For Tomorrow's Salads

Plant Extracts To Conquer Microbes

Tender leaves of deep-green, freshly harvested spinach—neatly displayed in sealed bags at the chilled-produce section of your local supermarket—may one day include a powerful new food-safety feature. That added protection might take shape as a five-thousandths-of-an-inch-thick piece of what's known as "edible film," made from a purée of spinach itself.

When slipped into the bag, the protective power of this little puréed spinach square or wedge would come from a potent antimicrobial compound chosen from nature's bounty of botanical bactericides. The antimicrobial would be added in tiny amounts during the puréeing process to provide a safe, effective, natural defense against pathogens like *E. coli* O157:H7, *Salmonella*, *Listeria,* and others.

Carvacrol, the predominant essential oil in oregano, would add a pleasant—and protective—accent to a spinach-purée film, for example. Already shown in lab investigations to be an effective weapon against several major foodborne pathogens, carvacrol currently flavors some

PEGGY GREB (K10168-1)

Tara McHugh, research leader of the ARS Processed Foods Research Unit in Albany, California, examines colorful fruit- and vegetable-based edible films. Antimicrobial edible films are now being tested against pathogenic bacteria.

PEGGY GREB (D1172-1)

ARS food technologist Carl Olsen (right) adds carvacrol, the main active antimicrobial compound in oregano essential oil, to a flavorful tomato-based mixture while technician Rachelle Woods spreads the mixture to form an antimicrobial edible film. Once the film dries, it will be tested against pathogenic bacteria, such as *E. coli* O157:H7.

popular salad dressings and seasoning mixes. Carvacrol vapors wafting from the wedge into the atmosphere inside the sealed bag would both season and safen the spinach.

Sound too good to be true?

Not so, say scientists at the ARS Western Regional Research Center in Albany, California, near San Francisco. The futuristic films they're developing would complement and supplement other food-safety strategies and tactics on the farm, at the packinghouse, and elsewhere along the way from field to fork.

In pioneering experiments, the California scientists are selecting plant extracts, such as carvacrol, to put in the experimental films and are then pitting the films against pathogenic bacteria such as *E. coli* O157:H7. Their investigations will help transform edible antimicrobial films from concept to reality.

Though wrinkles remain to be ironed out, their findings from films made with purées of Golden Delicious or Fuji apples provide proof that the concept is sound, that the botanical extracts are powerful, and that practical, affordable films are within technology's reach.

The experiments are the work of Tara H. McHugh and Wen-Xian Du of the center's Processed Foods Research Unit; Mendel Friedman of the Produce Safety and Microbiology Research Unit, also at Albany; Roberto J. Avena-Bustillos of the University of California-Davis, and others.

Initial Results Promising

Neither edible films—nor the idea of making them antimicrobial—are new. McHugh's work that led to the first-ever fruit-purée edible films, for instance, is based on a pending patent that she and coinventors filed in 2004. What is new is research from the Albany lab that shows, for the first time, that those same puréed-apple films—if enhanced with carvacrol—can kill *E. coli* O157:H7 in laboratory tests. Their suite of apple-purée studies can, the scientists point out, smooth the way to films that could be used to protect fresh-cut leafy greens—spinach, lettuce, and more.

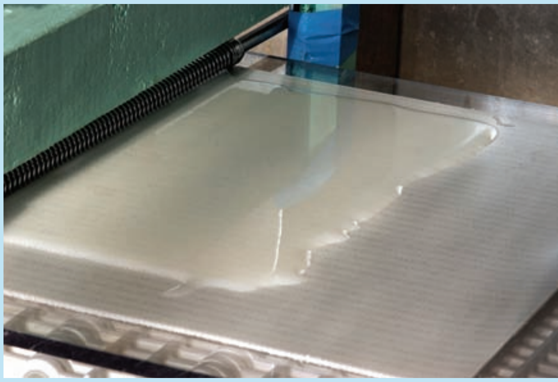
Hundreds of Compounds Scrutinized

Carvacrol was one of more than 200 botanical extracts that Friedman, a

PEGGY GREB (D1173-1)

Two antimicrobial edible film disks (the top two in the petri dish) repel *E. coli* O157:H7 growth in the agar surrounding them. Roberto Avena-Bustillos, a collaborating researcher from the University of California-Davis, uses a micrometer to measure the width of the clear agar zone around one of the two disks. The bottom two disks are controls.

PAUL PIERLOTT, USDA-ERRC/VGT (D299-1)



A biodegradable film made with casein, a milk protein, and glycerol, a byproduct of biodiesel production.

Biodegradable Films From Casein

You can't beat this wrap. At the ARS Eastern Regional Research Center in Wyndmoor, Pennsylvania, scientists have developed biodegradable films that can be used to protect fresh produce and other perishable foods.

Water-resistant, transparent, and edible, the films can protect a range of perishable products from moisture- and oxygen-induced damage.

"Barrier films with low-density polyethylene significantly reduce oxygen permeability," says research leader Peggy Tomasula, who helped develop the film-production technology. "This can protect the color and shape of a product for a long time and extend shelf life by preventing oxidation of lipids or diffusion of flavor compounds."

