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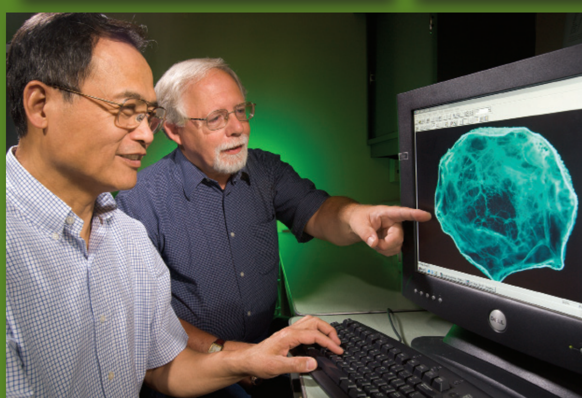
Agricultural Research Service

Eastern Regional Research Center
Research Highlights 2000-2010



Foreword

This Eastern Regional Research Center (ERRC) Research Highlights volume is a compilation of articles published from January 2000 to December 2010 in the *Agricultural Research* magazine, reporting research findings at the Center. Established by an Act of Congress in 1938, ERRC is one of four Regional Research Centers of the U.S. Department of Agriculture, Agricultural Research Service. ERRC researchers conduct studies to benefit producers of agricultural commodities, handlers and processors of food and nonfood products, Federal action and regulatory agencies, and the consumer.

ERRC is home to some of the Nation's leading scientists and engineers who help solve problems facing the United States today and tomorrow with fundamental, applied, and developmental research on agricultural commodities including milk, meat, poultry, hides, leather, wool, fats, oils, rendered protein, grains, fruits, vegetables, energy crops, and juices.

New knowledge and technology ensures an abundance of high-quality agricultural commodities and products at reasonable prices to meet increasing needs, and provides continued improvement in the standard of living for all Americans. These objectives are the basis for the following research:

- Development of rapid methods to detect human pathogens and harmful chemicals in foods.
- Development of novel methods to characterize human pathogens in foods.
- Development of microbial predictive modeling for food safety risk assessment and management.
- Development of effective and innovative intervention technologies to reduce or eliminate human pathogens in foods.
- Sustainable production of biofuels and co-products from grains and biomass.
- Sustainable agricultural practices.
- Development of new crop-derived food ingredients and nutraceuticals with health-promoting ability.
- Development of new dairy products with improved functionality and health benefits.
- Development of valuable biobased products from agricultural byproducts for biomedical, industrial, and commercial applications.
- Utilization of byproducts, particularly potential pollutants.
- Opening new domestic and foreign markets and expanding existing ones.

ERRC scientists have won numerous national and international awards for their work, including the 2007 designation by the American Chemical Society as a National Historic Chemical Landmark, recognizing ERRC scientists' development of the dehydration technology that resulted in reconstituted instant mashed potatoes.

For more information about ERRC, visit www.ars.usda.gov/naa/errc.

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Issued April 2012

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Front and back cover: The broad scope of scientific efforts in the Eastern Regional Research Center includes research on barley, dairy products, food safety, mycorrhizal fungi, nonthermal food processing, oysters, pectin extraction from citrus and sugar beets, produce-washing equipment, and wool-processing techniques.

Contents (continued)

Shaking Up the Future

Results are in . . . Adults love the taste of ARS' reduced-sugar, low-fat milk shakes. This shake for the new millennium is a remake of the low-sugar shake developed in the 1970s for USDA's school lunch program by retired researcher Virginia Holsinger, formerly with ARS' Dairy Products Research Unit in Wyndmoor, Pennsylvania.

The new shakes have less than half the sugar and about 10 percent of the fat of commercial shakes.

The concept of a low-sugar, low-fat shake was probably ahead of its time two decades ago. Now, however, since the introduction of low-fat cheese into the school lunch program and a push toward more nutritionally balanced meals, the shake's time may have finally come—in schools and elsewhere.

Last fall, ARS opened its doors to about 600 youngsters for a taste-testing of the chocolate shakes, which are more like soft-serve ice cream. Kids, with their "choosy" culinary tastes, weren't crazy about the shakes. Some comments: "Not sweet enough," "There's an aftertaste," and "Tastes like cereal."

"We're trying to reformulate the shakes and get rid of the cereal flavor," says Richard Konstance, a chemical engineer in the dairy unit.

Konstance is working with cooperative research and development agreement partner Devine Foods, Inc., of Philadelphia, Pennsylvania, to change the shakes and further develop them as a commercial product.

"Our goal is to provide a dessert that contains the same nutrients as an 8-ounce glass of chocolate milk. But it's easier to provide cartons of milk than milk shakes," Konstance says.

"It's more labor intensive to provide shakes because schools need a milk shake machine or soft-serve machine and someone to operate it," says Konstance. "A hard-pack version of the shake would be easier for schools to deal with, but there's a difference in formulating soft-serve and hardened ice cream."

"Ultimately, we want to create a product for young children that is healthful and great tasting," says Denise Devine, president of Devine Foods.

After getting kids' opinions, ARS took the shakes for another test drive—this time with adults, ages 22 to 52. The adults loved them! They thought they were just sweet enough and tasted better than many other dietary shakes.

What's more, the shakes are good for you. They are based on ARS technology and contain Devine's patented composition, which reduces fat and calorie content and adds fiber and nutrients. The fiber content is about 2 to 2.2 percent, which qualifies the shakes as a good source of fiber. A 10-ounce shake has as much calcium, vitamins, and minerals as a serving of milk and has fewer calories.

One more benefit: The shakes are significantly lower in lactose—good news for those who are lactose intolerant.

"These nutritious shakes should be available to consumers in the near future," says Konstance.—By **Tara Weaver-Missick,** ARS.

This research is part of New Uses, Quality, and Marketability of Plant and Animal Products, an ARS National Program (#306) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/cppvs.htm.

Richard P. Konstance is in the USDA-ARS Dairy Products Research Unit, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038; phone (215) 233-6600, fax (215) 233-6795, e-mail rkonstance@arserrc.gov.

The new ARS-developed **shakes** have less than half the sugar and about **10** percent of the **fat** of commercial shakes.

PEGGY GREB (K8876-1)

Chemical engineer Richard Konstance and president of Devine Foods, Denise Devine, discuss the nutritional value of the low-sugar, very low-fat shake and ways to improve and develop it into a commercial product.

Fabric To Dye For

Samples of wool/cotton crosswoven blends dyed in a single dye bath. The solid-color swatches were pretreated so that both the wool and cotton yarns would pick up the dye evenly. In the untreated fabrics, the cotton stayed largely undyed.

Peggy Greb (K9139-1)

Cotton may soon be afforded the same upscale recognition as wool, thanks to new technology. ARS chemists Jeanette M. Cardamone and William N. Marmer have found a way to make cotton more chemically similar to its high-end counterpart—wool—so that a fabric blend of the two can be easily dyed.

Dyeing a cotton/wool blend is difficult because the two fibers have different chemical makeups. Wool, which is sheep hair, is made of animal proteins, while cotton is made of plant cellulose—the main part of a plant's cell wall. Normally, when wool and cotton are blended together, two separate dye baths are required because the wool takes up most of the dye.

"The process we developed is a new single-bath dyeing procedure called union dyeing," says Cardamone. She and Marmer are with the Hides, Lipids, and Wool Research Unit at ARS' Eastern Regional Research Center (ERRC), in Wyndmoor, Pennsylvania. "This process helps textile manufacturers overcome a major technological barrier: dyeing all-natural blends to the same depth of shade in one step."

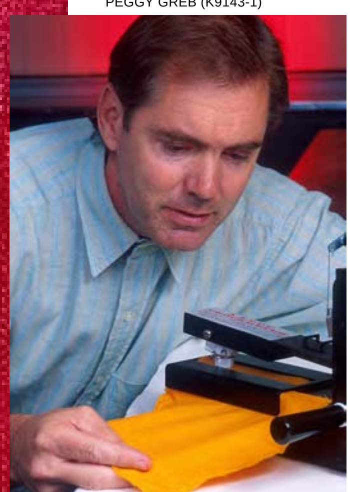
In the conventional procedure, Cardamone explains, cotton is dyed first and wool second. Wool is dyed in an acidic environment at high temperatures, and cotton is dyed in a nonacidic environment at lower temperatures. This difference requires that the wool and cotton be dyed either separately, or sequentially in one bath in which the pH and temperature levels are changed.

"Our improved method uses pretreatments to make the cotton as receptive to dye uptake as the wool," she says.

Opposites Attract

Cardamone and Marmer's simple approach is to reverse the chemical charge of cotton from negative to positive before dyeing; wool is already positive. To do this, they use cationic fixatives—positively charged ions—which are typically used after cotton is dyed to help it keep its color. Applying the fixatives before dyeing gives both fiber components of the fabric a positive charge. Since the dye is negatively charged—and opposites attract—the cotton and wool dye to a uniform shade because the dye is attracted equally to both fibers. This union-dyeing process uses one dye in one bath, under one set of conditions.

PEGGY GREB (K9143-1)



Technician Chet Sutton studies colorfastness of a union-dyed wool/cotton blend.

Cotton industry officials are excited about the new process. "This technology is easy to adopt," says John Turner, a senior chemist with Cotton Incorporated in Cary, North Carolina.

"It doesn't require elaborate equipment or expense. In the past, there was no satisfactory method for cotton mills to dye blends. This technology could potentially increase the use of cotton." Cotton Incorporated wants to expand the use of cotton and make it more profitable for cotton farmers and the textile industry.

PEGGY GREB (K9137-3)

Chemists Jeanette Cardamone and William Marmer study records of the effect of dyeing regimen on dye uptake.

"This process gives cotton an upscale market. A cotton/wool blend would have greater value than a 100-percent cotton item," Turner says. "And depending on the blend level, it could be cleaned in the washing machine." Cotton Incorporated has a research partnership with the ERRC scientists.

The wool industry also finds the concept appealing because it creates a new market for wool. Approximately 66,800 sheep producers raise 7.2 million sheep and lambs, producing about 49.2 million pounds of wool in the United States. Even so, the country still imports wool from Australia and New Zealand. This technology could help open more markets for American wool by increasing demand.

Keeping Colors Colorful

The ERRC scientists also use another method that helps make dyeing wool/cotton blends possible—a durable-press finishing resin. The resin treatment was originally developed by chemists at the ARS Southern Regional Research Center (SRRC) in New Orleans, Louisiana, to prevent wrinkling in 100-percent cotton. SRRC scientists further developed the technology to increase cotton's dyeability. SRRC chemist Eugene Blanchard collaborated with ERRC scientists on using the durable-press finishing resin treatment for cotton/wool blends.

Cardamone says resin treatments, which are alternatives to cationic fixatives, are important for good colorfastness in laundering. Colorfastness is a textile industry standard that determines how stable the color is in a garment. Good colorfastness means the garment won't fade after one washing. Resin pretreatment is best for garments that require excellent colorfastness. Cationic fixatives could be used for outerwear garments where colorfastness to washing is moderate but colorfastness to dry cleaning is high, notes Cardamone.

"Both pretreatment systems will effectively lead to union-dyeing of wool/cotton blends, but the treatment should be selected to accommodate the anticipated end use. A wool/cotton-blend fabric is ideal for multiseason apparel because wool provides inherent resiliency and warmth while cotton contributes comfort and coolness," says Cardamone. "These experimental textile treatments may broaden the market for cool-weather garments made of cotton/wool blends. In sweaters, for example, the blend's wool component retains body heat and imparts thickness, while cotton makes it comfortable to wear. Both natural fibers are great at wicking away moisture, too."

Through a cooperative research and development agreement between ERRC researchers and chemical specialties manufacturer Hercules, Incorporated, other pretreatment systems are being investigated.

Applied commercially, these technologies may cut textile dyeing costs—savings that can be passed on to consumers seeking versatile garments for spring and fall.—By **Tara Weaver-Missick,** ARS.

This research is part of New Uses, Quality, and Marketability of Plant and Animal Products, an ARS National Program (#306) described on the World Wide Web at http://www.nps.ars.usda.gov.

William N. Marmer and Jeanette M. Cardamone are with the USDA-ARS Eastern Regional Research Center, Hides, Lipids, and Wool Research Unit, 600 East Mermaid Lane, Wyndmoor, PA 19038; phone (215) 233- 6585 [Marmer], (215) 233-6680 [Cardamone], fax (215) 233-6795, e-mail wmarmer@arserrc.gov and jcardamone@arserrc.gov. ◆

PEGGY GREB (K9140-1)

Technician Kimberly Baxendale dyes samples of a wool/cotton blend fabric.

NEW TECHNOLOGY BOOSTS FIBER IN FOODS

PEGGY GREB (K9309-1)

Charles Onwulata, a food technologist, prepares ingredients for high-pressure homogenization, a process that can increase fiber content in fluid foods.

Americans fall short when it comes to eating enough fiber each day. So what are scientists doing to help? They have come up with a new technology that allows them to add more fiber to foods without changing their texture, says food technologist Charles I. Onwulata, who's in the ARS Eastern Regional Research Center's (ERRC) Dairy Products Research Unit, in Wyndmoor, Pennsylvania. "Until now, adding fiber to foods was difficult because it changed the qualities—particularly the texture and mouth feel—of the food," Onwulata says.

Onwulata has filed for a patent on the new technology, "invisible fiber," a process that uses milk protein to envelop the fiber and keep it from soaking up water. "The protein barrier makes the fiber 'invisible' to water. The fiber doesn't pull moisture out of the rest of the food product," he notes. "But the invisible fiber envelope will dissolve during digestion, allowing the fiber to perform its normal function in the gut. This new, encapsulated fiber can be incorporated into food products without changing texture or moisture. Many foods can be modified with the invisible fiber."

Traditionally, food manufacturers have increased fiber in foods in small amounts to avoid negative effects such as changes in texture, color, and mouth feel. In foods with high fiber content, the fiber absorbs water from its surroundings, giving the food a dry texture. Reducing the water-holding capacity of the fiber improves food quality and allows more fiber to be added without changing its texture, Onwulata says.

Onwulata and colleagues conducted baking studies to test the moisture level, protein content, color, and hardness of foods made with invisible fiber and compared them to recipes with regular fiber. Fiber affects these properties, and these properties determine consumer acceptability.

The scientists-turned-chefs baked cookies and muffins power-packed with invisible fiber. The invisible fiber improved the goodies' qualities.

ARS researchers are also working with industry to increase fiber in fluid foods. They had a cooperative research and development agreement with Verion, Inc., of Exton, Pennsylvania, to adapt a "dynamic pulse-pressure treatment" process for the food industry. The technology, which is patented by Verion, uses hydrostatic pressure—the force applied through water—to change the moisture, density, and melting properties of foods. The treatment was originally used for making pharmaceuticals.

"Scientists have known for a hundred years that high pressure can be used to process foods," says Onwulata. "Old methods take anywhere from 20 to 30 minutes to pressure-process foods. With dynamic pulse-pressure treatment, pressure-processed fluid foods like milk or slurries (pastelike fluid that can pass through a nozzle) can be processed in 1 second—faster than with anything else that's on the market," he says.

PEGGY GREB (K9310-1)

Chemist Renee Wildermuth bakes muffins and cookies containing "invisible fiber."

Foods react differently to pressure processing, since they have varying densities and abilities to dissolve in water. In studies at ERRC's dairy pilot plant, a small-scale processing facility, scientists tested several food ingredients, including whey proteins, corn starch, wheat bran fiber, and cellulose fiber.

They found that pressure treatment modified the molecular structure of the starches. Pressure-treated fiber had about a 40 percent reduction in its water-holding capacity. Microscopic images revealed that pressure treatment packed the fibers into small balls that were impervious to moisture.

"Once these technologies are fully developed and commercialized, the food industry will greatly benefit from them," Onwulata says. Many companies are interested in increasing the amount of fiber in their foods as a benefit to consumers. The recommended fiber intake is 20 to 35 grams per day. But on average, Americans get only about 15 grams per day. Studies suggest that fiber decreases heart disease, some cancers, high blood pressure, and diabetes.

Nutritionists still agree that eating a variety of grains, fruits, vegetables, and legumes is the best way to get fiber. Meanwhile, ARS researchers will continue to look at ways to boost fiber in other foods.—By **Tara Weaver-Missick,** ARS.

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Charles I. Onwulata is at the USDA-ARS Eastern Regional Research Center, Dairy Products Research Unit, 600 E. Mermaid Lane, Wyndmoor, PA; phone (215) 233-6497, fax (215) 233-6795, e-mail conwulata@arserrc.gov.

Foods With Fiber

Dietary fiber comes from plant cell walls. There are two main types of dietary fiber, and we need a combination of both. Soluble fiber (gums, mucilages, pectins) forms a gel and is found in fruits, dry beans and peas, vegetables, and some cereals. Insoluble fiber (cellulose, hemicellulose, lignin) passes through your digestive tract basically intact and is primarily found in whole-grain products, like wheat bread. Insoluble fiber helps get rid of food your body can't use. It also gives you that full feeling. Soluble fiber lowers blood cholesterol and helps regulate blood sugar. Here are just a few sources of fiber:

Food	Serving Size	Total Fiber (grams)
Apple, raw, with skin	1 medium	3.7
Baby lima beans, cooked	1/2 cup	5.4
Baked potato with skin	1 potato	4.8
Banana	1 medium	2.8
Black beans, cooked	1 cup	15.0
Broccoli, cooked	1 spear	1.1
Carrots, raw	1 carrot	2.2
Green peas, cooked	1 cup	8.8
Lentils, cooked	1 cup	15.6
Oat bran bagel	1 4-inch bagel	3.2
Orange	1 small	2.3
Peach	1 medium	2.0
Peanut butter, smooth	2 tablespoons	1.9
Popcorn, air-popped	1 cup	1.2
Shredded cabbage, cooked	1/2 cup	1.7
Spinach, cooked	1 cup	4.3
Whole-wheat bread	1 slice	1.9
Whole-wheat English muffin	1 muffin	4.4

Source: U.S. Department of Agriculture, Agricultural Research Service. 1999. USDA Nutrient Database for Standard Reference, Release 13. Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp.

PEGGY GREB (K9311-1)

Muffins and cookies made with invisible fiber.

Bioenergy Today

*Scientists worldwide are researching a wide variety of ways to improve **ethanol** and **biodiesel** production. The goals are to develop valuable **coproducts,** lower production costs and emissions, and enhance the performance of **biofuels.***

Taking the lead for the nation last year, the U.S. Department of Agriculture ordered all its locations nationwide to run vehicles and equipment on ethanol or biodiesel blends wherever feasible. Gasoline-operated vehicles and equipment will use a blend of at least 10 percent ethanol and 90 percent conventional gasoline. Diesel vehicles and equipment will use B20 biodiesel, a blend of 20 percent biodiesel and 80 percent regular diesel. The USDA fleet includes more than 700 "flex-fuel" vehicles, which use a blend of 85 percent ethanol and 15 percent gasoline.

The Department's action reflects the federal government's commitment to expanding its use of biofuels and biobased products to set an example for the private sector. The year 2000 also saw a groundbreaking for a $20 million National Ethanol Research Pilot Plant in Edwardsville, Illinois. When completed in January 2003, this will be the largest ethanol pilot plant in the country. The Agricultural Research Service is administering the federal government's contribution of $14 million toward the $20 million cost of construction. Industry considers the plant essential to meeting its goal of increasing annual ethanol production to 16 billion gallons over the next 10 to 15 years.

KEITH WELLER (K9835-17)

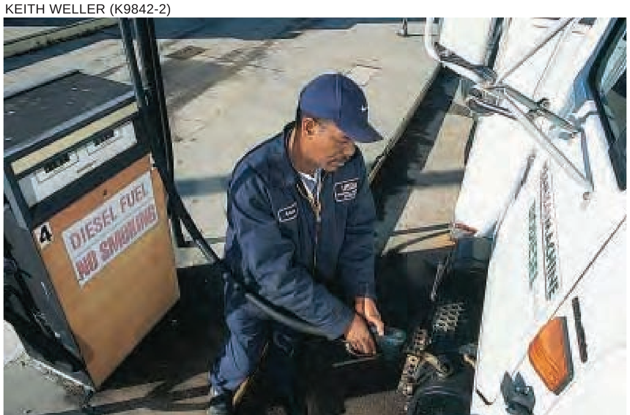
At the Beltsville Agricultural Research Center, quality assurance specialist David Johnson examines a sample of biodiesel while Greg Meyer, driver from a cooperating fuel company, fills a 20,000-gallon tank at a boiler plant, which heats BARC's dairy buildings.

The new plant is designed to enable researchers to develop the technologies required to improve the efficiency of ethanol production. The more efficient the production, the lower the costs—in terms of both energy and money—and the more competitive ethanol becomes. Since each crop has its own mix of complex sugars and starches, each requires its own techniques to be economically processed into ethanol. The challenge is to design methods that allow different crops to be processed in the same facility.

Throughout the United States and in several other nations, scores of scientists are researching a wide variety of ways to improve ethanol production. These researchers include those in ARS' national Bioenergy and Energy Alternatives research program. At two of ARS' regional research centers—in Illinois and Pennsylvania—their goals are to improve the conversion of agricultural plant materials into ethanol and valuable coproducts, lower production costs and fuel emissions, and enhance performance properties of biodiesel. The Western Regional Research

KEITH WELLER (K9842-10).

KEITH WELLER (K9842-2)



Above: In a biodiesel-powered tractor, animal caretaker Angel Santiago heads to a dairy barn at BARC. The center uses B20, a common biodiesel blend, in its entire fleet of over 150 diesel vehicles.

Left: High-voltage electrician Alvin Coates fills the tank of an aerial hydraulic lift bucket truck with soy-based diesel.

Biofuel *Basics*

Biodiesel is a clean-burning alternative fuel that can be made from materials such as vegetable oils, animal fats, and spent cooking greases. Typically, biodiesel is prepared by the reaction of fat or oil with alcohol under alkaline conditions. Soy-based biodiesel is the most commonly used form.

Ethanol is an alcohol-based fuel produced by fermenting sugars from crop starches. Currently, 95 percent of ethanol is produced from corn kernels. About 5 percent of U.S. ethanol is made from sugar- and starch-containing materials other than corn. These include wheat, barley, and sorghum grains; sugarcane; cheese whey; and wastes from paper mills, potato processing plants, breweries, and beverage manufacturers—or some combination of these materials.

Originally, most ethanol was made through **wet-milling,** which means the starch is separated from the corn germ and fiber and liquefied by cooking. The liquefying creates sugars in a form that can be fermented with yeast to produce ethanol and carbon dioxide. The ethanol is then removed from the slurry.

The number of ethanol plants has surged in the past few years, and **dry-milling** is now the method used for over half of the ethanol currently produced. In this process, kernels are ground to a fine powder, and all of it is cooked to liquefy it, without removing the germ or fiber. Different enzymes are added at different stages and temperatures as the mash cools, producing ethanol and carbon dioxide.—By **Don Comis,** ARS.

Center, in California, focuses its biofuel efforts on ethanol. In 1999 the national program was expanded to include the breeding of improved energy crops.

Seeing Is Believing

USDA alone last year used over 100,000 gallons of biodiesel fuel and expects to easily double that amount next year. This puts practical examples of biofuel use within driving distance of public and private organizations around the country.

Leading by example, USDA's Henry A. Wallace Beltsville (Maryland) Agricultural Research Center (BARC) uses B20 biodiesel in its entire fleet of 150 diesel vehicles, including a tour bus (see cover photo) for the ARS National Visitor Center, located on BARC grounds. The center also heats some buildings with B5 (5 percent biodiesel).

The success with biodiesel in vehicles has encouraged commercial and public fleets to adopt biodiesel, particularly in Maryland. The cities of Greenbelt, Takoma Park, and Ocean City have all recently adopted biodiesel fuel for their snowplows and other public-works vehicles and equipment. Greenbelt also uses biodiesel to run its Connector bus, which ferries Greenbelters on short trips within the city, filling gaps in the Washington, D.C., metropolitan area public transit system. These cities learned about biodiesel by sending representatives to BARC meetings.

KEITH WELLER (K7776-1)

To help lower the cost of ethanol production, ARS scientists have developed valuable coproducts from corn, such as A*maiz*ing Oil, which can lower blood cholesterol levels. Here, chemist Kevin Hicks checks the color and quality of a corn fiber oil sample.

Cheaper Ways To Make Ethanol

Scientists at ARS' Eastern Regional Research Center (ERRC), in Wyndmoor, Pennsylvania, are working on lowering ethanol's price per gallon on two fronts: developing coproducts to defray costs and lower-cost production techniques and materials.

They've developed a growing number of valuable coproducts of processing corn kernels for ethanol, such as *Amaiz*ing Oil, a new corn oil that can lower blood cholesterol levels, and a valuable food ingredient called *Zea*gen, a corn fiber gum. Both products were found in the fibrous hull that forms the kernel's outermost layer, and both are moving closer to the marketplace.

ERRC engineers have developed a radical alternative way to produce ethanol at a price expected to be significantly lower than is typical of conventional methods. It's called continuous fermentation with stripping. The method removes, or strips, ethanol contained in the escaping carbon dioxide, which is then recycled back to the fermentation vat. In the conventional process, when the ethanol level rises too high in fermentation vats, it slows the yeast's ability to produce more ethanol. The new method continuously strips ethanol from the fermentation broth, freeing the yeast to make additional ethanol.

The team has also developed a new process called pervaporation, which uses a membrane to filter ethanol out of the broth.

Says engineer Frank Taylor, "We are now looking for companies interested in taking our processes off the research bench and develop them further for commercial use."

Andy McAloon is the team's cost engineer. He developed a computer model that can estimate the cost per gallon of ethanol if a new process were used to produce it. "This guides our research so that we don't spend too much time on processes that would not yield a more competitive product," says Kevin Hicks, who leads the ERRC ethanol team. "It's expensive to test a process at the pilot-plant stage, so this model could screen out processes not likely to be practical."

Other researchers at ERRC are working to reduce the cost of biodiesel production. They are making biodiesel fuel from lower quality starting materials, such as soybean soapstock (see story on page 9).

Enzymes for Greater Efficiency

At ARS' Western Regional Research Center, in Albany, California, scientists are creating better enzymes that produce ethanol in a more cost-effective manner.

"About 10 to 15 percent of the energy required to make ethanol goes toward providing the heat to cook the starch," says chemical engineer George Robertson. "The more energy it takes to make ethanol, the less useful it is as a fuel alternative. So we're working on enzymes that can digest the starch and make ethanol production more efficient. That could open up the ethanol market to other grains, like wheat," Robertson says.

To construct these enzymes, the team uses a technique developed in the pharmaceutical industry called directed evolution. Using biotechnology, they take apart key plant genes and reconstruct them, introducing mutations.

The mutant genes are then inserted into yeast organisms, where they begin to make, or express, starch-digesting enzymes. The scientists then screen the yeast colonies for their enzyme-producing abilities and select the best ones for another cycle of gene mutation and selection.

"We can do various things to direct the evolutionary path, speeding up development of enzymes with desired characteristics," says chemist Dominic Wong.

In the laboratory, at 98.6˚F, their high-powered enzymes break down starch 50 times faster than the original enzymes. And the technique shows promise of making even better enzymes. The team plans to use similar approaches to develop new enzymes for use in biomass conversion.

Microbes, Too, Can Play a Role

Making biofuels, such as ethanol, economically from the whole crop instead of just the grain is the long-range goal of scientists in the Fermentation Biochemistry Research Unit at ARS' National Center for Agricultural Utilization Research, in Peoria, Illinois.

"But our starting point is researching fermentation of fiber in just the corn kernel," says ARS microbiologist Rodney J. Bothast, who leads the project. Currently, the kernel fiber is separated out and used as inexpensive cattle feed that is valued for protein, not fiber. If technology were developed to break down the different polymers in kernel fiber to simple sugars, about 10 percent more ethanol could be produced from each bushel of wet-milled corn.

Bothast collaborates with scientists at ERRC and in the Department of Wood Science at the University of British Columbia, Vancouver, in research on the physical and chemical pretreatment of fiber. Pretreatment frees the cellulose from hemicellulose, starch, and lignin components.

Cellulose fragments are more readily converted into sugars that can be fermented to make ethanol. The lignin component is not fermented but can be burned to produce energy.

"So far, the most effective way we've found to break down the fiber is to pretreat it with mild acid and then with alkaline hydrogen peroxide," says Bothast.

The pretreated fiber contains sugars, mainly arabinose and xylose and some glucose. Normally, ethanol-producing microbes eat the glucose first, leaving little appetite for the other sugars. Nancy N. Nichols, microbiologist, and Bruce S. Dien, chemical engineer, have developed genetically engineered microorganisms that consume the sugars at nearly equal rates.

These researchers are collaborating with others at the University of British Columbia, Purdue University, and Williams Energy Service, in Pekin, Illinois—the second largest ethanol producer in the country—to test these new microbes on kernel fiber converted to sugars by industrial processes.

KEITH WELLER (K7408-6)

Microbiologist Rodney Bothast (left) and technician Loren Iten add starter microorganisms to pilot-plant-size bioreactors in which ethanol is brewed from sugar mixtures derived from corn fiber.

SCOTT BAUER (K9844-1)

Scientists are creating enzymes that produce ethanol in a more cost-effective manner. Technician Tina Williams and chemist Charles Lee use an automated liquid handler and a microplate reader to measure enzyme activity.

SCOTT BAUER (K9843-1)

At WRRC, technician Sarah Batt uses a robot to pick yeast colonies and transfer them onto starch plates, where they'll be screened for desirable enzyme production.

Value-Added Products

As the scientists seek ways to increase ethanol production efficiency, they're mindful of coproducts that might help make ethanol crops more economically successful. For example, other microbes developed by Nichols and Dien convert the sugars derived from kernel fiber into lactic acid. Biobased companies use lactic acid to produce solvents and biodegradable plastics.

Badal Saha, an ARS chemist, and microbiologist Timothy Leathers have developed yeasts that convert the xylose derived from corn fiber into xylitol, a low-calorie sweetener. Xylitol, which has a minty-cool taste, is used in some mints and gum and sells for about $3 per pound. It's made from birch wood by an expensive, energy-intensive process.

Saha and Leathers have also discovered fungi that produce enzymes especially well suited for converting corn fiber into sugars. Use of enzymes decreases the amount of acid needed to convert corn fiber to sugars, and that makes ethanol an even more environmentally friendly fuel.

And Out on the Range . . .

Instead of making ethanol from the sugars and starches in plants, Ken Vogel, with ARS in Lincoln, Nebraska, is experimenting with using cellulose and hemicellulose from switchgrass as another source of ethanol. Vogel's hope is that farmers might be able to grow this native prairie grass on highly erodible soils —including those set aside for USDA's Conservation Reserve Program—harvest the grass periodically for ethanol production, and reap conservation benefits, such as reduced soil erosion and enhanced carbon storage.

Vogel and colleagues are breeding new switchgrasses for biofuel use. They're genetically improving the grass for conversion to ethanol and conducting on-farm trials to obtain economic information on production costs. Ron Follett, at Fort Collins, Colorado, is working with Vogel and ARS scientists at Mandan, North Dakota, to study carbon storage on lands grown for biofuel crops.

Plant geneticist JoAnn Lamb and colleagues at the ARS Plant Science Research Unit in St. Paul, Minnesota, are looking at alfalfa as another cellulose source for producing ethanol. They received $288,000 from ARS' new $2.4 million in funding for developing bioenergy crops.

They are breeding a new alfalfa variety specifically to double as a high-quality livestock feed and a bioenergy crop. They'll incorporate genes from southern European varieties to give the plant a thicker, almost woody, stem. This means more cellulose for ethanol production.

The humid East might prefer alfalfa to switchgrass as an ethanol source, but switchgrass is ideally suited for the arid West, because it needs very little rainfall to grow. Both alfalfa and switchgrass can also be burned to generate electricity.

There are obstacles to overcome when making ethanol from cellulose in plants like switchgrass or alfalfa, such as finding ways to convert the complex sugars in cellulose into simple ones that can be fermented to produce ethanol. Facilities to do this conversion will have to be built. Equipment for this purpose could be tested at the new Illinois pilot ethanol plant when it is up and running, as could equipment for the new continuous fermentation stripping process.—By **Don Comis,** ARS. **Ben Hardin** and **Kathryn Barry Stelljes,** both formerly with ARS, also contributed to this story.

