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The Authenticity of Olive Oil

Story on pages 4-7

Agricultural Research Service • *Solving Problems for the Growing World*

FORUM

Providing More—and Better—Food by 2050

The world in 2050 is predicted to have 9 billion people, who will differ in almost every way but one: They will all need to eat on a daily basis.

Their nutritional needs will be similar, too. Nutrition means eating, eating means food, and food means agriculture.

Many people speak of the increased food needed for 9 billion, but in reality, the increased need is for nutrition. Thus, the world of 2050 will force a melding of two disciplines that have diverged in recent years—agriculture and nutrition.

We need to change our focus. We need sustainable nutrition as much as sustainable agriculture, and this merger has implications for government policy, the food industry, and production agriculture, as well as for research by the Agricultural Research Service.

Feeding 9 billion people can be accomplished in several ways. One is to continue to produce and consume as we are presently doing, but to increase the scale. A second is to produce and consume more efficiently. A third is to change the nutritional needs—or perceived needs—of people.

U.S. agriculture after World War II seemed boundless, as farmland stretched from sea to shining sea. But in the intervening years, we built as if the land were limitless. Today, many market farms in the East are suburban home sites; many cattle ranches of the High Plains are resorts; and western agriculture is pressed by urban sprawl and limited water.

These issues are not unique to the United States. Europe reached our point perhaps 100 years ago, and the population explosion in Asia and Africa has saturated their land mass beyond the carrying capacity. One thing is clear: We will not feed 9 billion people by simply expanding the scale of the present system.

A second way to feed 9 billion is to produce more efficiently. One idea is to refine present means of production, but this is limited; for example, worldwide grain production increased by more than 3 percent from 1960 to 1970, but by less than 1 percent from 1990 to 2000. Certainly there will be more production-related discoveries in the future, but will they be enough to feed 9 billion? A new paradigm for "efficiency" is needed, and this is the reason for melding agriculture and nutrition.

Take, for example, our meat production processes, which many have criticized as an inefficient way of turning plants into too little nutrition and too much wasted fat. In fact, some demand an end to meat production and claim that it is an unethical approach to feeding the world.

But is it?

What if "efficiency" emphasized taking advantage of an animal's ability to utilize feeds unsuitable for humans? And what if we bred for leaner animals with "better" fats? Then animals would not compete with humans for food and, in fact, would convert these foodstuffs into nutritionally dense food with good fats and bioavailable protein, vitamins, and minerals. ARS is already doing this research, and a closer alliance of agriculture and nutrition will make the process more efficient and responsive.

Another example is research being done with micronutrient nutrition. The Green Revolution of the 1960s greatly reduced protein/calorie malnutrition of the world's poor. But because grains are primarily carbohydrates, many people remained deficient in vitamins and minerals. What if we developed high-yielding grains that are also enriched in vitamins and minerals? Then a person's nutrition would improve even if the food intake remained the same.

ARS is doing this as well. ARS has developed vitamin- and mineral-enriched rice and maize, and that has happened because of the partnership of the plant scientists with human nutritionists. But we will need many more such efforts to feed 9 billion.

A third means to feed 9 billion is to change people's nutritional needs. Agricultural demand is based on consumption, but more than half of the adult population in most developed nations is overweight or obese. If we substantially reduce obesity, then energy and nutrient needs in our country—and in virtually every other developed nation—will decrease. The concomitant decrease in the demand for food will leave more for the expanding population.

Reducing obesity-linked overconsumption will require changes in the kinds and amounts of agricultural products flowing into the food supply. If we understood how to increase consumer demand for foods such as fruits and vegetables, that would drive changes in agricultural production and food industry processing practices to deliver the products. For example, do we need to lower prices? Or improve taste and other sensory characteristics? We then need to implement these actions and inform the consumer.

To put our challenge in perspective, 2050 is in the future about the same as the U.S. Bicentennial is in the past—an event that seems like yesterday to many of us. To be ready in that short amount of time, we must prepare now.

Nine billion people will demand food every day, and providing it will be one of the biggest challenges ever faced by science. ARS will be there, and a closer alignment of agriculture and nutrition will be a big part of the solution.

John W. Finley
ARS National Program Leader
Human Nutrition
Beltsville, Maryland

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Solving Problems for the Growing World

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Editor: **Robert Sowers** (301) 504-1651
Associate Editor: **Sue Kendall** (301) 504-1623
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Photo Editor: **Tara Weaver-Missick** (301) 504-1663
Staff Photographers:
Peggy Greb (301) 504-1620
Stephen Ausmus (301) 504-1607

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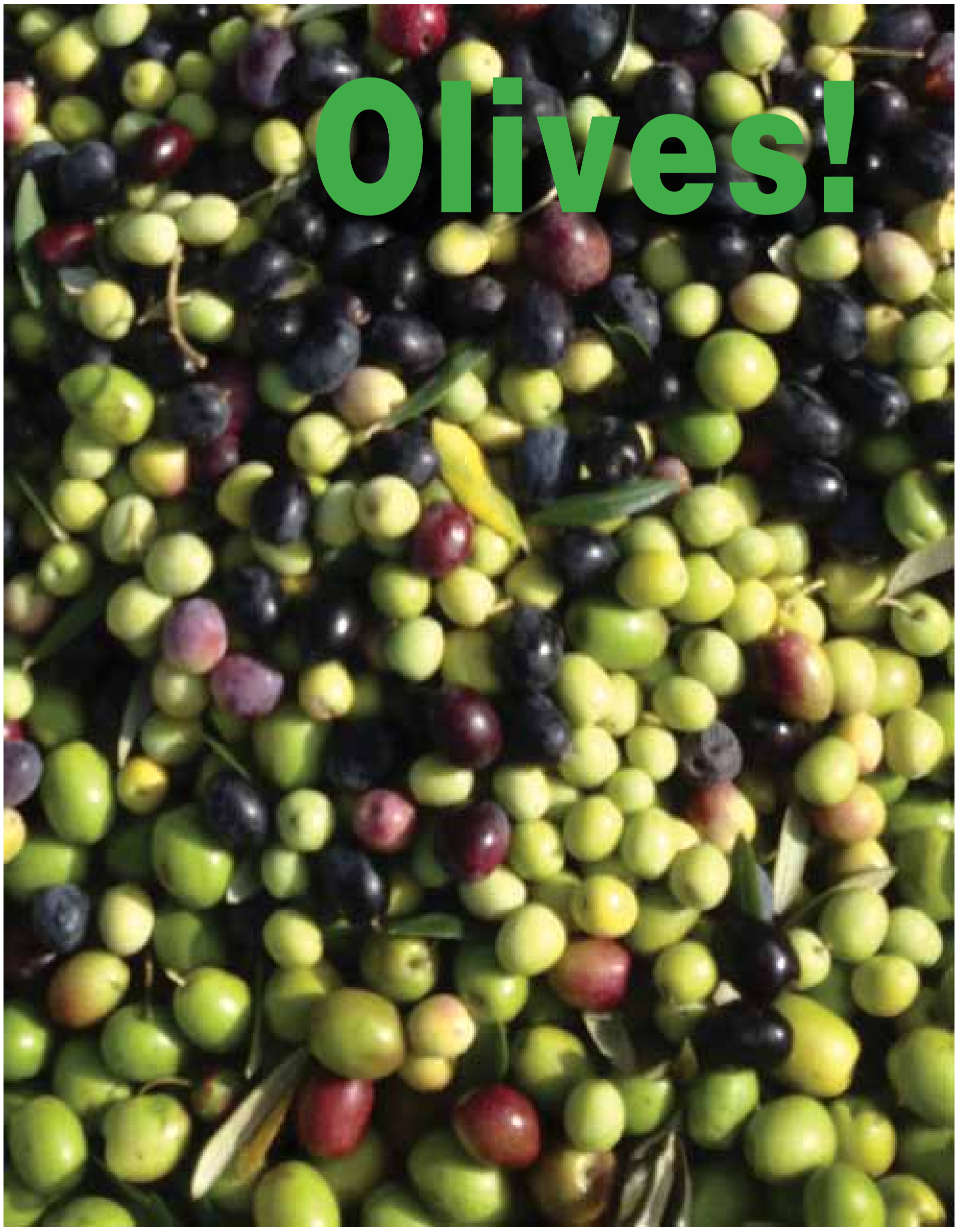
MICHAEL HEATON (D2861-1)

Sheep throughout the world are susceptible to ovine progressive pneumonia, a slow-acting, wasting disease. ARS scientists at three U.S. locations have teamed up to verify the gene that affects susceptibility to the virus that causes the disease. Story begins on page 12.

Cover: Research findings from ARS scientists in California may help strengthen America's olive oil industry. Recent developments include two analytical methods that could be used to assure the authenticity of olive oil. Story begins on page 4. Photo by Peggy Greb. (D2868-2)



Scientists Take a New Look at an Ancient Crop

Olives of various shapes, sizes, and colors. ARS scientists are helping to discover the secrets of a crop that is a lot more than just delicious. Once the oil is extracted, even the byproducts, or leftovers, have significant potential value to consumers.

Though the price makes you wince, you might just buy that bottle of your favorite olive oil anyway. Perhaps it's exactly what you want for the salad dressing you're making tonight and for your special stir-fry on the weekend.

But are you really getting what you paid for?

A bottle proclaiming that it is olive oil might actually include another, less-expensive vegetable oil derived from, for example, safflower or canola.

Mislabeling is of concern not just to shoppers, retailers, and chefs, but also to America's olive growers, olive oil processors, and more—especially those newly entering the U.S. olive oil market. California, which already produces the bulk of the nation's olives, is experiencing a resurgence of interest in producing more of this popular vegetable oil, even in the face of significant international competition: Today, about 98 percent of all olive oil consumed in the United States is imported.

Scientists at the Agricultural Research Service's Western Regional Research Center in Albany, California, are contributing research findings that may strengthen the domestic olive oil industry.

Talwinder Kahlon and Ken (Jiann-Tsyh) Lin, for instance, have developed analytical methods that can be used to assure the authenticity of olive oil. Rebecca Milczarek is investigating opportunities for making better use of the olive-milling byproducts that are left once the plump fruit, or "drupe," has been processed to extract its oil. Mendel Friedman and colleagues have shown the effectiveness of olive powder for a perhaps surprising task: keeping hamburger patties safe to eat.

PCR Test Helps Detect Undisclosed Oils

The assay that chemist Talwinder Kahlon and coinvestigators developed relies on PCR (polymerase chain reaction) technology to compare olive DNA to that of two other kinds of plants—canola and sunflower. Oil from these plants is sometimes mixed with olive oil.