The film-production process combines casein, a byproduct of dairy production, and glycerol, a byproduct of biodiesel production. The ERRC films have not been embedded with antimicrobial materials, Tomasula says, though this is one potential application.

Read more about this research in the May/June 2007 issue of *Agricultural Research*, online at www.ars.usda.gov/is/AR/archive/may07/whey0507.htm.—By **Laura McGinnis,** ARS.

chemist, analyzed in a globe-spanning study published in 2002. Other studies of the pathogen-fighting prowess of plant oils and oil compounds abound. But the methods used to prepare those compounds for assays vary widely, as do the assays themselves, the strains of any given bacteria that were used, and other scientific variables.

"These earlier studies gave us a wealth of data," says Friedman, "but there was no common basis of comparison for us to work forward from."

To remedy that, Friedman and co-researchers used new sample-preparation and assay methods that they invented. For even more consistency, they used the same bacterial strains, from the same suppliers, across the investigation.

Their exhaustive study put plant compounds—from everyday allspice to exotic frankincense—up against four big-time bacterial bad guys: *Campylobacter jejuni, E. coli, Salmonella,* and *Listeria.*

The study also delved into the relation of chemical structure to a bactericide's pathogen-quelling ability. The investigation was, at the time, the most extensive of its kind, according to Friedman.

Eight Top *E. coli* 0157:H7 Fighters

Food safety expert Mendel Friedman selected more than 200 natural plant oils and oil extracts for in-the-laboratory bouts with *E. coli* O157:H7. Some of the best botanicals were later singled out for advanced testing in all-natural antimicrobial films. Top-performers, ranked in order of their effectiveness, are:

Oregano
Thyme
Cinnamon
Palmarosa
Bay leaf
Clove bud
Lemongrass
Allspice

Source: Friedman et al., *Journal of Food Protection,* 2002, volume 65, pages 1545 to 1560.

Many of the compounds examined are already approved for food use—an important bonus in choosing candidates for the experimental films.

Top scores for compounds like oregano's carvacrol, citral from lemongrass, and cinnamaldehyde from cinnamon earned them a place in the subsequent tests of apple-purée films.

Vapors Go In, Out, Under

Importantly, some of the compounds Friedman studied—including carvacrol—have what's called a "vapor phase." Inside the enclosed environment of a packaged salad mix, carvacrol's protective vapors could find their way into folds and crevices—like those on a crinkly spinach leaf—that other protectants might not reach.

So how do you gauge a film's ability to fight a foodborne pathogen?

In several studies, extract-enhanced apple films were cut into small disks, each a half-inch in diameter. Then, the disks were put on agar gel teeming with *E. coli.* The samples, kept chilled, were checked at intervals to see the disks' effects on the growth and spread of the pathogen.

As an indicator of bactericidal strength, the researchers measured the zone around the disk in which no living *E. coli* could be detected. The approach is somewhat like measuring the size of the egg white surrounding the yolk of a fried egg.

The protective zone encircling oregano-impregnated apple purée disks was significantly larger than those of disks containing cinnamon or lemongrass oils, the scientists found.

A related study showed that it took nearly five times as much citral from lemongrass to get the same protective effect as oregano-derived carvacrol.

In their newest work, published in a recent issue of the *Journal of Agricultural and Food Chemistry,* Du, Friedman, McHugh, and coinvestigators designed a study to answer a key question about making films: Could the manufacturing process—batch or continuous—affect a film's antimicrobial performance?

PEGGY GREB (D1171-1)

Roberto Avena-Bustillos places an antimicrobial edible film into a small dish within a larger dish of spinach leaves inoculated with *E. coli* O157:H7. The larger dish is then sealed to evaluate the efficacy of antibacterial vapors released from the film.

For carvacrol, the antibacterial used in the study, the answer is: No. Carvacrol-enhanced apple purée films made with a batch process were about as effective in quelling *E. coli* as those made with a continuous process, according to preliminary results.

McHugh and Friedman estimate that antimicrobial film inserts for packaged leafy greens—a spinach-purée wedge, a colorful square of carrot-based film, or other innovative options—might be ready within a year or so to test and evaluate at fresh-produce packinghouses. The inserts would add a reassuring new leaf to the history of packaged, ready-to-eat salads in America.—By **Marcia Wood,** ARS.

This research is part of Food Safety (#108) and Quality and Utilization of Agricultural Products (#306), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

Tara H. McHugh, Wen-Xian Du, and Mendel Friedman are with the USDA-ARS Western Regional Research Center, 800 Buchanan St., Albany, CA 94710; phone (510) 559-5864, fax (510) 559-5851, e-mail tara.mchugh@ars.usda.gov [McHugh]; phone (510) 559-6148, fax (510) 559-5818, e-mail wen.xian.du@ars.usda.gov [Du]; phone (510) 559-5615, fax (510) 559-6162, e-mail mendel.friedman@ars.usda.gov [Friedman]. ✷