This research is part of Quality and Utilization of Agricultural Products (#306) and Bioenergy and Energy Alternatives (#307), two ARS National Programs described on the World Wide Web at http://www.nps.ars.usda.gov.

To reach scientists mentioned in this article, contact Don Comis, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; phone (301) 504-1625, fax (301) 504-1641. ◆

New Fuels From an Ancient Crop

Barley has been cultivated for thousands of years, yet it doesn't always make the list when energy experts discuss potential biofuel crops. And bio-oil—a liquid fuel generated when heat breaks down plant matter—is still a low-profile energy alternative. But research by Agricultural Research Service scientists at the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, could give a big boost to producing bio-oil from barley feedstocks.

As a renewable transportation fuel, bio-oil made from barley byproducts—or any other biofeedstock—has several advantages. The fuel can potentially be refined, stored, and distributed with the infrastructure already in place for the petroleum fuel industry. And perhaps in the future, consumers will be able to fill up their cars with gasoline or diesel derived from bio-oil without needing special adaptations in their fuel systems.

PEGGY GREB (D1985-1)

Chemical engineer Akwasi Boateng (right) and mechanical engineer Neil Goldberg (center) adjust pyrolysis process conditions while chemist Charles Mullen (left) loads the reactor with bioenergy feedstock.

ERRC lead scientist and chemical engineer Akwasi Boateng worked with chemist Charles Mullen, mechanical engineer Neil Goldberg, chemist Robert Moreau, and research leader Kevin Hicks on studies that evaluated the yields of bio-oil from barley straw, hulls, and dried distillers grains (DDGS). All three feedstocks are byproducts of fermentation of barley grain for ethanol—a biofuel option that is slowly gaining ground in the Middle Atlantic States and the Southeast, where farmers could cash in on the production of winter barley cover crops while continuing to raise corn and other food crops in the summer.

The researchers produced bio-oil from all three barley byproducts via "fast pyrolysis," an intense burst of heat delivered in the absence of oxygen. In the lab, a kilogram of barley straw and hulls yielded about half a kilogram of bio-oil with an energy content about half that of No. 2 fuel oil.

The energy content of bio-oil from barley DDGS—including DDGS contaminated with mycotoxins, which can't be used to supplement livestock feed—was even higher, about two-thirds that of No. 2 diesel fuel oil. But it was more viscous and had a shorter shelf life than the bio-oils produced from straw or hulls.

The fast-pyrolysis process also creates a solid byproduct called "biochar," which might improve the water-holding capacity and nutrient content of soils. Amending soils with biochar can sequester carbon in the soil for thousands of years.

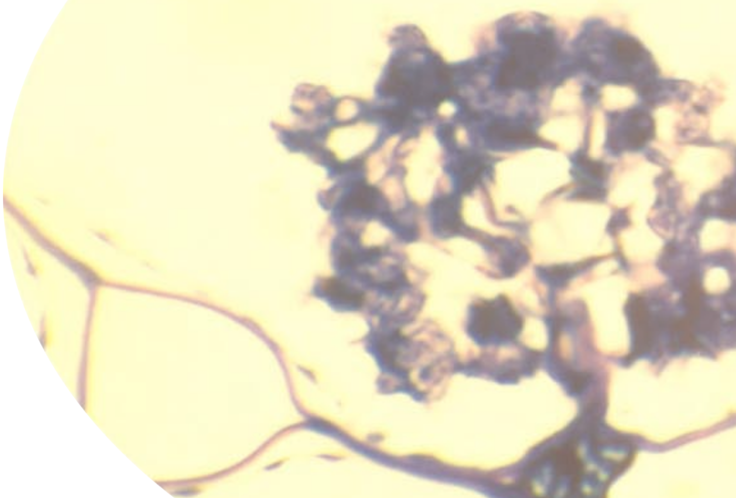
Based on these studies, the scientists suggest that colocating fast-pyrolysis units in commercial barley grain ethanol plants could be a win-win proposition for farmers in the Middle Atlantic States. Barley grain could be used to produce ethanol, and the byproducts could be used to produce bio-oil either for transportation fuels or for producing heat and power needed for the grain-to-ethanol conversion.

Growing winter barley would also help reduce soil erosion and nitrogen leaching, both major concerns for farmers in the Chesapeake Bay Watershed. Adding biochar as a soil amendment could further benefit food crop production and carbon sequestration efforts—all of which shows that even venerable old crops can be used to help solve current energy challenges.—By **Ann Perry,** ARS.

This research supports the U.S. Department of Agriculture priority of developing new sources of bioenergy and is part of Bioenergy (#213), an ARS national program described at www.nps.ars.usda.gov.

To reach scientists mentioned in this story, contact Ann Perry, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1628, ann.perry@ars.usda.gov. *

Soil Fungi Critical to Organic Success



Micrograph of fungal arbuscules (treelike structures) in the cells of leek roots.
(K9438-1)

One casualty of America's agricultural revolution were valuable native soil fungi that enabled crops to grow well with less water, nutrients, and pesticides.

Increased agricultural productivity has been largely dependent on high levels of chemical fertilizers and synthetic pesticides. There is a growing interest in reducing this dependency by encouraging biologically based systems to enhance productivity and product quality on farms.

That's exactly what ARS chemist Philip E. Pfeffer and his co-workers hope to accomplish by helping farmers reestablish the beneficial soil organisms called mycorrhizal fungi. Pfeffer is at the Agricultural Research Service's Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania.

Mycorrhizal fungi live within the roots of most plants in a mutually beneficial relationship (symbiosis). They help roots scavenge more nutrients and water from the soil in exchange for sugar to make the molecules they need to live and grow. These fungi extend long threads, called hyphae, outside the roots. The hyphae transport phosphorus and other nutrients into plant roots. Mycorrhizae also enable plants to use water more efficiently and resist pests.

Fields may eventually have colonies of beneficial microbes rivaling those of yesteryear.

PEGGY GREB (K9434-1)

At Stoneleigh Estate in Villanova, Pennsylvania, chemist Philip Pfeffer (left) and microbiologist David Douds inspect a tropical grass planted to produce compost-based inoculum.

Pfeffer and co-workers study the most common type of mycorrhizae, which are called endomycorrhizae because the fungi live inside—rather than between—root cells. They are also called arbuscular mycorrhizae because of the treelike structures (see photo above), or arbuscules, they build within the cells. The branches transfer nutrients to the plant cells in exchange for sugar for the fungi. The trunks of the arbuscules attach to the hyphae.

Today, farmers who grow row crops, like corn and soybeans, must rely on whatever soil fungi survived the decades of high chemical application that began when American agricultural production intensified in the 1950s.

Horticultural crop producers fare better because they can buy potting mixes with beneficial fungi added. Home gardeners can buy the fungi as soil inoculants from seed catalogs. But it's impractical for farmers to buy and apply the large quantities of fungi they'd need for farm fields.

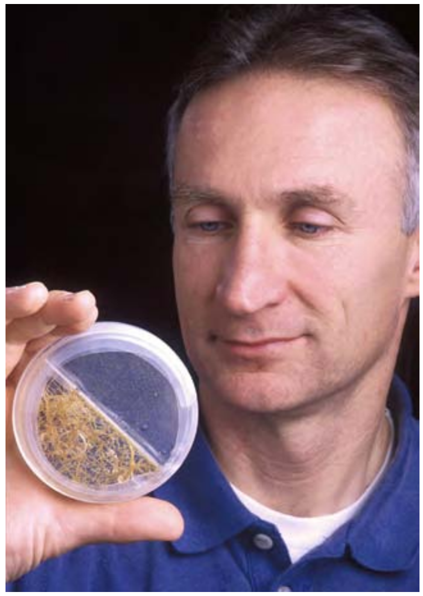
On-Farm Fungi Production

One member of the ARS team, David D. Douds, is supervising experiments to find practical ways for farmers to grow and apply their own mycorrhizal fungi. At the Rodale Institute Experimental Farm in Kutztown, Pennsylvania—a long-time proponent of organic farming—and at nearby Stoneleigh Estate, Douds has tried growing the fungi in compost. That would enable farmers to apply the fungi along with compost with no extra effort or cost.

Douds planted a tropical grass in the compost after inoculating its roots with arbuscular mycorrhizal fungi. He hoped the roots would harbor the fungi and spread them throughout the compost, but the fungi didn't spread well enough.

"We think the compost was so rich in nutrients that the grass roots didn't encourage the fungi to proliferate, because their help wasn't needed for getting nutrients," he says. This spring, Douds and his colleagues will try mixing the compost with a less nutrient-rich soil to see if that promotes fungal proliferation.

PEGGY GREB (K9435-1)



Microbiologist David Douds inspects carrot root culture system used to study in vitro interactions of the host plant and mycorrhizal fungi.

The researchers' goal is to have suppliers sell farmers colonized host plants for planting—not as a crop, but to start colonies of the fungi in soil, compost, or a compost/soil mix. Then, after the fungi have had time to multiply, farmers would apply the colonized soil in manure spreaders along with their compost.

"Instead of farmers having to buy and transport a whole field's worth of inoculum, they could buy a small fraction packed in with host seedlings. Then, they'd plant the mycorrhizal seedlings and increase the inoculum on their own," Douds explains. Farmers would eventually have crop fields with colonies of beneficial microbes rivaling those of yesteryear.

How Host Roots Communicate With Mycorrhizal Fungi

Another member of the ARS team, chemist Gerald Nagahashi, has found that plant roots release signals to encourage or discourage proliferation during at least two of the fungi's seven life stages. He found that in the first stage, when the fungal spores start growing hyphae in the soil, roots exude compounds that encourage prolific hyphal growth. This helps the fungus find the root, colonize it, and produce the arbuscules.

"We used carrot roots for these studies because they're a good model and can be grown easily in liquid culture," Nagahashi says. The scientists have improved techniques for growing the fungi with carrot roots in petri dishes and hope to do this someday without the roots.

PEGGY GREB (K9436-1)

Chemist Gerald Nagahashi transfers roots into liquid medium. Compounds released into the medium by these roots will be used for in vitro studies.

Nagahashi also did an experiment using light and found it triggered more fungal hyphal branching. "We predicted that exposing inoculum to light would increase colonization of corn seedlings by the fungi, and that's exactly what happened," he says.

The team is studying the basic physiology of the mycorrhizal fungi and their interactions with plants so they can find a way to grow fungal colonies without host plants. This would permit large-scale production of inoculum for field application.

"We also need to find out what nutrients and other conditions have to be met so the fungi grow through all seven life stages and multiply," Pfeffer says. "Using specially marked molecules and nuclear magnetic resonance spectroscopy to analyze a mycorrhizal system in a petri dish, we've learned a great deal about how carbon—in the form of carbohydrates—flows from the plant to the fungi. We've also learned that at certain stages of the life cycle—such as during spore germination—the fungi can take carbon directly from the soil without getting it from plants, which encourages us.

"However, we need to find out why the spores are unable to utilize this carbon to replenish their lipid stores, which are needed for completion of their life cycle," says Pfeffer. "We'd like to be able to feed the fungi glucose in the laboratory and have them reproduce in mass quantities in fermentation vats, as we do with bacteria and other fungi."

In collaboration with Yair Shachar-Hill and Peter Lammers at New Mexico State University, the ERRC team is also examining gene expression of these fungi at each life-cycle stage. With this information the researchers hope to turn on the mechanisms necessary for the fungi to complete their life cycle in the absence of the host plant.—By **Don Comis,** ARS.

This research is part of Soil Resource Management (#202) and Plant Biological and Molecular Processes (#302), two ARS National Programs described on the World Wide Web at http://www.nps.ars.usda.gov.

The ARS scientists mentioned in this article are at the ARS Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038; phone (215) 233-6400, fax (215) 233-6581, e-mail ppfeffer@arserrc.gov ddouds@arserrc.gov gnagahashi@arserrc.gov.

CORN COPRODUCT CUTS ETHANOL PRODUCTION COSTS

The United States is the world's leading corn-growing country, with more than 40 percent of global production. More than 9 billion bushels were produced in 2001—the third highest yield on record, according to USDA's National Agricultural Statistics Service.

Where does all this corn end up? Most is used in livestock feed, but it is also processed into many food and industrial products. These include starch, sweeteners, corn oil, beverage and industrial alcohol, and ethanol fuel.

Corn is an abundant and renewable resource, and the search for energy alternatives makes it a natural choice as a fuel source. The high starch content of corn can be converted to sugar and then fermented to ethanol fuel by brewer's yeast.

PEGGY GREB (K9840-1)

Chemist Nick Parris spraying zein-lipid mixture onto brown kraft paper. These mixtures can be used to replace waxes made from refined petroleum-based products.

Combining ethanol with gasoline lifts the octane level and makes a cleaner-burning fuel. In the 2000-2001 season, about 620 million bushels of corn were used in ethanol production, according to figures from USDA's Economic Research Service. This number could increase 10 percent in the 2001-2002 season. A federal tax exemption keeps ethanol economically competitive with petroleum fuel products but is due to expire in 2007.

Cheaper Zein Means Cheaper Ethanol

Ethanol production from corn creates a surplus of byproducts that are increasingly difficult to sell. Engineers at the Eastern Regional Research Center in Wyndmoor, Pennsylvania, saw the potential to lower production costs of ethanol by making these byproducts more valuable, which could create new markets. The researchers believed one approach was to develop a less expensive process to separate a valuable protein—zein—from corn.

Zein is the main storage protein in the corn endosperm and makes up more than half the total mass of the seed protein. It is currently extracted from corn gluten. It is used mostly as an edible, water-resistant coating for nuts, confectionery products, or pharmaceutical tablets. Little zein is sold because it sells for about $10 a pound.

"In the dry-milling ethanol process, zein is found in dried distillers grain—or DDG—which is mostly sold as a protein supplement in livestock feed," says James C. Craig, the recently retired former head of the Engineering Science Research Unit, where the project originated. But as ethanol production expands, the supply of DDG is expected to far exceed demand. "We believed we could develop a process to extract the zein at a cost that makes it attractive as a commodity."

The researchers engineered and built a pilot ethanol plant at ERRC to find ways to improve the economic return of commercial corn-fermentation plants. The team broke the cost barrier for affordable zein with a system for bulk extraction. Their approach was to use the ethanol as a solvent to extract zein from dry-milled corn.

"A key cost-savings in this process is that the solvent, ethanol, is already present, since it's the primary product," Craig says. "After fermentation, part of the ethanol produced can be recycled to the extraction step, used, and then returned downstream for separation."

This method gives corn-ethanol plant owners an option of producing a value-added coproduct, zein, which would provide more revenue and reduce the overall cost of ethanol production. Efforts are now under way to determine the maximum concentration of zein that can be directly extracted from corn.

The pilot plant work was carried out under the supervision of chemical engineer Leland C. Dickey. Pilot plants model commercial processes so that innovations can be evaluated in a realistic setting.

Finding Added Value in Corn Kernels

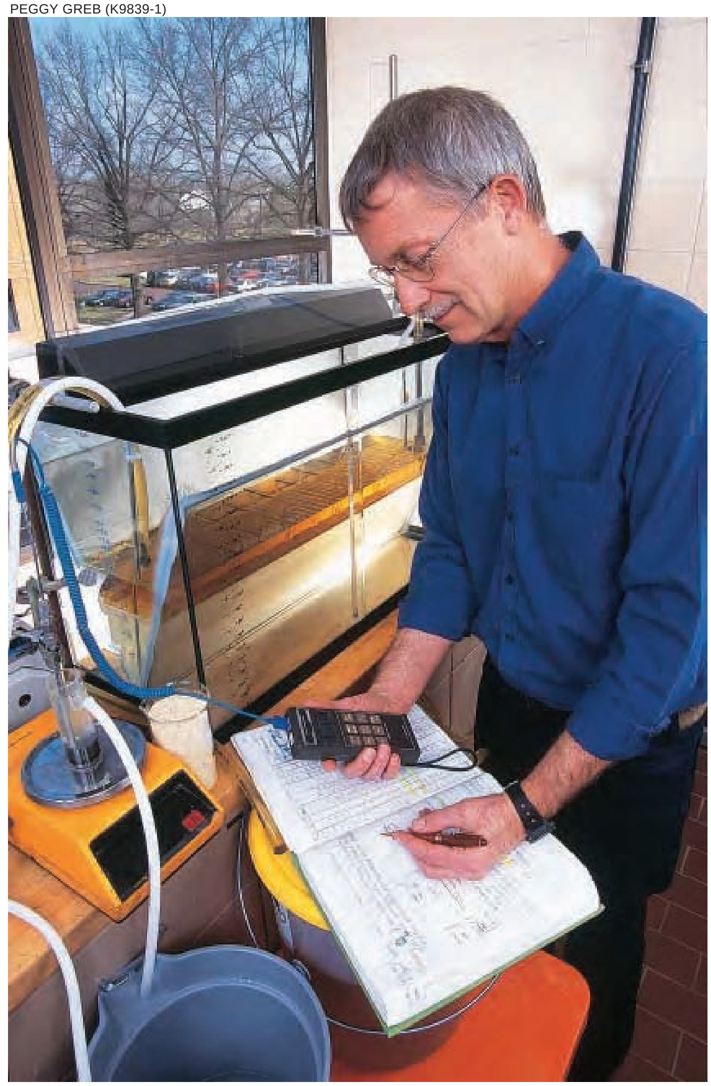
Traditionally, films made from commercial zein are too brittle and their tensile strength too low for most applications. Chemist Nicholas Parris is finding ways to improve the properties of zein isolated from ground corn.

Recently, ERRC scientists isolated a zein-and-lipid mixture from dry-milled corn that costs about $1 a pound to produce. While not as pure as the zein currently on the market, it is still well suited for many applications. The lipid in the mixture replaces refined petroleum-based products that are used to make wax paper and wax-coated packaging. The mixture is an excellent material for coatings, according to Parris, because the zein portion resists grease, and the fatty acids repel water. Because lipids eliminate use of paraffin wax, the paper can be recycled. Unlike petroleum-derived waxes, the zein-lipid mixture is biodegradable.

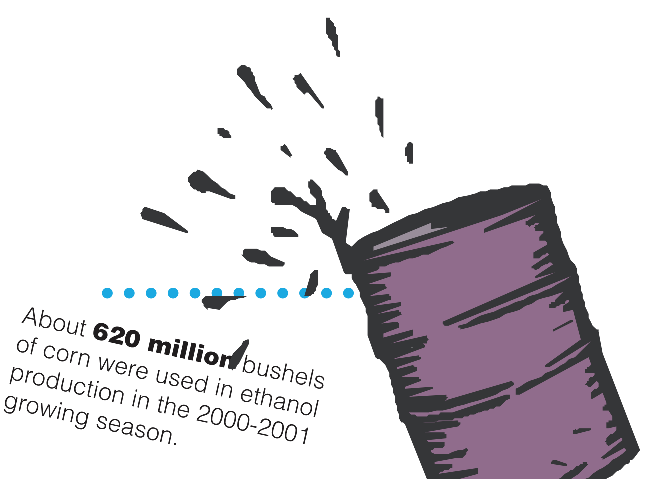
In the past, synthetic plasticizers have been used to improve the mechanical properties of films made from commercial zein. But Parris found that the presence of free fatty acids in the zein-lipid complex could have the same effect.

A computer simulation model was designed to make cost estimates for production from commercial plants. The models are based on data from ethanol producers, engineering firms, equipment manufacturers, and other sources. ARS cost engineer Andy McAloon provides support to scientists and engineers to determine research direction and the costs of possible alternatives to standard industry practices. He uses the program to predict the economic impact of the research.

PEGGY GREB (K9839-1)



Using a small-scale laboratory tank, chemical engineer Leland Dickey separates corn and extract liquid. The extract liquid will be further processed to separate out the zein.



ERRC researchers are seeking cooperators who have specific commercial requirements. The team can develop a process to extract zein with the purity and characteristics for a specific application at an affordable cost.—By **Jim Core,** ARS.

This research is part of Quality and Utilization of Agricultural Products (#306) and Bioenergy and Energy Alternatives (#307), two ARS National Programs described on the World Wide Web at http://www.nps.ars.usda.gov.

Leland C. Dickey is in the USDA-ARS Crop Conversion Science and Engineering Research Unit, Eastern Regional Research Center, 600 East Mermaid Ln., Wyndmoor, PA 19038-8598; phone (215) 233-6640, fax (215) 233-6406, e-mail ldickey@arserrc.gov. ◆

SCOTT BAUER (K7247-10)

All these products make use of soybeans. When ARS research develops new uses for agricultural commodities, it benefits both consumers and farmers.

For You, the Consumer

Abundant, affordable, safe, vast variety—it's the American way when it comes to shopping. What isn't found on any store shelf is an Agricultural Research Service brand name. But many products that people use every day could justifiably carry the stamp "Courtesy of ARS Research."

Pharmaceuticals, cosmetics, roach and termite controls, leather, shampoos, and processed foods, not to mention a proverbial cornucopia of fruits, vegetables, berries, meats, and nuts, all come to a store near you by way of ARS laboratories. Of course, there are essential stops along the way for technology transfer to the companies that turn out the actual products.

But without the work that ARS invests in research—often long-term, high-risk research—there are whole industries of products that might never have gotten off the ground. Some products make it tastier to balance your diet. Some keep you warm and fashionable. Some improve recreation and athletics, and others keep you safe and healthy.

STEPHEN AUSMUS (K10005-1)

Irradiated ground beef from Omaha Steaks.

Tasty Juice

Sixty years ago, orange juice came either freshly squeezed in season or canned—and tasting like the can. But in 1948, ARS scientists collaborated with Florida Citrus Commission researchers to perfect a method to produce a practical, flavorful frozen concentrate with all its vitamins and minerals. Today, U.S. consumption of orange juice from frozen concentrate, still made using these methods, comes to more than 1.07 billion gallons a year.

SCOTT BAUER (K7237-13)

An abundance of useful products from ARS research benefits consumers and farmers alike.

One product in that last category of safety and health to which consumers now have access as a result of ARS research is irradiated hamburger free of *Escherichia coli* O157:H7, bacteria that can cause serious, even life-threatening illness. Ground beef is especially vulnerable because more than just the surface of the meat may potentially have been exposed to bacterial contamination.

The term "irradiated" refers to treatment with ionizing radiation from gamma rays produced by cobalt and cesium atoms, machine-produced X-rays, or electron beams. Treated meat in no way becomes radioactive.

Omaha Steaks, for example, began offering irradiated hamburger patties and ground beef in November 2000. Today, all the 6.5 million pounds of ground beef they sell each year in their retail and bulk food service operations is irradiated.

Bruce Simon, president of Omaha Steaks, feels strongly that, with irradiation, research has developed a way to provide consumers with an extra measure of food safety for ground beef. "We know we were among the first beef companies to make use of irradiation. Whenever we can add another safeguard to make sure that there is less possibility that anyone, especially a child, could be exposed to *E. coli,* it's a step we just have to take," Simon explains.

Consumers won't see ARS' name where it says "Treated by Irradiation" on Omaha Steaks' hamburger packaging. But much of the research to prove irradiation an effective and safe method to sanitize ground beef came from the laboratory headed by ARS microbiologist Donald W. Thayer.

"When you look at the docket that was submitted to the Food and Drug Administration and the Food Safety and Inspection Service to get approval for irradiation of red meat, many of the papers they cite are from ARS," Thayer says. Final approval for irradiation treatment of meat came in February 2000.

Products that protect people from illness are not new accomplishments for ARS. In 1954, ARS developed N,N-diethyl-meta-toluamide for the Department of Defense to protect soldiers from disease-carrying insects. Now best known simply as DEET, it remains the most effective mosquito repellant available. About 230 products containing DEET are currently registered with the U.S. Environmental Protection Agency.

World Health Organization statistics report that mosquitoes spread about 4 million malaria cases, causing about 1 million deaths globally each year. These insects also spread dengue fever-related illnesses, which lead to 24,000 deaths annually.

More recently, the spread in the United States of West Nile virus, also mosquito borne, has focused new attention on DEET. West Nile virus has been reported in 36 states plus Washington D.C., and it is expected to spread to all 50 states.

The Centers for Disease Control and Prevention recommend that people in affected areas always wear an insect repellant containing DEET when they go outside during mosquito season as the best protection against being exposed to West Nile virus.

Some possible concerns about DEET posing a health risk have been raised. EPA has reviewed the data and determined "normal use of DEET does not present a health concern." Several changes have been made in the label directions to ensure DEET is safely applied, principally to avoid oversaturating skin or clothing and not to spray infants.

SCOTT BAUER (K7243-7)

Insect repellants made from DEET, an ARS-developed compound.

Many antibiotics could also bear the ARS stamp. In 1941, British scientists, overwhelmed by World War II, requested U.S. help to find a way to produce penicillin in mass quantities. The problem was given to Andrew J. Moyer and others at the National Center for Agricultural Utilization Research, then called the Northern Regional Research Center (NRRC), in Peoria, Illinois.

Work began in July 1941. By November, Moyer had succeeded in increasing the yield of penicillin by creating a better growth medium with the addition of corn steep liquor, an inexpensive byproduct of wet corn milling, and milk sugar. The team's development of deep vat techniques to grow the mold cultures, called deep fermentation, added the missing piece of the production puzzle.

Eight days after the bombing of Pearl Harbor, NRRC representatives met with U.S. drug companies, which agreed to attempt large-scale production of penicillin using the new methods. The combined work of many researchers, including ARS scientists, resulted in making penicillin available in mass quantities by June 6, 1944, just in time to treat Allied soldiers wounded on D-Day. Infection has always been one of the largest causes of death in war, more so than direct battle injuries.

Mass-produced penicillin saved many lives during World War II and continues to do so. And the improved growth media and deep-fermentation methods and their outgrowths have since been used in development of many other important antibiotics. In 1987, Moyer became the first federal government scientist inducted into the National Inventors Hall of Fame.

Filling the Cornucopia

Any time they shop for produce, people are very likely to be buying a result of ARS research. The agency's successes in breeding new fruit, vegetable, nut, and berry varieties have had an incredible impact on what consumers eat. New flavors, extended harvest seasons, increased growing range, and better shelf life are just a few of the improvements ARS researchers have made.

Almost all the blueberries and cranberries in commercial production were either developed by ARS or bred from ARS varieties. ARS also brought consumers supersweet strawberry varieties with longer shelf life. Southern-grown fresh peaches would probably not be readily available to consumers in eastern U.S. markets if ARS had not developed improved peach varieties as well as the Guardian rootstock.

Citrus—fresh and processed—also has ARS' fingerprints all over it. More than 80 percent of the citrus grown in the United States are rootstock or fruit varieties developed by ARS. When you buy a sweet red grapefruit, chances are pretty good that it will be a Flame grapefruit from ARS. Most of the early-season tangerines—about a $100 million annual retail product—are ARS varieties Sunburst and Fallglo.

Red seedless grapes were all but unknown to the U.S. consumer before ARS released the Flame variety in 1973. Release of another ARS variety, Crimson, in 1989 further increased this table grape's popularity. The two varieties alone, now grown extensively by both domestic and foreign producers, make up a major portion of today's consumer market.

Sweetpotato varieties released by ARS are the standard, keeping the price of this crop affordable, the quality high, and

SCOTT BAUER (K7229-21)

Strawberries, blackberries, and blueberries have all been improved by ARS research.

Lactose-Free Milk

One-quarter of all adults cannot digest dairy products. But ARS used a bacterium to produce an enzyme that breaks down the milk sugar responsible for the problem. Today, consumers have access to lactose-free products like milk, cheese, and ice cream as well as Lactaid tablets, which let lactose-sensitive people have dairy products. Lactose-free milk is now 1 percent of all fluid milk sales, about 40 million gallons a year.

the supply steady. The same can be said for cantaloupe, watermelon, broccoli, nectarines, plums, lettuce, peppers, onions, carrots...the list of fruits and vegetables that ARS research has improved is almost endless.

In addition to whole foods like apples and oranges, ARS has also developed many ingredients that people buy every day as part of processed foods. On a wide variety of food ingredient lists appear some form of the words “hydrolyzed oat flour” or “hydrolyzed oat bran.” A lot of that is actually ARS’ patented Oatrim, a calorie- and cholesterol-lowering replacer for shortening made from enzyme-treated oats and barley.

Oatrim has been licensed to several companies, including Quaker Oats, Inc., which uses Oatrim in foods like some Healthy Choice dinners. Oatrim production was estimated to be in excess of 20 million pounds in 1999, resulting in more than $1 billion in retail sales.

Nutrim, another ARS development similar to Oatrim, has recently led to a new, niche-market consumer product: gourmet vegan chocolate truffles. Mrs. Mudd’s, Inc., of Oceanside, California, worked out a way to make tasty chocolate truffles with a great mouth feel without any dairy ingredients. When mixed with water, Nutrim flows like heavy dairy cream or coconut cream.

“It took 2 years to work out the right formula,” says company president Jan Mudd. “But our dark chocolate truffles are certified organic and completely vegan, so they are the perfect choice for people who are watching their cholesterol or those who do not eat dairy products.”

Xanthan gum, discovered by ARS in the 1960s, is another food ingredient that appears in a whole host of consumer products. This natural product comes from fermenting corn sugar with the microorganism *Xanthomonas campestris.* Even a partial list of functions provides insight into why it is a ubiquitous ingredient: it thickens liquids, provides good cling, provides easy pourability and pumpability; controls crystallization, increases baking volume, provides temperature stability, and suspends active ingredients.

PEGGY GREB (K10141-1)

Gourmet vegan chocolates made with Nutrim, an ARS-developed product that can substitute for dairy products in baking.

©DONN JONES/TITANS

Tifsport, a tough turfgrass developed by ARS, is used for sports fields by some high schools, universities, recreation departments, golf courses, and professional sports teams. Here, the Tennessee Titans play the Washington Redskins on Tifsport in their Nashville stadium.

Salad dressings, instant soups, chocolate sauces, ice cream, cake mixes, yogurt, and squeezable chewing gum are just a few of the food products that make use of xanthan gum. It is also in products like toothpastes, cosmetics, rust removers, water-based paints, and antidiarrheal medicines.

Green Side Up

ARS is not an acronym you think of in connection with the NFL, PGA, or even your neighborhood soccer field. But ARS' bermuda grass varieties are taking over recreation fields and golf courses, especially in the southern part of the country. Tifsport, developed and released in 1997 by ARS plant geneticist Wayne Hanna in Tifton, Georgia, has a whole team roster of beneficial traits.

"We managed to breed in all the important traits—excellent cold tolerance, uniform dark green color, aggressive establishment, improved pest tolerance, earlier spring green-up, and an ability to tolerate frequent lower mowing," Hanna explains. "But most importantly, Tifsport can stand up to the stress and demands of big-time sports, to the wear and tear of football and soccer cleats, to the punishment of baseball spikes, and to constant heavy foot traffic."

Professional sports teams, universities, high schools, and local recreation departments across the South have installed Tifsport on athletic fields. Texas A&M recently planted about 97,000 square feet of Tifsport turf to give the Aggie soccer team a new playing field.

The NFL Tennessee Titans football team installed Tifsport when Adelphia Coliseum was built. A league-wide player

Neem Me Up

ARS researchers developed an environmentally friendly alternative to synthetic pesticides from the oil of neem tree seeds that controls about 20 different plant pests and diseases. For home gardeners, it is sold under the Green Light Company name. "It appealed to the organic garden market from the first; now it is being well accepted by all types of home gardeners," says Bing McClellan, Green Light director of marketing.

SCOTT BAUER (K7222-13)

Lactose-free milk and milk products, for people who cannot digest dairy products.

survey in 2001 rated the coliseum as the fourth best playing surface in the National Football League.

"Tifsport has great leaf texture and good dark green color. But the best thing is that the grass stands up well to use; it shears rather than divots so it lasts longer," explains Terry Porch, sports field manager for the Titans.

Southern golfers not only play on fairways planted with Tifsport; many also putt on Tifeagle, a triploid bermuda grass variety Hanna released in 1999. At last count, there are Tifeagle greens in 14 states and 7 countries. Tifeagle can be mowed to one tenth of an inch daily to provide a smooth, fast putting surface. The previous standard for southern golf greens, Tifdwarf, also an ARS development, can only be mowed to three sixteenths of an inch.