The test focuses on key regions of two genes, *matK* and *psbA-trnH,* that occur widely throughout nature—including in olive, canola, sunflower, safflower, and other everyday sources of edible vegetable oil.

The research team has shown that the DNA sequence of specific regions of these two genes provides a reliable basis of comparison and can be used to quickly detect the presence of the non-olive oils in an "olive oil" sample. The assay can identify the three oils at concentrations of 5 percent or higher.

The test requires only about one-fifth teaspoon of an oil sample. And, from start to finish, including various sample-preparation steps, the test takes only about 2 to 2½ hours to conduct. What's more, it is comparatively inexpensive and requires only the equipment and supplies that most DNA labs already have on hand.

With further work, the procedure could perhaps be used to identify other vegetable oils, such as avocado, hazelnut, soybean, or walnut, that are sometimes added to incorrectly labeled olive oil.

The idea of using PCR technology to detect specific plant DNA in olive oil is not new. But Kahlon and colleagues say their approach offers several improvements over other PCR-based assays. For one thing, their process relies on analyzing plastid DNA. The double layer of membranes in which this DNA is encased may protect it from damage that might otherwise skew test results, Kahlon says.

Another advantage: The olive, sunflower, and canola "DNA barcodes" that the team developed—to serve as the basis for comparing these plants' DNA—are based on not just a single olive tree or a particular sunflower or canola plant. Instead, each barcode is a broadly representative composite, referred to as "consensus DNA." The olive barcodes, for example, are representative of olive DNA from commercially grown olives and bottled olive oil, as well as from olive DNA sequences posted at an international online database maintained by the National Center for Biotechnology Information.

Kahlon and coinvestigators Shashi Kumar, formerly at Albany and now with the International Center for Genetic Engineering and Biotechnology, New Delhi, India; and Sanika Chaudhary, formerly at the University of California-San Francisco, documented their research in a 2011 article in the scientific journal *Food Chemistry* and are seeking a patent for the assay.

Triglycerides' Components: A Key to Authenticating Olive Oil

Olive oil is made up of triglycerides—molecules composed of three fats (technically, fatty acids). These fatty acids are the focus of another approach to detecting oils in olive oil that might not be listed on the label.

Each of the three fatty acids in a triglyceride is attached to what scientists describe as a "glycerol backbone," explains chemist Ken Lin. The bonds occur along the backbone at specific positions. For every type of triglyceride, the three fatty acids bond to glycerol in specific patterns.

The technology that Lin uses, ESI-MS (electrospray ionization mass spectrometry), enables scientists to glean details about variations in specific triglycerides of interest, referred to as "regioisomers." From that, the scientists can develop ratios of regioisomers that can be used to determine whether the sample contains any oil other than that extracted from olives.

The value of ESI-MS for analyzing plant fatty acids and animal fats has been recognized since at least 1994. But Lin's ESI-MS protocol helps make this application simpler.

Lin developed the protocol for use in his research with castor, a plant that produces an inedible, top-quality industrial oil. Olive oil came into the picture only tangentially: About 6 years ago, Lin chose it as a model for testing the assay.

Lin has described the ESI-MS assay in scientific articles that appeared in the *Journal of Agricultural and Food Chemistry* in 2008, the *Journal of the American Oil Chemists Society* (AOCS) in 2012, and in a 2010 addition to the AOCS Lipid Library, a reference maintained online for use by researchers worldwide.

Learning More About Milling Leftovers

Right now, most olive milling leftovers—skins, pulp, and pits—typically have only low-value uses. The two-phase process commonly used in the United States for olive milling produces these leftovers, referred to as "pomace." This wet, heavy goulash ranges in color from green to brown to black to purple, depending upon how ripe the olives were when they were harvested.

Pomace has an aroma somewhat like that of olive tapenade—a flavorful spread made of finely chopped or pureed olives, anchovies, capers, garlic, and olive oil.

"Mills produce about 38 pounds of pomace for every gallon of olive oil produced, so they are always facing the problem of what to do with it," says agricultural engineer Rebecca Milczarek. "Some olive mill operators pay to have the pomace shipped to sites where it dries

DE WOOD (D2862-1)

A protocol developed by chemist Ken Lin helps to simplify the use of ESI-MS (electrospray ionization mass spectrometry) to determine whether an olive oil sample contains oil other than that extracted from olives.

REBECCA MILCZAREK & CARL OLSEN (D2863-1)

Food technologist Carl Olsen places olive pomace into a pilot-scale microwave-convection drying system while agricultural engineer Rebecca Milczarek inserts a temperature probe into it. Milczarek is studying the drying dynamics of pomace to help reduce its weight and shipping cost.

outdoors for a number of weeks, and then it's sold as a cattle-feed ingredient."

Milczarek says one key to creating innovative, environmentally friendly, higher value uses for olive pomace is to develop techniques that millers can use to quickly and affordably dry it on-site.

Drying significantly reduces the weight of the pomace, making it cheaper and easier to ship. Small mills could ship their dried pomace to a centralized processing plant where, for instance, additional oil, or compounds for use in new foods, pharmaceuticals, cosmetics, or other products, might be extracted.

"Most small mills can't afford to buy their own extraction equipment," notes Milczarek, who began working with California olive growers and olive oil processors in 2010.

According to Milczarek, pomace typically is about 65 percent moisture—a level that would need to be lowered to about 10 percent before shipping the pomace to a processing plant. Once there, the pomace may require more drying, depending upon what component is being extracted. Milczarek is investigating the dynamics of drying pomace to determine precisely how long it takes to dry.

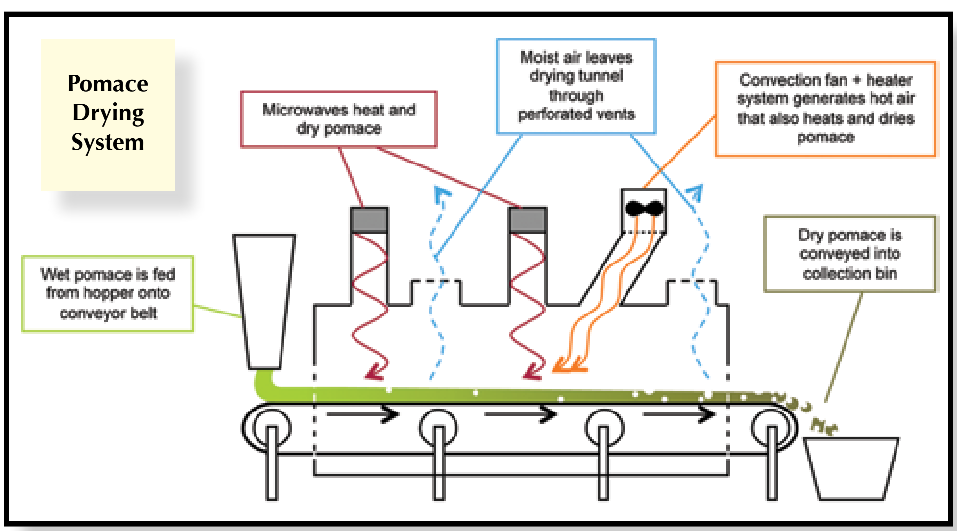
"To give an olive mill owner a science-based answer to that question," she says, "you need to know the 'diffusion coefficient' of water from the pomace. The coefficient depends on pomace characteristics and the drying system used. The higher the coefficient, the faster the rate at which it loses water and, theoretically, the less money you might have to spend drying it."

In preliminary experiments, Milczarek processed—in batches—about 12 pounds of fresh pomace, using a combination of microwave and convection (hot forced air) drying. The study is likely the first to take into account the fact that the pomace shrinks during drying. It may also be the first to keep the internal temperature of the pomace steady.

"These two aspects of our study helped us get more accurate values than those that have been reported in earlier work," Milczarek explains. "Our coefficients for the four internal temperatures that we used—104°, 122°, 140°, and 158°F—average about 28 percent lower than those published in previous studies, meaning that drying times may be longer than what earlier data had indicated.

"Longer drying times could affect the configuration of your drying system. In a commercial setup, the magnetrons that generate microwaves, and the heater and fan unit for convection drying, would be positioned in a drying tunnel that has a conveyor belt running through it. You may need a longer tunnel and may need to operate the conveyer belt at slower speeds so that the pomace will be sufficiently dry when it comes out of the tunnel.

"Longer drying times also add to energy costs. Nevertheless, the combination of convection and microwave drying is inherently more energy efficient than drying options that are based solely on convection."



REBECCA MILCZAREK (D2874-1)

Concept diagram of an industrial continuous microwave convection drying tunnel for olive pomace. Olive processors could use findings from ARS research to define key parameters of this system, such as the speed and width of the conveyor belt.

Milczarek collaborated in the research with Tara H. McHugh, leader of the Processed Foods Research Unit at Albany, to which Milczarek belongs; Annie A. Dai of the University of California-Davis; and Caio C. Otoni of Brazil's Federal University of Viçosa. Their 2011 article in the *Journal of Food Engineering* has details.

Olive Powder for Safer Burgers?

The potential of olive powder to keep foods safe to eat is getting a fresh look from Albany chemist Mendel Friedman and colleagues at the University of Arizona-Tucson.

An olive processing coproduct, olive powder was one of about two dozen plant extracts, spices, and herbs that the team evaluated for their potential to combat *Escherichia coli* O157:H7 and to retard formation of heterocyclic amines during cooking of hamburger patties.

E. coli O157:H7 is a leading cause of food sickness in the United States and is blamed for more than 73,000 cases of illness annually. In recent years, many *E. coli* outbreaks have been traced back to ground beef and have led to the introduction of stringent new food safety rules designed to reduce the occurrence of this microbe, and six of its relatives, in meats, poultry, and other foods.

Heterocyclic amines are of concern because they can inadvertently be formed when beef patties are cooked to the doneness recommended for killing unwanted microbes, such as *E. coli.* The two amines studied in the burger experiment, MeIQx and PhIP, are on the National Toxicology Program's roster of possible carcinogens.

For the study, high levels of *E. coli* O157:H7, along with the plant extract, spice, or herb of interest, were added to the ground beef patties. The patties were then cooked on a griddle until the meat's internal temperature reached 114°F, then flipped and cooked another 5 minutes until the internal temperature reached the recommended 160°F.

The amine data showed that olive powder reduced MeIQx by about 80 percent and PhIP by 84 percent.

