proven useful. Buchanan was on the team that first isolated the *Carnobacterium* class of bacteriocins 10 years ago. "Interestingly," he says, "despite the large amount of work in this field, we've seen a very limited number of users petitioning the FDA for approval of bacteriocins, and none in this class." He says any applications would be subject to normal FDA review of what would be classified as a food additive.

Veggie Wrap

The second new technology tackles the area of food packaging. Whether it includes the application of a bacteria killer or not, food packaging is essential for the related reasons of safety and economics. Each food has unique requirements to ensure the slowest possible aging. If the aging process can be slowed, then shelf life can be extended. The primary means of accomplishing life extension is refrigeration, sometimes combined with modifying the storage atmosphere or removing the ripening hormone, ethylene, for instance by continually passing air over the produce. By extending the time during

which food is fresh and safe to eat, packaging enables products such as fresh vegetables to be shipped greater distances—an important consideration in a time when food supplies must often travel across oceans and continents to reach market.

To meet these needs, a low-cost food film wrap that can precisely match the respiration rate of packed fresh produce has been developed by researchers from the polymer fabrication group at the Manufacturing Science and Technology unit of the Commonwealth Scientific and Industrial Research Organisation and the CRC for Food and Packaging. The film has been successfully tested on fresh-cut lettuce and stir-fry mixtures.

The film is aimed at solving a dilemma caused by the fact that fresh produce continues to respire after harvest, taking in oxygen and giving off carbon dioxide. Respiration depletes the food's store of starches and sugars, and results in aging.

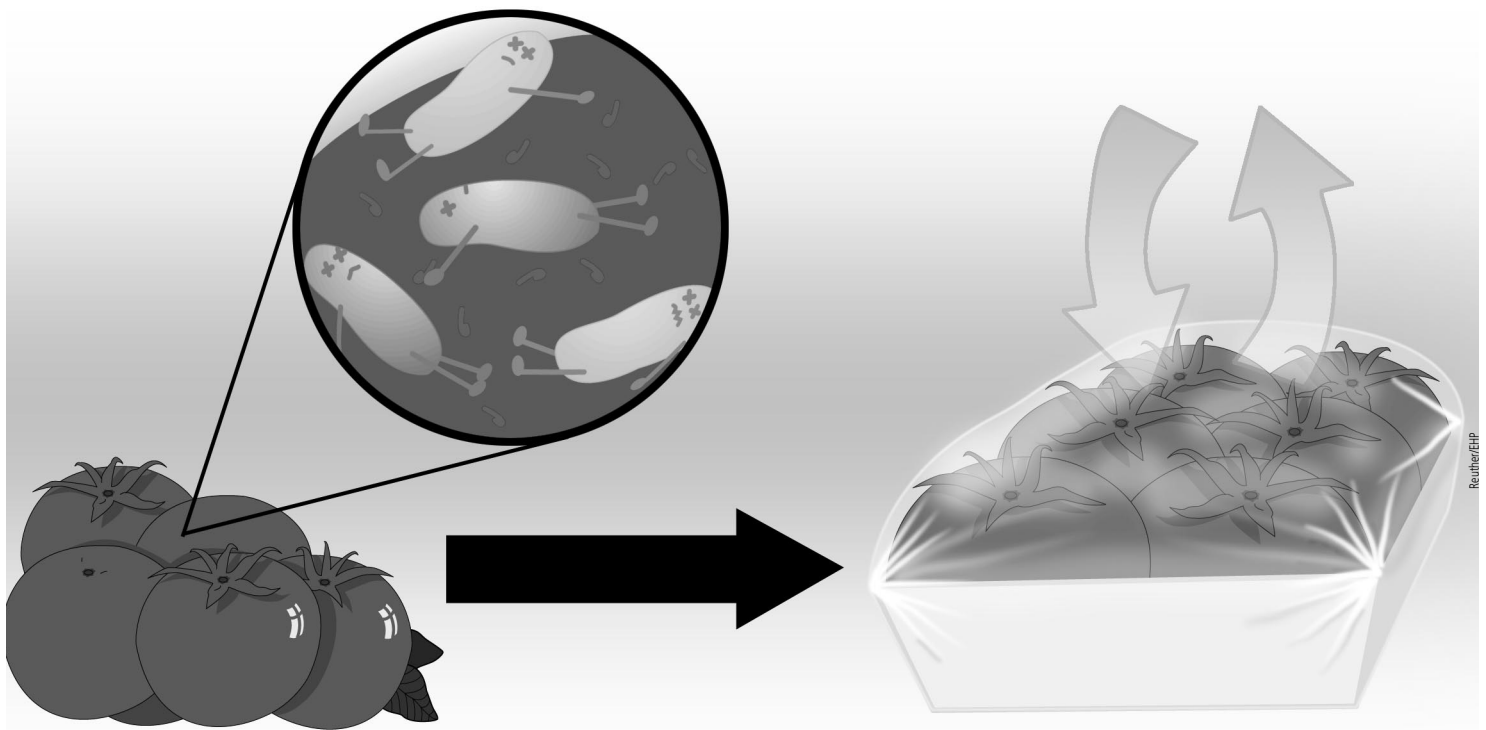
Modified atmosphere packaging (MAP) is a general technique that has been developed to create a beneficial atmosphere for storage within a polymer packaging film. Its goal is to modify the respiration rate of a particular food using the permeability of a film to control the atmosphere. When the food is