KEITH WELLER (K3560-3)

The beautiful blooms of crape myrtle are now common in many U.S. gardens because of improved cold tolerance and disease resistance provided by ARS.

ARS has been responsible for the bermuda grass standards for southern sports fields, golf courses, commercial landscaping, and home lawns since grass breeder Glenn Burton started the program in 1952. "Golfers were playing on sand or at best rough common bermuda grasses before ARS developed the improved triploid hybrid ones," Hanna says.

Gardeners also benefit from ARS research. Breeding successes like camellias with better cold tolerance, American elms resistant to Dutch elm disease, and later flowering magnolias have given landscapers and gardeners important new choices.

One ARS advance consumers buy in droves every year are crape myrtles developed to resist powdery mildew in the South and colder temperature in the North, making the shrubs one of the country's most popular. "Within its current hardiness zone range—which is now pretty much Baltimore south because of ARS introductions—there is hardly a major landscape project today that doesn't include one or more crape myrtles. ARS changed the crape myrtle world," says Marc Byers of Byers Wholesale Nurseries of Huntsville, Alabama.

Byers sells tens of thousands of crape myrtles each year and a significant percentage are ARS-developed varieties. Natchez, released by ARS in 1978, continues to be the standard by which all other white crape myrtles are judged. Twenty-four years is a long time for any one woody plant variety to remain the standard for landscapers, Byers points out.

Permanent Research

You may even be wearing ARS research as you read this article. In 1958, wash-and-wear cotton clothes hit the consumer market. Since the 1950s, ARS scientists have played key roles in the research to develop durable press treatments, usually called permanent press, that give cotton and cotton blends wrinkle resistance. And durable press plays a key role in the continued popularity of cotton and cotton-blend fabrics.

"Trousers represent the largest single apparel market for cotton today, and I estimate that when we exclude jeans, more than half of all mens', boys', and ladies' trousers are made from durable press-treated fabrics," says Andrew Jordan, director, technical services for the National Cotton Council. "ARS' work was fundamental to putting together the technology."

Other ARS research on cotton has reached the consumer in the form of fiber-reactive dyestuff groups that allow cotton to be dyed more bright colors and better flame-retardant treatments, including new-on-the-market, fire-resistant cotton carpet.—**J. Kim Kaplan,** ARS.

To reach people mentioned in this article, contact Kim Kaplan, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5128; phone (301) 504-1637, fax (301) 504-1648, e-mail kaplan@ars.usda.gov. ★

Scientists Seek To Sanitize Fruits and Vegetables

PEGGY GREB (K9821-1)

Engineering technician Andrew Cuthbert (foreground) gathers apples exiting a flatbed brush washer within a containment chamber in ERRC's new biosafety level-2 pilot plant. Behind him, mechanical engineer Joseph Sites (middle) and food microbiologist Bassam Annous operate the equipment.

Agricultural advances, improved distribution, and increased imports have made it possible for people in every state to enjoy fresh fruits and vegetables year-round. Americans know the health benefits of a diet high in produce and are taking advantage of this increased availability and variety.

But occasionally, raw produce can become contaminated with disease-causing organisms such as *Escherichia coli* O157:H7, *Salmonella,* or *Listeria monocytogenes*. Although these instances are relatively uncommon, media reports and public awareness in the last few years have increased because scientists and public health agencies have become better at detecting, reporting, and determining causes of foodborne illness.

When produce is being grown, harvested, packed, and shipped, it can pick up dust, soil, microorganisms, or chemical contaminants. Consumers should wash fruits and vegetables to remove those substances from surfaces.

A Clean Break

Whatever the source of contamination, more than one solution may be needed. One area of concern is that conventional washing methods remove or kill only between 90 and 99 percent of bacteria attached to the surfaces of produce. Scientists at the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, are searching for practical ways to overcome these current limitations.

The scientists are conducting experiments on commercial-type produce washing and sanitizing equipment to reduce bacterial populations on fresh and minimally processed fruits and vegetables. The tests use produce artificially contaminated with harmless bacteria similar in behavior to disease-causing organisms. This research is being conducted in a unique BSL-2 (short for biosafety level 2) pilot plant at the ERRC, says Gerald M. Sapers, a food technologist with ERRC's Food Safety Interventions Research Unit.

Future studies will be carried out on produce contaminated with actual disease-causing organisms within a containment structure equipped with its own steam decontamination system. A small-scale prototype was designed, built, and validated by a team of scientists and engineers from Pennsylvania State University and ERRC.

"Early tests with the prototype containment system have been very successful, and installation of a full-scale unit at ERRC is nearing completion," Sapers says.

Paul Walker, a professor of agricultural and biological engineering at Penn State, came to ERRC on a 15-month sabbatical to design, build, and evaluate research-grade produce-washing equipment for the BSL-2 pilot plant as part of a joint venture between Penn State and ARS, says Sapers. Walker oversaw the design and construction of the prototype and full-scale containment systems as well as the newly designed, commercial-scale washing equipment the lab received from Penn State. This equipment is operated by computer and permits precise control of all experimental variables. Joseph Sites, an ERRC mechanical engineer, manages the pilot plant, designs equipment, and conducts experiments.

Sapers and food microbiologist Bassam Annous are developing new washing and sanitizing treatments in the laboratory and then testing them in the pilot plant. Current trials are performed primarily with apples and cantaloupes, using nonpathogenic surrogates for human pathogens.

Denise Riordan, a former research associate, and Annous compared nonpathogenic strains of *E. coli* with harmful

strains to find surrogates with similar traits.

ARS uses pilot plant programs to serve as a bridge between invention and commercialization. Industries form partnerships with ARS scientists to further evaluate research that shows promise in the laboratory. Effective technology can then be transferred to produce packing and processing industries.

Sapers says that the one-of-a-kind equipment developed in this program allows improvements in conventional methods as well as novel approaches.

"The equipment must be suitable for use in a commercial produce packing or processing facility," he explains.

One of the major problems that the ERRC team is addressing is the ability of bacteria to attach firmly to produce surfaces, often in inaccessible locations, and survive conventional washing and sanitizing methods.

Packinghouses use chlorine and other sanitizers to reduce microbe levels, but conventional sanitizers are not able to penetrate skin crevices, creases, or pockets to destroy pathogens very effectively. The trick is to find an agent that will reach the pathogens without damaging the appearance and texture of the product.

Is the Solution in the Solution?

Scientists at ERRC have confirmed the limited capabilities of conventional washing methods, and are now looking for methods that increase the safety of produce while keeping the sensory qualities consumers expect in their fruits and vegetables. Their results with experimental sanitizing treatments have been mixed.

Washing apples in a brush washer, even when the apples were sprayed with very hot water, was found to be ineffective. Sapers says that total immersion in a sanitizing solution is superior to brush washing. However, he cautions that improper use of this "dump tank" method for washing can lead to cross-contamination of the submerged produce.

Experimental hydrogen peroxide and hot water treatments were applied to apples in a dip tank at different temperatures. Temperatures exceeding 60˚C could not be used without causing discoloration. While such treatments were able to eliminate up to 99.9 percent of the bacteria, they still did not achieve the total kill (99.999 percent) desired by the Food and Drug Administration.

Sapers says other experimental methods being studied involve steam treatments or application of sanitizer solutions under vacuum—for better surface penetration.

"This would be a new approach for commercial facilities," he said. "One challenge for researchers is to find ways to keep the speed of the processing line up to par, even when new sanitizing operations have been incorporated into the process."

Another method they are trying in Wyndmoor involves treating apples and other produce with acetic acid and hydrogen peroxide vapors. Sapers said there were large population reductions in inoculated apples with some vapor treatments but also some product discoloration. Work in this area is continuing.

Food technologist Gerald Sapers (left) and technician Donyel Jones evaluate apples after an antimicrobial wash treatment.

Yet another approach involves the use of an abrasive paste to grind pathogens off produce surfaces while being careful not to bruise or puncture the product.

The pilot plant program will become fully operational when the larger containment structure is in place, permitting the scientists to experiment with real pathogens.—By **Jim Core,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at http://www.nps.ars.usda.gov.

Gerald M. Sapers is in the USDA-ARS Food Safety Interventions Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038-8598; phone (215) 233-6417, fax, (215) 233-6406, e-mail gsapers@arserrc.gov. ◆

New Milling Methods Improve Corn Ethanol Production

A golden kernel of corn is a rich source of many food and industrial products, one of which is ethanol. Ethanol production in the United States grew from 175 million gallons in 1980 to a record 2.8 billion gallons in 2003. This boost in ethanol demand has created a significant new market for corn.

"The United States is producing more ethanol from corn and other domestic, renewable resources than ever before," says Kevin Hicks, research leader in ARS's Crop Conversion Science and Engineering Research Unit. "Almost 10 percent of the U.S. corn crop is used to make fuel ethanol. That's good for America's farmers. Ethanol is also good for the environment because its use reduces greenhouse gas emissions."

Hicks and colleagues at the ARS Eastern Regional Research Center (ERRC), in Wyndmoor, Pennsylvania, are developing new ways to reduce the costs of producing this important fuel and other corn products. They're also creating computer models to help researchers and ethanol producers estimate how different techniques might affect the bottom line.

STEPHEN AUSMUS (K11276-1)

Food technologist David Johnston (left) and research leader Kevin Hicks check fermentability of enzymatically milled corn.

Alternative Dry-Grind Techniques

The dry-grind process is the most common method used to produce fuel ethanol. In it, the whole corn kernel is ground and converted into ethanol. This method is relatively cost effective and requires less equipment than wet milling, which separates the fiber, germ (oil), and protein from the starch before it's fermented into ethanol. But the cost of making fuel ethanol must be lowered even further before ethanol can compete favorably with gasoline.

To further lower costs for the dry-grind process, Frank Taylor, a chemical engineer at ERRC, and Vijay Singh, a professor from the University of Illinois at Urbana-Champaign, developed and patented a process that permits low-cost recovery of nonfermentable corn components, such as germ and fiber. These can be sold as valuable co-products for food or feeds. The process treats corn kernels with anhydrous ammonia gas to loosen up the kernel components so they can be easily separated and recovered.

Taylor and colleagues also developed another modified dry-grind process called continuous fermentation with ethanol stripping. It allows more efficient production of ethanol in smaller, less expensive fermentors. (See "Bioenergy Today," *Agricultural Research*, April 2002, p. 4.)

Wet Milling Made Easier

In the wet milling process, corn is separated into its four basic components: starch, germ, fiber, and protein, which are each made into different products. The advantage of wet milling is that, besides ethanol, valuable co-products such as corn oil are also produced. The disadvantages are that the equipment is expensive and the process uses hazardous sulfur dioxide.

During conventional wet milling, corn is steeped for 24 to 36 hours in water and sulfur dioxide to begin the separation of the starch and protein connections. Then the corn is coarsely ground to break the germ loose from other kernel components. Later, the starch is separated out and converted into sweeteners or ethanol.

ERRC food technologist David B. Johnston and Singh developed a faster, cheaper way to break down the starch and protein connections. They are using several commercially available protease enzymes to do the job. This process requires much less sulfur dioxide during the steeping stage of wet milling.

The new method includes a 6-hour soaking in water of the corn kernels before milling. After about 3 hours of soaking, the enzymes are added. Then normal wet-milling steps are resumed.

Johnston says that in laboratory and pilot-scale trials, the enzyme method separated starch and proteins faster and yielded starch equal to or greater than the conventional process.

"With sulfur dioxide, there are regulations on emissions, and costlier equipment for processing is required," Johnston says. "Protease enzymes are also expensive, but we can lessen use of harmful materials in milling and produce the starch more quickly, using less energy."

Predicting Costs

So, how might innovations like these affect the ethanol producer's bottom line? The Wyndmoor scientists recently completed several computer models to help answer that question.

Andy McAloon, head cost engineer of the Process and Cost Simulation Group, says the models estimate the cost per gallon to produce ethanol with various processes. The models, which run on Aspen Plus software, are based on data from commercial plants and current costs for equipment, materials, labor, supplies, grain, and utilities.

McAloon and chemical engineer Winnie Yee worked with researchers to create the models, which analyze how new methods affect ethanol production costs. They provide support to scientists and engineers to determine research directions and predict the costs of possible alternatives to standard industry practices.

"These models can save researchers and producers time and money by simulating the many variables involved in production instead of having to actually go through the complete process each time they want to adjust a system," McAloon says.

The scientists have just released what they believe is the first publicly available corn wet milling process and cost model. It was developed by McAloon, Yee, and food technologist David Johnston, with cooperation by the Corn Refiners Association. Johnston and co-workers plan to use it extensively in their work with protease enzymes.

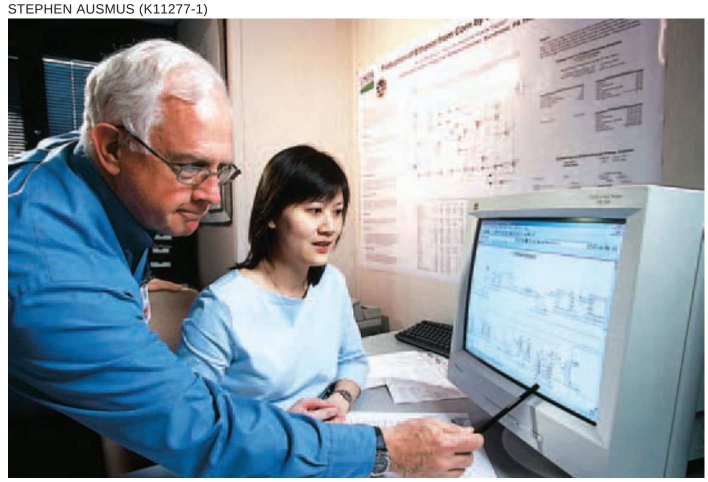
Another model, made by Yee, McAloon, and Taylor, helps estimate costs for making ethanol by dry grind processes. For example, they showed that the ARS-developed continuous fermentation and stripping procedure could save 3 cents per gallon. Users can examine countless other possibilities—such as reclaiming wasted heat, converting some of the fiber to ethanol, or pulling out the germ before fermentation—to see how they would affect the cost of making a gallon of ethanol.

The researchers recently updated their 25-million-gallon-a-year model for dry-grind ethanol producers to a 40-million-gallon version, which is available to anyone doing research or in the initial phases of building a plant.—By **Jim Core,** ARS.

This research is part of Quality and Utilization of Agricultural Products (#306) and Bioenergy and Energy Alternatives (#307), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

Kevin B. Hicks is in the USDA-ARS Crop Conversion Science and Engineering Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038; phone (215) 233-6579, fax (215) 233-6406, e-mail khicks@errc.ars.usda.gov. ★

STEPHEN AUSMUS (K11277-1)



Using a new computer model, cost engineer Andy McAloon and chemical engineer Winnie Yee discuss different ethanol production techniques and how they might affect costs of producing the fuel.

STEPHEN AUSMUS (K11278-3)

Chemical engineer Frank Taylor prepares to test fermentability of corn that has been pretreated with a new "ammoniation" process.

High-tech tests—simpler, cheaper, faster.

New Detection Methods Improve Food Safety

By developing new detection techniques and processes that incorporate principles from many different scientific disciplines, Agricultural Research Service scientists in Wyndmoor, Pennsylvania, are improving food safety.

Regulatory agencies and the food industry need fast, automated, cost-effective analytical methods that are accurate, reliable, and safe and that minimize waste. Several of the research unit's chemists are using advanced technologies to develop methods to screen, detect, and confirm multiple chemical residues—such as veterinary drugs and pesticides—and pathogenic bacteria and their toxins in food products.

Shu-I Tu, research leader of the Microbial Biophysics and Residue Chemistry Research Unit at the Eastern Regional Research Center, oversees development of such techniques. He says the laboratory works with state-of-the-art scientific instrumentation and biosensor-based methods that can detect chemical signals and provide information about specific biological activities.

"Many procedures we use have been modified from diagnostic systems used for medical analysis," Tu says. "We are taking several different approaches to prevent distribution of contaminated foods."

One method they use, fluorescence spectroscopy, involves recording optical spectra from molecules absorbing and emitting light. There are several ways to attach highly fluorescent probes to make biological targets—such as pathogenic bacteria—glow. Fluorescence spectroscopic methods, which rely on monitoring changes in the wavelengths and intensity of the signal, have been used for decades. Researchers use changes in the emission spectrum to achieve valuable insight into the concentration and behavior of the emitting molecules.

Detecting low levels of protein or DNA targets in or on a sample is sometimes difficult and prone to errors because specific fluorescence signals can be low or masked by interferences. One approach to improve detection and isolate the desired spectroscopic signal is to use what's known as time-resolved fluorescence (TRF) and luminescence (TRL) reagents. Time-resolved spectroscopic techniques help reduce background noise and increase sensitivity.

Scientists Monitor Food for Residues

Some veterinary drugs are not approved for use in food-producing animals. Others, such as beta-lactam antibiotics, have tolerance levels—maximum amounts of a given chemical or its breakdown products allowed to remain in or on food commodities—established by the U.S. Food and Drug Administration (FDA). Time-consuming and laborious methods are currently used for regulatory purposes to ensure that these drugs are not present in samples.

Chemist Guoying Chen has developed a prototype of a portable, suitcase-sized device to detect contaminants, such as tetracycline antibiotics, in meat, milk, and fish. The 25-pound prototype was designed for regulatory-agency investigators to take directly to a site for field analysis. Its user-friendly custom software is already completed and will run in a Microsoft Windows environment.

The filter-based fluorometer uses TRL to detect trace amounts of target chemicals by removing interference from fluorescent background signals given off by other organic substances present in a meat sample. The system requires 1-5 g of meat from which to extract the antibiotics present and concentrate them into liquid solution. Testing can be conducted on site and results provided on the spot. A quick change of filters

STEPHEN AUSMUS (K11684-1)

Chemist Steve Lehotay prepares extracts of fruits and vegetables for analysis of pesticide residues.

allows the user to move from one targeted drug to another. The device is capable of analyzing key antibiotics in chicken and beef at slaughterhouses, and it could be used to check for contaminants in liquids, such as milk, water, or urine.

Marilyn Schneider, another chemist in the research unit, is interested in analyzing animal-derived foods for veterinary drug residues. She is developing assays using natural fluorescence, as well as TRF, to screen for the presence of fluorescent antibiotics. Schneider takes advantage of the fluorescence of a fluoroquinolone antibiotic called enrofloxacin to first screen muscle extracts for its presence. After two reagents are added to this solution, the same sample can be screened for tetracycline antibiotics, eliminating the need for a separate extraction.

QuEChERS stands for "quick, easy, cheap, effective, rugged, and safe."

In another approach, Schneider and Chen use a buffer to extract samples from chicken, clean the samples by solid phase extraction, add TRL reagents, and then measure the samples for tetracyclines using TRL.

"These screening assays are alternatives to biological assays used by regulatory agencies," Schneider says. "The fluorescence assay is very rapid and detects the drugs when they are at or above the tolerance level. TRL methods require more cleanup, but have greater sensitivity."

Chemist Steven Lehotay, along with a previously visiting scientist, Michelangelo Anastassiades, developed the QuEChERS method, which stands for "quick, easy, cheap, effective, rugged, and safe" and is pronounced "catchers." The streamlined approach makes it easier and less expensive for analytical chemists to examine fruits and vegetables for pesticide residues. Lehotay says the method reduces procedural steps—and that lessens the chance for a mistake. A single, easy-to-clean Teflon tube is the only item to be washed and reused, eliminating all the glassware used in conventional methods. Furthermore, less than 10 mL of solvent waste is generated—much less than the 75-450 mL generated by other methods.

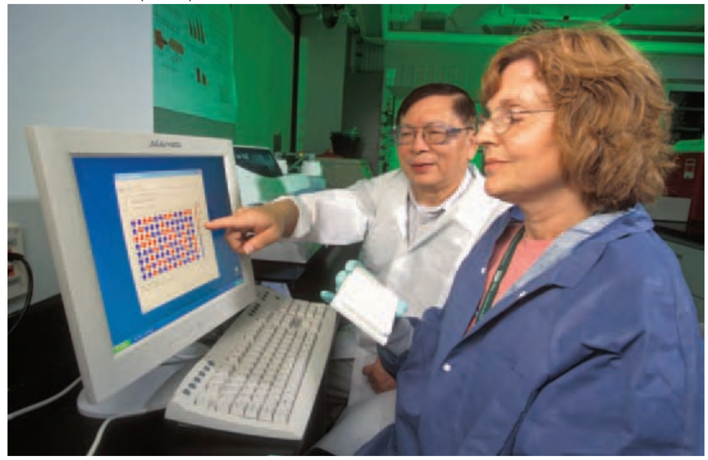
"Several monitoring laboratories, including a few in FDA, are evaluating QuEChERS for use in routine monitoring and other approaches designed to safeguard the food supply," Lehotay says. He and colleagues are now working to adapt the concept to analyze meats for veterinary drugs.

Chemist Keith Fagerquist, along with Lehotay and chemist Alan Lightfield, developed a method to measure and confirm beta-lactam antibiotics in pork and cattle kidney tissue. The method—which uses liquid chromatography/tandem mass spectrometry—is fast and looks for multiple residues in tissue samples, where antibiotics tend to concentrate the most.

Methods Detect Pathogens and Toxins in Food

Antibodies are protein molecules that bind to antigens—such as bacteria—and remove them from the body. Researchers can use antibodies to isolate pathogens or chemicals in food products as well.

STEPHEN AUSMUS (D009-1)



To detect *E. coli* O157:H7 in foods, chemist Shu-I Tu and microbiologist Marsha Golden use immunomagnetic capture and time-resolved fluorescence.

STEPHEN AUSMUS (K11685-1)

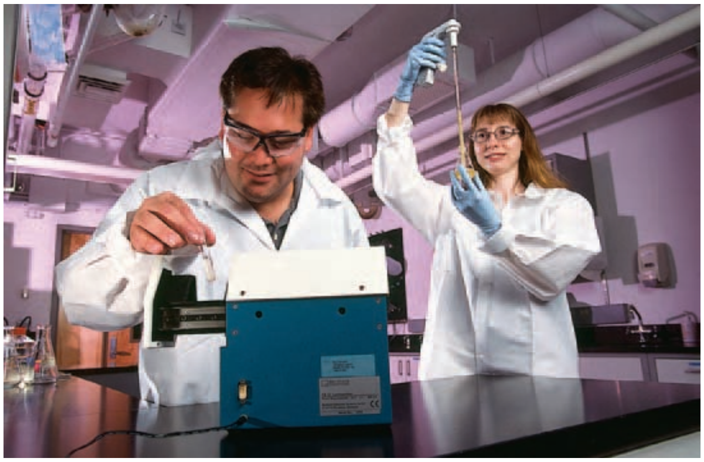
Chemist Marjorie Medina prepares egg samples for analysis of *Staphylococcus aureus* enterotoxins by surface plasmon resonance.

Andrew Gehring, another chemist in the unit, is working with Tu on a procedure that combines immunomagnetic capture with TRF to simultaneously detect *Escherichia coli* and *Salmonella* in ground beef, ground turkey, alfalfa sprouts, and seeds. The procedure uses magnetic beads that are coated with pathogen-specific antibodies. The antibodies bind to the bacteria, and the magnetism pulls them out of complex mixtures of food. Once extracted, the bacteria can be more easily detected.

Gehring is also developing a luminescence-based method coupled with an ELISA (enzyme-linked immunosorbent assay) to detect and confirm *E. coli* O157:H7. An ELISA is a sensitive laboratory test that uses antibodies and enzymes to detect and measure specific antigens in samples. Gehring's test can be completed in 8 hours and can detect 1-10 bacteria per gram of ground meat. USDA's Food Safety Inspection Service (FSIS) would ultimately like to be able to detect 1 bacterium in 25 grams of meat.

The search for fast, inexpensive analytical food safety methods that are accurate, reliable, and safe.

STEPHEN AUSMUS (K11687-1)

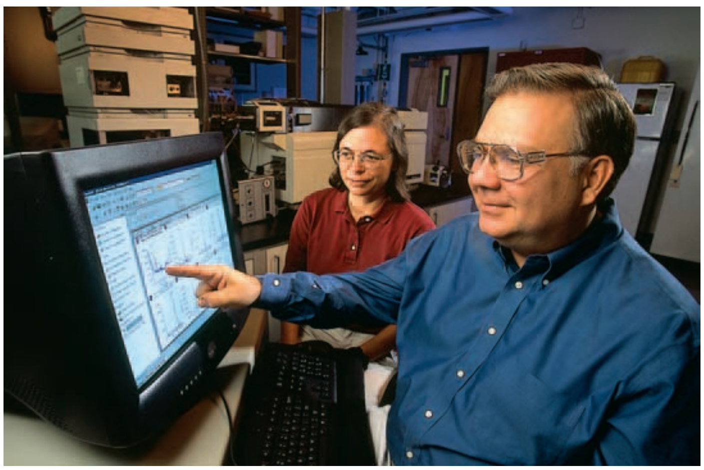


Chemist Andy Gehring inserts a meat sample into a luminometer to check for the presence of bacteria as technician Sue Reed prepares other samples for analysis.

STEPHEN AUSMUS (K11688-1)

The tube held by technician Sheri Mazenko contains concentrated immunomagnetic beads coated with antibodies that bind to specific bacteria, making it possible to detect contaminants in food.

STEPHEN AUSMUS (K11686-1)



Chemists Marilyn Schneider and Alan Lightfield analyze veterinary drug data from the liquid chromatograph/tandem mass spectrometer.

STEPHEN AUSMUS (K11689-1)

Chemist Guoying Chen tests a portable fluorometer he designed and built to screen for drug residues in food extracts.

Chemist Marjorie Medina developed a biosensor immunoassay using surface plasmon resonance (SPR) to detect *Staphylococcus aureus* enterotoxin A (SEA) and B (SEB)—toxins that cause gastroenteritis—in foods such as ham, milk, and eggs. Conventional heating and processing kills the bacterium but not its toxins. Bacteria produce toxins under stressful conditions, such as when they are too crowded or denied food or when they're fighting back against antibiotics.

"SPR uses light reflected off thin metal films," Medina explains. "Toxin molecules in the sample bind to the sensor surface, and the refractive index at the surface changes. The time it takes for a response from the interaction provides a measure of how much toxin, if any, is actually present in the food sample."

Medina says that FSIS is interested in an alternative to the conventional method to detect enterotoxins in whole eggs. Her semi-automated method has several advantages over other methods and may detect multiple bacterial toxins in a single food sample.

Medina also developed a latex particle agglutination assay for detection of SEA and SEB that causes the toxins to clump together. The method takes advantage of an antibody's ability to bind to a unique antigen in pathogen cells. The assay is simpler to use than other methods and can detect as little as 10 parts per billion of toxin per gram of sample.—By **Jim Core,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

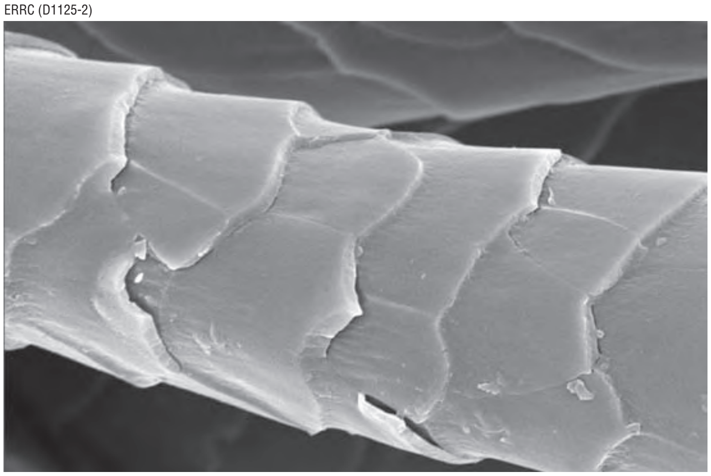
Shu-I Tu is in the USDA-ARS Microbial Biophysics and Residue Chemistry Research Unit, Eastern Regional Research Center, 600 East Mermaid Ln., Wyndmoor, PA 19038-8598; phone (215) 233-6466, fax (215) 233-6581, e-mail stu@errc.ars.usda.gov. ★

Protecting U.S. Troops With Fireproof Wool

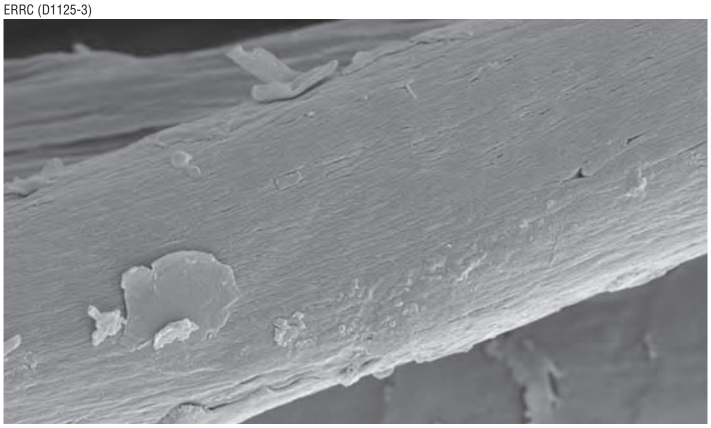
ERRC (D1125-1)

Biopolished wool, developed at the ARS Eastern Regional Research Center in Wyndmoor, Pennsylvania, is displayed as an army neck gator. A new ARS-developed polymer can be added to this fabric to make it flame retardant.

ERRC (D1125-2)



ERRC (D1125-3)



Scanning electron micrographs of untreated wool fiber (top) and wool fiber treated by the ARS biopolishing, shrinkproofing method (bottom). Magnified about 2,500x.

Though natural, untreated wool is scratchier than synthetic fabrics, wool is less susceptible to burning. This makes it an ideal fabric for uniforms worn by members of the military, firefighters, and others whose occupations expose them to fire.

"Wool burns with a self-extinguishing flame and produces a soft ash that dissipates and will not lodge in open wounds," says Jeanette M. Cardamone, a chemist at the ARS Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania. "Synthetic materials, on the other hand, form hot, molten beads that can drip into a wound and cause trauma."

In ERRC's Fats, Oils, and Animal Coproducts Research Unit, Cardamone has discovered and patented a heat-resistant material that can be incorporated into wool and other fabrics to match the flame resistance of commercial firefighters' uniforms. The material was developed at the request of the U.S. military—one of the largest markets for domestic wool—to offer U.S. troops protection against fire-related injuries.

In an earlier project, Cardamone developed biopolished wool that is both machine washable and itch free. Nine companies have expressed interest in licensing the patent for this technology, and the ARS Office of Technology Transfer has issued two licenses for it. This wool already has many desirable properties, so it's a natural choice for developing a fabric with improved flame retardancy.

Working with visiting scientist Anand Kanchagar, Cardamone improved the flame retardancy of the biopolished wool by treating it with a heat-resistant polymer that is stable, easy to process, and highly tolerant of extreme temperatures. Unlike some popular flame retardants, the ARS material does not use the heavy metal zirconium, which can present a health hazard during processing. While natural, synthetic, and blended fibers can be treated with the polymer, wool is particularly suitable because of its innate fire resistance.

Early tests show that the burning behavior of the polymer-treated ARS wool compares to a 50/50 blend of wool and Nomex—the fabric currently used in protective firefighting gear.

The scientists are experimenting with different methods to enhance the wool's heat-resistant and flame-retardant properties.

Cardamone says that the flame-retardant treatment should be durable to laundering.

Increasing the value and versatility of domestic wool would benefit the U.S. sheep industry, which produces about 40 million pounds of raw wool a year. In addition, U.S. consumers—including the U.S. military and many law-enforcement agencies, which are required to use domestic wool in their uniforms and equipment—would enjoy the benefits of home-grown, soft, shrinkproof, fire-retardant wool.—By **Laura McGinnis,** ARS.