Overall, olive powder was the most effective of the plant extracts (olive, apple, and onion powders) that were tested.

Friedman notes that followup studies are needed to pinpoint the compounds in olive powder that are responsible for these effects and to determine whether the amount added in the experiments alters the burgers' taste.

The ability of olive extracts to kill foodborne pathogens has been reported in earlier studies conducted at Albany, Tucson, and elsewhere. However, the *E. coli* and amines study, reported in a 2012 issue of the *Journal of Agricultural and Food Chemistry,* may be the first to show olive powder's performance in concurrently suppressing three targets of concern—two major amines and a pervasive *E. coli.*

Friedman collaborated in the work with University of Arizona coinvestigators Yelena Feinstein, Cody M. Havens, Liliana Rounds, and study leader Sadhana Ravishankar.—By **Marcia Wood,** ARS.

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

Talwinder S. Kahlon, Jiann-Tsyh Lin, Rebecca R. Milczarek, and Mendel Friedman are with the USDA-ARS Western Regional Research Center, 800 Buchanan St., Albany, CA 94710; (510) 559-5665 [Kahlon], (510) 559-5764 [Lin], (510) 559-5656 [Milczarek], (510) 559-5615 [Friedman], talwinder.kahlon@ars.usda.gov, jiann.lin@ars.usda.gov, rebecca.milczarek@ars.usda.gov, mendel.friedman@ars.usda.gov. *

UNIVERSITY OF CALIFORNIA-DAVIS OLIVE CENTER (D2864-1)

Fully mature olives growing in a California orchard. The state's 2012 olive harvest of 320 million pounds was worth about $130 million.

STEPHEN AUSMUS (K9988-1)

If used in hamburger patties, olive powder has potential to suppress the foodborne pathogen *Escherichia coli* O157:H7 and to retard the formation of heterocyclic amines that can form during cooking.

Getting Hooked on Farmed Salmon

A Good Source of Omega-3s

TANIA DAVIS (D2866-1)

A salmon fillet (high in omega-3 fatty acids), purple potatoes (loaded with antioxidants), and asparagus.

Nearly 85 percent of seafood enjoyed by U.S. consumers is imported, and almost half of that is farm raised through a process called "aquaculture," according to the National Aquaculture Association, based in Pine Bluff, Arkansas. In 2011, the United States imported $16.6 billion of seafood and exported $5.4 billion, a difference of $11.2 billion.

As the world's population grows and demand for seafood increases, many seafood species are overfished or fully exploited. That means the world's oceans cannot turn out more fish than are currently being produced. As the ceiling is being reached on seafood availability from the wild, U.S. producers of farm-raised seafood are working hard to help fill today's growing demand for seafood in a sustainable way. Aquaculture is a process for raising aquatic species—both marine and freshwater—in a captive environment under controlled conditions.

Salmon: A Great Catch for Nutrition

Agricultural Research Service scientists at the Grand Forks [North Dakota] Human Nutrition Research Center (GFHNRC) conducted studies involving one popular U.S. farmed fish, Atlantic salmon. They wanted to learn more about the omega-3 fatty acids in the fish.

TROUTLODGE, INC. (D1376-1)

Atlantic salmon is one of the major species grown in aquaculture, a process for raising aquatic species in a captive environment under controlled conditions. Recent ARS studies should help alleviate concerns about whether farm-raised salmon have less available omega-3s than salmon caught in the wild.

Two omega-3 fatty acids, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), are abundant in oily fish such as salmon, tuna, mackerel, and herring. Some data has shown that consuming 250 milligrams of EPA and DHA—the amount in less than 3 calories of polyunsaturated fatty acids (PUFAs) per day—is associated with reduced heart-disease risk.

The GFHNRC scientists and their colleagues published two papers that examine the role of eating farmed Atlantic salmon as a means to increase intake of omega-3 fatty acids among consumers. The studies were led by GFHNRC nutritionist Susan Raatz and physiologist Matthew Picklo. They collaborated with William Wolters, who heads the ARS National Cold Water Marine Aquaculture Center in Franklin, Maine.

"Atlantic salmon is an excellent source of EPA and DHA," says Raatz, "and people can easily increase their consumption of omega-3 PUFAs by including more seafood in their diets." These new studies provide data of interest to those who have concerns that farm-raised salmon has less available omega-3s than salmon caught in the wild.

Retaining Healthy Omega-3s in Fish

While eating seafood rich in omega-3 fatty acids is known to reduce risk of heart disease, it has not been known whether the baking process causes loss of omega-3s in farm-raised Atlantic salmon. The researchers at Grand Forks demonstrated that baking salmon to the proper temperature does not decrease the content of beneficial omega-3 fatty acids in salmon.

SUSAN RAATZ (D2869-1)

At the Grand Forks [North Dakota] Human Nutrition Research Center, physiologist Matthew Picklo performs fatty acid analysis of a sample of human blood plasma to determine the effects of salmon consumption.

The study also examined the extent to which the baking process for Atlantic salmon produces oxidized fatty acids. "Some people think that when you bake fish, it's possible to oxidize the healthful fatty acids, which would leave compounds that are not good for you, like toxic omega-3 oxidation byproducts," says Picklo. "We looked at the extent to which baking would alter the fatty acids in salmon."

The researchers found that baking actually decreases the presence of fatty acid oxidation products. "Preparing the fish based on restaurant and safety guidelines—to a tender-but-safe 145˚F—rather than overcooking to a tough, chewy fish was key," says Raatz.

The 2011 paper was published in the *Journal of Agricultural and Food Chemistry*.

Measuring Blood Levels of Omega-3s

In another study, the scientists evaluated a group of 19 healthy human volunteers who were provided 3 different portion sizes of farm-raised Atlantic salmon. Each volunteer was assigned to consume two weekly servings of one of the three portion sizes of salmon for a 4-week period. After a "blood-clearing" break of 4 to 6 weeks, a different portion size was served, followed by another break, after which the third portion size was served, so that each volunteer had consumed all three portion sizes.

The raw weights of the salmon portions fed to the volunteers were 90 grams (about 3.2 ounces), 180 grams (about 6.3 ounces), and 270 grams (about 9.5 ounces).

The Institute of Medicine (IOM) and the *Dietary Guidelines for Americans* recommend consuming 8 ounces of seafood weekly, but at this time the IOM has not established a recommended dietary allowance for EPA or DHA.

Blood was collected from each of the 19 subjects to mark fatty acid levels and other heart disease risk indicators at the beginning and end of each treatment.

The results showed that EPA blood levels doubled after the volunteers consumed the 6.3-ounce portions and increased nearly threefold after they consumed the 9.5-ounce portions. Also, based on the blood indicators, DHA levels were elevated by about 50 percent, regardless of portion size.

"We found that eating 6 ounces twice weekly was adequate when it comes to raising blood levels of EPA and DHA," says Raatz. "The volunteers did not have to eat a lot of fish to see the benefit."

"We showed that consuming farm-raised salmon was an excellent way to increase omega-3 fatty acids in the blood to levels that corresponded to reduced heart disease risk," says Picklo.

The American Heart Association recommends consuming fish twice a week to reduce risk of heart disease.

At this time, only the U.S. states of Maine and Washington produce farm-raised salmon, says Sebastian Belle, executive director of the Maine Aquaculture Association based in Hallowell, Maine.

"On average during the past 5 years, Maine has produced about 26 million pounds of farm-raised salmon," says Belle. Significantly, in 2011, both U.S.-imported wild and farmed Atlantic salmon reached nearly 400 million pounds.

"It's important to understand the health benefits of Atlantic salmon produced through aquaculture," says Wolters, who collaborated with GFHNRC on both of these salmon studies. "A lot of the seafood consumers find at local grocery stores is farm raised."

The study was published in the *Journal of the Academy of Nutrition and Dietetics* in February 2013.—By **Rosalie Marion Bliss,** ARS.

This research is part of Human Nutrition, an ARS national program (#107) described at www.nps.ars.usda.gov.

Susan Raatz and Matthew Picklo are with the USDA-ARS Grand Forks Human Nutrition Research Center, 2420 2nd Ave., Grand Forks, ND 58202; (701) 795-8294 [Raatz], (701) 795-8380 [Picklo], susan.raatz@ars.usda.gov, matthew.picklo@ars.usda.gov.

William Wolters is with the USDA-ARS National Cold Water Aquaculture Center, 25 Salmon Farm Rd., Franklin, ME 04634; (207) 422-2713, bill.wolters@ars.usda.gov. *

MATTHEW PICKLO (D2867-1)

ARS nutritionist Susan Raatz (right) and cook Doris Zidon prepare farm-raised salmon fillets for a research study at the Grand Forks nutrition center.

STEPHEN AUSMUS (D1910-33)

IVAN HILTPOLD (D2858-1)

Left: Adult female western corn rootworm, *Diabrotica virgifera,* on a corn leaf. Above: Beneficial nematodes that can attack and kill corn rootworm must be mass-produced before distribution. Here, a cadaver of a waxworm larva (right) serves as a host "factory" for nematodes feeding within it. Inset: A microscopic view of the thread-like nematodes (white arrows point to several; the gray arrow points to a nerve of the insect host) inside the cadaver.

Nematode-Filled Capsules Tested Against Corn Rootworms

Each spring, the western corn rootworm *(Diabrotica virgifera)* awakens from its winter slumber to wreak havoc on corn crops across the United States. The pest emerges in larval form, hatching from small white eggs deposited beneath the soil and causing significant feeding damage to the grain crop's roots. The toll on U.S. farmers: an estimated $1-2 billion annually in yield losses and chemical control.

European growers face a similar threat from the pest, which was first reported in a corn field near the Belgrade international airport in Serbia (formerly Yugoslavia) in 1992, but is presumed to have arrived a decade earlier. Since then, the insect has spread over Eastern Europe and partially over Western Europe. In response, scientists from the United States and Europe have been pooling their expertise and resources to launch a multifaceted counterattack. (See "Rooting Out Rootworm Resistance," *Agricultural Research,* September 2010.)

On the biological control front, for example, a team of scientists from the Agricultural Research Service and the University of Neuchâtel (UniNE), in Switzerland, is field-testing different formulations to apply beneficial roundworms that prey on the pest. The roundworm, a species of entomopathogenic nematode known as *Heterorhabditis bacteriophora,* poses no danger to humans, pets, or livestock. But its lethality to rootworms may give corn growers another option for protecting their crops—together with use of insecticides, rotations with nonhost crops like soybean, and *Bt* corn.

