# Food Safety

Responsibility for the continuing safety of America's food supply is shared widely among farmers and ranchers, food handlers and processors, private laboratories, and Federal, State, and local agencies. Food safety is also a responsibility of people who prepare meals in the home, in restaurants, and in institutions. It has long been an objective of much of the research conducted by the Agricultural Research Service, and chemists, food technologists, and microbiologists at the regional centers carry out some of the most significant food safety investigations in the country. Their areas of concern include mycotoxins, including aflatoxin; plant toxins; salmonellosis, botulism, and other types of food poisoning; and chemical residues in food, including pesticides and antibiotics.

***Aflatoxin.*** Of all the naturally occurring toxins caused by fungi, the most dangerous is aflatoxin, the name of a group of closely related toxins. They first came to world attention in 1960, when a mysterious "Turkey X Disease" in England killed 120,000 turkeys and other poultry. Cause of the deaths was traced to contaminated peanut meal from Brazil, which was found to contain a potent toxin resulting from the growth of certain molds on crops. The substance was given the name *aflatoxin*. Since that time, researchers in the United States and several other countries have learned a great deal about this toxin and have shown that it is a carcinogen in Fisher rats, ducks, rainbow trout, and other animals in the laboratory. It is also suspect as a human carcinogen. Tolerances of the toxins in foods have been the subject of intense regulatory debate. For many years, a limit of 20 parts per billion set by the U.S. Food and Drug Administration for all commodities except milk was recognized worldwide. Many countries today, however, are imposing even lower limits in foods for human consumption.

Scientists now know that aflatoxins are a product of either of two soil fungi, *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin can contaminate U.S. crops of corn, cottonseed, peanuts, and tree nuts. At first believed to develop only in stored grain and oilseeds or in dead or dying plants in the field, it was soon discovered in developing seed crops as well. Aflatoxin contamination of these crops has resulted in serious losses to growers, food handlers, and processors, particularly in hot, dry crop years, when the mold flourishes after plants are weakened by stress.

Scientists at three ARS regional laboratories have made important contributions for nearly three decades to the world's knowledge of aflatoxin, and research continues today at the Northern and Southern centers. The first comprehensive study on the effects of feeding aflatoxin to livestock and poultry was published in 1971 by a Western lab scientist in cooperation with researchers in New Orleans and Peoria and the University of California. The team established the levels of aflatoxin required to produce recognizable growth effects in swine, beef, and dairy cattle and in broilers and laying hens. The effects on animals of ingesting aflatoxin in feeds may be acute, causing sudden death, or prolonged, resulting in poor weight gains, reduced productivity, and suppression of immunity to disease.

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Other ARS scientists conducted the first studies documenting that aflatoxin contaminates corn, particularly in the South. Researchers developed rapid screening tests to detect aflatoxin in corn and found, for example, that corn kernels contaminated with aflatoxin fluoresce with a telltale greenish-yellow glow when exposed to ultraviolet light. This test, known as BGY, proved simple, economical, and fast to perform and could be used for screening corn on the farm and at grain elevators. More than 3,000 crystalline and liquid standards for determining aflatoxin levels were distributed by Peoria researchers to other investigators.

In New Orleans, several significant improvements were made in methodology to detect aflatoxin in cottonseed. These methods first cut analysis time from 4 days to 3 hours. Further research led to even faster quantitative assays, and a chemical means was found to enhance the fluorescence of the already highly fluorescent aflatoxin and to increase the sensitivity of the detection method. The Peoria lab conducted the validation studies that resulted in the analytical method for determining aflatoxin in corn being accepted by the Association of Official

Analytical Chemists (AOAC). Other sensitive analytical methods were developed at this laboratory, including techniques for detecting and measuring aflatoxins in milk and in animal tissue. Researchers at the Peoria center were also instrumental in the evaluation and subsequent development of commercial immunochemical test kits for aflatoxins. These kits are widely used today by researchers, grain dealers, and USDA and FDA regulatory agencies.

Once the contamination of grains and oilseeds was recognized, scientists searched for ways to detoxify animal feedstuffs. At the Western and Southern labs, researchers received a patent in 1969 for the ammoniation process for detoxification of aflatoxin. Southern lab scientists found that treating cottonseed meals with gaseous ammonia under heat and pressure for less than an hour of contact time reduced aflatoxin by from 99 to 100 percent without harming the meal for animal feed. A related process, developed by Peoria scientists, detoxified aflatoxin in whole corn under ambient pressure. Neither the high-pressure ammoniation process developed at SRRC for feed meals nor the ambient-pressure process developed at NRRC for detoxifying corn has ever received the needed approval from the Food and Drug Administration. They have been approved, however, in several States.