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

Jeanette M. Cardamone is in the USDA-ARS Fats, Oils, and Animal Coproducts Research Unit, Eastern Regional Research Center, 600 E. Mermaid Ln., Wyndmoor, PA 19038-8598; phone (215) 233-6680, fax (215) 233-6795, e-mail jan.cardamone@ars.usda.gov. ✷

ARS Continues Advances in

Irradiation

of Ready-To-Eat Foods

We are surrounded by radiation. It's a natural part of the electromagnetic spectrum, and we've harnessed its power for our benefit.

Ionizing radiation is widely used around the world today as a safe and effective nonthermal way to pasteurize fruits and vegetables, ground beef, poultry, and spices. Agricultural Research Service scientists in Wyndmoor, Pennsylvania, are discovering more about how this valuable technology can be used to improve food safety.

Whether from gamma rays, electron beams, or x-rays, irradiation inactivates bacteria such as *Escherichia coli* O157:H7, *Salmonella, Listeria,* and other microorganisms and parasites that cause some 250 types of foodborne illnesses. Symptoms of infection can include nausea, vomiting, abdominal cramps, and diarrhea.

Irradiation also inactivates food-spoilage organisms, including bacteria, molds, and yeasts. Irradiation modifies the genes and cell membranes of microorganisms, killing or severely injuring them. It can lengthen the shelf life of fresh fruits and vegetables by delaying ripening and inhibiting sprouting.

Irradiation's effectiveness against food-spoilage organisms was first shown in France in the 1920s. In the mid-1960s, the U.S. Food and Drug Administration (FDA) approved use of low-dose ionizing radiation for killing or sterilizing insects in wheat and wheat flour and for inhibiting sprouting in potatoes. In 1983, FDA approved using irradiation to sterilize spices.

In 1981, research programs for food irradiation were transferred from the U.S. Army to USDA's Eastern Regional Research Center (ERRC) in Wyndmoor. Since then, ERRC scientists have conducted genetic toxicology testing of irradiated foods and supervised completion of the Raltech Study, the most complete and comprehensive experiments ever conducted on the toxicological safety of irradiated foods. These studies found no increased risk of cancer, birth defects, or other harm from consumption of irradiated foods.

Thanks in part to ERRC research efforts, FDA approved irradiation of poultry in 1990 and of fresh and frozen red meats, including beef, lamb, and pork, in 1997. ERRC research also established that ionizing radiation could inactivate *E. coli* O157:H7 in red meat and poultry. Irradiated ground beef and poultry are now sold in more than 4,000 supermarkets across the United States.

The 2002 Farm Bill allowed, on a voluntary basis, irradiated ground beef into the National School Lunch Program, which USDA oversees. In 2003, Xuetong Fan, a food technologist at ERRC, led taste panels where participants were asked to evaluate various sensory characteristics of irradiated meats in comparison with untreated products. The project was started to support two USDA agencies, the Food and Nutrition Service and the Agricultural Marketing Service, in their efforts to assure parents and school leaders of the continued quality of school lunches. States were permitted to offer school districts irradiated ground beef beginning in January 2004.

STEPHEN AUSMUS (K11710-1)

So that study panelists focus only on taste, flavor, and texture, food technologist Xuetong Fan presents samples in a specially lit room that helps mask visual differences between samples. This study evaluates sensory properties of irradiated ground beef for the National School Lunch Program.

Microbiologist Christopher H. Sommers is the lead scientist for food irradiation in ERRC's Food Safety Intervention Technologies Research Unit. His research is directed at improving the safety and shelf life of raw meat and ready-to-eat (RTE) meat products. Sommers has done extensive work with ionizing radiation to eliminate *Listeria monocytogenes* from RTE meats.

"Although RTE meats are already cooked, FDA and the meat industry recommend reheating for some products, because they can become contaminated between cooking and packaging. Pathogenic bacteria such as *L. monocytogenes*, if present on the product, can sometimes multiply during refrigerated storage," Sommers says. FDA is reviewing, but has not yet approved, irradiation for RTE meats such as hotdogs, bologna, and deli turkey and ham.

Currently, the RTE industry uses thermal and other nonthermal techniques to inactivate, if not completely kill, microorganisms. But those treatments don't always prevent subsequent growth of bacteria under refrigeration. At least 90 million pounds of RTE meat products have been recalled since 1998 due to *L. monocytogenes* contamination. Sommers believes that for ground meats and some RTE products, irradiation may be the only effective means of treatment, since it can be used after packaging.

The radiation resistance of *L. monocytogenes* and other pathogens depends on the product's formulation and the genetic characteristics of the contaminating strain. Sommers has established the radiation doses needed to inactivate *L. monocytogenes* in a variety of RTE meat products, and he's determined the radiation resistance of *L. monocytogenes* strains associated with foodborne illness.

Sommers has also completed studies on the roles of food additives in inhibiting growth of injured pathogens in food products during long-term refrigerated storage.

Food additives are commonly used to extend the shelf life of meat products. Sommers says certain ones make *Listeria* more sensitive to radiation. For instance, he determined that a mixture of salts of acetic acid (vinegar) and lactic acid in bologna formulations decreased the radiation dose needed to inactivate 99.999 percent of *L. monocytogenes* inoculated onto the meat from 3.0 to 2.5 kiloGrays (kGy). The combined treatments also prevented growth of spoilage microorganisms for 2 months. "Ionizing radiation, when combined with common food additives, has the potential to significantly reduce the incidence of listeriosis associated with consumption of RTE meats in the United States," Sommers says.

Fan and colleagues demonstrated that irradiation promoted production of several volatile sulfur compounds associated with unpleasant odors that can sometimes occur in irradiated or overcooked RTE turkey meat. Adding antioxidants to RTE formulations had a very limited effect in preventing production of the compounds. In additional work with Fan and A.P. Handel from Drexel University in Philadelphia, Sommers found that the combination of common additives and mild heating reduced levels of sulfur compounds in RTE turkey meat by more than 50 percent compared to irradiation alone.

Sommers collaborates with colleagues at ERRC in related research, including irradiation of foodborne pathogens in fruits, vegetables, and juices. He's also examining ways to control other major pathogens, such as *E. coli*, *Salmonella*, and *Yersinia*, in other meat products. *Yersinia* infection most often occurs from eating raw or undercooked pork products, such as chitterlings. *Y. enterocolitica* is of great concern to researchers

STEPHEN AUSMUS (K11708-1)

At ERRC, microbiologist Glenn Boyd places a batch of hotdogs into the gamma radiation source to rid them of foodborne pathogens.

and the meat industry because, like *L. monocytogenes*, it is capable of growing at refrigeration temperatures and in high-salt environments.

To determine the radiation resistance of *Y. enterocolitica* in different commercial practices, Sommers and co-researchers studied the pathogen in ground pork at different temperatures. They were particularly interested in the radiation dose needed to control *Y. enterocolitica* in subfreezing temperatures. There are fewer undesirable effects on some physical characteristics, such as texture and color, of meat products at subfreezing temperatures than those irradiated at refrigeration temperatures at the same dose. But it takes a larger dose to pasteurize frozen meat than nonfrozen meat. They were able to determine the range of radiation doses required to treat the same product at different temperatures—as commercial processors do.

Research on Fruits and Vegetables Improves Safety and Quality

Brendan A. Niemira, a microbiologist and plant pathologist at ERRC, focuses on methods to irradiate fruits, vegetables, juices, and meat substitutes of vegetable origin, such as soy. He is interested in finding the ideal dose of irradiation that increases safety but does not affect a given product's quality.

Since late 2002, government regulations have permitted use of irradiation on imported fruits and vegetables to meet phytosanitary quarantine requirements, which refers to controlling organisms that affect plant health. Higher radiation doses could be used to inactivate foodborne pathogenic bacteria as well. The National Food Processors Association has petitioned FDA to allow the higher doses on fruits and vegetables. FDA is currently evaluating the petition.

Grapes, for instance, have recently been suspected in *Salmonella* outbreaks. Niemira and Fan tested red and white whole grapes and their juices after purposely inoculating them with the pathogen. The textures of both grapes were not significantly affected by irradiation doses of up to 1 kGy—the maximum dose approved for fresh fruits and vegetables. Although the color of red grapes and red grape juice was influenced by treatments, white grapes and white grape juice held up very well.

"These results demonstrate that each product responds differently to irradiation and has to be treated differently to ensure it remains appealing to the consumer," Niemira says.

Niemira also recently studied the effectiveness of gamma irradiation in inactivating *Listeria* and *Salmonella* placed on the surface of lettuce. He studied four closely related but distinct types of lettuce: Boston (butterhead), iceberg (crisphead), green leaf, and red leaf (variants of looseleaf). The studies showed that the radiation sensitivity of *L. monocytogenes* was similar on the four lettuce types, but the sensitivity of *Salmonella* was slightly different. Complexity of the leaf surface was the underlying factor. Although irradiation was effective at reducing pathogen numbers, subtle differences between lettuce types influenced the sensitivity of bacteria present on their surfaces. Niemira says radiation doses would have to be tailored for each type of lettuce to be effective.

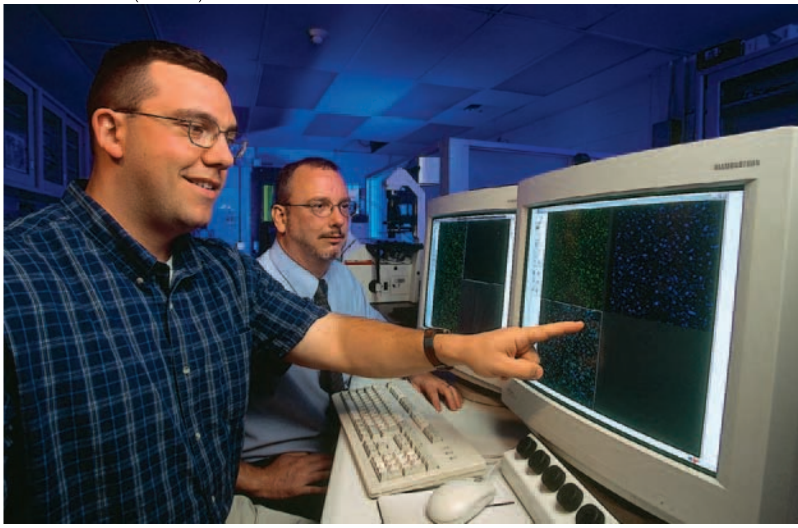
The key to using irradiation is to eliminate foodborne pathogens without affecting product quality. Fan wants to assess the extent of irradiation's effect on product quality and to develop novel ways to minimize any adverse effects. He examines factors such as chemical composition, nutritional qualities, aroma, flavor, and texture in a variety of foods.

Major quality changes in some vegetables appeared to be tissue browning and decreased shelf life from loss of tissue integrity, which, in some instances, gives a soggy appearance. Because fruits and vegetables contain so much water, they are especially susceptible to the effects of irradiation and other nonthermal treatments.

STEPHEN AUSMUS (K11706-1)

Molecular biologist Chris Sommers (left) and microbiologist Glenn Boyd vacuum-seal hotdogs to get them ready for irradiation.

STEPHEN AUSMUS (K11707-1)



Food technologist Ethan Solomon (left) and Chris Sommers examine fluorescent micrographs of bacteria suspended in biofilms exposed to ionizing radiation.

Fan found that irradiation and heat pasteurization of apple and orange juice led to increased levels of several volatile sulfur compounds and aldehydes. Studies have shown that compounds such as aldehydes might contribute to off-odors in heated and irradiated foods. Fan believes conducting the treatments at lower temperatures, adding antioxidants, and combining irradiation with other treatments could help reduce undesirable quality effects on juices.

Fan teamed up with collaborators to develop a method to treat sliced apples used in fruit salads. They were interested in reducing tissue browning, which happens when plant tissue is cut during processing. They found that dipping apple slices into a calcium ascorbate (a form of vitamin C) solution before exposure to a low-dose gamma radiation treatment improved microbiological safety and inhibited tissue browning.

Fruits and vegetables are rich in antioxidants, which help protect us against cancer and heart disease. These beneficial effects are partially due to high amounts of phenolic compounds. Fresh and fresh-cut fruits and vegetables are living organisms, capable of synthesizing novel antioxidants in response to stresses even after they are picked and processed. Fan showed that irradiated iceberg and romaine lettuce and endive developed a higher antioxidant capacity than nonirradiated vegetables during a postirradiation cold-storage period. The higher antioxidant capacity was mostly due to the increased content of phenolic compounds. Vitamin C content in the fresh-cut vegetables, however, was not significantly affected by irradiation doses as high as 1 kGy.

STEPHEN AUSMUS (K11709-1)

Using a texture analyzer, food technologist Xuetong Fan and chemist Kimberly Sokorai evaluate an apple before processing and irradiation treatments to ensure initial product quality.

"In addition to the beneficial effect of foodborne pathogen inactivation, irradiation of fresh-cut fruits and vegetables may result in a product with enhanced antioxidant capacity," Fan says.—By **Jim Core,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

Christopher H. Sommers, Brendan A. Niemira, and Xuetong Fan are with the USDA-ARS Food Safety Intervention Technologies Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038; phone (215) 836-3754 [Sommers], (215) 836-3784 [Niemera], (215) 836-3785 [Fan], fax (215) 233-6445, e-mail csommers@errc.ars.usda.gov, bniemira@errc.ars.usda.gov, xfan@errc.ars.usda.gov. ★

Poultry Pathogen *Models for Predictive Microbiology*

PEGGY GREB (K11896-1)

In a modeling study to predict growth of *Salmonella* on chicken, food technologist Thomas Oscar uses an automated counting system to count pathogen colonies (the black dots) on an agar medium.

Food technologist Thomas P. Oscar wants to make poultry as pathogen-free as possible. His research focuses on modeling growth and survival of *Salmonella* and *Campylobacter*—the two most prevalent bacterial food pathogens—on chicken, the most consumed meat product in the United States.

Oscar and technician Jacquelyn B. Ludwig are a two-person team at a research laboratory on Maryland's Eastern Shore. The ARS Poultry Food Safety Research Laboratory is part of the Microbial Food Safety Research Unit of the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania. But it's physically located on the University of Maryland Eastern Shore (UMES) campus in Princess Anne—in the heart of the Delmarva Peninsula, one of the country's leading poultry-producing regions.

1.4 million cases of salmonellosis food poisoning occur annually

Their research is part of a growing field—known as predictive microbiology—that forecasts the behavior of foodborne pathogens in response to environmental conditions encountered in food production and processing operations. Models predict how long it takes for pathogens to grow under certain conditions and how fast they grow once they start. Models help processors make proper decisions about food safety and help regulatory agencies and scientists learn more about the conditions in which pathogens, such as *Salmonella*, thrive.

An estimated 1.4 million cases of salmonellosis food poisoning occur annually in the United States, according to the Centers for Disease Control and Prevention, and about 10 percent of those cases are from poultry. Most people recover without treatment, but some get diarrhea so severe that they have to be hospitalized. If the infection spreads from the intestines to the bloodstream, it can cause death unless the patient is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are most at risk.

Real-World Competition

"One way to better assess food safety," Oscar says, "is to develop pathogen models that take into account real-life competition from other microbes present in food. These models would be more realistic, make better predictions, and help better ensure that the food industry doesn't needlessly dispose of safe food."

Previously, Oscar says, models were often developed in broth with no other microbes present. Researchers thought this would allow them to accurately predict pathogen behavior in food. Recent findings by Oscar and ERRC colleagues Mark L. Tamplin, microbiologist, and Andy Hwang, food technologist, indicate that this is not always the case.

The ARS researchers will produce more realistic models by using a system Oscar developed to rate performance of current models. Called the "acceptable prediction zone method," it establishes criteria for verifying and validating models as having acceptable or unacceptable predictive records, classifies them to show which are best, and then pinpoints any changes that are needed. Models are evaluated under specific conditions to find which ones meet established standards and demonstrate a level of performance that could be used with confidence in the food industry to predict food safety.

Oscar thinks most current broth models are "overly fail-safe," meaning they predict much higher pathogen numbers than would be present in real food with microbial competition. Still, he says, it's better to over-predict the growth rate of a pathogen than to under-predict it. His laboratory is working on two promising techniques to improve the models.

One method is to introduce a jellyfish gene into *Salmonella* to make the bacteria fluoresce, or glow, so they'll be easier to detect among other microorganisms on raw poultry. At first, the foreign DNA slowed *Salmonella* growth, making the models inaccurate. But Oscar and colleagues Dwayne Boucaud, assistant professor and microbiologist at UMES,

PEGGY GREB (K11900-1)

Technician Jacquelyn Ludwig grinds chicken in preparation for modeling experiments of *Salmonella*.

and Kalpana Dulal, a graduate student, are working on a new way of introducing the foreign DNA that will allow normal growth.

Oscar has had more immediate success with the second approach. He and his colleagues found that a strain of antibiotic-resistant *Salmonella* can be a valuable tool for modeling pathogen growth. *Salmonella typhimurium* DT104 is known to be resistant to five different antibiotics. The researchers put four of those five antibiotics in an agar medium they were using to determine the amount of *Salmonella* in a food sample. The antibiotics killed off competing microorganisms on the agar plates and left just the *Salmonella*. The scientists were able to follow the surviving *Salmonella* and model its growth on raw poultry meat, showing how it behaves in a real food environment. The DT104 strain helped confirm their theory that models developed in the absence of microbial competition do not accurately predict pathogen behavior in foods that contain other spoilage organisms.

"One of the biggest hurdles we face in developing models in food with other microorganisms has been not having a valid marker pathogen strain that we could follow in the presence of other microorganisms," Oscar says. "The DT104 strain, which exists in nature, has allowed us to jump over this hurdle and holds great promise for developing better *Salmonella* models for the food industry."

Oscar's study doesn't end with *Salmonella*. Along with Kisun Yoon, assistant professor at UMES, and graduate student Candace Burnette, he has new findings on *Campylobacter*, the leading cause of bacterial food poisoning worldwide.

The three scientists recently found that *C. jejuni* died faster at room temperatures than at refrigeration temperatures. The data collected in the study was used to develop one of the first predictive models for *C. jejuni*. The new model will be included in ERRC's Pathogen Modeling Program, a package of models available on the World Wide Web at www.arserrc.gov/mfs/pathogen.htm. The availability of these and other food safety models should accelerate use of models by food industries and researchers interested in the field of predictive microbiology.—By **Jim Core,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

Thomas P. Oscar is with the USDA-ARS Poultry Food Safety Research Laboratory, Room 2111, Center for Food Science and Technology, University of Maryland Eastern Shore, Princess Anne, MD 21853; phone (410) 651-6062, fax (410) 651-8498, e-mail toscar@errc.ars.usda.gov. ✷

PEGGY GREB (K11904-1)

Graduate student Kalpana Dulal (left) and microbiologist Dwayne Boucaud, of the University of Maryland Eastern Shore, examine expression of green fluorescent protein by *Salmonella* on agar medium.

Researchers Study Microbial Threats to Shellfish Safety

Shellfish can remind us of treasured times—going clam digging with the family, slurping down oysters on the half shell at a raw bar with friends, or shucking them yourself in the hopes of finding a pearl. Unfortunately, eating them is often discouraged today because of environmental contamination.

In hopes of reversing this trend, scientists at the Microbial Safety of Aquaculture Products Center of Excellence in Dover, Delaware, are conducting research to provide safer shellfish. A field location of the ARS Eastern Regional Research Center's Microbial Food Safety Research Unit in Wyndmoor, Pennsylvania, the Center of Excellence is a partnership between ARS and Delaware State University, one of the 1890's Historically Black Colleges and Universities. ARS's Centers of Excellence are intended to foster complementary research on problems of national and regional concerns and to enhance cooperative research. Established in 1999 on the university's campus, the Dover worksite is the only ARS laboratory studying bacterial and viral safety of shellfish.

> Mortality rates for immunocompromised people infected with *Vibrio vulnificus* exceed 50 percent.

According to Gary P. Richards, a microbiologist and the center's lead scientist, shellfish are filter feeders, so they concentrate pollutants, including human pathogens, from seawater.

"The laboratory focuses on developing rapid, cost-effective, and practical methods to detect microorganisms in oysters, clams, and mussels and is evaluating processing strategies to eliminate these potentially harmful pathogens," Richards says.

Oysters, clams, and mussels are considered aquaculture species because of the amount of management that goes into maintaining productive molluscan shellfish beds. These shellfish are a concern to Richards and his colleague, microbiologist David H. Kingsley, because bacterial and viral pathogens can become concentrated within edible shellfish tissues. Many types of viral and bacterial pathogens that grow in the gut of infected people may contaminate water and food. Shellfish live along the shore, where they are subject to contamination with pathogens from improperly treated municipal waste, leaking septic systems, floodwaters, runoff, or overboard discharge of boat wastes. They may also become contaminated by unsanitized hands and surfaces at harvesting, processing, and preparation facilities.

From a food-safety standpoint, three groups of pathogens are of greatest concern to the molluscan shellfish trade: The noroviruses (formerly known as the Norwalk-like viruses), hepatitis A virus, and *Vibrio* bacteria.

Noroviruses, the leading cause of nonbacterial gastrointestinal illness in the United States, have gained recent notoriety because of several outbreaks on cruise ships. There are an estimated 9.2 million cases of norovirus infection caused by food in the United States annually. Norovirus illness comes on rapidly, lasts only a few days, and is rarely life threatening.

The second agent is hepatitis A virus. It is far less prevalent than norovirus—about 23,000 cases of hepatitis A are reported each year in the United States. But this virus infects the liver and can be more serious than norovirus infection. Although many illnesses are mild and asymptomatic, some require hospitalization or lead to death. Contamination of shellfish beds by direct exposure to human fecal wastes can readily lead to hepatitis A and norovirus infections.

The third group of human pathogens are bacteria of the genus *Vibrio*. Perhaps the best-known *Vibrio* infection is cholera, which sickens many people in underdeveloped countries through contaminated food and water. In the United States, *V. cholerae* is not a problem, but two other vibrios are of concern: *V. vulnificus* and *V. parahaemolyticus*. These bacteria are naturally found in shellfish and seawater, particularly when water temperatures are warm.

"Consumption of *V. vulnificus*-contaminated oysters is not a problem for healthy individuals," says Richards, "but that's not the case for sick, elderly, or immunocompromised people, especially those with liver disease or diabetes. These groups should avoid eating raw shellfish because of the widespread presence of *V. vulnificus* in the marine environment."

Mortality rates for those who acquire a *V. vulnificus* infection exceed 50 percent, with rapid disease onset and death often within 3-4 days. This bacterium is also a flesh-eating organism, which can produce major disfigurement in those who survive infection.

The other *Vibrio* pathogen found in the United States is *V. parahaemolyticus,* which causes a gastrointestinal illness that is generally not life threatening. Illnesses from *V. parahaemolyticus* have resulted in the closure of shellfish beds on the Atlantic, Pacific, and Gulf coasts of the United States and have led to major economic hardships for the shellfish industry.

Bacterial Virulence Factors

Deaths from *V. vulnificus* continue to occur among immunocompromised oyster consumers. To better understand how these and related bacteria invade the human host, Richards focused

STEPHEN AUSMUS (K11763-1)

John Ewart (left), an aquaculture/fisheries specialist (University of Delaware), and ARS microbiologist Gary Richards examine freshly harvested oysters on board the Center for the Inland Bays' work boat before transport to the laboratory.

on identifying *Vibrio* enzymes that may enhance bacterial invasiveness. Leading a research group involving scientists from Delaware State University and the National Institutes of Health, Richards recently discovered and characterized an enzyme in *V. vulnificus* and identified it as phosphoglucose isomerase with a novel lysyl aminopeptidase activity.

The presence of this enzyme activity signifies a potential mechanism that may help the spread of *Vibrio*. He also detected the enzyme in virtually all species of *Vibrio* tested to date, but not in non-*Vibrio* pathogens. The enzyme is capable of metabolizing substances found in human tissues and the bloodstream. Such metabolism produces peptides that act on the blood vessels. These could account for the low blood pressure and rapid spread of *V. vulnificus*—a hallmark of *Vibrio* infection in humans.

Richards also developed a quick and simple enzyme-based assay that will allow vibrios to be readily detected in food, water, and clinical samples. This assay is being evaluated collaboratively with the Haskin Shellfish Research Laboratory, Rutgers University, in New Jersey to detect vibrios in oysters and seawater.

Virus Methods Development

Recently, Richards, working with the Centers for Disease Control and Prevention (CDC), developed a rapid method to detect a broad range of noroviruses. He combined a technique known as real-time reverse transcription-polymerase chain reaction (RT-PCR) with universal primers developed by CDC and was able to detect most norovirus types circulating in the world today.

According to Richards, "This method should be particularly useful to clinical, environmental, and food-testing laboratories, because, for the first time, analysts will be able to rapidly test for a wide spectrum of noroviruses in a single reaction tube."

Other recent research in this area involved detection of noroviruses in stools of individuals who had no symptoms of illness. The results suggest that healthcare workers and food handlers could unknowingly spread noroviruses in the workplace, and highlight the importance of good hygienic practices, particularly handwashing, to reduce the threat of food contamination and enteric virus illness.

Kingsley and Richards have also developed a way to extract viral RNA from shellfish. This method permits relatively rapid purification of hepatitis A virus and norovirus genetic material from within shellfish tissues. To detect the virus's genetic material, RT-PCR is then used to amplify the viral RNA. This extraction method is being evaluated by state, federal, and foreign laboratories to measure its performance, determine its cost-effectiveness, and consider it for possible adoption in virus-testing programs.

STEPHEN AUSMUS (K11765-1)

In studies to measure virus uptake and depletion rates, technician Gloria Meade inoculates a tank of oysters with hepatitis A virus.

Intervention Strategies

Some shellfish consumers prefer to eat shellfish raw or only lightly cooked, so the shellfish industry is interested in methods that can inactivate pathogens in their products without cooking. Kingsley is studying a way to sanitize raw shellfish and other virus-contaminated foods by using high-pressure processing (HPP), in which foods are subjected to extremely high pressure. The advantage of this technology is that no heat or chemicals are involved, permitting shellfish and other foods to retain their raw, uncooked flavor and character.

"HPP is being used commercially, for example, to pasteurize fruit juices in Japan and to treat sliced deli meats in Spain," Kingsley says.

In the United States, some in the oyster industry are already using HPP to facilitate shucking, eliminate *Vibrio* contamination, and extend product shelf life. These factors make HPP desirable to the processor and consumer, but the initial costs of the HPP units have prevented their widespread use.

Kingsley, working collaboratively with researchers from the U.S. Food and Drug Administration, tested the ability of HPP to inactivate hepatitis A virus from oysters. They found that 1-minute treatments of oysters greatly reduced hepatitis A virus populations.

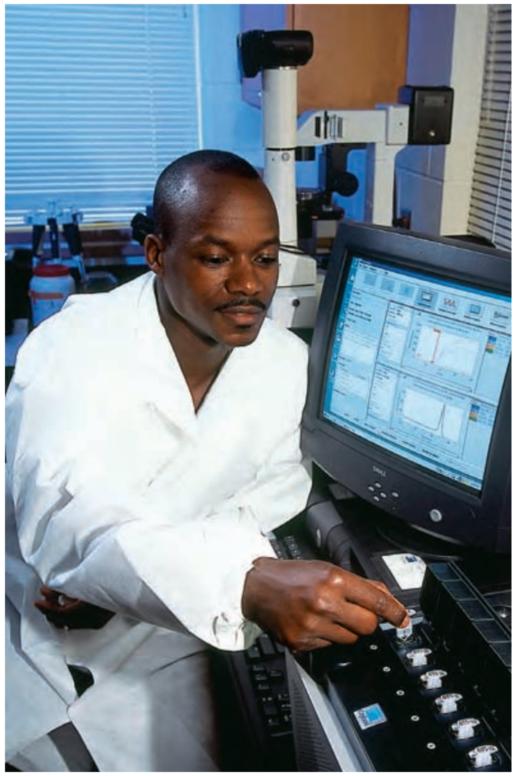
Since it is not currently possible to replicate norovirus in the laboratory, Kingsley and Richards, in collaboration with researchers at the University of Delaware, used feline calicivirus, a cat virus that is genetically related to norovirus, to demonstrate that noroviruses may be sensitive to HPP. Kingsle hopes to find a way to directly study norovirus response to HPP in the future. Shellfish processors may be more willing to invest the capital needed to perform HPP once it's been conclusively shown to inactivate norovirus.

"Our research is of direct benefit to state and federal regulatory agencies who can use the improved methods and to the industry and seafood processors who can use new and innovative processing strategies to reduce contamination in seafoods," Richards says. "We will continue to evaluate these methods and seek partners to help validate them as we pursue new ways to enhance seafood safety."—By **Jim Core,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

Gary Richards and David Kingsley are with the USDA-ARS Microbial Safety of Aquaculture Products Center of Excellence, Delaware State University, James W.W. Baker Center, Dover, DE 19901; phone (302) 857-6419, fax (302) 857-6451, e-mail grichards@errc.ars.usda.gov, dkingsley@errc.ars.usda.gov. ◆

STEPHEN AUSMUS (K11766-1)



Technician Michael Watson loads shellfish extracts into a real-time RT-PCR machine to detect the presence of norovirus RNA.

STEPHEN AUSMUS (K11762-1)

At the ARS Microbial Safety of Aquaculture Products Center of Excellence, microbiologist Gary Richards injects *Vibrio* extract into a chromatograph to isolate enzymes that may influence bacterial invasiveness.

Make Whey for Progress

New Uses for Dairy Byproducts

The average American consumes more than 30 pounds of cheese every year. Every pound produced creates an estimated 9 pounds of whey, the liquid byproduct that remains after the curds, or solids, coagulate.

Where does all the whey go? It's used in a range of products such as candy, pasta, baked goods, animal feed—and even pharmaceuticals.

Since its inception, ARS's Dairy Processing and Products Research Unit at the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, has investigated uses for whey and other dairy byproducts. Today, thanks in part to ERRC research, cheesemakers have markets for over 1 billion pounds of whey every year.

PEGGY GREB (D793-1)

Food technologist Charles Onwulata inspects molded dairy bioplastic made from surplus whey proteins.

New research shows that whey can also be used to create eco-friendly products. For example, using a process called "reactive extrusion," food technologist Charles Onwulata supplements polyethylene—a common non-biodegradable plastic—with whey proteins.

Reactive extrusion involves forcing plastic material through a heating chamber, where it melts and combines with a chemical agent that strengthens it before it's molded into a new shape. Onwulata showed that by combining dairy proteins with starch during this process, it's possible to create a biodegradable plastic product that can be mixed with polyethylene and molded into utensils.

Working with laboratory chief Seiichiro Isobe, of the Japanese National Food Research Institute, Onwulata created a bioplastic blend by combining whey protein isolate, cornstarch, glycerol, cellulose fiber, acetic acid, and the milk protein casein and molded the material into cups. Onwulata observed that dairy-based bioplastics were more pliable than other bioplastics, making them easier to mold.

Bioplastic blends can replace only about 20 percent of the polyethylene in a product, so resulting materials are only partially biodegradable. But Onwulata and his colleagues are currently applying this process to polylactide (PLA), a biodegradable polymer.

"Blending dairy-based bioplastics with PLA could eventually allow producers to make completely biodegradable materials," he says.

In a separate project, research leader Peggy Tomasula and her colleagues have developed technology to create biodegradable films from byproducts of both dairy processing and biofuels production. Tomasula found that combining casein with water and glycerol—a byproduct of biodiesel production—produces a water-resistant film that can be used as an edible coating for groceries and other products.

"We use carbon dioxide as an environmentally friendly solvent to isolate dairy proteins from milk, instead of harsh chemicals or acids, which can be difficult to dispose of," Tomasula says. Carbon dioxide is a byproduct of the glucose fermentation that is used to make ethanol, and she says it makes the edible film more water resistant.

The resulting food coatings are glossy, transparent, and completely edible. Like traditional food packaging, edible films can extend the shelf life of many foods, protect products from damage, prevent exposure to moisture and oxygen, and improve appearance. By using renewable resources instead of petrochemicals, the scientists can create more biodegradable products and reduce waste.—By **Laura McGinnis,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

Peggy M. Tomasula and Charles I. Onwulata are in the USDA-ARS Dairy Processing and Products Research Unit, Eastern Regional Research Center, 600 E. Mermaid Ln., Wyndmoor, PA 19038-8598; phone (215) 233-6703 [Tomasula], (215) 233-6497 [Onwulata], fax (215) 233-6795, e-mail peggy.tomasula@ars.usda.gov, charles.onwulata@ars.usda.gov. ✸

PEGGY GREB (K11893-1)

Wool

Biopolishing Process Scratches the Itch Factor

From the agency that brought you permanent-press cotton and permanent creases in wool trousers in the 1960s comes an exciting new breakthrough.