initially packaged in the film, the atmosphere within is a standard air mix. But the flow of oxygen and carbon dioxide across the film barrier soon equalizes into an atmosphere that is optimal for the food inside.

"Most traditional plastic is not permeable enough for high-respiratory foods like broccoli," says Gregor Christie, the research director of the CRC for Food and Packaging. "It's important that the oxygen level in the package never falls to anaerobic conditions. If the polymer is too impermeable, conditions may allow for the growth of harmful bacteria to unacceptable levels."

Over the past five years, Christie and his colleagues have developed a unique MAP technology that uses activated diffusion and capillary gas flow principles to improve and control gas flow across the film. "Our aim is to have a better way of getting gas into the package," Christie says.

The film is made by traditional manufacturing processes, but is impregnated with an inert particle that Christie will identify only as a food ingredient. Heat or the weight of rollers during production activates the particles. This process thins the film around the particles and alters the interface between the particles and the polymer matrix, creating portals that allow the capillary flow of gases



Tests have shown that piscicolin 126, when applied to some foods, reduces viable counts of *Listeria monocytogenes*—a major culprit in food poisoning—to below detection limits.

Scientists from CSIRO Manufacturing Science and Technology and the CRC for Food and Packaging have developed a porous film wrap that maintains an optimal balance of gases within food packages, keeping the food fresh en route to faraway consumers.

between the particle and the film and thus increasing the overall permeability of the film.

The permeability of one basic type of film can be varied by changing the heat, roller weight, or other factors involved in manufacture. For example, raising the temperature at which the activation process occurs increases the permeability of the film. The packager can therefore tailor the film to maximize the life span of a particular product.

The film itself is a common commercial polymer that is heat-sealable, printable, glossy, and clear. Christie says the MAP approach will work with any mono- or multilayer polymer, and will enable a package manufacturing company to reduce or eliminate the need to keep a large variety of plastic on hand since it could simply activate a single stock according to the respiratory requirements of the produce to be packed.

“I don’t know if it’s revolutionary, but it may be an evolutionary advance,” says George Sadler, a professor of food production at the Illinois Institute of Technology and a scientist with the U.S. National Center for Food Safety and Technology in Summit-Argo, Illinois, which is a consortium of food companies, university scientists, and the FDA. “If it’s cleverly done, this approach can establish a microstatic balance that inhibits

the growth of microorganisms. You never want to take packaging to an anaerobic level because you run the danger of producing off-flavored fermentation by-products, and create an environment suitable for the growth of anaerobic organisms including [those that cause] botulism.”

Sadler notes that the general concept of breathable film has been around since the 1920s, with the initial Australian interest tied to finding a means of shipping lamb carcasses overseas. He thinks that the current feature of tailoring the permeability of the film to match the food will be a big plus for this version of MAP.

The CRC for Food and Packaging is now in the midst of transferring the MAP technology to industry, with at least four companies looking at licensing it for various uses in addition to food packaging, including medical and industrial applications. If the commercial version for food packaging proves as promising as it seems, then fresh produce can be readily shipped to Asia, and agriculture Down Under should prosper.

W. Conard Holton

Suggested Reading

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