In Peoria, scientists subsequently developed a trickle ammonia process, an on-farm method that allowed the drying of high-moisture grain with unheated air. Periodic treatment with anhydrous ammonia, fed to the wet corn through an ordinary garden hose, retarded the growth of molds while the corn was slowly dried. Use of unheated air saved fuel. This process did receive Federal approval from the Environmental Protection Agency.

Western lab scientists began in the early 1970's to help the California tree nut industry detect and remove aflatoxin. Among other things, WRRC researchers found that contaminated almonds fluoresce with a characteristic purple color under ultraviolet light. They developed reliable sampling procedures and advised the industry that aflatoxin was associated with damaged nutmeats, which could be removed during processing. WRRC findings enabled processors and growers to improve their processing techniques, resulting in an acceptable product.

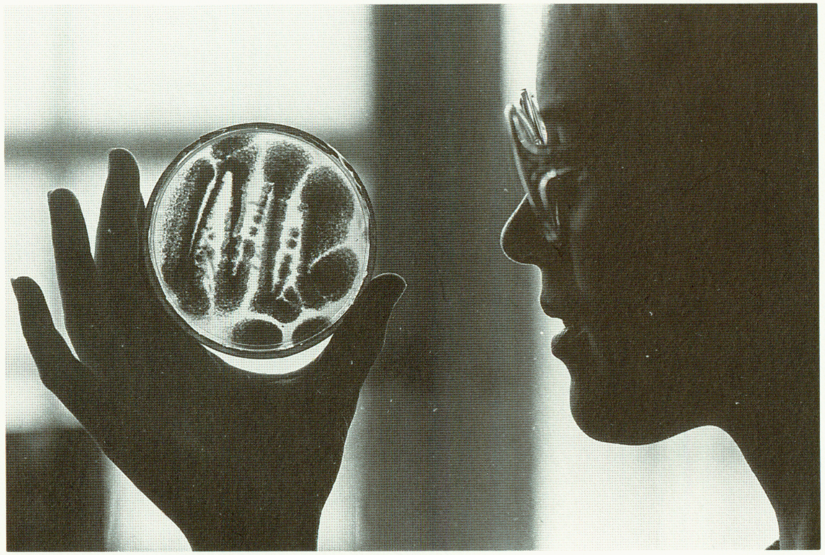
Regional researchers have learned more about aflatoxin every year, searching for some means to control the molds in the field or to prevent *Aspergillus* from manufacturing the toxin. They discovered how the fungi survive in field soils in the form of hardened resistant structures called sclerotia. They have demonstrated that germinated sclerotia at the surface of the soil may be the primary source of mold infecting a corn crop. They have learned how soil-dwelling insects commonly known as sap beetles play a role in spreading the fungus by carrying it from the soil to the ear of corn. They also found that the beetles were attracted mostly to ears damaged by the corn earworm. Understanding how the fungus is spread may help reduce its prevalence by encouraging better management of crop residues in the soil.

Meanwhile, Southern lab scientists in 1986 discovered two enzymes in *Aspergillus* that are essential to production of aflatoxin. They are trying now to identify the gene or genes responsible for making at least one of these enzymes, hoping to remove or alter them through genetic engineering so that the fungi will be unable to produce aflatoxin. The capability to identify and thus assay these genes is also important to ensure the safety of naturally occurring non-aflatoxin-producing strains of *A. flavus* or *parasiticus* that could be used for biocontrol.

***Other mycotoxins.*** Unfortunately, aflatoxin is not the only dangerous toxin produced by molds. Peoria scientists have studied several others, including mycotoxins produced by *Fusarium* molds, long known to be responsible for many types of plant diseases. Recent research has focused on how and why *Fusarium* makes certain types of poisons called trichothecene toxins. Also under study are the chemicals that a plant produces to defend itself when attacked by *Fusarium* and the chemical combat that results between the mold and the plant.