Researchers at the ARS Eastern Regional Research Center in Wyndmoor, Pennsylvania, have developed a biopolishing method that makes scratchy wool feel silky smooth. Not only does it remove the itch factor, it also bleaches the wool to a high level of whiteness and alters the surface of wool fibers to make them shrink-proof.

According to Jeanette Cardamone, a textile chemist in the center's Fats, Oils, and Animal Co-products Research Unit, their method increases smoothness and shine on the fabric's surface by removing fiber-yarn ends projecting from it. This contributes to a pleasing feel, which increases wool's appeal for fashion uses.

The process involves two steps. First, an activated peroxide bleach is used to whiten the wool fibers and remove the protective lipid barrier that surrounds them. This step bleaches the wool at lower temperatures and in half the time as conventional techniques, which cuts processing costs, according to Cardamone. And because it removes the lipid layer, the peroxide treatment also makes the fibers more receptive to dye.

"High-temperature dyeing is traditionally used with wool because of the lipid barrier to dye uptake," Cardamone says. "Although wool has resilient properties, those high temperatures weaken the fiber. Our process lets wool be dyed at lower temperatures, preserving its strength."

The second step—enzyme treatment—is what makes machine-washable wool a reality. The surface of a woolen fiber is covered with microscopic scales, somewhat like scales on a fish or shingles on a roof. Wool shrinks during machine-washing because the heat and pressure lock the scales in place. The enzyme treatment "digests" the scales so they can't become locked. This controls shrinkage without loss of strength or elastic recovery.

The same lipid layer that makes wool resistant to dye uptake would usually protect the scales against such an enzyme attack. But the bleaching step removes that protection.

"No damage is done to the underlying fiber structure, and the fabric's mechanical properties are not changed, because the enzyme activity is limited to the outside layer, or cuticle," Cardamone says. An additive is used in both the bleach pretreatment and the enzyme treatment to keep the enzymes out of the fiber's inner structure.

The process can be applied to everything from loose fibers to yarn, fabric, or completed garments.

Good News for Wool Industry

Washable wool has been available for about 35 years, but the processing required for it is not done in the United States. Wool fiber and fabric are conventionally made shrinkage resistant by a process called "chlorination." Like the new ARS method, chlorination alters the fiber's cuticle. But chlorination is not allowed in the United States because it produces chemicals of environmental concern. So we import all our shrinkage-resistant wool.

In the United States, wool is mainly a byproduct from breeds of sheep raised for meat and ranges in quality from fine to coarse. The new method could help the U.S. wool industry compete against countries like Australia, which produces a fine, soft wool from Merino sheep.

The U.S. military is interested in using biopolished wool, especially for manufacture of underwear for our troops. Cardamone says underwear garments currently being used by the military contain synthetic fibers that can burn and melt into wounds during combat situations. Wool, on the other hand, produces a self-extinguishing flame and a dissipating ash when burned.

By law, in fact, uniforms must be produced domestically or in a country with which the United States has a free-trade agreement. The Berry Amendment to 2004's Defense Federal Acquisition Regulation Supplement and the Buy American Act require the U.S. government—in particular, the Department of Defense—to procure (generally) only U.S.-manufactured textiles and apparel.

Mill trials are under way specifically to meet military needs. The woolen-mill industry is interested in licensing the process, but if it is used for the military, no licensing is required. Military field trials are also ongoing.

Concurrently, textile mills are applying the process to new and existing wool fabric lines to evaluate its commercial value as a replacement for imported, chlorinated wool textile products. All processing can be completed by wool mills within the United States, promoting use of domestic wool.

PEGGY GREB (K11877-1)

Technician Guoping Bao prepares solutions for treatment of wool fabrics with an ARS-developed process for whitening, biopolishing, and shrinkage prevention.

PEGGY GREB (K11875-1)

Visiting scientist Anand Kanchagar (left), from the University of Georgia, and textile chemist Jeanette Cardamone examine the effect of ARS processing on wool fabric captured by image processing. The processed fabric held by Kanchagar has a biopolished, smooth surface, whereas the unprocessed fabric held by Cardamone has a fuzzy, wrinkled surface.

ARS filed a patent on the technique. The American Wool Council, a division of the American Sheep Industry Association, provided partial funding for the research.—By **Jim Core,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

For further information on U.S. Patent Application No. 10/730,208, "Methods of Improving Shrink-Resistance of Natural Fibers, Synthetic Fibers, or Mixtures Thereof, or Fabric or Yarn Composed of Natural Fibers, Synthetic Fibers, or Mixtures Thereof," contact Jeanette Cardamone, USDA-ARS Fats, Oils, and Animal Co-products Research Unit, Eastern Regional Research Center, 600 East Mermaid Ln., Wyndmoor, PA 19038; phone (215) 233-6680, fax (215) 233-6795, e-mail jcardamone@errc.ars.usda.gov. ✷

Getting to the Root of the Solution

PEGGY GREB (D365-1)

Technician Joe Lee (left) and microbiologist David Douds examine pot cultures of bahiagrass and arbuscular mycorrhizal fungi in the greenhouse.

It might be surprising to learn how little is actually known about the role of one of nature's tiniest and most effective gardeners.

Mycorrhizal fungi are organisms that live inside and outside root cells and help them reach for nutrients by extending long threads called "hyphae" into the soil. The hyphae act as extensions of the root system. The plant, in exchange, gives the fungi glucose and possibly other organic materials they need to grow.

The most common mycorrhizal fungi, arbuscular mycorrhizal (AM) fungi, colonize most crop plant roots and enhance nutrient uptake, mainly phosphorus. AM fungi have been shown to enhance disease resistance, increase soil's stability against erosion, maintain soil pores for air and water infiltration, improve soil fertility, and increase concentrations of organic matter in the soil.

Unfortunately, AM fungus populations in soils have been diminished by modern agricultural practices. But they can still make important contributions to productivity in systems where little or no chemical fertilizers and pesticides are used, such as in organic farming.

Although home gardeners can buy potting mix with beneficial fungi added, it's impractical for farmers to buy and apply the amount they would need for their fields. A team of ARS scientists at the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, is examining the feasibility of on-farm mass production of AM fungus inoculum already native to a site or introduction of nonnative AM fungi by way of colonized host plants. By learning more about AM fungus physiology and finding ways to grow colonies without host plants, they hope to make the fungi a practical option for producers.

Researchers in ERRC's Microbial Biophysics and Residue Chemistry Research Unit take a three-pronged approach to mycorrhizal research. David D. Douds, a microbiologist, heads the on-farm inoculum production. Philip E. Pfeffer, a chemist, is mainly investigating the fungi's metabolism and molecular biol-

PEGGY GREB (D366-8)

Technician Aisha Abdul-Wakeel places samples into a gas chromatograph while chemist Philip Pfeffer examines mass spectral data to measure the amount of isotopically labeled nitrogen that has been delivered from the mycorrhizal fungus to the host plant.

ogy. Gerald Nagahashi, a chemist/cell biologist, is studying the fungal life cycle.

Pfeffer and Douds collaborated with Michigan State University and New Mexico State University scientists to show the symbiotic relationship between mycorrhizal fungi and plants in nitrogen recycling. Nitrogen is an essential part of proteins, nucleic acids, and other cellular components of living organisms.

Though the scientists had identified enzyme activities in AM fungi and genes involved in nitrogen absorption and metabolism, little was known about how nitrogen is transported from fungus to plant root and in what form it is transferred.

Using nuclear magnetic resonance, mass spectrometry, and gene expression measurements, they recently established that nitrogen is transported as the amino acid arginine. It takes a novel metabolic route from external hyphae to fungal structures located in plant roots. It is then broken down by the fungus to ammonium, which is transferred to the host plant.

"Nitrogen availability often determines plant growth," Pfeffer says. "Microorganisms have a central role in supporting life, because they are involved in most aspects of nitrogen availability. We've shown that mycorrhizal fungi may have a much more significant role in the worldwide nitrogen cycle than previously believed."

One Step at a Time

Currently, researchers cannot cultivate the fungus without a host. The fungus cannot complete its life cycle without the organic nutrients or other stimuli it receives from roots. Nagahashi and his colleagues have been focusing on events that must occur before the fungus can colonize a host plant.

"We developed a bioassay that has allowed us to determine that chemical compounds exuding from the host's roots, root caps, and root border cells induce fungal hyphal branching," Nagahashi explains. "This is the earliest structural change in the fungus after spore germination. The increase in branching creates a greater potential for the fungus to find the host root surface, where it can attach."

Nagahashi and Douds used in vitro sterile culture techniques to investigate how certain environmental factors—root compounds, blue light from the sun's spectrum, and carbon dioxide—affect AM fungal growth. They found that these three factors can all work independently to promote growth of AM fungi but were even more effective when combined to operate in synergy. The most effective treatments involved the joint use of chemical compounds with one of the environmental factors.

The AM fungus life cycle's first three steps have been completed in laboratories without a host root. Nagahashi and his colleagues are currently focused on the fourth step—formation of "penetration hyphae," which enter and develop into a network of intercellular hyphae inside the root.

"This step leads to host root colonization," Nagahashi says. "We have developed another bioassay that may allow us to test for host compounds, or interactions between host and fungus, which lead to this step's completion."

Identifying these compounds could eventually be used to provide researchers with pure fungus spores, the development of which is the life cycle's final step, and allow them to be cultivated away from host plants.

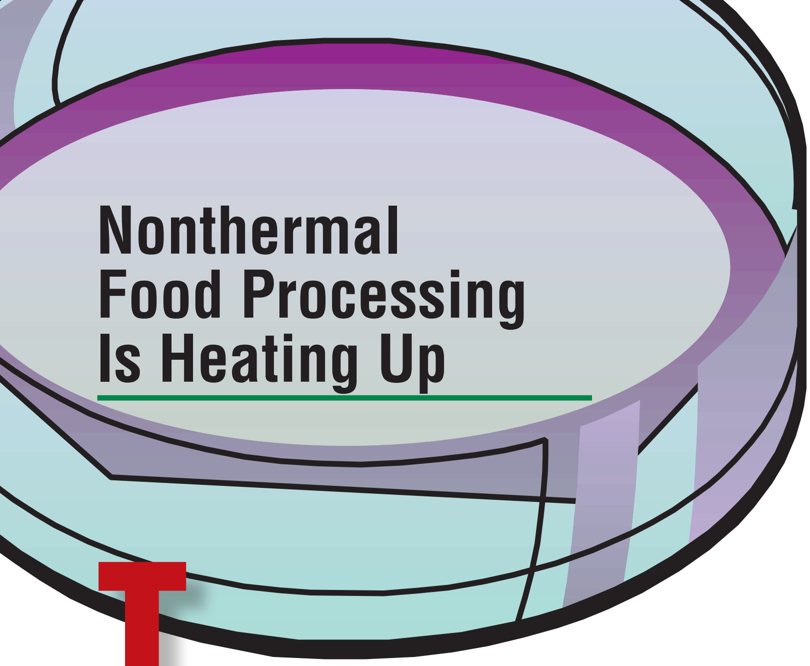
AM Fungi Thrive on Organic Farms

Douds collaborates with researchers at the Rodale Institute in Kutztown, Pennsylvania, which for more than 25 years has compared conventional and low-input, or organic, farming systems at the same site. Some of Douds's work with mycorrhizal fungi was conducted in Rodale's Farming Systems Trial, a comprehensive study of organic farming techniques that began in 1981 on 6.2 hectares, which makes it the longest running comparison study of its kind in the United States.

Of particular interest to Douds were results showing that plots farmed with two organic systems had greater AM fungi spore populations and produced greater plant root colonization than did soils from a conventional corn-soybean rotation system.—By **Jim Core,** ARS.

This research is part of Soil Resource Management (#202) and Plant Biological and Molecular Processes (#302), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

David D. Douds, Philip E. Pfeffer, and Gerald Nagahashi are with the USDA-ARS Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038; phone (215) 233-6469, fax (215) 233-6581, e-mail ppfeffer@errc.ars.usda.gov, ddouds@errc.ars.usda.gov, gnagahashi@errc.ars.usda.gov. ✷



Nonthermal Food Processing Is Heating Up

The food industry wants to ensure the safety of its products while maintaining quality. But while the maxim "heat kills germs" still holds true for food sterilization, scientists are exploring alternative treatments for lowering foodborne pathogen levels. Today, new technologies that are faster, cheaper, and less disruptive to quality than traditional thermal processing are increasingly common.

Under the guidance of research leader Howard Zhang, scientists in the Food Safety Intervention Technologies Research Unit—part of ARS's Eastern Regional Research Center (ERRC) at Wyndmoor, Pennsylvania—have investigated several nonthermal processing technologies. These include high-pressure processing, pulsed electric fields, radio-frequency electric fields, ultraviolet light, and irradiation.

"Some of these nonthermal techniques were developed here, but we have also tested and improved other technologies developed elsewhere," Zhang says. "Our work has improved food safety by enabling the food industry to make better decisions about how to reduce or eliminate foodborne pathogens."

For Liquids, High-Pressure and Electric Fields

High-pressure processing (HPP) treatment involves applying 80,000-130,000 pounds per square inch of pressure to a sample. Imagine the pressure in the greatest depths of the ocean, then multiply that by 10. Applying that extreme pressure for 2-5 minutes will kill most vegetative microorganisms—those that grow in foods under normal storage conditions.

ERRC researchers found that HPP treatment at 25˚C can significantly reduce *E. coli* populations in tomato juice and liquid whole eggs. Their research also suggested that multiple brief high-pressure treatments were more effective than one lengthy treatment using continuous pressure for the same total amount of time.

While HPP can eliminate vegetative microorganisms, it is not effective at killing microbial spores. And at 5-10 cents per pound, it is too pricey to be practical.

"We hope to develop pressure technology that is cheaper and more effective against spores," Zhang says.

Another technology that ERRC scientists have improved uses pulsed electric fields (PEF) to kill yeasts, molds, and vegetative bacteria in liquid foods. These intensive electric pulses break down the cell membranes of the microorganisms.

One study explored the feasibility of using PEF in making shelf-stable salad dressings. A model salad dressing for the study was formulated by Kraft Foods. ARS scientists inoculated the dressing with *Lactobacillus plantarum*, a very heat-resistant spoilage bacterium. The sample was then treated either by PEF alone or by PEF followed by mild heat. While PEF treatment alone significantly reduced *L. plantarum,* the dressing retained microbial shelf stability only when refrigerated. PEF plus mild heat exposure, however, produced shelf stability at room temperature.

Another study subjected applesauce samples to PEF followed by mild heat treatment. The processed applesauce was aseptically packed into plastic cups and stored at room temperature. Evaluations indicated that the processed applesauce maintained high sensory quality during 470 days of storage. That's a longer shelf life than is currently used in commercial practice. This research demonstrated that following PEF with mild heat may be used in producing high-quality, shelf-stable applesauce products.

Like the PEF process, radio-frequency electric field (RFEF) technology subjects liquid foods to high electric fields. But this procedure—developed at ERRC—uses a continuous alternating current power supply, which costs less than a PEF pulse generator.

In one study, ARS scientists applied RFEF for 3 seconds at 60˚C to an apple juice sample inoculated with *E. coli*. The electrical cost of RFEF processing was about 1 cent per deciliter (10.5 quarts), and the procedure was more effective than conventional heating under the same conditions.

UV or Not UV? Ultraviolet Light and Irradiation

ERRC has also investigated ultraviolet (UV) light and irradiation technologies as alternatives or complements to thermal processing.

Scientists used UV processing on an apple cider sample

PEGGY GREB (D616-1)

Food microbiologist Dike Ukuku (left) and engineer Joseph Sites evaluate effects of high-pressure processing on microbial stability of tomato juice and liquid eggs.

PEGGY GREB (D617-1)

In studies of methods to pasteurize liquid foods such as apple cider without using heat, research leader Howard Zhang (left) and chemical engineer David Geveke develop and evaluate pulsed electric field and radio frequency electric field treatments.

that had been inoculated with bacteria. Heat and chemical treatments can destroy bacteria within cider, but they generally alter the flavor. The UV treatment reduced populations of *E. coli* and *Listeria innocua* by more than 99 percent without changing the liquid's flavor. Scientists believe UV processing has potential to improve the safety of apple cider and extend its shelf life without diminishing its quality.

Could UV processing provide safe liquid egg products too? Extensive thermal pasteurization, though effective for other types of food, damages the functional properties of egg whites, so USDA's Food Safety and Inspection Service asked ERRC to investigate.

ERRC researchers combined UV and heat treatments to kill *E. coli* in liquid egg whites. Whites infected with *E. coli* O157:H7 were processed using a simple UV apparatus consisting of a low-pressure mercury lamp surrounded by a coil of UV-transparent tubing. The scientists found that UV treatment at room temperature can significantly reduce the heat subsequently required to pasteurize liquid egg whites.

Irradiation exposes food to a low level of ionizing radiation to kill bacteria, molds, yeasts, parasites, and other microorganisms that can lead to food spoilage and illness if untreated. ERRC is the only federally funded and operated food irradiation research facility in the United States.

Over the last 25 years, studies have shown that eating irradiated foods poses no increased health risks for consumers. ERRC research findings have enabled federal regulatory agencies to establish standards to ensure safety and quality of irradiated products like fruit, vegetables, juice, meat, and meat substitutes. At 3-5 cents per pound, irradiation is slightly more expensive than thermal processing, but its success in deactivating spores and other microorganisms has made it an option for the food industry.

Foodborne pathogens are varied and numerous, but thanks in part to contributions from ERRC scientists, knowledge of pathogen prevention improves constantly, and with it, the safety of the nation's food supply.—By **Laura McGinnis,** ARS.

This research is part of Food Safety, an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

Howard Zhang is with the USDA-ARS Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038; phone (215) 233-6583, fax (215) 233-6406, e-mail howard.zhang@ars.usda.gov. ✷

PEGGY GREB (D618-1)

Food microbiologist Kathleen Rajkowski evaluates how well a pulsed ultraviolet light treatment inactivates bacterial pathogens on the surface of test media.

Fueling America—
Without Petroleum

PEGGY GREB (D768-1)

Chemical engineer John Nghiem (left) and research leader Kevin Hicks monitor a new process for converting barley into fuel ethanol.

The demand for alternatives to petroleum-based fuels is steadily rising.

Corn and soybeans—the dominant feedstocks for ethanol and biodiesel production in the United States—grow well in the central regions of the country. But are these the only available sources? What options exist for U.S. growers in other regions? How can corn and soybean feedstocks be improved?

Scientists at the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, are answering these and other questions about renewable fuels production. Their research focuses on four major areas: biodiesel, ethanol, thermochemical processes, and cost analysis.

"For years, ERRC has been a committed participant in alternative fuels research," says center director John Cherry. "This is a particularly exciting time, because so much of our research work is being adopted and used by industry."

Biodiesel: From Grease to Glycerol

What do animal fats, rendered materials, and restaurant grease have in common? Besides ready availability and limited marketability, they're all subjects of ERRC biodiesel research headed by research leader Bill Marmer. Scientists in his group have demonstrated that products of the rendering industry can be used as low-cost feedstocks for biodiesel production.

Biochemist Mike Haas and biologist Karen Scott are working with the Philadelphia Fry-o-Diesel company to demonstrate that trap grease—waste grease that restaurants and food companies collect from their drains—can be converted into a clean-burning, renewable fuel source.

Haas and Scott helped characterize trap-grease samples, advised the company on operation design, and analyzed the products of trial runs. They have successfully produced fatty acid methyl esters, the chemical compounds that make up biodiesel, from the grease. The esters are being tested to determine whether they meet accepted biodiesel standards.

These researchers are also developing a method to produce biodiesel directly from oil-bearing materials, including soybean flakes and rendered products. The oils or fats in the feedstock are treated with 18 percent methanol, forming biodiesel as the extractant. This would eliminate the need to isolate the oil before converting it to fuel, thereby reducing production costs, and would expand the amount of available fuel feedstocks.

Another objective of biodiesel research is to find uses for glycerol, a coproduct of biodiesel production.

"For every 100 pounds of biodiesel produced, you get 10 pounds of glycerol," says chemist Tom Foglia. "Current markets are saturated."

Concerned that increased biodiesel production could result in a hyperglutted glycerol market, ERRC researchers are investigating alternative uses for the compound. Molecular biologist Dan

Enzymes could improve the speed, efficiency, and cost of barley-based ethanol production.

Solaiman and microbiologist Rick Ashby have found that crude glycerol can be used to support microbial cell growth and production of polyester biopolymers, which can be used as plastics or adhesives, and biosurfactants, which are used in detergents or as antimicrobial agents. This is particularly important because crude glycerol is less marketable than pure glycerol.

In related studies, chemist Victor Wyatt demonstrated that glycerol could be used to produce a new class of prepolymers for making such products as coatings, resins, foams, and agents for remediation of polluted environments.

These alternative uses for glycerol have proved successful on a trial scale. Now the scientists are testing them at an industrial level through a cooperative research and development agreement with an international consumer products company.

Ethanol: Beyond the Corn Belt

Affordable, available, and easy to work with, corn is the main feedstock for ethanol in the United States. As ethanol production increases—USDA chief economist Keith Collins estimates that our country could produce 12-13 billion gallons in 2009—so does the demand for suitable feedstocks.

To avoid overburdening the corn market, ethanol producers have two options: increase conversion efficiency or use an alternative crop. Several ERRC research projects have demonstrated how these can be done.

Food technologist David Johnston is investigating new processes using protease enzymes from microbial and fungal sources to produce ethanol more efficiently. In trials, Johnston found that adding enzymes during fermentation sped up the process and increased ethanol yields.

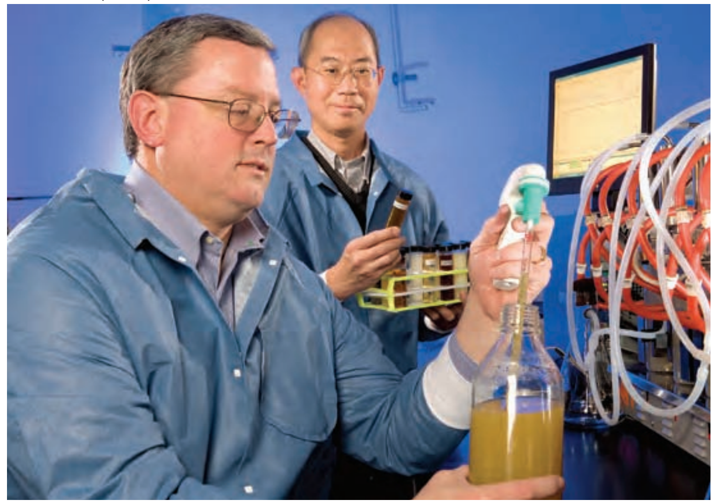
"The enzymes make more nutrients available for the yeast. They expedite the fermentation process and can also make it easier to separate liquid from solids after the ethanol has been removed," Johnston says. "This is important because the more efficiently you separate the free liquid from the solids, the more energy efficient the process can be."

Corn isn't the only available feedstock for ethanol. Research leader Kevin Hicks is collaborating with biotechnology company Genencor International; Virginia Tech, in Blacksburg, Virginia; and members of the barley industry to explore barley's potential as a feedstock in regions of the United States where corn is not the principal crop.

Hicks estimates that barley grown in North America could supply about 1 billion gallons of ethanol per year. The crop is well suited to the Mid-Atlantic, where it could be grown as a winter crop in rotation with soybeans and corn in 2-year cycles.

Currently, barley yields less ethanol than corn does, and the ethanol from barley is more expensive. Barley's physical properties—an abrasive hull and low starch content—impede production efficiency. But Hicks and his colleagues are overcoming these hurdles with research.

PEGGY GREB (D770-1)



Microbiologist Rick Ashby (left) and molecular biologist Dan Solaiman monitor bacterial growth and production of a biopolymer for use in plastics and other products. The bacteria are growing in a nutrient broth containing glycerol, a coproduct of biodiesel production.

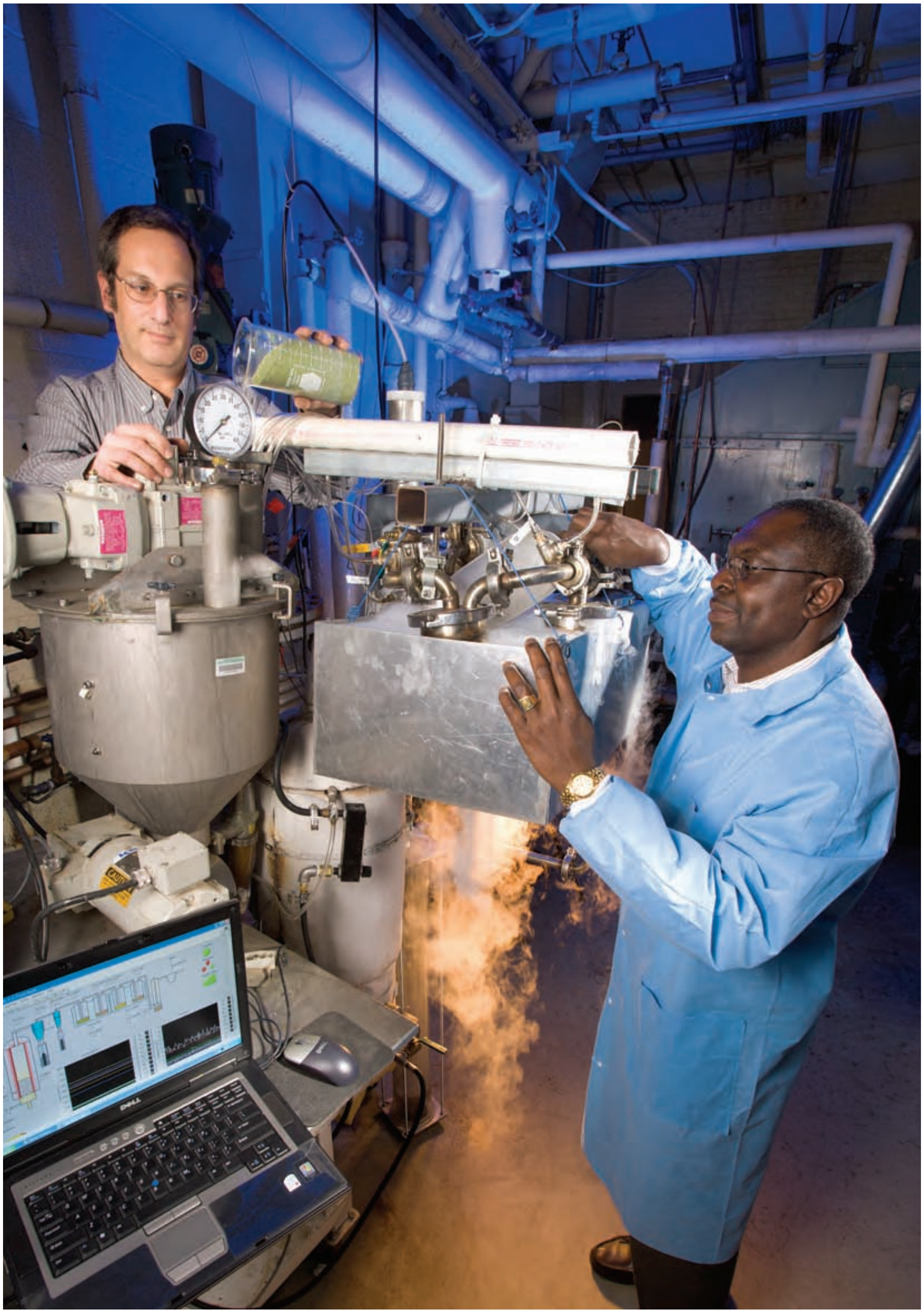
With Genencor, the researchers are developing new enzyme technology that could improve the speed, efficiency, and cost of barley-based ethanol production.

They also collaborated with Virginia Tech researchers to develop barley varieties with higher starch content and a loose hull that generally falls off during harvest or grain cleaning. Initial studies suggest that such varieties have promise as a feedstock.

PEGGY GREB (D769-1)

Biologist Karen Scott prepares a sample of trap grease for conversion to biodiesel. In the foreground are samples of distilled (left) and crude (center) biodiesel from trap grease.

PEGGY GREB (D767-1)



Engineers Neil Goldberg (left) and Akwasi Boateng operate a fluidized-bed thermochemical reactor they designed and built for converting crop residues into renewable bio-oils and hydrogen fuels.

Biomass—materials such as sawmill waste, straw, and cornstalks—is heated in a reactor and converted into liquid (bio-oil) and synthetic gas.

In one study, for example, a hull-less barley produced 2.27 gallons of ethanol per bushel, whereas hulled barley produced 1.64 gallons per bushel.

The scientists are now studying which conditions will promote the most cost-effective production of barley-based ethanol.

Breaking Down the Biomass

There are two main processes, or "platforms," for making fuels from biomass: sugar and thermochemical conversion. The sugar platform involves breaking down complex carbohydrates in the biomass—materials such as sawmill waste, straw, and cornstalks (stover). Then, yeasts metabolize, or consume, the simple sugars to make alcohol.

Breaking down those complex carbohydrates requires a lot of energy, Hicks says, and special microorganisms are required to convert some sugars into ethanol. And, ironically, the process creates a lot of carbon dioxide—the greenhouse gas that's helping to spur the biofuels movement.

The thermochemical platform involves heating the biomass in a reactor and converting it into liquid (bio-oil) and synthetic gas (gaseous fuels comprising carbon monoxide, hydrogen, and low-molecular-weight hydrocarbon gases such as methane and ethane). Chemical engineer Akwasi Boateng has led much of the ERRC research on this process.

In a study with research leader Gary Banowetz and colleagues in Corvallis, Oregon, Boateng converted grass seed straw into synthetic gas using small-scale gasification reactors. Built to serve a farm or small community, these reactors could provide an environmentally friendly and economic use for the 7 million tons of straw produced by the grass seed industry every year in the Pacific Northwest.

Neither the sugar platform nor the thermochemical platform has been perfected yet, Hicks cautions.

"Each one has technical and economic hurdles that must be solved through research," he says. "We're trying to compare the processes and determine which, if perfected, would give the most useful energy from a given amount of biomass. We're working with international experts to make intelligent decisions on where to focus our efforts."

A Model Approach: Cost Analysis

Price is one of the major factors inhibiting the spread of biofuels. Reducing production costs would make them more competitive with petroleum-based fuels—but where can scientists cut costs?

Engineers Winnie Yee and Andy McAloon create technical models to guide research efforts toward economically feasible processes. With the models, they analyze every aspect of a biofuel production process and determine where cost-cutting would be most effective. This allows researchers to pinpoint the exact steps in the process that need to be modified.

PEGGY GREB (D777-1)

Engineers Andrew McAloon (left) and Winnie Yee (right) explain the economic advantages of a new fuel ethanol process to ERRC director John Cherry.

"It's important to know that our research makes economic sense, that these processes will be competitive enough for industry to accept them," McAloon says.

Haas used one of McAloon's models to analyze his efforts to create biodiesel from soy flakes. The model estimated that by first drying out the moist flakes, Haas could reduce the amount of methanol required later, thereby reducing the cost per gallon from $2.83 to $2.66. Haas and his colleagues are currently working to reduce that cost even further to a point of commercial competitiveness.

For about 10 years, ERRC has been providing these technical models for ARS scientists. Developing a model from the ground up is time-consuming, McAloon says, but once developed it can be modified to meet the needs of a specific product or process. Within the past year alone, he estimates, ERRC has produced several hundred copies of their models for researchers within ARS and around the world.

"Our scientists are approaching biofuels research from many different angles that allow us to come up with comprehensive solutions," Cherry says. "We've made some great discoveries here at ERRC that have helped improve biofuel production, and I'm confident that we'll see even more improvements in the future."—By **Laura McGinnis,** ARS.