Building a Better Mouse Trap

Delivering beneficial nematodes to the fight isn't always easy, though. "A standard way to apply the nematodes is to spray them over the fields. This is done because the natural populations are not high enough to control the insects on their own. However, spraying is labor-intensive, water-consuming, and costly," says Ivan Hiltpold, a visiting scholar from Switzerland who is now working with Bruce Hibbard, an entomologist and rootworm expert in ARS's Plant Genetics Research Unit in Columbia, Missouri.

In an alternative approach, Hiltpold and UniNE professor Ted Turlings, along with Hibbard and ARS entomologist Wade French, are experimenting with ways to encapsulate *H. bacteriophora* within an algal-based sugar polymer. The test

capsules, which were soft enough for the nematodes to escape from, were deposited by hand beneath the soil surface.

During 2011 field trials conducted in corn fields in Columbia, “we used half a liter of water to form the capsules, but about 2,000 liters with spraying,” says Hiltpold. He is investigating the technology in parallel with a 2-year assignment with Hibbard. A report describing the team’s findings appeared online in May 2012 and later in print in the journal *Plant and Soil.*

Although the encapsulated nematodes (about 2,700 per capsule) reduced rootworm damage to corn roots better than spray formulations, refinements to the capsules proved necessary, including determining optimal thickness of capsule walls and storage conditions. During laboratory experiments, for example, twice as many nematodes escaped capsules stored at room temperature (around 77˚F) than at 45˚F. “We’re now working on a new, harder polymer shell that will dissolve in soil over time, releasing the nematodes,” says Hiltpold.

The team is also examining whether coating capsules with rootworm attractants and feeding stimulants will increase the pest’s likelihood of encountering the nematodes. “That worked pretty well in the lab, but hasn’t proved true in the field yet,” says Hiltpold.

Amplifying Corn’s Chemical SOS Signals

Fortunately, the nematodes are adept hunters. Hiltpold’s and Turlings’s studies of *Heterorhabditis* show it’s something of a “subterranean bloodhound” when it comes to tracking chemical cues, or scents, that diffuse through soil, particularly the volatile compound *(E)*-beta-caryophyllene, or EβC. When chewed on, corn plant roots release the compound, which attracts hungry nematodes in the surrounding soils.

Upon locating the offending rootworm, the nematodes wriggle inside the rootworm and release symbiotic bacteria, which multiply and then kill the insect by poisoning its blood. The nematodes then feed on the bacteria and mate, spawning new generations that eventually burst out of the dead insect in search of new hosts—starting the cycle over again and sparing the plant further harm. Such “tritrophic interactions” (in which one organism benefits from another’s interference of a third organism) have been a key focus of Hiltpold’s studies at UniNE’s Institute of Biology.

IVAN HILTPOLD (D2859-1)

IVAN HILTPOLD (D2859-1)

Encapsulated within an algal-based sugar polymer capsule are thousands of beneficial *Heterorhabditis bacteriophora* nematodes. After the capsule is deposited into the soil near corn plants, it’s soft enough for nematodes to escape from and requires substantially less water to apply than spray applications of nematodes.

An Integrated Approach

Hiltpold notes that European corn varieties normally produce the EβC root signal. But the trait is missing in most U.S. varieties—save for an experimental strain that has been modified with an EβC-producing gene introduced from oregano. In earlier lab and field trials, the ARS-UniNE team demonstrated the success of the approach in restoring the corn strain’s nematode-signaling capacity.

Whether by conventional breeding or biotechnological means, fine-tuning corn’s ability to release a distress signal could ultimately complement the use of encapsulated nematodes—broadening the arsenal of weapons brought to bear on the rootworm and diminishing its costly damage. Backed by genetics-driven studies of the pest’s populations, movements, and ability to develop resistance to *Bt* corn, researchers could be closer to shutting down the pest’s avenues of attack and flight, as well.—By **Jan Suszkiw,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Plant Genetic Resources, Genomics, and Genetic Improvement (#301), two ARS national programs described at www.nps.ars.usda.gov.

Bruce Hibbard is in the USDA-ARS Plant Genetics Research Unit, Curtis Hall, University of Missouri, Columbia, MO 65211; (573) 882-6281, bruce.hibbard@ars.usda.gov. *

LANILA DEMARTA (D2857-1)

Visiting scientist Ivan Hiltpold examines corn plants for damage from western corn rootworm in a greenhouse at ARS’s Plant Genetics Research Unit in Columbia, Missouri.

Viral Infection in Sh

The discovery of a gene associated with a persistent viral infection that causes an incurable disease—ovine progressive pneumonia (OPP)—in sheep has led to the development of a genetic test that can be used to help reduce the impact of the disease.

A slow-acting, wasting disease, OPP affects millions of sheep worldwide and is one of the most costly diseases to producers in North America. Previous research revealed that 36 percent of sheep operations and 24 percent of sheep tested in the United States were infected with ovine progressive pneumonia virus (OPPV)—lentivirus strains that target the immune system and cause OPP.

Once infected, sheep are infected for life. Infected ewes are about 20 percent less productive, having fewer lambs and that also weigh less than lambs born to uninfected ewes. In addition to pneumonia, they show signs of wasting, lameness, and "hard bag" syndrome, in which udders become hard and contain barely any milk. Infected ewes are often culled—removed from the flock.

Scientists at the Agricultural Research Service Roman L. Hruska U.S. Meat Animal Research Center (USMARC) in Clay Center, Nebraska, along with their colleagues at the Animal Disease Research Unit in Pullman, Washington, and the U.S. Sheep Experiment Station near Dubois, Idaho, have found and verified that the gene—*TMEM154*—affects susceptibility to OPPV infection in sheep.

A Journey to Discovery

Research to find a genetic link began in the late 1990s, when scientists recognized that the rate of OPPV infection varied among breeds of sheep in the same flocks at USMARC.

Technician Jacky Carnahan and molecular geneticist Michael Heaton collect a blood sample for DNA analysis.

PEGGY GREB (D642-1)

"In one breed, 80 percent of the animals were infected, whereas in another breed, 15 percent were infected," says microbiologist Michael Heaton, who's in USMARC's Genetics, Breeding, and Animal Health Research Unit. "They were being raised together, but the proportion that got infected was different for each breed."

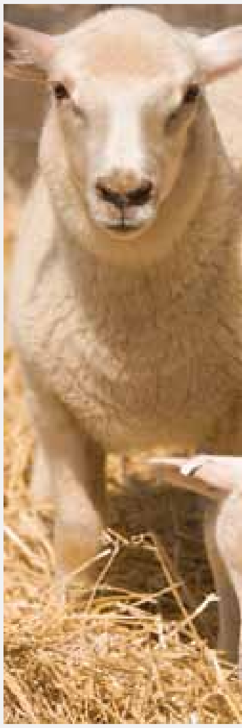
This finding suggested that breed made a difference and that genetics were a major factor in the susceptibility of sheep to OPPV, Heaton says. The team began studies of the USMARC flock, testing the sheep for candidate genes that could be involved in infection.

After 10 years of research, scientists were finally able to design a genome-wide study, thanks to the development of the Ovine SNP50 BeadChip by the International Sheep Genome Consortium. The researchers genotyped animals using blood samples collected over the years and discovered a gene, *TMEM154,* that influences OPPV infection. They also found several different forms, or "haplotypes," of the gene. Although the function of this gene is still unknown, the three major forms—haplotypes 1, 2, and 3—were found in 97 percent of the more than 8,000 sheep tested.

Gene Type Matters

"Sheep in the United States typically have some combination of these three major forms of the *TMEM154* gene," Heaton says. "Each animal will have two haplotypes—one inherited from its mother and one from its father."

Haplotypes 2 and 3 were strongly associated



Lambs produced at the U.S. Meat Animal Research Center to have specific combinations of gene haplotypes.

PEGGY GRE

heep Linked to Gene

MICHAEL HEATON (D2860-1)

At ARS's U.S. Meat Animal Research Center, ewes are bred to produce lambs with a gene that influences ovine progressive pneumonia virus (OPPV) infection. These lambs are naturally exposed to OPPV and monitored for infection throughout their lives.

with OPPV infection and considered to be highly susceptible forms of *TMEM154*. Only one copy of either haplotype 2 or 3 was needed to increase the risk for OPPV infection. In contrast, ewes with two copies of haplotype 1 were significantly less likely to be infected.

EB (D643-1)

"Rams that have two copies of haplotypes 2 or 3 clearly carry the most risk," Heaton says. "If you are selecting rams within your flock or buying new rams, you might want to avoid those. But our research indicates that rams with two copies of haplotype 1 seem to provide an advantage."

Although these results are promising, OPPV is highly adaptable and affects flocks differently, Heaton adds. It is uncertain whether selection for *TMEM154* haplotype 1 will reduce the incidence of infection in all production environments.

"We are now conducting research to learn more about these three most common forms of the gene—which ones are less susceptible, which ones are more susceptible, and if one form of the gene is dominant relative to other forms of the gene," says USMARC geneticist Kreg Leymaster.

Testing for Risks

With these findings, scientists were able to develop a genotyping test in collaboration with GeneSeek, a Neogen Corporation Company based in Lincoln, Nebraska. The test, which is now available for commercial use, indicates which sheep have the highest genetic risk for becoming infected if exposed to the virus.

Diagnostic tests have been available for some time to determine whether sheep are infected with OPPV, Leymaster says. "But they don't tell us whether a sheep is more or less susceptible to the disease. What our test has done is add a genetic component that previously wasn't available."

For example, a producer can make a flock free of the disease by eliminating infected sheep, but that doesn't mean that the flock would be resistant to the disease if an infected animal were brought in, he adds.

"Producers could use the marker we've made available to make a flock genetically less susceptible to disease, and therefore, decrease the risk of animals becoming infected again over time," Leymaster says.

The ultimate goal is to give producers tools that allow them to choose breeding stock that do not have genetic risk factors, he says, so they can reduce the prevalence of OPPV and eventually eradicate it from flocks.

What's critical is that producers understand the incidence of the disease in their flock, Leymaster says. Not all flocks are infected. Producers can look at their own unique management system, take the information provided from this research, and decide whether it fits into their system.

Adverse production conditions like high animal density, indoor housing with poor ventilation, and moist climates may enhance virus transmission and overcome sheep genetic resistance, the scientists say.

"We don't want to oversell these findings, but at the same time, we want producers to consider how they might use this to their advantage," Leymaster says. "We're continuing our research and will be able to contribute additional information in the future. I'm optimistic that the industry will be able to successfully address this major disease problem."—By **Sandra Avant,** ARS.