This research is part of Bioenergy and Energy Alternatives, an ARS National Program (#307) described on the World Wide Web at www.nps.ars.usda.gov.

To reach scientists mentioned in this article, contact Laura McGinnis, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705; phone (301) 504-1654, fax (301) 504-1486, e-mail laura.mcginnis@ars.usda.gov. ✷

Pectin Extraction From Citrus and Sugar Beets

Low-Value Pectin's a Natural for Making High-Value Products

At ARS's Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, researchers in the Crop Conversion Science and Engineering Research Unit are at work on pectin, a polysaccharide component in the cell walls of fruits and vegetables.

Polysaccharides are polymers made up of many simple carbohydrates (sugars) linked together into long, continuous molecules. Pectin is currently valued for use as a gelling and thickening agent, beverage stabilizer, and fat substitute.

Most commercial pectin is obtained by extraction from citrus peels, but sugar beet pulp is also rich in pectin. About 1.5 million tons of dry beet pulp—an enormous untapped source of a valuable polysaccharide—are generated annually by U.S. processors.

At present, most pulp is dried and sold as animal feed at little profit because of the costly energy required to dry it for storage and shipment.

But sugar beet pectin has different chemical features than citrus pectin, so it could find new uses, especially in industrial products. Beet pulp is also rich in other highly functional cell wall polysaccharides that could be isolated and put to use. To increase profits for sugar beet growers and processors, the Wyndmoor researchers are working on new processes to efficiently isolate beet pectin and associated polysaccharides and find higher value uses for them.

Extraction of pectin from plant material now takes an hour or more using conventional heating; care must be taken not to break down its structure—which provides thickening properties—by overheating. To save time and reduce cost, Marshall L. Fishman, an ERRC chemist, developed microwave and steam-injection techniques to heat fruit peels with acidified water in pressure-resistant containers.

"Using these 'flash extraction' methods, pectin can be extracted in just 10 minutes," Fishman explains. "They use less energy and yield higher quality pectin than conventional heat extraction." Fishman is now adapting flash extraction techniques to remove pectin from sugar beet pulp—which is even more heat-sensitive—at lower temperatures than those used to extract pectin from citrus.

Brett Savary, a plant physiologist formerly at ERRC, evaluated enzymes from citrus fruits and sugar beet roots for use in modifying the properties of pectin and other cell wall polysaccharides. He has been using new proteomic technologies to rapidly identify and characterize these enzymes.

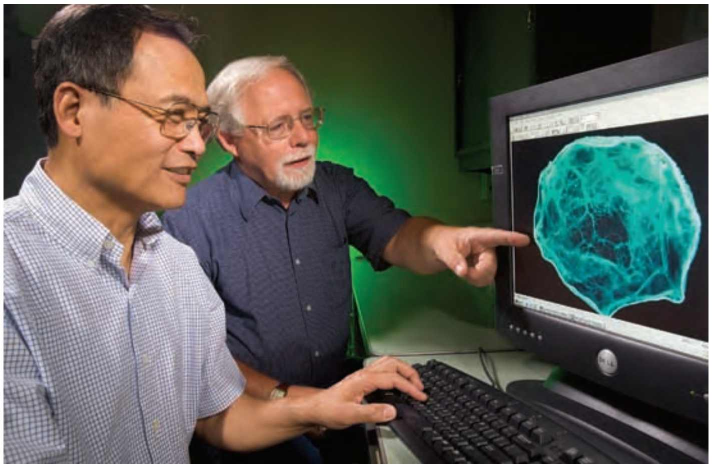
With cooperators at the ARS Citrus Products Laboratory in Winter Haven, Florida, Savary discovered a distinctive citrus enzyme that may be useful for commercial processing of pectin. They are filing a patent on the discovery and partnering with a U.S. manufacturer to produce the enzyme and determine its usefulness for manufacturing pectin with improved properties in food applications.

The group is also looking at using enzymes to help dry out

PEGGY GREB (D702-1)

Chemists Marshall L. Fishman (left) and Hoa K. Chau prepare orange peels for flash extraction of pectin using microwave heating under pressure.

PEGGY GREB (D704-1)



Chemists LinShu Liu (left) and David Coffin examine a microsphere made from pectin and zein. It is used as a controlled drug-delivery system.

PEGGY GREB (D705-1)

Technician Andre White (left) and plant physiologist Arland Hotchkiss analyze the pectic fragment composition of a prebiotic fraction from orange peel.

beet pulp after sugar extraction, which would reduce processors' energy costs. It may be possible to combine enzymes with Fishman's flash extraction technologies to efficiently extract arabinan, another cell wall polysaccharide that could also be commercially produced from sugar beet pulp. With effective recovery of valuable soluble polysaccharides, much of the remaining material is an enriched form of cellulose that's readily digestible by microbes and could be fermented to produce ethanol.

Products With Medical Uses

Recently, ERRC chemist LinShu Liu developed a material from natural crop polymers, including pectin, which can be used in biomedical materials, such as those used in human tissue regeneration. The new, pectin-based material forms three-dimensional structures with better physical and mechanical properties than the tissue replacer in current use.

Other potential medical applications of these novel pectin materials include prosthetic medical devices and scaffolding for bone or cartilage repair, since pectin and other polysaccharides have properties that allow human cells to bind to them and grow. A patent application has been filed, and ARS has signed a cooperative research and development agreement with a private company to further develop the materials.

Liu has combined pectin and zein—the main storage protein in corn seeds—and found that the combination can be used in a colon-specific drug-delivery system. It doesn't degrade until it reaches the large intestine, allowing for controlled release.

In another human health application, plant physiologist Arland T. Hotchkiss and cooperators demonstrated for the first time that pectin fragments from orange peel have prebiotic properties. Prebiotics are nondigestible food ingredients that increase growth of beneficial probiotic bacteria in the large intestine. Probiotic bacteria stimulate health and help prevent growth of foodborne pathogens.

Orange pectin prebiotics are also being evaluated by collaborators as animal-feed ingredients. This may allow reduction or elimination of antibiotics that are often added to feed.

Industrial Uses, Too

Microbial fermentation of sucrose or starch is used to manufacture a polymer known as "polylactic acid," or PLA, and its copolymers.

At present, PLA is more expensive than petroleum-derived thermoplastics, and its polymers are stiff and brittle in thicker materials. Liu is testing sugar beet pulp as an inexpensive filler to reinforce PLA. The pectin and other polysaccharides in the pulp add tensile strength. Compression heating forms them into a water-resistant, thermoplastic composite material well suited to manufacturing diverse products, such as office supplies, automobile parts, and lightweight construction materials. The improved properties are achieved at lower cost, and the material is biodegradable.

Further refinements of these ERRC-developed pectin-processing techniques should lead to more economical carbohydrate-based alternatives to imported petroleum-based products.—By **Jim Core,** formerly with ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

To reach scientists mentioned in this article, contact Linda Tokarz, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; phone (301) 504-1658, fax (301) 504-1486, e-mail linda.tokarz@ars.usda.gov. ✷

New Methods for Ensuring Food Safety

Good news for fans of raw cookie dough. Researchers at ARS's Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, have filed a patent on technology that can further protect pasteurized liquid eggs from food safety threats. These threats include both naturally occurring spoilage bacteria and pathogens such as *Salmonella enteritidis*, the primary cause of egg-related foodborne illness in the United States. The technology has also been successfully applied to milk.

But don't go running for that dough just yet. The U.S. Food and Drug Administration (FDA) still cautions against consuming any raw, unpasteurized eggs or products that contain them.

Despite adherence to pasteurization protocols prescribed by the U.S. Department of Agriculture, illnesses related to consumption of raw egg products still occur. About 40,000 cases of salmonellosis are reported in the United States each year. The new ARS technology, developed by ERRC scientists Sudarsan Mukhopadhyay, Peggy Tomasula, and John Luchansky, may help reduce that number.

"Current pasteurization technology is not adequate to remove all pathogens effectively from egg products," says Tomasula, research leader of the ERRC Dairy Processing and Products Research Unit. "Though pasteurization eliminates heat-sensitive pathogens, some heat-resistant microorganisms can survive and spoil liquid egg whites."

Consumers can avoid illness by properly handling and cooking eggs before consumption. But Tomasula, along with lead scientist Luchansky of the Microbial Food Safety Research Unit and chemical engineer Mukhopadhyay, has found that new technology can compensate for the shortcomings of thermal pasteurization.

The technology, called "crossflow microfiltration membrane separation" (CMF), removes more pathogens than thermal pasteurization does. And it does so without affecting the eggs' ability to foam, coagulate, and emulsify—meaning that CMF-treated eggs could be safely substituted for pasteurized eggs in products where those characteristics are desired, such as angel food cake and mayonnaise. In a pilot-scale study, CMF was shown to remove about 99.9999 percent of inoculated *S. enteritidis* from unpasteurized liquid egg whites.

The technology can also be used to remove *Bacillus anthracis* spores from egg whites. This finding adds to previous work in which ERRC researchers used CMF to remove 99.9999 percent of *B. anthracis* spores inoculated into fluid milk. Microfiltration can also protect milk from more common bacterial pathogens, potentially extending its shelf life.

PAUL PIERLOTT (D1452-1)

Microbiologist George Paoli inspects antibody-coated magnetic beads and biologist Chandi Wijey analyzes DNA samples in their efforts to develop immunological, microbiological, and genetic-based methods for detection of *Yersinia pestis* in food.

Though effective in its own right, CMF works best when treated as an accompaniment to pasteurization, not a replacement for it, says Tomasula. Combining the two processes significantly reduces the pathogen load.

Plague Defense

Using antibody- and DNA-based technology, ARS researchers in the ERRC Microbial Biophysics and Residue Chemistry Research Unit have developed a variety of methods for targeting dangerous pathogens in food. They've used these technologies to detect foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*. Recently, microbiologist George Paoli and his colleagues have applied some of the same detection techniques to *Yersinia pestis*, the bacterium that causes bubonic plague.

"Many methods used to detect more common foodborne pathogens can be extended or modified for detecting organisms that are a concern with respect to food safety," Paoli says.

The biological makeup of individual bacterial species varies considerably. Though the methods used to detect them are similar, they have to be altered to suit specific pathogens. Because antibodies bind to specific targets, antibody-coated immunomagnetic beads (IMBs) can be used to target and remove specific pathogens. The trick to IMB technology is finding an antibody that attaches to one, and only one, target—for example, a *Y. pestis* antigen. Paoli and his colleagues have developed IMBs to capture *Y. pestis,* and preliminary tests in milk samples have been successful.

In the early stages of testing the IMB technology, ERRC scientists found that their beads could pick *Y. pestis* out of a food mixture, but they also captured *Staphylococcus aureus*. Further research showed that the binding of *S. aureus* to the IMBs could be overcome by plating on media that were selective for growth of *Yersinia*. Use of these plates, along with the IMBs, significantly reduces the danger of false-positive results in samples that might also contain *S. aureus*.

The team is also using genetic methods that rely on a common DNA test known as the "polymerase chain reaction," or PCR. The group has developed PCR-based methods to detect *Y. pestis* and differentiate it from other foodborne bacteria, including two closely related *Yersinia* species, *Y. enterocolitica* and *Y. pseudotuberculosis*. They have also developed a PCR method that targets genes related to the virulence of *Y. pestis*. This test could be used to determine a particular strain's potential public health impact.

The scientists are currently working to combine the different areas of this research—the IMB detection, microbiological plating media, and genetic detection and typing of *Y. pestis*—to develop a complete set of tools for detection of *Y. pestis* in food, as part of a holistic approach to food security.

Warming Up to Food Security

PAUL PIERLOTT (D1451-1)

At ERRC, special project team members sample steaks to search for pathogens both before and after cooking on a commercial gas grill. Left to right: technician Brad Shoyer, food technologist Anna Porto-Fett, and microbiologists John B. Luchansky, Vijay Juneja, and Jeffrey Call.

PAUL PIERLOTT (D1450-1)

ERRC chemical engineers Sudarsan Mukhopadhyay (left) and Peggy Tomasula examine a ceramic membrane module for use in microfiltration of liquid egg whites to eliminate potential pathogens such as *Salmonella enteritidis.*

The likelihood of *B. anthracis* or *Y. pestis* entering the U.S. food supply is very small—significantly smaller than the relatively low risk of contamination by *Salmonella* or other naturally occurring pathogens. Should that remote possibility occur, however, one of the easiest, most effective food safety tools—heat—lies in the hands of the consumer.

Previous ERRC studies have established and validated the use of thermal and nonthermal technologies to reduce the risk of foodborne pathogens. Luchansky and colleagues Vijay Juneja and Anna Porto-Fett recently led studies in which they applied thermal technology to ground beef inoculated with *B. anthracis* and *Y. pestis*—with encouraging results.

Patties made from inoculated ground beef were cooked on an open-flame gas grill and a "clam-shell" style electric grill. Both models are commonly used by consumers and the food industry. In both studies, the scientists found that cooking the ground beef on commercial grills, at the heat and time levels recommended by USDA and FDA, noticeably reduced the overall levels of both pathogens on or in the meat.

Research efforts like these ensure that U.S. consumers and food-industry professionals have the most advanced technology and information available to protect against potential pathogen threats.—By **Laura McGinnis,** ARS.

This research is part of Food Safety, an ARS national program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

To reach the scientists mentioned in this article, contact Laura McGinnis, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; phone (301) 504-1654, fax (301) 504-1486, e-mail laura.mcginnis@ars.usda.gov. ✷

From Lubricants to Packaging Materials

ARS Scientists Just Say "No" to Petroleum

Screech!

Few sounds are more nerve-wracking than the scraping of metal on metal. It's the job of lubricants to prevent that annoying noise by making sure that—when metal touches metal—everything moves smoothly and quietly.

Machines that have moving parts—your car's engine or the hydraulic pump of a huge earthmover, for instance—almost always require lubricants. Most lubricants are made of so-called "base oil" that's blended with additives to boost performance.

In the United States, the demand for additives, already at nearly 2 billion pounds a year, is expected to increase at a respectable 2 percent a year for the next 5 years. That's why Sevim Z. Erhan and colleagues have developed a new process for making biobased additives, ones suitable for use in formulating greases; crankcase, two-cycle, and marine engine oils; and hydraulic, transmission, and drilling fluids.

The additives would be made from the predominant fat molecules—triglycerides—in natural oils of familiar crops like soybean, corn, or canola or from lesser-knowns like pennycress, camelina, and crambe. Fully biodegradable, these vegetable-oil-based additives are just slightly darker, and somewhat thicker, than the clear, light-yellow cooking oils used in home kitchens across America.

The additives would provide an alternative to today's petroleum-based products—and a potentially profitable market for growers in the Midwest and elsewhere.

Erhan did the work while a chemist at the ARS National Center for Agricultural Utilization Research in Peoria, Illinois. She's now director of the agency's Eastern Regional Research Center in Wyndmoor, Pennsylvania.

The new-age additives, says Erhan, offer many benefits and, to date, no downside. "You can use them with either biobased or conventional lubricants," she points out. And the novel additives meet all the standard criteria for a top-notch, antifriction, antiwear additive, namely, impressive viscosity and liquidity, high flashpoint, and stability despite temperature extremes.

In small-scale laboratory tests to evaluate wear and friction, for example, the plant-oil additives performed as well as or better than commercial petroleum-based additives. And since they're fully biodegradable, proper disposal is fast, easy, and inexpensive.

What's more, the additives can be produced with the same equipment that chemical companies already have in place for making traditional additives.

It's no wonder that several leading makers of petroleum-lubricant additives have already expressed an interest in the scientists' process for making the biobased additives.

Erhan and coinvestigators Brajendra K. Sharma of Pennsylvania State University-University Park, and Atanu Adhvaryu, formerly with the university, describe the technology in their U.S. patent, issued in 2007. Importantly, the procedure and the resulting additives are eco-friendly. "No harsh solvents are used, and very

NCAUR (D1643-1)

At the NCAUR lab in Peoria, chemist Sevim Erhan (now center director at the Eastern Regional Research Center in Wyndmoor, Pennsylvania) formulates a biodegradable lubricant using an ARS patented biobased antiwear additive.

Wrapping Up With Dairy-Derived Ingredients

Speaking of saying "no" to petroleum-based products, many food manufacturers want to incorporate renewable materials into their operations to help reduce food-packaging waste.

Most food packages are made of multilayer films that are thin, continuous sheets of synthetic polymers. But consumers and food retailers are concerned about the waste generated during the manufacture of such packaging. Many, it seems, are interested in replacing petroleum-based packaging with biobased packaging.

At the ARS Eastern Regional Research Center in Wyndmoor, Pennsylvania, scientists are developing strong, biodegradable dairy-based films that are better oxygen barriers than petrochemical-based films. "We are involved with finding the technology to improve dairy films so that they can eventually perform as plastics," says research leader Peggy Tomasula, with the center's Dairy Processing and Products Research Unit.

PEGGY GREB (D1642-1)

Close-up of the additive being placed onto ball bearings before friction test at right.

PEGGY GREB (D1641-1)

Visiting chemist Brajendra Sharma uses a standard four-ball test method to measure friction and wear properties of vegetable-oil-based antiwear additives. The additives can be used in either biodegradable or conventional lubricants and greases.

little energy and water are required," says Erhan.

In brief, the vegetable oil is allowed to react with several chemicals, for various periods of time, including a 4-hour stint at a boil. Savvy chemists would summarize the process as "reacting triglyceride oils with thiols to yield poly (hydroxy thioether) derivatives."

Plant Starch—Not Polystyrene

Just as the environmentally sound additives might help lessen America's dependence on petroleum, so too might starch-based foams replace polystyrene products. An example: the rigid, custom-fit foam pieces used inside cardboard cartons to keep computer monitors securely in place during shipping and storage. Those foam pieces could just as easily be made from wheat, potato, or corn starches instead of petroleum.

That's according to Gregory M. Glenn, a plant physiologist with the ARS Western Regional Research Center in Albany, California.

Glenn and co-inventor Simon K. Hodson, formerly with EarthShell, LLC, of Lebanon, Missouri, have patented two "green" processes for making top-performing, starch-based foams for a multitude of everyday products—shipping liners, disposable dinnerware, and more.

These biofoams, which look like familiar polystyrene foam goods, are strong, durable, and versatile, Glenn says. They can be manufactured to the same range of densities as conventional foams and can be die-cut or molded into a seemingly limitless array of shapes, sizes, and thicknesses.

The starch-derived foams aren't waterproof, however, so a moisture barrier needs to be added. But such barriers are nothing new and, in fact, can be derived from plant sources, such as corn, so that the finished biofoam product would still be made exclusively from renewable, biodegradable natural resources.

The Shape of Things To Come

Glenn has worked with both of the new processes in small-scale experiments at

She wrote "Using Dairy Ingredients To Produce Edible Films and Biodegradable Packaging Materials," a book chapter in the soon-to-be-published *Dairy-Derived Ingredients: Food and Nutraceutical Uses,* by London-based Woodhead Publishing.

The chapter focuses on films made from dairy proteins, with an emphasis on those based on casein and whey, the major proteins found in milk. It also covers research efforts to improve the proteins' mechanical and barrier properties so that these natural materials could eventually be used in a variety of applications.

As a dairy ingredient, casein shows good adhesion to different substrates. But while casein is an excellent barrier to oxygen, carbon dioxide, and aromas, it is a weak barrier to moisture. "The water-soluble nature of these proteins is a big problem," says Tomasula. "Much of the research on edible casein films to date is directed to improving their water-vapor barrier properties. The use of dairy-based films as part of multilayered packaging systems will be the focus of future research."—By **Rosalie Marion Bliss,** ARS.

PEGGY GREB (D1639-1)

Food technologist Artur Klamczynski (left) and plant physiologist Greg Glenn prepare batches of starch-based dough which will be further processed by extrusion to form heat-expandable pellets.

his laboratory. Both technologies rely on an extruder—a standard piece of equipment—to heat and mix a dough-like blend of starch and other all-natural compounds.

For one process, the extruder squeezes out long strings of the dough, referred to as a "thermoplastic melt," that are later cut into small beads, about half the size of a marble. At various points in the process, the beads puff and expand. That happens, for instance, when the beads are put into the cavity of a heated mold, to press them into the desired shape.

Expanded beads will eventually touch one another, creating a strong matrix that's much like the bead matrix of polystyrene foams.

The second process that Glenn and Hodson developed results in a mix that emerges from the extruder as a continuous hollow tube. As it moves, it is slit along one length so that instead of being rounded, it opens flat, just like a book that's opened to lie flat on a table.

The now-flat foam is then rolled up—like a bolt of cloth—and stored. Later, it can be heated, unrolled, and die-cut into the desired product.

To enhance the strength of the foams, natural fibers—from a softwood such as southern pine—can be added to the mix before it goes into the extruder, reinforcing the foam for heavy-duty jobs.

The mounting environmental costs of landfills increasingly stuffed with non-biodegradable polystyrene may eventually make Earth-friendly, biobased foams the preferred choice for tomorrow.—By **Marcia Wood,** ARS.

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

To reach the scientists mentioned in this article, contact Marcia Wood, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; phone (301) 504-1662, fax (301) 504-1486, e-mail marcia.wood@ars.usda.gov. ✷

SCOTT BAUER (K5052-5)

For Sustainability Smarts: The National Agricultural Library's "Sustainable Agriculture Information Tools" Website

You're interested in learning more about sustainable farming, but you don't want to spend a lot of time surfing the web.

No worries.

Expert librarians at the National Agricultural Library in Beltsville, Maryland, and Washington, D.C., have done the homework for you, compiling handy resources at www.nal.usda.gov/afsic/sustain.shtml.

Scroll down to the "Learn More" headings to find an interesting potpourri of clickables, all leading you to reliable, authoritative resources. All are carefully vetted by the pros at the library's Alternative Farming Systems Information Center.

Get backgrounded quickly—and become fluent in the lingo—by skimming "Sustainable Agriculture: Definitions and Terms."

Check out the comprehensive "Sustainable Agriculture Automated Database Searches." Anywhere from thousands to millions of documents were sifted and winnowed to find those most relevant and of the highest quality.

Curious about scientific journals? You'll want to view the library's top picks, posted at "Sustainable Agriculture: Top 10 Research Journals."

Have college-age kids or grandkids who might be interested in studying sustainable ag? They'll definitely want to read "Educational and Training Opportunities in Sustainable Agriculture," which covers everything from the nation's longest-running undergraduate degree program in organic farming (Washington State University-Pullman) to the University of New Hampshire's new "eco-gastronomy" program, an eclectic blend of nutrition, food, and farming.

Updated frequently, the library's sustainable agriculture website is widely regarded as among the best of its kind.—By **Marcia Wood,** ARS.

ARS National Research Program on Obesity

Obesity is a growing epidemic in the United States that is leading to spiraling health problems and rapidly rising health care costs. ARS has unique expertise and facilities to carry out a cohesive research program on obesity prevention and energy metabolism, both through its national program in Human Nutrition and in coordination with ARS crop and animal breeding and new product and food-processing research.

The agency's obesity research program rises from an interlocking tripod of research areas: learning what people eat, what the body needs, and how to modify what we eat to be more beneficial. Results from each of these areas influence research in the others. A major facet of the research program is finding ways to prevent obesity in people.

ARS's six human nutrition research centers are home to carefully controlled human studies as well as community studies, which provide information from diverse populations at various stages of growth and physiology. This special expertise forms a core capability of ARS's obesity and human nutrition program.

In addition, ARS scientists at other locations across the country focus on developing new foods and food ingredients that may help solve the obesity problem. Their goal is foods that farmers can competitively produce, with taste, texture, and flavor that consumers accept. These are critical factors that must go along with nutritional enhancement.

To leverage resources for obesity research, ARS partners with other agencies, from USDA's Center for Nutrition Policy and Promotion to the Department of Health and Human Services' National Center for Health Statistics as well as many universities.

To further program coordination, ARS is developing a new obesity initiative that will build on the agency's capabilities to address the many facets of this growing problem. A particularly important facet will be conducting multicenter studies with adults and children to better understand biological factors underlying the propensity to gain weight and to test dietary and physical activity strategies to prevent unhealthy weight gain.

Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University
Boston, Massachusetts
www.ars.usda.gov/naa/hnrca

Children's Nutrition Research Center
Houston, Texas
www.ars.usda.gov/spa/cnrc

Western Human Nutrition Research Center
Davis, California
www.ars.usda.gov/pwa/davis/whnrc

Grand Forks Human Nutrition Research Center
Grand Forks, North Dakota
www.ars.usda.gov/npa/gfhnrc

Beltsville Human Nutrition Research Center
Beltsville, Maryland
www.ars.usda.gov/ba/bhnrc

Lower Mississippi Delta Nutrition Intervention Research Initiative
Little Rock, Arkansas
www.ars.usda.gov/spa/littlerock/deltaniri

Arkansas Children's Nutrition Center
Little Rock, Arkansas
www.ars.usda.gov/spa/littlerock/acnc/

U.S. Plant, Soil and Nutrition Laboratory
Ithaca, New York
www.ars.usda.gov/naa/ithaca/uspsnl

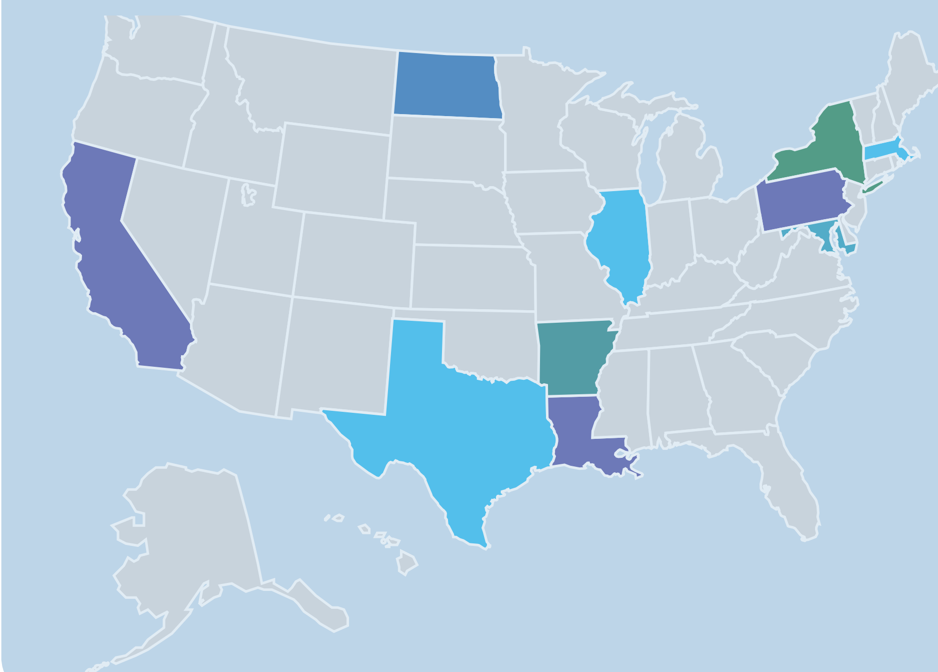
Eastern Regional Research Center
Wyndmoor, Pennsylvania
www.ars.usda.gov/naa/errc

Southern Regional Research Center
New Orleans, Louisiana
www.ars.usda.gov/msa/srrc

Western Regional Research Center
Albany, California
www.ars.usda.gov/pwa/wrrc

National Center for Agricultural Utilization Research
Peoria, Illinois
www.ars.usda.gov/mwa/ncaur

Locations of ARS Centers Doing Research on Obesity



IN-DEMAND FISH: Making Sure They're

Popular fish like salmon, catfish, and tilapia are coming under the close scrutiny of Agricultural Research Service food-safety scientists Andy Hwang and Kathleen Rajkowski. They're discovering more about how to prevent foodborne pathogens from contaminating these and other delicious, good-for-you seafood. Both scientists are based at the ARS Eastern Regional Research Center in Wyndmoor, Pennsylvania.

Hwang, a food technologist, has completed a series of studies in which he's simulated—in his laboratory—commercial processes used today for preparing smoked salmon. A gourmet treat, smoked salmon is typically sold in vacuum packages that have a refrigerator shelf life of about 3 to 8 weeks, according to Hwang. Trouble is, pathogenic microbes like *Listeria monocytogenes* can live at refrigerated temperatures, so it's important to get rid of these harmful microbes before the product leaves the processing plant.

Smoked salmon, pricey and, when properly prepared, delicate in texture, is often served in thin slices with bagels and cream cheese or as an appetizer, stacked on toast-type crackers with red onion and a splash of lemon juice. Too, some sushi bars feature smoked salmon surrounded by sticky rice and snugly wrapped in seaweed.

Hwang is looking for ways that processors can protect the pleasing flavor and texture of smoked salmon while reducing or eliminating contamination by *L. monocytogenes* or other foodborne pathogens.

PEGGY GREB (D1966-1)

Food technologist Andy Hwang and technician Stacy Raleigh study the effect of smoking temperature on survival of *Listeria monocytogenes* on smoked salmon.

At the Smokehouse

Smoked salmon is typically prepared by using what's known as "wet brining" or "dry brining" to cure the raw fillets before smoking. Fish cured with a wet brine are soaked in a solution of water, salt, and sugar, which preserves the fish, helps it retain moisture, and enhances its flavor. The brine may also include spices or liquid smoke, like the kind home chefs use for a backyard barbeque.

With dry brining, the salt and other compounds are rubbed on the fillets and later rinsed off before the fish is smoked.

The smoking process takes place in special smoking ovens in which wood chips are burned to smoke the cured fillets. Most processors opt for cold smoking, which uses temperatures of 68˚F to 86˚F to smoke—but not cook—the fillets. Cold-smoking takes about 3 to 4 days.

Hot-smoking, a lesser-used option for salmon, uses temperatures of about 140˚F and takes about 6 to 10 hours. Hot-smoking cooks the fish, giving it a different taste and texture than cold-smoked fish.

Always Safe To Eat

Many Combinations Tested

In a series of experiments, Hwang and colleagues Shiowshuh Sheen and Vijay Juneja at Wyndmoor exposed cooked salmon samples, prepared with various concentrations of salt and smoke compound (from burning wood chips or liquid smoke), to midrange temperatures—between 104˚F and 131˚F. "The temperatures were higher than those used for cold-smoking but not quite as warm as hot-smoking," explains Hwang. "We wanted to provide a range of alternative smoking temperatures for processors to consider and to show them the level of *Listeria* inactivation they might be able to achieve at various temperatures and various combinations of salt and smoke compound."

The scientists cooked the fillets for the tests to kill any existing microbes before inoculating the fish with *Listeria*. Not surprisingly, smoking temperature was the single most important factor for inactivating the microbe. "Every 9˚F increase in temperature resulted in a 10-fold increase in rates of inactivation of the *Listeria*," Hwang reports.

The researchers used data from the study to create a new, first-of-its-kind formula, or mathematical model, for food processors and their food-safety consultants to use in choosing the optimal combination of temperature and concentrations of salt and smoke compound.

"Users can plug into the model the salt concentration, smoke-compound concentration, and smoking temperature of their choice to predict what effect this combination may have on *Listeria* levels," says Hwang. "Salt and smoke-compound concentrations and smoking temperature affect taste, texture, and other key qualities of the smoked fish, so processors often have their own unique combination of these three factors. We constructed the model to accommodate a wide range of choices."

The team's 2009 article in the *Journal of Food Science* has details.

Now, Hwang intends to test these laboratory findings at a smokehouse and monitor the safety of the smoked salmon as it makes its way through the distribution chain, from wholesaler to retailer to restaurant or home.

And as a followup to a preliminary study that he and Sheen described in another 2009 *Journal of Food Science* article, Hwang wants to discover more about the extent to which other microbes—benign or harmful—can colonize the fillets and help or hinder *Listeria's* survival.

Powerful Tactics That Don't Require Heat

Meanwhile, colleague Rajkowski, a food microbiologist, is determining how to prevent certain foodborne pathogens from contaminating fish fillets. She's using tilapia and catfish fillets for this research. "Even though foodborne illnesses are not commonly associated with either of these fish," says Rajkowski, "we chose them for our research because they are the two most commonly consumed kinds of fish fillets in the United States today."

Microbes that she's studying include not only *Listeria* but also *Salmonella, Shigella, Staphylococcus*, *Pseudomonas*, and *Escherichia coli* O157:H7.

In one study, Rajkowski is determining the correct cooking times and temperatures for packaged tilapia fillets. Instructions for cooking fillets are sometimes based on visual determination—what the fish looks like.

"Instructions might require you to know what the fillet looks like when it 'flakes easily with a fork,'" she says. "Not everyone knows what's meant by that. We want to provide science-based cooking instructions that are precise and easier for everyone, even beginning cooks, to follow."

Rajkowski is continuing research on heat-free ways to reduce levels of harmful microbes. Overheating can easily ruin the taste and texture of fish.

In an early experiment with both frozen and thawed tilapia and catfish fillets, Rajkowski artificially inoculated the fillets with *L. monocytogenes* and then determined the amount of ionizing radiation needed to reduce the pathogen's population by 90 percent. The dosages required to achieve that level of safety were nearly the same for both kinds of fish, Rajkowski found. Published in the *Journal of Food Protection* in 2008, the study was the first to identify the dosages needed to effectively reduce *Listeria* in these popular fish products. Her results were similar to those that reduce *Listeria* in ground beef.

PEGGY GREB (D1964-1)

Food microbiologist Kathleen Rajkowski places a frozen catfish fillet into a device for surface decontamination by pulse UV treatment.

Rajkowski is also testing the effects of ultraviolet (UV) light in combating another pathogen, *Shigella sonnei*. Like *Listeria*, *Shigella* can cause gastrointestinal illness. For one investigation, Rajkowski applied a solution of *S. sonnei* to the surface of frozen tilapia and then exposed the samples to UV light. The treatment resulted in a 99-percent reduction of the pathogen. In contrast, tests with small samples of fresh tilapia showed that the UV treatment did *not* kill the pathogen. But exposing the fillets to pulsating beams of high-intensity UV light reduced the pathogen by 99 percent. Rajkowski documented the study in 2007 in *Ice World Journal*.

Fish that Hwang and Rajkowski are investigating are a good, low-fat source of high-quality protein. That's reason enough to make sure these fish, and others from farm and sea, remain pathogen-free and safe for us to eat.—By **Marcia Wood,** ARS.

This research supports the USDA priority of ensuring food safety and is part of Food Safety, an ARS national program (#108) described at www.nps.ars.usda.gov.

Cheng-An (Andy) Hwang and Kathleen Rajkowski are with the USDA-ARS Eastern Regional Research Center, 600 E. Mermaid Ln., Wyndmoor, PA 19038; (215) 233-6416 (Hwang), (215) 233-6440 (Rajkowski), andy.hwang@ars.usda.gov, kathleen.rajkowsi@ars.usda.gov. *

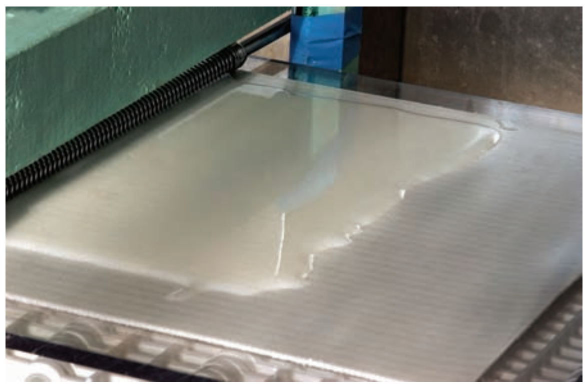
Edible, Water-Resistant Film from Milk Protein

A new process to continuously make films from a milk protein could lead to edible, water-resistant coatings on several products commonly found in grocery-store dairy aisles.

The process uses the unique characteristics of casein (pronounced *KAY-seen*), a milk protein that is the chief nutritional ingredient in cheese. It is also used as a food supplement and as a functional component in nonfood products, including adhesives, finishing materials for paper and textiles, and paints.

Casein's natural structure gives it the ability to form water-resistant and biodegradable films or coatings. Casein films could be produced as stand-alone sheets or as thin coatings that could adhere directly to a product. Either form would act as a barrier to outside substances while protecting a product from damage or contamination. The edible film locks in moisture, so it may be used to coat dairy food products, such as cheese, or as part of a laminate in packaging for cottage cheese or yogurt. Flavorings, vitamins, or minerals could also be added to the coatings.

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



A continuous biodegradable protein film begins to form using the new ARS film-making process.

The process uses an ARS-patented method developed by Peggy Tomasula, research leader in ERRC's Dairy Processing and Products Research Unit in Wyndmoor, Pennsylvania. Her extraction method separates casein from milk with high-pressure carbon dioxide.

Tomasula found that if this casein is mixed with water and glycerol and left undisturbed to dry, it forms a water-resistant, flexible, filmlike material.

"It's been difficult to obtain films, fibers, and molded materials with acceptable mechanical properties from casein," Tomasula said. "That's because moisture can dissolve it. The new material remains intact when exposed to water, unlike other protein-based films patented in the past."

And until now, it hasn't been known how to make such films on a continuous, large scale.

Michael Kozempel, who recently retired as a chemical engineer from ERRC, and Tomasula developed a pilot-plant process to produce the film continuously. Kozempel determined which feeding mechanism would spread the casein solution uniformly and which belt material would allow easiest removal of the dried films. He also specified drying conditions and temperatures for making the films in 3 hours.

"This process makes the films continuously, and it can be modified for other proteins," Kozempel says. "It establishes the feasibility of commercial production of biodegradable polymer coatings made from dairy products."

Kozempel also showed that up to 20 percent of the casein can be substituted with nonfat dry milk, which reduces the cost of the films with little loss in physical properties.

ARS has filed a patent application on the continuous-production process and is interested in finding business partners to move it to market.—By **Jim Core,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

Peggy Tomasula is in the USDA-ARS Dairy Processing and Products Research Unit, Eastern Regional Research Center, 600 East Mermaid Ln., Wyndmoor, PA 19038; phone (215) 233-6703, fax (215) 233-6795, e-mail ptomasula@errc.ars.usda.gov. ✷

The edible film locks in moisture, so it may be used to coat dairy food products, such as cheese, or as part of a laminate in packaging for cottage cheese or yogurt.

Hulled winter barley ready for harvest.

Boosting Barley for Bioenergy

Agricultural Research Service (ARS) scientists Kevin Hicks and David Marshall want winter barley to become a prime-time player in bioenergy production.

"The 2007 Energy Independence and Security Act requires production and use of 36 billion gallons of renewable transportation fuels by 2022. Today we only make 9 billion," says Hicks. "We see winter barley as the perfect biofeedstock for making biofuels on the East Coast."

So Hicks and others in the ARS Crop Conversion Science and Engineering Research Unit in Wyndmoor, Pennsylvania, are developing new sustainable technologies to convert varieties of hulled and hull-less winter "energy" barley into fuel ethanol. This initiative also includes Virginia Polytechnic Institute and State University scientists Carl Griffey, Wynse Brooks, and Mark Vaughn, who are supervising ongoing research efforts to develop improved varieties of hulled and hull-less barley.

Their combined efforts could help farmers from southern Pennsylvania to South Carolina develop a profitable 2-year rotation of winter barley, corn, and soybeans. Winter barley is grown on seasonally fallow land. It acts as a cover crop by protecting soil and nutrients and preventing migration of fertilizers from crop fields to the Chesapeake Bay—which is why the Chesapeake Bay Commission supports the development of winter barley as an energy crop. And since the field would otherwise be left fallow, producing biofuels from winter barley would not interfere with food production.

Now, too, there is a major marketing opportunity for growers of winter barley. Osage Bio Energy, headquartered in Glen Allen, Virginia, is well under way in constructing the first major barley-to-ethanol production facility in the United States.

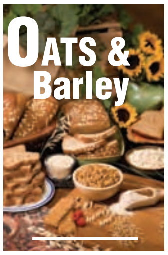
Meanwhile, in Raleigh, North Carolina, Marshall coordinates the regional winter barley testing nursery, which has the best experimental lines from both public and private winter barley breeding programs in the United States. He and other scientists in the ARS Plant Science Research Unit are just a few years into making crosses between hull-less barley and barley with resistance to Ug99, a stem rust that can inflict crop losses of up to 100 percent.

Once the researchers have developed robust lines that contain both traits, they'll begin breeding for traits to enhance ethanol production, such as starch content. "In several years, we hope to release barley varieties with traits for enhanced agronomic performance, good grain-to-ethanol qualities, and good resistance to stem rust," Marshall says.—By **Ann Perry,** ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301), Bioenergy and Energy Alternatives (#307), and Quality and Utilization of Agricultural Products (#306), three ARS national programs described at www.nps.ars.usda.gov.

Kevin B. Hicks is with the USDA-ARS Crop Conversion Science and Engineering Research Unit, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038; (215) 233-6579, kevin.hicks@ars.usda.gov.

David S. Marshall is in the USDA-ARS Plant Science Research Unit, North Carolina State University, 3411 Gardner Hall, Raleigh, NC 27695; (919) 515-6819, david.marshall@ars.usda.gov. ✷



New Database Helps Monitor Food Pathogens

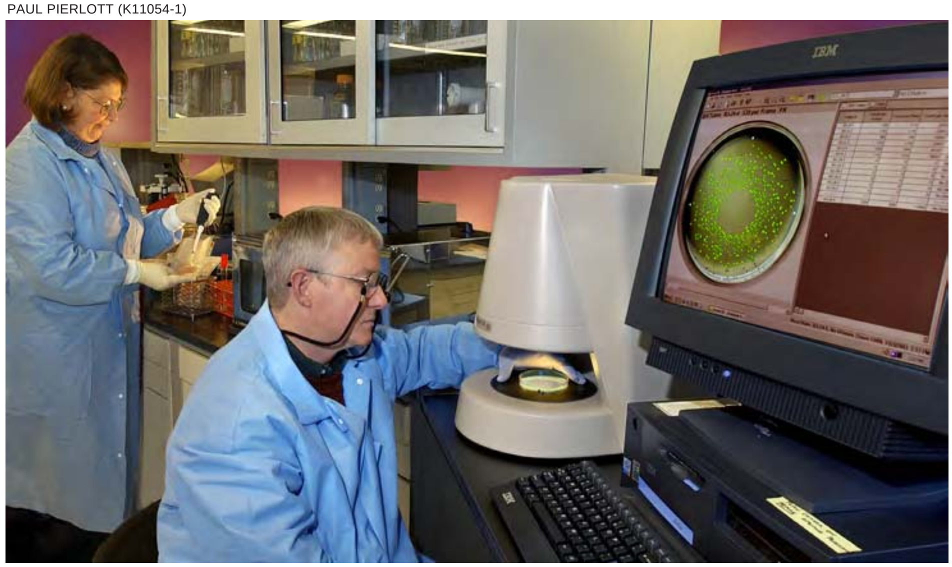
A new Agricultural Research Service initiative will help facilitate research cooperation among scientists studying a key food safety issue—how pathogenic bacteria respond to different environmental conditions in food.

Scientists in the Eastern Regional Research Center's (ERRC) Microbial Food Safety Research Unit in Wyndmoor, Pennsylvania, and the United Kingdom's Institute of Food Research (IFR) have formed the world's largest online database of predictive microbiology information. Predictive microbiology is a growing field that estimates behavior of microorganisms in response to environmental conditions, including food production and processing operations from the farm to the table. The database, called ComBase, is designed to help make risk assessments and model development easier. It was released at the fourth International Predictive Modeling in Foods Conference in June 2003 and can be found on the World Wide Web at wyndmoor.arserrc.gov/combase.

For more than 15 years, ERRC has developed mathematical models of the behavior of bacterial pathogens in food. In February 2002, ERRC established the Center of Excellence in Microbial Modeling and Informatics (CEMMI) to help generate interest in forming partnerships that advance use of predictive models of microorganisms in food. This "virtual laboratory" can be found at www.arserrc.gov/cemmi.

CEMMI's objective is to link the expertise of its members to researchers in the food industry, government, and academia. According to coordinator Mark L. Tamplin, the center hopes to improve the way predictive models are developed and then applied to various food-processing situations, while ensuring that users properly interpret results. Predictive microbiology can also benefit the risk-assessment community by solving gaps in research data and enhancing uniformity in experimental designs, he says.

PAUL PIERLOTT (K11054-1)



Microbiologist Benne Marmer (left) and technician Tod Stewart use computer-assisted laboratory methods to record growth of *Listeria monocytogenes* bacteria on ready-to-eat meat products. This information is analyzed in ComBase and then converted to a model in the ARS Pathogen Modeling Program software.

ERRC's Pathogen Modeling Program software, a research and instructional tool for estimating the effects of multiple variables on growth, inactivation, or survival of foodborne pathogens, has been available for download since 2002 at www.arserrc.gov/mfs/pathogen.htm. That program and the United Kingdom's Food MicroModel, produced by IFR and the Foods Standards Agency, are both major software packages used to describe bacterial responses to food environments. By combining their efforts and using ComBase to organize the thousands of existing data sets in the hands of scientists and in the literature, ARS and IFR have expanded the uses for this vast amount of information.

ComBase, a CEMMI project, already contains around 25,000 growth and survival data set records. The software lets scientists simulate a food environment by entering data—such as temperature, acidity, and available water—and then retrieves all records that match those search criteria. Tamplin says microbiologists in academia, government, and industry are submitting data to ComBase, thus eliminating unnecessary repetition of experiments among laboratories, improving models, and standardizing data sources. More than 1,000 people accessed ComBase data in the first 4 months following its launch. Tamplin says the ultimate success of ComBase relies on collaborators' willingness to deposit more data.

"Development of models depends on available data," he says. "We're appealing to professional journals to ask authors to submit all their raw data with their articles, much as they already do for papers about genomic sequences. This would help keep the database timely and offer users the most reliable information."—By **Jim Core,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

Mark L. Tamplin is with the USDA-ARS Microbial Food Safety Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038; phone (215) 836-3794, fax (215) 233-6581, e-mail mtamplin@errc.ars.usda.gov. ★

Cheese, Please

Recreating Unique Properties of Hispanic Cheeses

America's growing Hispanic population craves the various types of cheese available in their native countries. Raw milk, which is used to produce these cheeses, gives them distinctive flavors, textures, and cooking properties. Even though some American companies are producing Hispanic-style cheeses from pasteurized milk (a U.S. requirement for cheeses aged less than 60 days), these do not exhibit the full range of properties of cheeses made from raw milk.

Researchers at ARS' Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, are studying Hispanic cheeses to help producers meet the increasing demand for them. Total Hispanic-style cheese production in the United States jumped 52 percent from 1996 to 2001, when more than 102 million pounds were sold, according to USDA's National Agricultural Statistics Service. In fact, it is one of the fastest growing U.S. food markets, outpacing the growth of the cheddar and mozzarella markets.

PEGGY GREB (K10121-1)

properties of Hispanic-style cheeses to establish their texture profiles.

It's Flavor, Not Fire

Hispanic-style cheese does not mean hot-and-spicy cheese; other ingredients make Hispanic dishes hot. In general, the cheeses are white or off-white and moist. They taste like fresh milk. They become soft and creamy when heated but do not lose shape, run, or separate into greasy solids and liquids. Most cheeses are fresh, though some are aged. Many are mild tasting and crumbly. Others are harder and have stronger flavor.

Chemist Diane L. Van Hekken and colleagues in ERRC's Dairy Processing and Products Research Unit are studying selected Hispanic cheeses made in Mexico to better understand how specific processing techniques result in their desirable qualities. They are looking at the cheeses' chemical and physical makeup to learn how these properties relate to flavor; texture, such as chewiness and stringiness; and function, such as the ability to melt or be sliced. They want to find ways to duplicate these characteristics by following U.S. practices and standards. Then, they hope to apply the findings to improving cheese-processing techniques in general.

According to Van Hekken, there is not a lot of literature available for researchers on the characteristics of these cheese types, and so a main objective is to compile needed references.

In Search of Authenticity

The researchers are looking at four specific cheese types. The first, Queso Blanco, may be the most popular cheese south of the border. It is soft and won't melt. Panela is mild, sweet, and crumbly. Asadero is a smooth, yellow cheese that is somewhat tangy and good for baking. And last, Van Hekken's research team traveled to Mexico twice, where they are working with a collaborator to examine Mennonite-style cheeses from the state of Chihuahua. These semihard cheeses—named after the Mennonite settlers who introduced them to the region—are similar to Queso Quesadilla and Menonita found in the United States.

"All these cheeses have been developed for specific purposes," Van Hekken explains. "People can't cook a Mexican-style dish, for example, with American-style cheese and expect it to taste authentic. Restaurants that want their dishes to be traditional know this, and they search for the right cheeses."

A sensory evaluation board (taste panel) at ERRC has been working since May 2001 to define the flavor profiles of both raw and pasteurized cheeses. MaryAnne Drake, a professor with North Carolina University's Department of Food Science, helped initiate training for panelists to become human instruments and to use a common terminology in describing what they taste.

The researchers also hope to improve the shelf life of Hispanic-style cheeses, which will expand their marketability here and in foreign markets and ensure high food-safety standards.—By **Jim Core,** ARS.

This research is part of New Uses, Quality, and Marketability of Plant and Animal Products, an ARS National Program (#306) described on the World Wide Web at http://www.nps.ars.usda.gov.

Diane L. Van Hekken is in the USDA-ARS Dairy Processing and Products Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038-8598; phone (215) 836-3777, fax (215) 233-6795, e-mail dvanhekken@arserrc.gov. ★

Biofuel Gets Less Expensive

Being stuck in a traffic jam may be a bit more bearable if the aroma of fresh french fries was in the air, rather than the noxious stink of fossil fuel exhaust.

When scientists at the Eastern Regional Research Center (ERRC) recently tested biodiesel fuel produced from soybean soapstock—an abundant but underused byproduct of vegetable oil refining—they found its composition, engine performance, and emissions to be comparable to those of biodiesel fuels presently on the market, which are made from highly refined edible oils. The french-fry aroma, another typical trait of current biodiesel fuels, was an added bonus.

Biodiesel is the term given to diesel engine fuels made from agricultural fats and oils. There is much interest in the use of biodiesel throughout the world because it is made from renewable resources and reduces air-polluting emissions by diesel engines.

ARS research chemist Michael J. Haas and biologist Karen M. Scott teamed with research scientist Scott Bloomer—then at the international agricultural company Cargill in Minneapolis, Minnesota—to develop a chemical method that converts all forms of fatty acids found in lipids of soybean soapstock into simple methyl esters. The researchers have applied for a patent on the method.

PEGGY GREB (K9841-1)

Biologist Karen Scott and biochemist Michael Haas prepare a reactor of soybean-derived soapstock for conversion to biodiesel.

Haas, Thomas A. Foglia, and other ARS researchers with ERRC's Hides, Lipids, and Wool Research Unit in Wyndmoor, Pennsylvania, are interested in using lower value lipids derived from animal fats, vegetable oils, and recycled greases as raw materials for biodiesel production. They want to make biodiesel more attractive economically while reducing use of imported fossil fuels and increasing use of renewable, agriculture-based products.

The french-fry aroma, another typical trait of current biodiesel fuels, was an added bonus.

Many commercially available biodiesel fuels are made from refined soy oil and are then added to diesel, typically at levels of 20 percent of the mixture's volume. According to Haas, studies show that biodiesel, used alone or in such blends, can provide much needed lubrication to fuel systems while also reducing production of polluting exhaust emissions. Because of these benefits, Haas points out, there is strong interest in this country and around the world in developing methods to produce biodiesel from fats and oils.

Originally, methods were designed to produce biodiesel from highly refined oils. Now Haas, Scott, and Bloomer have modified the technology to allow use of lower value, less pure lipids, such as soapstock, as starting materials. This could increase availability of biodiesel while decreasing its cost.

Soybean oil soapstock is a plentiful and relatively inexpensive byproduct of edible-oil refining. About 100 million pounds are produced in the United States every year, and it can be had for one-tenth or less the cost of refined vegetable oil, Haas says. Currently, it is used mostly as a cheap source of fat in livestock feeds. However, by implementing processes such as the one developed by Haas and Bloomer, the agents in soapstock could one day serve as the source of more than 6 million gallons per year of diesel engine fuel or fuel additives and in other applications such as cleaning agents and organic solvents.

Other ARS scientists have patented another method to develop biodiesel and lubricants, as well as fuel and lubricant additives. This enzymatic method, invented by ERRC researchers Foglia, William N. Marmer, and Lloyd A. Nelson, uses animal fats, vegetable oils, rendered fats, and restaurant grease to produce the fatty acid esters that can be used as biodiesel and lubricants. Because conversion of grease, in particular, was very effective and inexpensive, grease-derived biodiesel could be used in conjunction with soybean oil derivatives to reduce costs.—By **Jim Core,** ARS.

This research is part of Bioenergy and Energy Alternatives, an ARS National Program (#307) described on the World Wide Web at http://www.nps.ars.usda.gov.

Michael J. Haas is in the USDA-ARS Hides, Lipids, and Wool Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038; phone (215) 233-6459, fax (215) 233-6795, e-mail mhaas@arserrc.gov. ◆

New Methods for Detecting *Listeria*

Quick, accurate, cost-effective methods for detecting pathogenic bacteria are essential to ensuring a safe food supply. ARS scientists at the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, are improving methods to detect foodborne pathogens like the deadly *Listeria monocytogenes*.

The Centers for Disease Control and Prevention estimates that listeriosis, the disease caused by *L. monocytogenes* infection, affects around 2,500 people every year and kills about 500. Newborns, seniors, pregnant women, and individuals with compromised or weakened immune systems are particularly susceptible.

Most methods for detecting harmful foodborne bacteria rely on antibodies, proteins used by the immune system to fight infections and foreign bodies. Because antibodies are highly specific, researchers can use them to identify and locate specific pathogens.

Antibodies vary in their degree of specificity. For example, current antibody-based methods for detecting *L. monocytogenes* can't distinguish it from the mix of nonharmful bacteria found in most foods, says Shu-I Tu, research leader of the ERRC Microbial Biophysics and Residue Chemistry Research Unit.

A molecular method called "phage display" uses bacteria and bacterial viruses, or phages, to quickly select antibodies to detect pathogens. Microbiologist George C. Paoli and chemist Jeffrey D. Brewster have used phage display to isolate an antibody fragment that binds specifically to *L. monocytogenes*.

"This may be the first antibody to demonstrate the high degree of specificity required to accurately detect this pathogen," Paoli says.

The researchers' success demonstrates that antibody phage display can be used to select antibodies for pathogen detection—even where traditional methods have proved inadequate. Paoli is also using phage display to select antibodies targeting other difficult-to-detect bacterial pathogens related to food safety and security.

Paoli has since characterized the interaction of the antibody with the bacterium and has identified the part of the *L. monocytogenes* cell to which this antibody binds. Paoli and postdoctoral research associate Lynn Kleina also linked the antibody fragments to microscopic magnetic beads, creating an *L. monocytogenes*-specific immunomagnetic bead (IMB).

Because the antibodies bind to specific targets, the anti-*L. monocytogenes* IMBs can be used to capture just *L. monocytogenes* from the bacterial mix found in food. This is the first step in developing a significantly improved detection method for *L. monocytogenes.* This work will allow researchers to develop much-needed rapid tests for the pathogen, reducing the likelihood that contaminated food will reach consumers.

The Microbial Biophysics and Residue Chemistry Research Unit has an impressive history of pathogen detection research. To learn more about this work, see "New Detection Methods Improve Food Safety," *Agricultural Research,* January 2005, pp. 15-17.—By **Laura McGinnis,** ARS.

This research is part of Food Safety, an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

Shu-I Tu is in the USDA-ARS Microbial Biophysics and Residue Chemistry Research Unit, Eastern Regional Research Center, 600 E. Mermaid Ln., Wyndmoor, PA 19038; phone (215) 233-6466, fax (215) 233-6581, e-mail shu-i.tu@ars.usda.gov. ✷

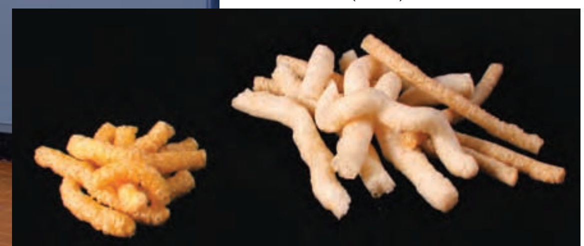
PEGGY GREB (D615-1)

From left, microbiologist George Paoli, postdoctoral research associate Lynn Kleina, and chemist Jeffrey Brewster examine colonies of *Listeria monocytogenes* captured with immunomagnetic beads coated with single-chain antibodies specific for the pathogen.

PAUL PIERLOTT (D041-1)

Left: In a modern pilot plant at the Eastern Regional Research Center in Wyndmoor, Pennsylvania, food technologist Charles Onwulata works on development of processed foods and creation of unique biopolymers with production-scale extruders and an injection molding machine.

PAUL PIERLOTT (D041-2)



Inset: Whey-protein enriched corn curls. ARS scientists have developed a method to modify whey proteins, making them more compatible with starch and therefore easier to puff.

Creating Novel Foods and More From Agricultural Products

The Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, recently announced the opening of the Center of Excellence in Extrusion and Polymer Rheology (CEEPR).

The goal of the new center is to create value-added, novel foods, food ingredients, and nonfood or industrial biodegradable products from underused agricultural materials such as whey or corn byproducts. Current research projects cover a wide range of products, including snack foods, cheeses, meal replacement bars, pet foods, texturized proteins or meat substitutes, aquaculture feed, and nonfood materials such as films.

Scientists at ERRC work to create processes for converting corn and other grains into food and nonfood products. The waste streams from these processes will provide working materials for CEEPR projects.

The center features a modern pilot plant where new products can be developed from concept to prototype and eventually to full market production through technology-transfer collaborations. For example, they will develop processed foods and create unique biopolymers with production-scale extruders and an injection molding machine at ERRC.

Extrusion is a process of converting raw materials into new forms. The materials are first made into a semisolid mass, then forced through a die's restricted opening to create new shapes. According to Charles Onwulata, a food technologist in ERRC's Dairy Processing and Products Research Unit and CEEPR's coordinator, the food industry uses rheology equipment, which helps determine the form, deformation, and flow properties of melted materials and the texture of resulting products. As Onwulata says, "The rheology of the product in the extruder affects the structure and texture of the finished product."

Knowledge of polymer rheology is essential in maintaining uniform textures in molded products such as, for example, an ice cream sandwich. ERRC's polymer work will use injection molding to create bioplastics from agricultural wastes.

Onwulata says they are forming partnerships with industry, other research agencies, and universities. In the past, Onwulata has collaborated with two sister agencies at the U.S. Department of Agriculture. He worked with the Animal and Plant Health Inspection Service to develop an insect feed and with the United States Agency for International Development to develop an extruded instant emergency food product needing no further cooking. Onwulata is also developing new ways to use whey proteins in enhancing the nutrition content of extruded crunchy snack foods.—By **Jim Core,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

Charles Onwulata is in the USDA-ARS Dairy Processing and Products Research Unit, Eastern Regional Research Center, 600 East Mermaid Ln., Wyndmoor, PA 19038; phone (215) 233-6497, fax (215) 233-6795, e-mail conwulata@errc.ars.usda.gov. ◆

Washing and Sanitizing Techniques Aim To Make Produce Safer

Fresh fruits and vegetables are rich in essential vitamins and minerals, a quality that contributes to healthier diets. But several outbreaks of food poisoning in recent years have been linked to cantaloupes, sprouts, lettuce, tomatoes, and other fresh produce. Conventional commercial washing and sanitizing methods to remove microbial contaminants from produce surfaces have been found to be marginally effective.

Scientists at ARS's Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, are conducting experiments to understand how these microorganisms survive and grow on produce surfaces even after exposure to sanitizing solutions like chlorine.

Says Bassam A. Annous, a microbiologist in ERRC's Food Safety Intervention Technologies Research Unit, "Gaining knowledge of factors that permit survival of microbial contaminants during washing and sanitizing will help us develop new technologies for removing or inactivating microbes on both fresh and minimally processed produce."

The human pathogen *Salmonella* is often the culprit behind produce-related outbreaks of foodborne illness. It's an important concern in ensuring microbiological safety of fresh-cut cantaloupe. The bacteria quickly cling to the rind and form a "biofilm"—a mass of microbes attached to a surface and to each other by bacterial polymers (complex sugars). Then, *Salmonella* cells present on the rind can be transferred to the melon's internal tissues during cutting.

Annous, along with postdoctoral researcher Ethan Solomon, biologist Angela Burke, food technologist Dike Ukuku, and biologist Peter Cooke, recently gained new insight into how *Salmonella* forms this biofilm on cantaloupe surfaces. *Salmonella* cells produce fimbriae (hairlike structures) and cellulose that help them attach, colonize, and survive on the melon's surface. Once attached to the outside of the rind, the cells grow and excrete polymers, forming the biofilm, which shields the bacteria from harsh sanitizing solutions.

The researchers will collaborate with scientists at the University of Navarra, in Spain, to determine how *Salmonella* develop fimbriae as they grow and how they use them to cling to surfaces. They will also determine the role of specific bacterial polymers involved in surface attachment and biofilm formation by using *Salmonella* cells that are defective in producing fimbriae and/or cellulose.

But scientists are already finding ways to outsmart the bacerium. For instance, Annous and Joseph Sites, a mechanical engineer, recently developed a commercial-scale surface-pasteurization treatment that resulted in a 5-logs (99.999 percent) reduction in a population of *Salmonella* Poona on the surface of artificially contaminated cantaloupe. The process involves immersing melons in water at 169˚F for 3 minutes to kill the pathogens, then sealing each melon in a plastic bag before rapid cooling in an ice-water bath. The plastic bag prevents the fruit from potential recontamination in the cold water. The treatment not only enhances the safety of the fruit, but also extends its shelf life by reducing native microflora that may cause spoilage. And it did not harm melon quality.—By **Jim Core,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

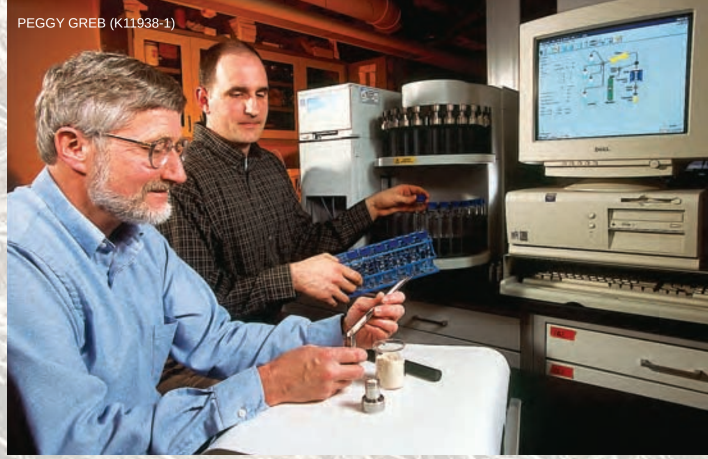
PAUL PIERLOTT, USDA-ERRC/VGT (D321-1)

Surface pasteurization of cantaloupes using a commercial-scale dump tank (designed at ARS's Eastern Regional Research Center) in ERRC's biosafety level-2 pilot plant.

Bassam A. Annous is in the USDA-ARS Food Safety Intervention Technologies Research Unit, 600 East Mermaid Ln., Wyndmoor, PA 19038-8598; phone (215) 233-6797, fax (215) 233-6406, e-mail bannous@errc.ars.usda.gov. ✷

New Varieties and Techniques Make Barley Better For Fuel

PEGGY GREB (K11938-1)



Chemist Robert Moreau (left) and biologist Mike Powell prepare samples to analyze edible oils and nutraceuticals in barley fractions using HPLC-MS methods.

PEGGY GREB (K11935-1)

Chemical engineer Gerry Senske (left) and research leader Kevin Hicks carry out a fermentation of hull-less barley to produce fuel ethanol.

New varieties of barley could help solve two national problems: energy dependence and obesity. That's because some parts of barley are excellent for making fuel ethanol, while other parts are nutritious and naturally low in calories.

High starch content is needed to produce ethanol, while soluble fibers such as beta-glucan and other types of fiber have been found to possess health benefits for people. Now scientists are developing ways to maximize the benefits found in some special barley varieties.