This research is part of Animal Health, an ARS national program (#103) described at www.nps.ars.usda.gov.

Michael Heaton and Kreg Leymaster are in the USDA-ARS Roman L. Hruska U.S. Meat Animal Research Center, State Spur 18D, Clay Center, NE 68933; (402) 762-4362 [Heaton], (402) 762-4172 [Leymaster], mike.heaton@ars.usda.gov, kreg.leymaster@ars.usda.gov. *

Technician Jacky Carnahan determines the quality and amount of DNA used for gene haplotype determination.

PEGGY GREB (D641-1)

Cited for More Than 60 Years of Flavor Research

The cornerstone-laying ceremony, October 27, 1939, for the Western Regional Research Center (WRRC), in Albany, California. WRRC is the only research center in the 21-year history of the American Chemical Society's National Historic Chemical Landmarks Program to receive two awards for its research.

It's a unique scientific niche that touches the lives of everyone.

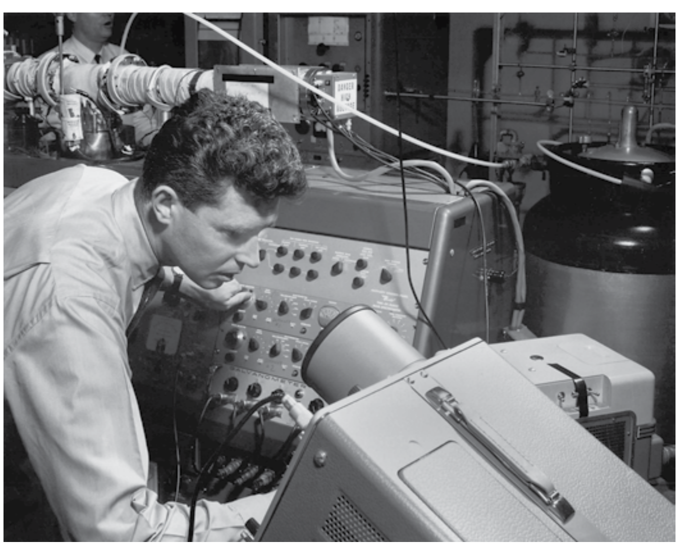
Flavor chemistry is one of several areas of research conducted at the Agricultural Research Service's Western Regional Research Center (WRRC) in Albany, California. The WRRC's achievements in flavor chemistry have earned it the distinction of being named a 2013 National Historic Chemical Landmark by the American Chemical Society (ACS). The ACS is the world's largest society of chemists, and its prestigious National Historic Chemical Landmarks program grants landmark status to seminal achievements in the history of chemical science in the United States that have had a significant impact on the chemistry profession and the public.

The WRRC research being cited by the ACS revolutionized the field of flavor chemistry by identifying major flavor compounds and developing both techniques to evaluate flavor constituents and analytical tools and methods to study flavor. The award reflects a huge body of research by a team focused on determining the chemical "essence" of flavors and odors.

"We're extremely honored to be receiving such an award, and I think it shows the significance of the work that has been done here in the past and that we continue to build on," said Howard Zhang, director of the WRRC.

The WRRC is the only research center in the 21-year history of the ACS program to receive two historical landmark awards for its research. It also received the award in 2002 for frozen foods research, known as the "Time-Temperature Tolerance studies."

An event to mark the designation of flavor chemistry research as a National Historic Chemical Landmark will take place at WRRC later this year.



USDA-ARS (D2871-1)

Bill McFadden operates the mass spectrometer portion of the gas chromatography/mass spectroscopy apparatus first reported at WRRC. (1963)

More information about the Landmarks program is available at www.acs.org/landmarks.

The Importance of Flavor

Having an ample supply of processed food ensures longer storage times and a more stable food supply, but ensuring quality presents its own set of challenges. The increased marketing of processed foods began in the years after World War II, but it prompted concerns about the loss of flavors that accompanied preservation by drying, freezing, and canning. An estimated 10,000 new processed foods are introduced each year, and almost all of them require flavor improvements.

Ensuring quality flavors is important. Frozen foods, snack items, soft drinks, prepackaged meals, and confections together represent a $600 billion processed-food industry. Studies have shown that appearance and packaging play a major role in whether someone will purchase a new food product, but flavor plays a big part in whether they buy it again.

What gives food its flavor is an extremely complicated balancing act. Cooking a

French fry produces an aromatic blend of compounds that gives the fry much of its flavor. When a health-conscious public expresses a need for fries cooked in "healthier" oils, the specific oil used can alter the flavor. The same is true with vitamins and minerals used to fortify breads, cereals, and pastas. Chemical structures of compounds in cooking oils and vitamins, even when those compounds are present at levels of only a few parts per million, can have major effects on taste. Highly sensitive tools are needed to separate and characterize very low concentrations of the flavor constituents.

New technologies and analytical methods, developed at WRRC, have met that need and have led to an impressive list of accomplishments. We may not see the results, but they are evident in what we eat every single day. They have also helped to lay the foundations for numerous advances in modern analytical chemistry and have spilled over into other scientific areas, such as food safety and human health, according to Bill Orts, research leader for WRRC's Bioproduct Chemistry and Engineering Research Unit. The work includes many "firsts."

Gas Chromatography

WRRC scientists Keene P. Dimick and Lloyd Ingraham designed and built one of the world's first practical gas chromatographs in 1953 and, with colleague Joseph Corse, used it to separate and identify the volatile flavor components of strawberries. Thanks to work by WRRC chemists, gas chromatography quickly became an essential tool for studying flavor and aroma.

Sophisticated gas chromatography methods were developed at WRRC to measure food quality through flavor, including detection of off-flavors. WRRC scientists Ron Buttery and Roy Teranishi were the first to analyze aroma compounds in 1961 by injecting the vapor collected above a food into a gas chromatograph. The method, termed "static headspace analysis," is useful for tracking rancidity in stored foods. It became standard throughout the food industry and is also widely used to measure blood alcohol levels, detect residual solvents in pharmaceutical products, and analyze organic compounds in soils.

WRRC researchers have conducted many fundamental studies on off-flavors. Buttery and co-workers identified bis(2-methyl-3-furfuryl) disulfide, one of the most potent odorants and known as the compound responsible for the odor of vitamin B1 (thiamin). The compound has an intense savory, meaty aroma and is an important component of beef gravy, high-heat skim milk powder, cooked brown rice, and soybean protein. It is also an off-flavor in stored orange juice.

WRRC scientists identified geosmin as the compound responsible for a musty off-flavor in dry beans, and they found that a "cinnamonlike" off-odor present in baked bread and wheat flour was from coumarin, traced to clover seeds inadvertently contaminating the wheat flour.

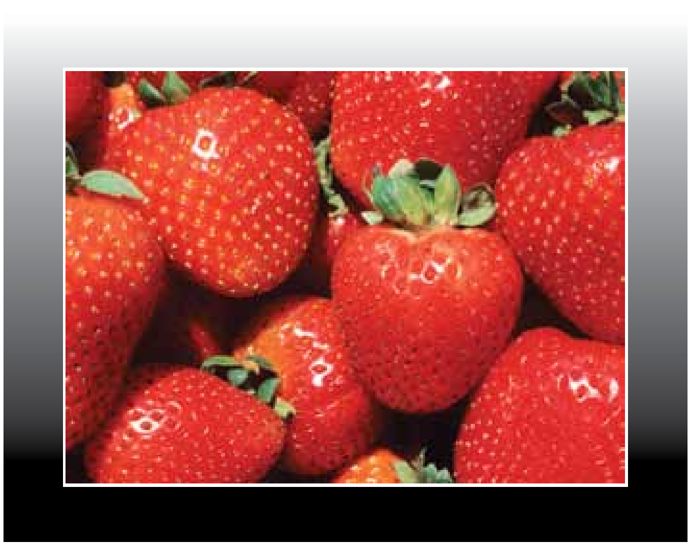
In the 1960s, Teranishi and WRRC scientist William H. McFadden combined sophisticated gas chromatography (GC) with mass spectroscopy (MS) and used their "capillary temperature programmed GC-MS technique" to identify complex mixtures of volatile chemical compounds, such as those that give flavor to strawberries. The technique was quickly picked up by scientists around the world and is now used routinely in chemical research. WRRC scientists used it to identify key aroma components in oranges, apples, bread crust, bell peppers, potatoes, corn tortillas, artichokes, and red beets.

Nuclear Magnetic Resonance

WRRC researchers built a nuclear magnetic resonance instrument in the late 1940s, several years before commercial models became available, and used it to determine the moisture content of different foods. Albany researchers modified it in the 1960s, and using a "time averaging computerized system" designed at WRRC, they were able to measure various compounds from as little as 50 micrograms of material, a far smaller sample size than previously needed.

Working with bell peppers, Buttery used the instrument to identify methoxypyrazines, an important class of odorants. In a landmark paper, he isolated and identified the "character impact compound" in bell peppers as 2-isobutyl-3-methoxypyrazine. The compound has been shown to be a key flavor constituent of certain wines as well as peas, green coffee, certain cheeses,

Separating volatile strawberry flavor compounds was not possible until WRRC scientists created one of the first practical gas chromatographs.



BRIAN PRECHTEL (K9189-1)

Roy Teranishi samples the headspace over fresh grapes just before injecting a sample into a gas chromatograph. (1963)

USDA-ARS (D2872-1)

chili peppers, and other varieties of peppers. Scientists now synthesize it, using methods developed by Buttery, and it is marketed in the United States and Europe as a food flavor.

Buttery and a co-worker synthesized eight related new alkylmethoxypyrazines, and that work led to the discovery of a new group of very significant flavor compounds. One of the synthesized compounds, 2-methoxy-3-isopropylpyrazine, is a major contributor to the earthy aroma and flavor of potatoes.

Aromatic Rice and Orange Oil

Buttery was the first to identify a flavor compound, 2-acetyl-1-pyrroline (ACPY), a major contributor to the flavor of aromatic rice, which commands a premium price because of its unique aroma. After WRRC researchers published their work, ACPY was identified as a major contributor to the flavor of bread, cooked beef, popcorn, and sweet corn products.

WRRC scientists were the first to identify alpha- and beta-sinensal as major contributors to the character and quality of orange oil, sweet orange oil, and mandarin oil. Alpha-sinensal has an intense orange scent accompanied by distinct floral notes, and beta-sinensal has a metallic-fishy odor that can lead to objectionable odors in higher concentrations.

WRRC scientists have shown that the main flavor compound in aromatic rice, prized for its aroma and taste, is also in some other foods.

COURTESY OF ANNA MCCLUNG, ARS (D2875-1)

USDA-ARS (D2870-1)

Ron Buttery, shown here adjusting a gas chromatography apparatus in 1959, was the first to identify the compound primarily responsible for the flavor of aromatic rice.

Volatiles as Breath Detectors and Odor Thresholds

In collaboration with Nobel Prize winner Linus Pauling, Teranishi carried out the first scientific studies of breath analysis in the early 1970s, demonstrating that human breath contains more than 200 volatile organic compounds. Today, breath analysis is used for blood alcohol testing and as a preliminary test for detecting certain cancers, diabetes, asthma, and *Helicobacter pylori* infection.

Although early flavor research focused mainly on identification of volatiles, attention was also paid to their sensory importance. Odor thresholds of volatile compounds (the concentration at which the compound is detectable) can vary widely and make a big difference in how a food smells and tastes. Ethanol, for instance, has an odor threshold of 100 milligrams per liter, while the character impact compound of bell peppers has an odor threshold of just 0.000002 milligrams per liter. WRRC scientists have determined the odor thresholds of hundreds of volatiles, and the results have been important to flavor researchers around the world.

Progress: From Hundreds of Compounds to Thousands

Efforts that began at the WRRC in the 1940s continue today. The progress made may be most evident in some key numbers from the field of flavor chemistry. Before the 1960s, researchers had to process tons of food to isolate sufficient samples for sensory and chemical studies, and so they had correlated only about 500 compounds to specific sensory properties. But by the 1990s, with help from new technologies and methods, scientists had identified and characterized more than 7,000 flavor/odor components.

Says Tara McHugh, research leader of WRRC's Food Processing Research Unit, "Processed foods are an important part of the nation's food supply, and the technology developed here will continue to help us ensure that the supply is safe, marketable, and of high quality and that the food tastes good to even the most discriminating palates."—**Dennis O'Brien,** ARS.

To reach scientists mentioned in this article, contact Dennis O'Brien, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1624, dennis.obrien@ars.usda.gov. *

In foods like bell peppers, volatiles are important to flavor research, and WRRC researchers have identified hundreds of such compounds.

DAVID BUTLER (D2133-1)

Dogs, Pine Trees, and Carbs

Carbohydrates from a perhaps surprising source—pine trees—may have beneficial effects on dogs' digestive-system health, according to collaborative studies by Agricultural Research Service, university, and corporate scientists.

The carbs, predominantly a group known as "GGMOs"—short for galactoglucomannan oligosaccharides—are a key ingredient in Previda, an all-natural dietary ingredient marketed to makers of pet food, aquaculture feed, and other animal-nutrition products.

ARS chemist Neil P.J. Price began his studies of these fiber-rich carbs in 2007, working under the auspices of a cooperative agreement with Temple-Inland, a Texas-based producer of lumber, fiberboard, and other wood products made from loblolly, longleaf, and other kinds of pine trees often referred to as "southern yellow pine."

GGMOs come into the spotlight in the latter stages of Temple-Inland's processing of the pine. When the trees are cut into lumber, wood chips remain. These are treated with heat, high pressure, and hot water to separate them into cellulosic fiber (for making fiberboard products) and hemicellulosic carbs—sometimes called "wood sugars"—which were the focus of Price's studies.

Temple-Inland had been selling the hemicellulosic mixture as a cattle-feed ingredient, and for other end uses, for about three decades before deciding to seek new ideas for value-added applications. In his laboratory at the ARS National Center for Agricultural Utilization Research in Peoria, Illinois, Price used proton magnetic resonance, mass spectrometry, and other analytical techniques to scrutinize the hemicellulosic carbs in samples from Temple-Inland's Diboll, Texas, operations.

His analyses, which at the time were apparently the most detailed of the hemicellulose mixture, pointed to the carbs' potential as a pet-food ingredient and paved the way for collaborative studies with a team led by George C. Fahey, Jr., a University of Illinois emeritus professor of animal sciences.

Studies Lead to New Pet-Food Ingredient

Today, hemicellulosic carbs from southern yellow pine make up 60 to 70 percent of Previda, a light-tan, fine-textured, spray-dried powder with a pleasant rum-and-raisin aroma.

STEPHEN AUSMUS (D2845-14)

STEPHEN AUSMUS (D2844-6)

In one experiment, GGMOs from southern yellow pine were purified in Price's lab, then added, in place of dietary cellulose, to a high-quality kibble at the rate of 0.5, 1, 2, 4, or 8 percent. A control kibble contained no added GGMOs. The kibble was then fed to six healthy female dogs. Analyses of the dogs' fecal samples showed that increasing the percentage of pine GGMOs in the kibble was, in general, associated linearly with several significant indicators of a healthy lower digestive system.

"At some of these dietary concentrations, we saw an increase in populations of beneficial *Bifidobacterium* bacteria species, an increase in concentrations of short-chain fatty acids, and a decrease in pH," says Fahey.

Hemicellulose extract, such as that in Previda, is already listed as a safe ingredient in a "gold-standard" registry maintained by the Association of American Feed Control Officials, a nonprofit organization that sets standards for the safety and quality of animal feed and pet food. The fact that no harsh chemicals are used in processing the wood chips from which Previda hemicellulosic carbs are extracted "is a further 'plus' for the safety of this product," says Temple-Inland chemist Anne C. Hopkins.

Price, Hopkins, and Fahey, along with former University of Illinois animal sciences graduate student Trevor A. Faber and other collaborators, published their findings in the *Journal of Agricultural and Food Chemistry* in 2010 and 2011 and in the *Journal of Animal Science* in 2011.—By **Marcia Wood,** ARS.

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

Neil P.J. Price is in the USDA-ARS Renewable Product Technology Research Unit, National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, IL 61604; (309) 681-6246, neil.price@ars.usda.gov. ✻

Top photo: Chemist Neil Price prepares fraction samples collected by technician Trina Hartman for further analysis with a mass spectrometer. Bottom: Price analyzes sugars using a mass spectrometer to investigate which oligosaccharide sugars are present. The process involves several steps.

Nourishing America's Preemies

Scientists Confront the Challenges of IV Feeding

Each year, more than a half-million infants are born prematurely in the United States. Many of these preemies, particularly those whose tiny digestive systems are simply too underdeveloped to handle mother's milk or infant formula, may need to be nourished exclusively via intravenous feeding, known as "total parenteral nutrition," or TPN.

TPN solutions, usually administered at the hospital for anywhere from a few days to a month or more, provide essential nutrients broken down into a very basic form. This liquid is gently and continuously infused into the infant's bloodstream, completely bypassing the digestive tract.

"TPN helps save the lives of newborns and supports their growth and development, especially of the brain," says Douglas G. Burrin, an Agricultural Research Service physiologist at the Children's Nutrition Research Center in Houston, Texas, and a faculty member at Baylor College of Medicine, also in Houston.

But preemies who are on TPN for longer than 2 weeks may develop complications that might affect their health later in life. Since 1998, Burrin and colleague Barbara Stoll, who is also with the nutrition center and the college faculty, have worked with teams of scientists in the United States and abroad to discover more about the unwanted side effects of TPN and to develop new, safe, effective ways to prevent these unintended consequences or, at the very least, to minimize their impact.

The TPN-linked problems that their research targets include poor control of blood sugar, slowed growth of the digestive tract, and onset of a constellation of disorders referred to as "parenteral nutrition-associated liver disease."

In these investigations, Burrin and colleagues use piglets as their laboratory animal model.

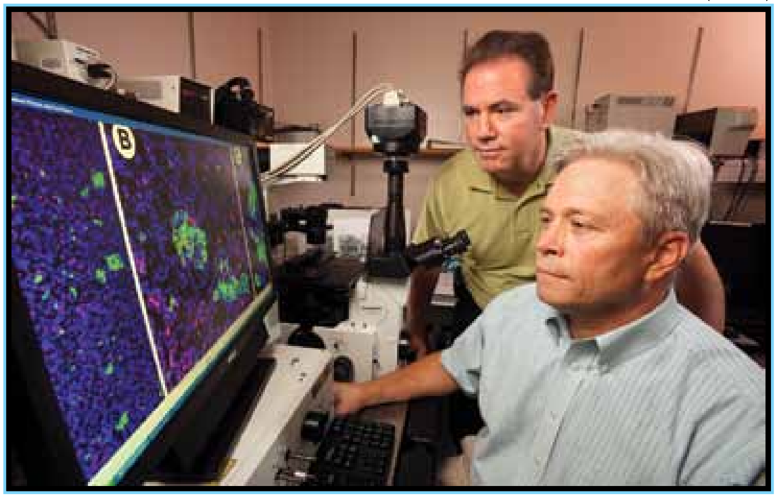
Why Piglets?

Nutrition researchers worldwide recognize that the pig digestive tract is very similar to that of humans. Also, the size and body composition, that is, the amount of fat and amount of lean tissue, of an infant piglet "are typically comparable to those of a human preemie," says Burrin. "Infant lab mice or newborn lab rats are simply too small for this research."

MARK BOGGESS (D2865-1)

Compared to human preemies, infant piglets are similar in size and body composition and have similar digestive tracts. These healthy piglets are about an hour old.

STEPHEN AUSMUS (D2848-3)



At the Children's Nutrition Research Center in Houston, Texas, ARS physiologist and professor of pediatrics Doug Burrin (left) and associate professor of pediatrics Darryl Hadsell examine a microscope image of pancreatic beta cells obtained from piglets fed by total parenteral nutrition.

Does TPN Increase Risk of Insulin Resistance?

In 2005, scientists elsewhere suggested an association between premature birth and increased risk of insulin resistance, a disorder common in type 2 diabetics.

A 2010 study by Burrin, Stoll, and others brought TPN into this picture. Their research provided the first evidence, in a newborn-lab-animal model, of a significant association between TPN feeding and several indicators of insulin resistance.

In people, and in piglets, insulin—a hormone—largely controls the amount of sugar (glucose) in the bloodstream. Insulin is produced exclusively by unique "beta cells" in the pancreas. As it circulates through the body, insulin triggers cells to remove glucose from blood and use it for energy. But resistance to insulin can lead to a buildup of unhealthy levels of glucose in the blood.

Insulin resistance was 40 percent greater in the TPN-fed piglets than in their orally fed counterparts, the scientists found. Also of concern: Proliferation of beta cells was 30 percent less in the TPN piglets.