Researchers at the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, want to reduce the cost of fuel ethanol and make it available nationwide. Most ethanol production facilities use corn as starting material, or "feedstock," and not surprisingly, they're located in the Corn Belt, not on the East and West Coasts, where demand for fuel ethanol is increasing. It's too costly for most coastal states to ship corn from the Midwest to a local ethanol plant, so it makes sense for these "corn-deficient" states to use locally grown grains as alternatives. Barley, which grows well in these regions, may be the answer—once a couple of problems are solved.

"Barley isn't usually thought of as a good candidate for fuel ethanol feedstock because it typically has an abrasive hull and a lower starch content than corn," says Kevin Hicks, research leader of ERRC's Crop Conversion Science and Engineering Research Unit. "And it contains a troublesome polysaccharide, beta-glucan, that makes barley mash too viscous to mix, ferment, and distill economically."

Low starch content in most barley varieties—50 to 55 percent compared to corn's 72 percent—results in lower ethanol yield. ARS researchers are helping to create new barley varieties with higher starch content. They're also developing new milling processes that will separate low-starch barley kernels into a starch-enriched stream for efficient ethanol production and a protein- and fiber-enriched fraction for making valuable food and feed co-products.

Research is needed to help growers select the best varieties for ethanol production and to solve technical problems related to milling and fermenting barley. ARS also wants to expand the feed applications for barley distillers dried grains with solubles (DDGS), the main co-product from "dry grind" ethanol production

In the process of finding solutions to all these needs, ARS researchers may have also found some ways to provide the food industry with nutritious ingredients.

Better Varieties for Ethanol Production

ERRC researchers examined more than 100 new lines of barley being developed by collaborators at Virginia Polytechnic Institute and State University for suitability in ethanol production and have narrowed their focus to several promising varieties. Lines being developed at the ARS Small Grains and Potato Germplasm Research Unit in Aberdeen, Idaho, are also being evaluated.

The scientists are looking at malt, hulled, and hull-less barley varieties suitable for growers in various parts of the country.

Most barley not selected for brewing is used in animal feed and sells for well under $2 a bushel, according to Hicks. Producers would like to see a higher return for their crop.

Barley hulls are very abrasive and cause expensive wear and tear on grain handling and milling equipment. Removing the hull and other nonstarch components of the kernel before fermentation for ethanol would greatly improve the production process. A combination of milling, grind-

Starch and **soluble fibers** are key in ARS's efforts to use barley to produce ethanol and enhance the nutrition in food products.

Chemical engineer Jhanel Wilson and agricultural engineer Rolando Flores mill barley kernels into starch-enriched fractions for ethanol production and low-starch fractions for food and feed.

PEGGY GREB (K11940-1)

ing, and size-fractionation techniques could accomplish this. But perhaps an even better idea is to use barley varieties that are naturally hull-less, avoiding the need for "pearling"—a process that removes the hull and makes the grains smaller and rounder. As Rolando Flores, an ERRC agricultural engineer, explains, pearling removes the hull, divides the fiber into several fractions, and separates the protein from the starch to produce ethanol without wasting low-starch barley co-products.

Hull-less varieties lose their hull during harvesting. They have more starch and protein but less fiber than hulled varieties. Several Virginia hull-less lines look promising in ERRC's program.

Hull-less barley solves the "hull" problem but not the beta-glucan viscosity issue. With hulled varieties, fractional milling techniques such as pearling help by removing beta-glucan-enriched fractions before fermentation. But these techniques don't remove all the beta-glucan. So, for both hull-less and hulled varieties, beta-glucanase enzymes are used in fermentation to dramatically break down high-viscosity beta-glucans into low-viscosity oligosaccharides, solving the beta-glucan problem, according to Dennis O'Brien, an ERRC chemical engineer.

Nutrition for Feed and Food

Animal feeds rich in beta-glucan cause nutritional problems in poultry. Beta-glucan in DDGS limits their application for use as feed mainly to ruminants, such as cattle. But high-protein DDGS fractions with low-levels of beta-glucan would make useful ingredients in poultry, swine, and fish feeds. The ERRC team is making progress in this area.

Barley fractions that are high in beta-glucan might also find use in certain foods. Beta-glucan has been found to lower cholesterol levels and blood pressure and improve immune function in human studies.

Scientists with the Beltsville (Maryland) Human Nutrition Research Center's (BHNRC) Diet and Human Performance Laboratory will study the possibility of using beta-glucan and other soluble fibers not being used in ethanol production. Future studies at BHNRC will include human feeding studies designed to test the health-promoting effects from foods prepared with the ERRC barley co-products.

"In our previous human feeding studies, consumption of soluble fiber from barley significantly improved several risk factors for cardiovascular disease and diabetes," says Kay Behall, a BHNRC chemist.

Robert A. Moreau, an ERRC chemist on the project, says fractions remaining after milling are also low in starch, so they're low in carbohydrates and high in dietary fiber, oil, and protein, which makes them ideal nutraceuticals—ingredients added to food for their health benefits. Barley fractions could be added to pasta, breads, pastries, and snack foods to increase their fiber content and reduce starch.

Moreau and cooperators at the University of Helsinki in Finland found that phytosterols in grains such as barley and rye are concentrated in the outer layers, or "fines," of kernels. Some studies have found that these plant sterols might contribute to a reduced risk of coronary heart disease. Currently, sterols are used to enrich products such as margarine and orange juice.

ARS researchers are using pearling to remove plant sterols and fiber-enriched outer layers of hull-less barley. "We found that fines removed from kernels contained two to three times as many phytosterols per unit weight as did whole kernels. These fines could serve as a new source of phytosterols," Moreau says.—By **Jim Core,** ARS.

This research is part of Quality and Utilization of Agricultural Products (#306) and Bioenergy and Energy Alternatives (#307), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

Kevin Hicks is in the USDA-ARS Crop Conversion Science and Engineering Research Unit, Eastern Regional Research Center, 600 East Mermaid Ln., Wyndmoor, PA 19038-8598; phone (215) 233-6579, fax (215) 233-6406, e-mail khicks@errc.ars.usda.gov. ✷

SCOTT BAUER (K5267-6)

New Method Simplifies Biodiesel Production

Oil from soybeans is the usual starting material for biodiesel.

ARS scientists in Wyndmoor, Pennsylvania, have modified biodiesel production technology. Their method eliminates a step—and an air-polluting chemical—from the process of synthesizing the fuel.

Michael Haas, a biochemist with the Eastern Regional Research Center's Fats, Oils, and Animal Coproducts Research Unit, and colleagues developed the new approach.

In this country, soybean oil is the most prevalent starting material for biodiesel, though other vegetable oils, animal fat, and waste grease are used too. But soybean oil's relatively high cost results in biodiesel being expensive, which discourages wider adoption of this desirable, renewable fuel.

In biodiesel production, hexane, a colorless, flammable liquid derived from petroleum, is traditionally used to extract the oil from the soybeans. But hexane is an air pollutant, and its release is regulated by the U.S. Environmental Protection Agency. Working with ERRC biologist Karen M. Scott and chemist Thomas A. Foglia, Haas eliminated hexane from the process simply by skipping the oil-extraction step that relies on it. Instead, Haas explains, soybean flakes are incubated with methanol and sodium hydroxide—the same agents that would be used to process extracted oil.

"In the new method, soybean flakes are incubated in alkaline methanol, eliminating the need to isolate and purify the oil before transesterification." ("Transesterification" is a reaction between fats and alcohol that forms the simple fatty acid esters that are biodiesel.) The lipids don't have to be isolated first because transesterification occurs in the raw soy flakes containing the oil.

Next, when the researchers collaborated with Andrew McAloon, a process modeler/cost engineer at their facility, to estimate and compare costs, they hit a snag. Without even accounting for the cost of the soy flakes or soy oil, a gallon of biodiesel produced by their new process was estimated to cost $3.14—versus 38 cents per gallon if produced by the conventional process.

The researchers then noticed that their new method used considerably more methanol than is typically needed in biodiesel synthesis. They reasoned that the moisture naturally present in soybeans, as much as 10 percent in soy flakes, could be the reason behind the high methanol requirement. They found that by drying the flakes before starting the biodiesel synthesis, they could greatly reduce the required methanol volume. As a result, the estimated cost went down to $1.02 per gallon.

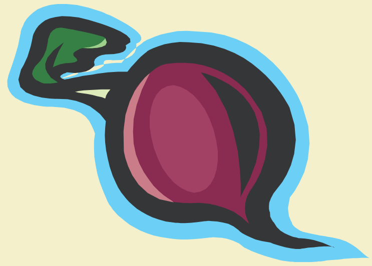
Haas and his colleagues are presently refining their economic model to account for income from selling the lipid-free, protein-rich flakes left after the biodiesel reaction for use as animal feeds and to account for cost differences between refined-oil and flaked-soybean starting materials.

ARS has filed a patent application on the process. Haas is exploring use of this new method to produce biodiesel from the lipids in corn co-products from ethanol plants that use corn as a starting material. He's also investigating the suitability of canola seeds and meat and bone meal.—By **Jim Core,** ARS.

This research is part of Bioenergy and Energy Alternatives, an ARS National Program (#307) described on the World Wide Web at www.nps.ars.usda.gov.

Michael J. Haas is in the Fats, Oils, and Animal Coproducts Research Unit, USDA-ARS Eastern Regional Research Center, 600 East Mermaid Ln., Wyndmoor, PA 19038; phone (215) 233-6459, fax (215) 233-6795, e-mail mhaas@errc.ars.usda.gov. ✸

QuEChERS Method Catches Pesticide Residues



QuEChERS (pronounced *catchers*) is not a cool, new way of spelling the position we usually associate with masked, padded, often grimy players toiling behind home plate during baseball and softball games.

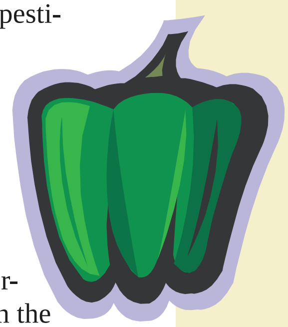
It's actually a catchy name for a new approach to analyzing pesticide residues in fruits and vegetables.

Steven J. Lehotay, an ARS chemist at the Microbial Biophysics and Residue Chemistry Research Unit, Eastern Regional Research Center in Wyndmoor, Pennsylvania, and a visiting scientist, Michelangelo Anastassiades, from a government laboratory in Stuttgart, Germany, developed the QuEChERS method, which stands for quick, easy, cheap, effective, rugged, and safe. It can be used with a wide range of pesticides and food types.

Current methods of extracting pesticide residues from food samples and preparing them for analysis are time consuming, expensive, and labor intensive. The new, streamlined approach makes it easier and less expensive for analytical chemists to examine food.

Routine monitoring serves to enforce laws, protect the consumer, provide data for risk assessment and pesticide reregistration, ease international trade, market residue-free products, and help verify organic food labeling.

The U.S. Department of Agriculture and other organizations started the Pesticide Data Program in May 1991 to test commodities in the U.S. food supply for pesticide residues.



Using QuEChERS, a single chemist can prepare a batch of 10 previously chopped samples in about 30 minutes with $1 worth of materials per sample. This gives at least fourfold lower material costs and fourfold greater sample throughput per analyst than traditional methods. Lehotay says the method combines different steps, which means there is less chance for error.

A single, easy-to-clean Teflon tube is the only item to be washed and reused, eliminating all the glassware used in conventional methods. Furthermore, less than 10 milliliters of solvent waste is generated—much less than the 75-450 milliliters generated by other methods.

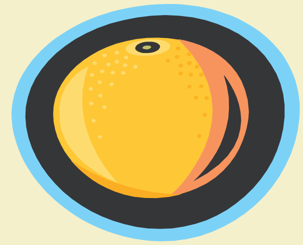
Key to the new approach is the development of a rapid procedure called dispersive solid-phase extraction. This technique quickly removes water and nontarget compounds with magnesium sulfate and a primary-secondary amine sorbent.

More than half the produce samples tested in the United States typically do not have measurable residues, and less than 1 percent of tested samples exceed tolerance levels, according to the U.S. Food and Drug Administration (FDA). Consumers should always wash, peel, or cook produce to help remove residues.

The term "tolerance" is used to describe the maximum amount of a given pesticide or its breakdown products allowed to remain in or on food commodities. The U.S. Environmental Protection Agency sets tolerance levels in the United States, and state and federal monitoring programs enforce these legal limits.

Several monitoring laboratories, including a few in the FDA, are evaluating QuEChERS. Lehotay believes that it will someday substantially increase monitoring rates and lower costs of pesticide residue analysis.—By **Jim Core,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.



Steven J. Lehotay is in the USDA-ARS Microbial Biophysics and Residue Chemistry Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038-8598; phone (215) 233-6433, fax (215) 233-6442, e-mail slehotay@arserrc.gov. ★

Unraveling the Listeria Genome

If knowledge is power, Agricultural Research Service scientists are gaining the upper hand on *Listeria monocytogenes*.

Researchers at the ARS Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, and the Institute for Genomic Research in Rockville, Maryland, sequenced four strains of *Listeria* related to foodborne listeriosis outbreaks—an important step toward developing a management strategy for this deadly bacterium.

"Researchers in the ERRC Microbial Food Safety Research Unit have completed whole genome sequencing and comparison of four genomes, which allowed us to significantly improve our understanding of *Listeria*," says research leader John B. Luchansky. In addition to finding serotype-specific and strain-specific genome sequences, they found that these four *Listeria* strains have largely similar genetic content and organization.

The team believes a few unique regions could account for epidemiological and antigenic differences among strains. Understanding these differences will improve scientists' ability to assess the risk posed by contamination of food by this bacterium.

They also confirmed that *Listeria* bacteria have 15 genes in a regulatory protein family known as "Crp/Fnr," considerably more than most bacteria have. Luchansky and molecular biologists Darrell Bayles and Gaylen Uhlich are investigating whether these sequences influence the bacterium's virulence or persistence in the food supply.

The scientists have estimated the percentage of the genome for which there is no known function and identified specific genes that warrant further investigation. They've also started to pursue proteomics and genomics studies, Luchansky says. This involves comparing different strains of bacteria—or the same strain growing under different environmental conditions—to determine whether there are differences in the level of gene or protein expression under the conditions tested.

Knowing more about the bacterium will enable regulatory agencies and the food industry to make better informed decisions about safety standards and control strategies, Uhlich says. And uncovering the genetic information that defines *Listeria's* survival, growth, persistence, and ability to cause disease will help scientists better understand the bacterium's virulence, its presence in the environment, and its persistence in the food chain.

"Ultimately, we hope to learn enough about *Listeria* to prevent contamination, decrease prevalence, and reduce disease," Bayles says. "This research puts us in a better position to make scientifically sound decisions about managing the threat of foodborne listeriosis."—By **Laura McGinnis,** ARS.

John Luchansky, Darrell Bayles, and Gaylen Uhlich are with the USDA-ARS Eastern Regional Research Center, 600 E. Mermaid Ln., Wyndmoor, PA 19038-8598; phone (215) 233-6620 [Luchansky,] (215) 233-6678 [Bayles], (215) 233-6740 [Uhlich], fax (215) 233-6581, e-mail john.luchansky@ars.usda.gov, darrell.bayles@ars.usda.gov, gaylen.uhlich@ars.usda.gov. ✷

More than 31 million children participate in the National School Lunch Program during the school year.

Celebrating 15 Years of a Healthy School Lunch Option

How about cheesy pepperoni, Hawaiian pineapple and ham, or vegetarian pizza, all piping hot and ready to enjoy? These pizzas are made with reduced-fat mozzarella cheese and are offered at the Crossroads Café—the food-service installation at Camas High School in Camas, Washington.

One technology for making tasty-but-healthy cheese was invented by Agricultural Research Service (ARS) scientists at the Dairy and Functional Foods Research Unit in Wyndmoor, Pennsylvania. It became available to schools in 1995 and is used to provide a low-fat alternative to high-fat cheese when making pizzas. The ARS team included chemists Michael Tunick, Edyth Malin, and James Shieh, physical science technician Brien Sullivan, and Peter Cooke (no longer with ARS). Other team members, Virginia Holsinger and Phil Smith, are deceased.

In 2009, more than 31 million children participated in the USDA National School Lunch Program in more than 101,000 schools and residential childcare institutions. With National School Lunch Week being observed each October, now is a good time to celebrate all healthy cheese options—produced using a variety of manufacturing methods—that are available in school lunches.

Starting in 1992, the team began exploring new ways to cut mozzarella's fat content without sacrificing its flavor or stretchy texture, especially as a pizza topping. They worked on modifying the network of the milk protein casein. The result was a mozzarella with improved storage life and only 10 percent fat—about half the fat content of regular mozzarella.

Just as important, pizza-eating students give the cheese a thumbs-up, according to school food-service director Sarah Winans with the Crossroads Café.

Martha Henry, director of food service for all schools in Tennessee's Maryville City School District, agrees. "We find that pizza is one of school kids' favorite lunches," she says. "Reduced-fat mozzarella cheese allows the students to enjoy pizza while reducing their dietary fat intake."

The USDA Farm Service Agency's Kansas City Commodity Office in Missouri began buying lower fat mozzarella cheeses in the early 1990s. Since 2000, that office has been buying lower fat mozzarellas exclusively. More than 500 million pounds of lower fat mozzarella cheeses—worth more than $800 million—have been purchased for school-related programs, according to program analysts David Leggett and Michael Buckley with the USDA Food and Nutrition Service in Alexandria, Virginia.

This research supports the USDA priority of improving children's nutrition and health.—By **Rosalie Marion Bliss,** ARS.

Michael H. Tunick is with the USDA-ARS Dairy and Functional Foods Research Unit, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038-8598; (215) 233-6454, michael.tunick@ars.usda.gov. *

Science Update

Toward Safer Fresh-Cut Tomatoes, Melons

Most of the fresh-cut produce market's 20-percent annual growth is in vegetables. While retailers have long sought a way to offer consumers fresh-cut tomatoes and melons, they haven't pursued that market beyond local or regional fresh-cut processing because of product quality problems and food safety concerns caused by inadequate cold temperatures during distribution.

In the past, melons and tomatoes have been associated with Salmonella, which heads the list of common causes of food-borne illnesses. About 40,000 cases of salmonellosis are reported annually, according to the federal Centers for Disease Control and Prevention in Atlanta, Georgia.

In a few years, supermarkets may sell packaged fresh-cut tomatoes, melons, and other fruits if researchers find novel methods for killing or removing disease- causing bacteria.

Now ARS scientists have entered into a 2-year cooperative research and development agreement with EPL Technologies, Inc., of Philadelphia. Pennsylvania, to develop novel methods for killing or removing disease-causing bacteria from fresh-cut fruits. Under the agreement, the scientists will seek alternatives to common sanitizing agents, such as chlorine, that are used to wash fresh-cut foods. This research should allow fresh-cut manufacturers to expand their markets and make healthy fresh-cut products available to a larger group. Successful introduction of these products will be a major boon to growers and shippers. Gerald M. Sapers, USDA-ARS Plant Science and Technology Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6417, e-mail gsapers@arserrc.gov.

Using Microwaves To Extract Pectin

Among other food uses, pectin acts as a gelling agent in fruit jams and jellies and as a texturizer in premixed yogurt. A natural ingredient, it's mostly obtained from citrus pulp, peel, and albedo—the white material between the outer peel and fruit—and from apples and sugar beets. To extract pectin from fruit, industry uses a conventional heating process that takes an hour or more per batch. Overheating sometimes breaks down the pectin, reducing its quality.

Now scientists have found a way to use microwave technology to extract this valuable food ingredient, which is largely imported and sells for $6 to $8 per pound. A faster, nondestructive method could reduce the cost of domestic production and provide a market for what is currently a low-value byproduct of U.S. food processing. A patent application on the technology has been filed. Marshall L. Fishman, USDA- ARS Plant Science and Technology Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6450, e-mail mfishman@arserrc.gov.

Corn Extract for Waxing Paper

Zein is a corn protein making up about half of the protein found in the corn ker- nel. Unlike other corn proteins, which are water soluble, zein repels water, making it an ideal coating material. It was discovered when researchers, at work on reducing the cost of distilling ethanol from corn, isolated it as a zein-lipid mixture. They found it had good grease resistance and water barrier properties.

Extracting such mixtures from ground corn should cost about $1 to $2 per pound. The zein coating would be suitable for most packaging material requiring waterproofing, such as boxes for perishable fruits, vegetables, or fish. Nicholas Parris, USDA-ARS Engineering Science Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6453, e-mail nparris@arserrc.gov.

Bacteria Turn Fats and Oils Into Plastics

Microbial fermentation is turning vegetable oils and animal fats into biodegradable industrial materials. Certain microorganisms, when grown on fats and oils, can produce poly(hydroxyalkanoate) polymers, or PHAs. When fed excessive carbon and deprived of other nutrients, bacteria like Pseudo- monas resinovorans produce these plasticlike substances as a survival mechanism. The polymers are either rigid or elastomeric, depending on their chemical structure, the organism that produces them, and the fat or oil feedstock.

Since PHAs break down naturally over time, they are suitable for a wide range of environmentally friendly medical and consumer products ranging from adhesives to plastics and films.

Scientists want to control the PHA properties to make them suitable for diverse industrial uses. The properties vary with the fatty acid composition of the feedstock source. Richard D. Ashby, Daniel K.Y. Solaiman, USDA-ARS Hides, Lipids, and Wool Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6483 [Ashby] -6476 [Solaiman], e-mail rashby@ars errc.gov, dsolaiman@ arserrc.gov.

Virtual Laboratory Furthers Food Safety

Predictive microbiology is a growing field that estimates the behavior of mi- croorganisms in response to environ- mental conditions. These include the food production and processing opera- tions that occur from farm to table. Now, mathematical models 15 years in the making are being used to estimate the behavior of foodborne bacteria that can cause illness or disease in consumers. They have been collected on one web site that functions as a virtual laboratory to help facilitate cooperation among researchers studying one key food safety issue: how pathogenic bacteria in food behave under varying environmental conditions. The site is called the Center of Excellence in Microbial Modeling and Informatics, or CEMMI, for short. It can be accessed at http://www.arserrc.gov/ cemmi.

Researchers hope that the center will improve the way predictive models are developed and applied. It should help define existing gaps in research data and enhance uniformity in experimental designs. It will network laboratories, researchers, model designers, and industry, thus facilitating the solving of contemporary food safety and quality problems. Mark L. Tamplin, USDA-ARS Microbial Food Safety Research Unit, Wyndmoor, Pennsylvania; phone (215) 836-3794, e-mail mtamplin@arserrc. gov.

Increasing Fiber in Cereals and Snacks

Americans average only about 15 grams a day of dietary fiber, even though the recommended intake is 20 to 35 grams. Studies suggest that adequate fi- ber can decrease the risk of heart disease, some cancers, diabetes, and high blood pressure. One easy way to increase the general population's fiber intake might be to add it to favorite snack foods, which now contain less than 3 percent fiber.

Until now, adding plant fiber during the extrusion process by which many cereals and snacks are formed has caused undesirable textural changes. But by adding dairy proteins such as whey or casein as binders, ingredients hold together better, making for a more acceptable product that may eventually contain as much as 10 percent fiber. Other work is testing use of milk protein to envelop fibers and keep them from soaking up water when used in foods. Reducing water-holding capacity of fiber can improve food quality. Charles I. Onwulata, USDA-ARS Dairy Products Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6497, e-mail conwulata@ arserrc.gov.

Getting More Out of Corn—More Economically

After 100 years of doing it the same way, corn refiners will soon have another option that can potentially lower costs and shorten the time needed to produce starch, oil, and other co-products. In laboratory and pilot-scale trials, this new method yields as much or more starch as the conventional process.

Relying on protease enzymes to break down starch and protein and using less sulfur dioxide, the method requires just a 6-hour pretreatment of corn kernels be- fore milling. This replaces the 24 to 36 hours of conventional steeping in water and sulfur dioxide that customarily start the breakdown process. Researchers have applied for a patent on the method. David B. Johnston, USDA-ARS Crop Conversion Science and Engineering Research Unit, Wyndmoor, Pennsylvania; phone (215) 836-3756, e-mail djohnston @arserc.gov.

Protective, Edible, Milk-Based Film

A patent has been issued for a method to modify milk proteins to make water-resistant films that may one day coat or package foods. It uses highly pressurized carbon dioxide to remove the protein known as casein from milk. The main protein in milk, casein solidifies when milk acidifies. It is already used as a food supplement and as an ingredient in adhesives, finishing materials for paper and textiles, paints, and other nonfood products. But because moisture can dissolve casein, it's been difficult to use in films, fibers, or molded materials and get acceptable mechanical properties.

The new extraction method capitalizes on casein's natural structure to form water-resistant films and coatings that can act as barriers between products and outside substances. The films can be formed either as stand-alone sheets or as thinner coatings that adhere directly to the product. Both can lock in moisture and remain intact when exposed to water.

Such edible coatings might be used on dairy food products such as cheese or be incorporated into packaging material. Flavorings, vitamins, or minerals could be added to the coating to enhance them. Researchers are still evaluating these and other potential uses, but the patent is available for licensing. Peggy M. Tomasula, USDA-ARS Dairy Processing and Products Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6703, e-mail ptomasula@arserrc. gov.

Seeking Quick Checks for *E. Coli*

Sometimes it's really important to know which strain of *Escherichia coli* bacteria might be causing illness in a patient. Several strains, such as O157:H7, are known to provoke severe gastrointestinal problems including bloody diarrhea and hemorrhagic colitis and can lead to serious health complications, including kidney failure. Experts use serotyping to distinguish between *E. coli* strains and determine how potentially dangerous a particular one might be. But the laboratory procedure is labor intensive and time consuming.

Now scientists are developing tests using both conventional and real-time PCR (polymerase chain reaction) methods. These chemical procedures generate enough genetic material to allow identification and study of various *E. coli* strains. The researchers want to find ways to detect and identify specific *E. coli* serogroups and increase knowledge of each one's potency. Pina M. Fratamico, USDA-ARS Microbial Food Safety Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6525, e-mail pfratamico@errc.ars.usda.gov.

More Protein in Snacks

Snack foods are a major dietary component for many U.S. consumers. Often these crunchy favorites are made from high-starch products such as corn flour. On average, these between-meal items consist of 3 to 5 percent protein, with the rest mostly carbohydrates, fats, and sweeteners.

Now a way has been found to increase the protein in foods such as breakfast cereals, corn puffs, cheese curls, and energy bars by up to 35 percent by adding whey proteins left over from cheesemaking. It wasn't easy for food technologists to figure out how to add more whey without reducing the crunchiness of the end product. But through trial and error, they found the right temperature and moisture at which to blend corn flour with whey protein isolate so that it would run through a twin-screw extruder and achieve the desired shape and consistency. This technology is available for licensing. Charles I. Onwulata, USDA-ARS Dairy Processing and Products Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6497, e-mail conwulata@arserrc.gov.

Orange Peel's Got Curative Powers

There's more good stuff lurking in an orange than just its vitamin C-rich juice. Studies show that carbohydrates in its peel have health-promoting effects. One of them, pectin, has prebiotic properties. Prebiotics are nondigestible foods or nutrients that increase growth of beneficial, probiotic bacteria in the large intestine, where they stimulate health and help curb foodborne pathogens.

Prebiotic carbohydrates, also known as oligosaccharides, are found in certain fruits and vegetables and are beginning to be used in food products and animal feeds. Now, antiadhesive prebiotics have been discovered that may prevent patho- gens from binding to intestinal walls. Work is under way to find new, cost-effective methods to extract pectic prebiotics from orange peel, a low-value, abundantly available processing by-product. A commercial partner's being sought to further develop and commercialize this technology. Arland T. Hotchkiss, USDA-ARS Crop Conversion Science and Engineering Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6448, e-mail ahotchkiss@arserrc.gov.

Itch-Free, Machine-Washable Wool

ARS has developed a better way to produce bleached, itch-free (biopolished), machine-washable wool suitable for use in military apparel and in commercial textile product lines where synthetic fibers are traditionally used. The work was headed by ARS chemist Jeanette M. Cardamone. The new process is an alternative to conventional chlorination processing, which is not environmentally friendly. The military is especially keen to have itch-free, machine-washable, shrink-resistant wool for clothing because it would be more comfortable and breathable than synthetics. In addition, this specially processed wool burns to a soft ash with a self-extinguishing flame, while synthetics melt and drip-burn to a hard bead that can lodge in open wounds. ARS washable wool technology could increase the demand for domestic wool. This work was conducted at the Fats, Oils, and Animal Coproducts Research Unit, Eastern Regional Research Center, Wyndmoor, Pennsylvania.

A new biopolishing wool-processing technique can make wool clothing more comfortable. Even the U.S. military is interested.

Food Safety Help for Processors

A new Internet resource could be invaluable to food processors—especially, to smaller meat and poultry processing companies. The information it provides can answer food safety questions and help food processors make science-based decisions to produce safe and wholesome products for consumers. Called the Predictive Microbiology Information Portal, or PMIP, it offers information on research, regulations, and resources related to *Listeria monocytogenes* in ready-to-eat foods. Soon it will be expanded to include other pathogen and food combinations. To help ensure the safety of food processing methods, a searchable database allows users to find information that can be used to develop plans for Hazard Analysis and Critical Control Point inspection.

Developed in collaboration with Rutgers University and Decisionalysis Risk Consultants, Inc., of Ottawa, Ontario, the portal includes a tutorial with instructions on using and interpreting predictive models. It links to the ARS Pathogen Modeling Program and ComBase, which provide diverse resources associated with databases, regulatory requirements, and food safety principles. The PMIP Web address is www.ars.usda.gov/naa/errc/mfsru/portal. *Vijay K. Juneja, USDA-ARS Microbial Food Safety Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6500, e-mail vijay.juneja@ars.usda.gov.*

Sustainable Corn Production Supports Advanced Biofuel Feedstocks

Researchers have found a cost-effective, energy-efficient, and environmentally sustainable method to use corn stover for gener- ating an energy-rich oil called “bio-oil” and for making biochar to enrich soils and sequester carbon. The team used fast pyrolysis to transform corn stover and cobs into bio-oil and biochar. They found that the bio-oil captured 70 percent of the total energy input, and the energy density of the bio-oil was 5 to 16 times that of the feedstock. This suggests it could be more cost effective to produce bio-oil through a distributed network of small pyrolyzers and then transport the crude bio-oil to central refining plants to make “green gasoline” or “green diesel,” rather than transporting bulky stover to a large centralized cellulosic ethanol plant. About 18 percent of the feedstock was also converted into biochar, which contains most of the mineral nutrients in the corn residues. Amending soils with this biochar would return those nutrients to the soil, reduce leaching of other nutrients, help build soil organic matter, and sequester carbon. Charles Mullen, USDA-ARS Crop Conversion Science and Engineering Research Unit, Wyndmoor, Pennsylvania; (215) 836-6916, charles.mullen@ars.usda.gov.

Tanning Innovation Might Boost Meat Safety

Microorganisms present on the hair of food animals at the time of slaughter—such as Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes—can contaminate meat and sometimes cause illness in consumers. Research shows that removing hairs from cattle hides before skinning significantly reduces the threat of meat contamination. Now, a 10-year-old hair-removal method has been improved to reduce both processing costs and environmental impact. The patented process begins with spraying a sodium sulfide solution on the carcass hide, which breaks protein bonds within hair fibers and allows their easy removal. Then a sulfide-neutralizing agent is applied. Packers can now remove most of the hair, split the hide, and send the top layer for tanning and the rest for other uses. This saves time and money, allows early-stage inspection of the hide’s grain layer, and reduces shipments of low-quality hides to tanners. The method is being implemented by Future Beef Operations, LLC, in a new Kansas plant. Andrew G. Gehring, USDA-ARS Hides, Lipids, and Wool Research Unit, Wyndmoor, Pennsylvania; phone (215) 233-6491, e-mail agehring@arserrc.gov.





Eastern Regional Research Center
USDA-ARS
600 East Mermaid Lane
Wyndmoor, PA 19038
Phone: (215) 233-6400
Fax: (215) 233-6777
www.ars.usda.gov/naa/errc