"The beta cells that you produce during infancy and childhood have to last you a lifetime," says Burrin, "so anything that interferes with normal beta-cell production could be detrimental to your later health. "

GLP-1 Levels: How Important Are They?

Followup research, reported by Burrin, Stoll, and others in 2012, again indicated that insulin

resistance was significantly greater in TPN-fed piglets, compared to piglets that were put on other feeding regimens.

In addition, the study suggested that reduced production of GLP-1 (glucagon-like peptide 1), a hormone secreted in the digestive tract, may help explain the difference in insulin resistance among the research piglets.

Previous research has shown that GLP-1, a "gut hormone," circulates through the body and helps lessen insulin resistance. It belongs to a class of hormones known as "incretins." Their insulin-regulating role, dubbed the "incretin effect," has led to development of synthetic incretins now used in treatment of type 2 diabetes.

Says Burrin, "We found that plasma levels of GLP-1 were significantly lower in the TPN piglets than in any of the other piglet groups. That makes sense, given that there was no food in the TPN piglets' digestive tracts to stimulate secretion of GLP-1.

"We are continuing to investigate GLP-1 in our research because we think there's much more to be learned about it in the context of preventing or reducing insulin resistance in TPN infants."

Small Doses of Bile Acid May Blunt Liver Disease

Gut hormones were also key in a study of a potential new approach to preventing parenteral nutrition-associated liver disease (PNALD).

PNALD is an umbrella term that encompasses several conditions, including cholestasis, which results from a buildup of excess bile acids in the liver, and steatosis, which—as its "fatty liver" nickname implies—occurs when there is too much fat in the liver.

There is no well-established, science-based cure for PNALD. In severe cases, this disease can lead to liver failure and the need for a liver transplant—a major surgery that, though not new, is still regarded as having considerable risks.

A team of Burrin, Stoll, and coinvestigators showed that giving newborn TPN-fed piglets a very small dose of a natural bile acid three times a day helped combat PNALD.

The acid, CDCA (chenodeoxycholic acid) is one of the major bile acids that are produced in the liver, then secreted—via the gall bladder and bile duct—into the upper digestive tract to help the body digest fat.

According to Burrin, the CDCA study is the first to demonstrate the use of small doses of this bile acid, delivered directly to the upper digestive tract, to control PNALD in a newborn-lab-animal model.

TPN piglets that were given CDCA had significantly lower levels of serum bilirubin, a biomarker of cholestasis, than did the TPN-fed piglets that were given a placebo. In addition, levels of serum bile acids, which are another biomarker of cholestasis, and levels of liver triglycerides, an indicator of steatosis, were nearly normal in the CDCA-treated TPN piglets.

The team also found that CDCA stimulated growth of mucosa, the inner lining of the intestine. That is significant. Explains Burrin, "TPN often has the exact opposite effect: It can lead to atrophy of the mucosa." The shriveling and shrinking of the intestine that result can diminish the body's ability to digest and absorb nutrients.

The researchers are now taking a closer look at the basic mechanisms responsible for the beneficial effects of the CDCA regimen. CDCA's ability—as shown in mice—to indirectly induce secretion of key gut hormones such as GLP-2 (glucagon-like peptide 2) and FGF19 (fibroblast growth factor 19) "may provide an explanation for some of our piglet findings," Burrin says.

For example, previous research, including that by Burrin and Stoll, has shown that GLP-2 boosts growth and proliferation of intestinal mucosal cells in piglets and in adult humans. In this newer study, reported in 2012, three factors—GLP-2 levels, better mucosal growth, and CDCA treatment—appeared to be interrelated. That's because TPN piglets that were treated with CDCA had higher levels of GLP-2 in their plasma. Those same piglets had better mucosal growth than the other TPN piglets.

FGF19 may also be an important part of the picture. In mice, FGF19 has the protective effect of suppressing production of bile acids in the liver, in response to signals sent from specialized bile-acid-sensing cells in the gut. CDCA-treated TPN piglets had healthier bile acid levels, and more FGF19 in their plasma, than did the other TPN-fed piglets.

Piglets that were not on a TPN regimen had the highest plasma FGF19 levels. "We regard this finding as strong evidence that TPN suppresses secretion of FGF19, which results in disruption of normal regulation of bile acids. We think this suppression is a key piece of the puzzle of why and how TPN can cause liver disease."

Burrin and Stoll, along with Teresa A. Davis, Darryl L. Hadsell, and David D. Moore, all of the nutrition center research staff and Baylor College of Medicine faculty, and other collaborators, have documented their TPN findings in peer-reviewed articles in the *Journal of Nutrition,* the *American Journal of Parenteral and Enteral Nutrition,* the *American Journal of Physiology: Gastrointestinal and Liver Physiology,* and other scientific publications. The American Society for Parenteral and Enteral Nutrition, the National Institutes of Health, the American Liver Foundation, and ARS funded the studies.—By **Marcia Wood,** ARS.

This research is part of Human Nutrition, an ARS national program (#107) described at www.nps.ars.usda.gov.

Douglas G. Burrin is with the USDA-ARS Children's Nutrition Research Center, 1100 Bates St., Houston, TX 77030; (713) 798-7049, doug.burrin@ars.usda.gov. ✻

STEPHEN AUSMUS (D2850-10)

Research instructor of pediatrics Barbara Stoll (left) and assistant Liwei Cui fill parenteral nutrition bags with formulas to nourish infant piglets.

Mapping the Way to Even Healthier Rice

With the aid of high-tech tools, Agricultural Research Service and collaborating scientists are closing in on the genes in rice that regulate the uptake and storage of important minerals—a pursuit that could bolster the nutritional value of this cereal grain crop as a staple food for roughly half the world's population.

Ultimately, the team envisions biofortifying rice using traditional plant breeding methods to develop new varieties whose kernels (grain) boast exceptionally high concentrations of essential minerals, including zinc, iron, and calcium.

Rice is a mainstay of the human diet for good reason. It is a rich source of energy, free of gluten (which causes allergic reactions in some individuals), easy to digest, low in fat, and packed with vitamins, minerals, and other nutrients. Some key elements, however, like iron, are lost when the bran on brown rice is stripped off during milling to produce so-called white rice, notes Shannon Pinson, a plant geneticist with the ARS Dale Bumpers National Rice Research Center in Stuttgart, Arkansas.

"More than 70 percent of the white rice eaten in the United States is 'enriched,' meaning that thiamine, niacin, iron, and folic acid have been added to the outside surface of the uncooked white kernels to bring the overall nutritional level up to or higher than that of the whole grain (brown rice) for these vitamins," write Pinson and coauthors in an article describing their work in the October 2010 issue of the newsletter *Texas Rice.*

But in developing countries where rice is a mainstay, fortifying the grain after milling may not be a viable option. Additionally, the soils in which the crop is grown may be lacking in certain essential minerals. For populations that rely on rice as a staple food, low levels of iron, zinc, or other minerals can lead to nutritional deficiencies that manifest as fatigue, poor immune system function, and other symptoms. Indeed, more than 3 billion people worldwide suffer from iron or zinc deficiencies in their diets.

To address these concerns, Pinson and her ARS and university colleagues focused their studies on three different groups, or "populations," of rice—with the most diverse represented by 1,643 lines (called "accessions") collected from 114 countries around the world. In this diverse group, the researchers encountered rice accessions whose grains contained up to nine times the amount of minerals normally observed in standard U.S. varieties.

"We've crossed these accessions with the U.S. cultivar Lemont and are now evaluating the seed of second-generation plants for extreme grain concentrations of these minerals," says Pinson.

Her chief collaborators are David Salt of the University of Aberdeen, United Kingdom (and adjunct professor at Purdue University); Mary Lou Guerinot of Dartmouth College; Lee Tarpley of Texas A&M AgriLife Research, and Ratnaprabha Chittoori, a graduate student being co-mentored by Pinson and Tarpley.

The group began the project in 2007. The National Science Foundation is supporting the effort, which includes mapping the approximate locale of the genes on rice's 12 chromosomes and developing marker data to easily detect them during future breeding efforts.

The collection they're working with, the USDA Core Collection, was developed by Wengui Yan at the USDA-ARS Dale

Rice growing in a flooded field at the Texas A&M AgriLife Research Center, in Beaumont, Texas. Scientists are studying the rice under both flooded and unflooded field conditions to see how differences in soil chemistry caused by flooding affect mineral accumulation in rice grains.

STEPHEN AUSMUS (D2842-3)

In search of traits that might influence plant uptake of certain minerals, ARS plant geneticist Shannon Pinson (right) and graduate student Ratnaprabha Chittoori examine roots from a rice plant removed from a field study in Beaumont, Texas.

STEPHEN AUSMUS (D2839-3)

STEPHEN AUSMUS (D2840-8)

Shannon Pinson and AgriLife plant physiologist Lee Tarpley (foreground), and technicians Richard Chase and Jerri Daniel (background) examine diverse rice lines found to have high concentrations of specific minerals in their grain.

Bumpers National Rice Research Center in Stuttgart, Arkansas, in collaboration with the National Plant Germplasm System's National Small Grains Collection, which is maintained in Aberdeen, Idaho, by ARS and includes more than 17,000 total rice accessions.

Based on data from side-by-side comparisons conducted under controlled field conditions in Beaumont, Texas, the team identified 40 rice accessions whose grain contained high levels of minerals important not only to human health, but also to that of the rice plant. Take, for example, calcium.

"Calcium strengthens the plant cell wall and decreases permeability of cell membranes, which, in turn, can lead to increased resistance to diseases and environmental stresses," explains Pinson.

Also of interest are rice accessions with low grain levels of certain elements that are toxic to plants and people, such as arsenic and cadmium. Rice with low levels of arsenic or cadmium may have genes that sequester or bind these toxic minerals in leaves instead of grain or that curtail their absorption from contaminated soils or irrigation water—a concern, for example, in parts of Bangladesh where rice is grown as a staple crop and high arsenic levels in water are indigenous.

In the Beaumont studies, the team compared mineral uptake in rice plants grown in flooded and nonflooded fields. Flooding, Pinson explains, changes the soil chemistry and converts some elements into forms more available for mineral uptake and others into less available forms. By studying plants grown in both soil conditions, the team was able to identify even more genes and to gain knowledge about how the genes function in the plant to affect element uptake and accumulation.

To date, the team has identified 127 genes clustered in 40 different chromosome regions that correlate to high concentrations of particular minerals. They have also identified genes affecting other grain features, including shape, and found that grain element accumulation is largely independent of these other grain quality attributes.

Other findings are that:

- there is a wide range of mineral concentrations among rice accessions from around the world.
- mineral levels fluctuated more when rice was grown in nonflooded fields than in flooded ones.
- rice with the highest grain levels of certain minerals, like molybdenum (important for plant nitrogen nutrition; high levels help rice cope with acidic soils), sometimes originated from the same geographic region of the world.
- natural gene variation may be responsible for some of the highest mineral levels in grain.
- plant maturity influences the amount of mineral absorbed and where it is stored—in leaves, bran, or grain, for example.

The team is also developing molecular marker data to quickly identify high-mineral rice plants without growing them to maturity during breeding operations. There is early evidence that high mineral levels (calcium, for example) in the leaves of seedlings of some rice varieties may correlate with high mineral levels in the grain at maturity. The team is investigating whether root architectural or physiological traits, which are expected to affect rates of mineral uptake from the soil, are causing differences that carry through to differences in grain concentrations. These seedling assays could offer a way for rice breeders to save time and money in selecting top-performing plants for use in developing elite commercial varieties. Much work has yet to be done toward that end, but the stage is set.

In addition to ensuring that consumers get more of the essential minerals needed for their sustained health and well-being, biofortified rice could mean expanded markets for U.S. growers, both domestically and abroad. Lastly, the approach could serve as a useful model for biofortifying other important grains that feed the world.—By **Jan Suszkiw,** ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement, an ARS national program (#301) described at www.nps.ars.usda.gov.

Shannon Pinson is in the USDA-ARS Dale Bumpers National Rice Research Center, 2890 Highway 130 East, Stuttgart, AR 72160; (870) 672-9300, ext. 228, shannon.pinson@ars.usda.gov. *

New Bioenergy Yeast Is an Overachiever

There's a lot that can be done with a corn cob after the kernels have been removed. Farmers leave the cobs on the field to boost soil quality. Enterprising cooks use the cobs to make jelly. In China, the sugar xylose is extracted from the cob for industrial uses.

Now Agricultural Research Service molecular biologist Zonglin Lewis Liu and his colleagues have identified a yeast strain for ethanol production that breaks down and ferments the sugars in corn cobs left behind after xylose has been extracted. And this takes place without the addition of a costly enzyme, a breakthrough that could help make cellulosic ethanol production a cost-effective proposition.

The new strain of yeast, *Clavispora* NRRL Y-50464 (Y-50464), can tolerate cob-derived compounds that interfere with yeast growth and fermentation rates. It is able to grow rapidly at 37˚C, unlike its parent strain, which grows best at 30˚C. That means it can thrive at the higher temperatures needed to optimize rates of "simultaneous saccharification and fermentation" (SSF), a one-step process in cellulosic ethanol production that combines releasing and fermenting feedstock sugars.

Liu worked with technician Scott Weber and supervisory microbiologist Michael Cotta on this project. All three researchers are in the Bioenergy Research Unit at the National Center for Agricultural Utilization Research in Peoria, Illinois.

The team started the project with a strain of yeast that had been identified in earlier studies on the use of sweet sorghum as a biofeedstock for ethanol production. Then, in the laboratory, they placed this strain in environments that would promote the expression of genetic traits that favor SSF, including adapting to higher temperatures and tolerating cob-derived compounds that interfere with ethanol production. This resulted in the selection of Y-50464.

The team compared how quickly Y-50464 and another yeast strain, Y-12632, could release and ferment the sugar in corn cob residues after the xylose had been extracted. Liu's star newcomer yeast was able to grow and reach its highest cell density in less than 24 hours after the test began. The comparison yeast didn't grow at all.

In a followup test, the scientists added the enzymes cellulase and beta-glucosidase—which are often used to break down residues and extract sugars—and observed that Y-50464 reached its peak ethanol production rate of 25.7 grams per liter 5 days after the experiment began. But the yeast produced *more* ethanol—26.6 grams per liter in 5 days—without the addition of beta-glucosidase. In contrast, Y-12632 showed a maximum ethanol production of 22.9 grams per liter after 6 days with the enzyme assistance. But without the enzyme, its production rate dropped to 11.9 grams per liter.

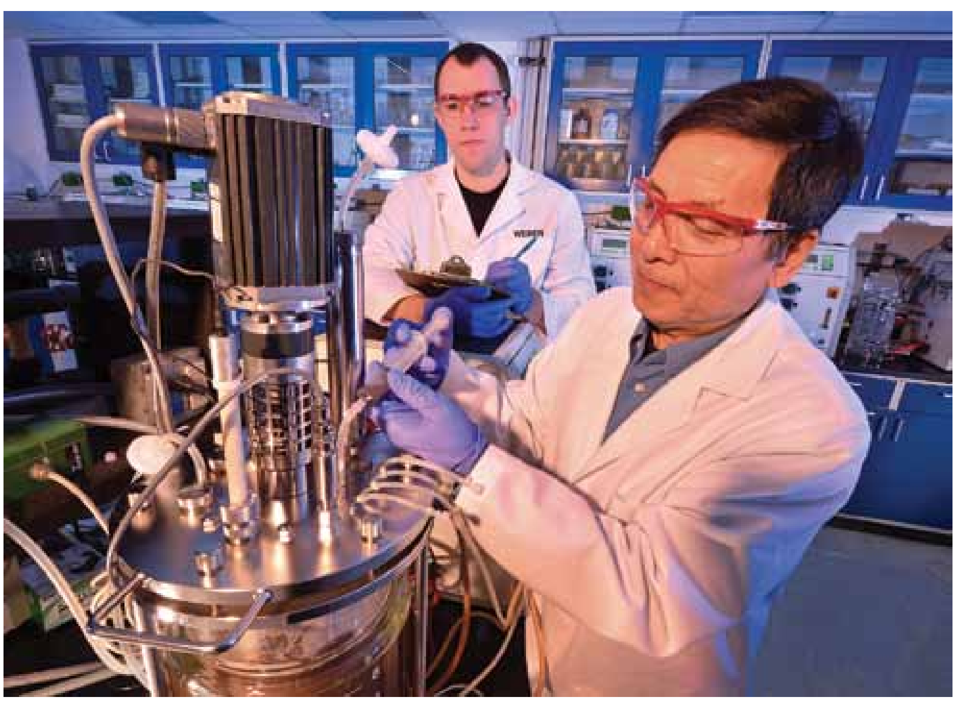
Liu and his colleagues sampled protein extracts from both yeast strains to look for evidence of enzyme activity within the yeast itself. They observed significant enzyme activity in the Y-50464 extracts, but did not see any indications of enzyme activity in the Y-12632 extracts. After additional testing, Liu's group confirmed that Y-50464 contains a new form of beta-glucosidase.

"This is a unique strain of yeast because it produces an innate beta-glucosidase, one of two enzymes needed for ethanol production from cellulose," Liu says. "Now we have a valuable strain of yeast we can use in the next generation of biocatalyst development. We hope to enrich the desirable characteristics of this strain with additional enzymes, which would bring us closer to the bioprocessing we need for advanced biofuels production."

Results from this work have been published in *Bioresource Technology and Bioenergy Research.*—By **Ann Perry,** ARS.

This research is part of Bioenergy, an ARS national program (#213) described at www.nps.ars.usda.gov.

Zonglin Lewis Liu is in the USDA-ARS Bioenergy Research Unit, National Center for Agricultural Utilization Research, 1815 North University St., Peoria, IL 61604; (309) 681-6294, zlewis.liu@ars.usda.gov. *



FRED ZWICKY (D2856-2)

Molecular biologist Z. Lewis Liu (right) and technician Scott Weber add a new yeast strain to a corn cob mix to test the yeast's effectiveness in fermenting ethanol from plant sugars. The yeast produces an enzyme that helps release and degrade biomass sugars, and that could help reduce ethanol production costs.

The Agricultural Research Service has about 100 labs all over the country.

Locations Featured in This Magazine Issue

Locations listed west to east.

Map courtesy of Tom Patterson, U.S. National Park Service

Albany, California
9 research units ■ 230 employees

Pullman, Washington
6 research units ■ 136 employees

Small Grains and Potato Germplasm Research Unit, Aberdeen, Idaho
1 research unit ■ 57 employees

U.S. Sheep Experiment Station, Dubois, Idaho
1 research unit ■ 10 employees

Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska
6 research units ■ 117 employees

Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota
2 research units ■ 51 employees

Children's Nutrition Research Center, Houston, Texas
1 research unit ■ 7 employees

Columbia, Missouri
3 research units ■ 86 employees

Stuttgart, Arkansas
2 research units ■ 56 employees

National Center for Agricultural Utilization Research, Peoria, Illinois
7 research units ■ 226 employees

National Cold Water Marine Aquaculture Center, Franklin, Maine
1 research unit ■ 8 employees

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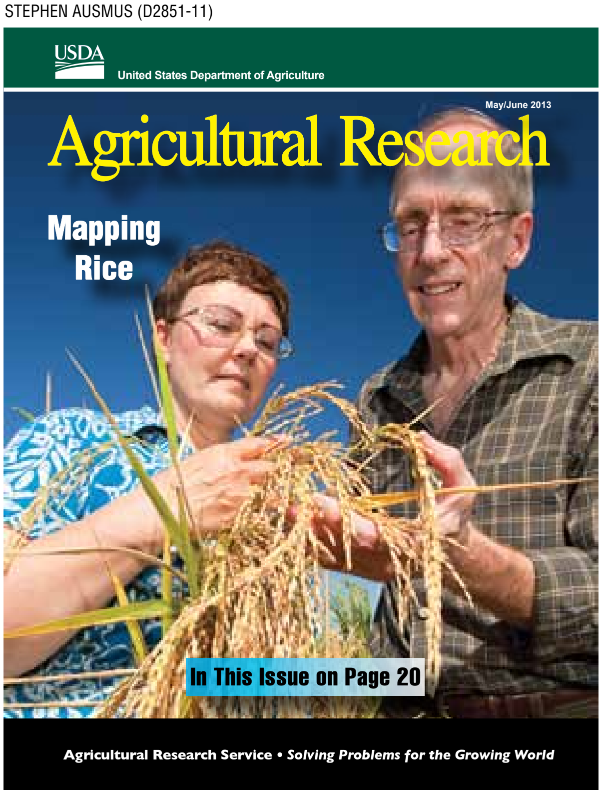
STEPHEN AUSMUS (D2851-11)



PEGGY GREB (D959-1)