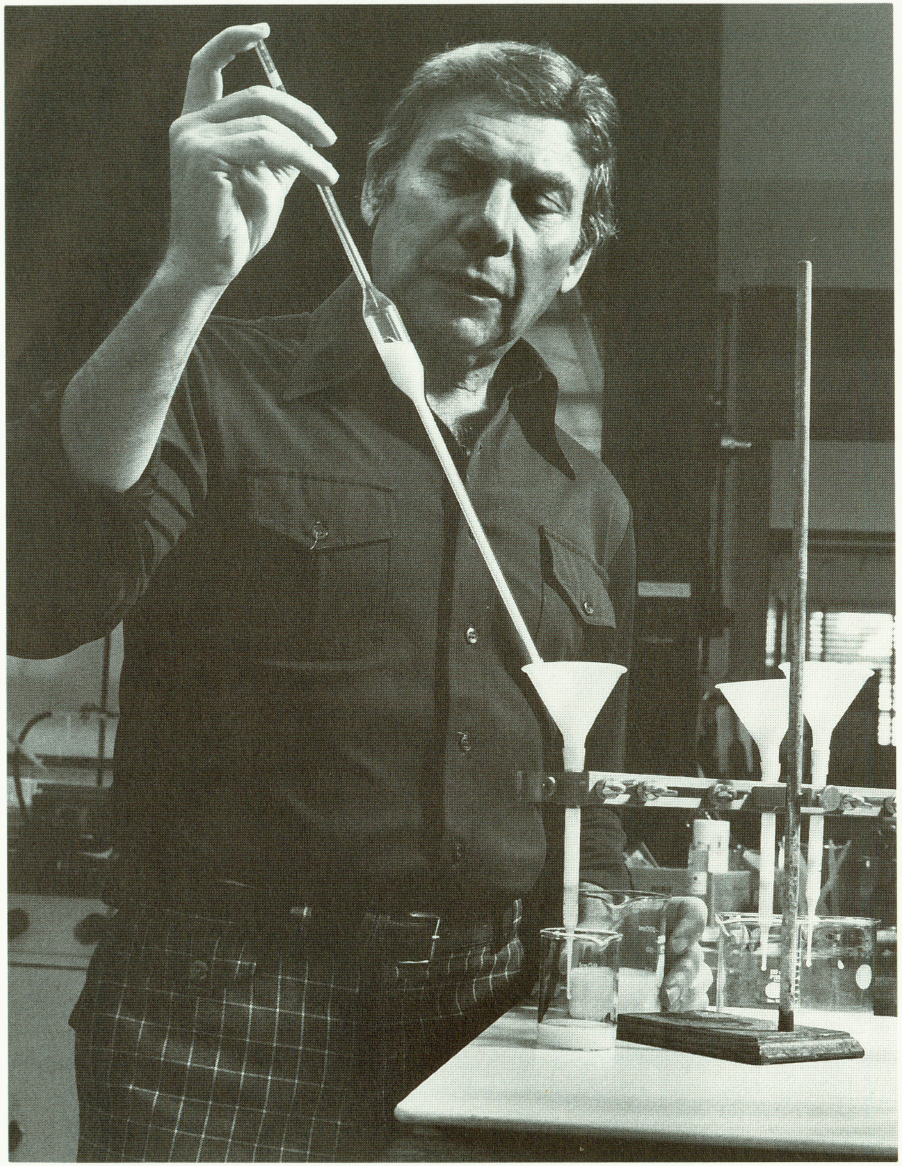
Work with *Fusarium* has demonstrated that the manufacture of mycotoxins is an extremely complex process that can be understood only after long and ingenious laboratory study. To produce one toxin, labeled T-2, the fungus carries out 13 or 14

*An SRRC researcher examines a mold transferred from a corn kernel under ultraviolet light, looking for characteristic fluorescence associated with aflatoxin.*



*Magnified view of kernel of corn infected with* Aspergillus flavus*, a fungus that produces aflatoxin.*

*In Peoria, microbiologist Donald T. Wicklow transfers sap beetles from overwintered corn to petri dishes containing a fungal growth medium. Also called picnic beetles, the insects carry* Aspergillus *fungi from the soil to corn ears.*



*Chemist Daniel P. Schwartz tests milk for traces of the antibiotic chloramphenicol (CAP) in an inexpensive yet sensitive procedure developed by his group at ERRC. Use of the drug in dairy cattle and meat animals is illegal.*

separate steps, of which the sequence of all but 4 has been determined by NRRC researchers. It is necessary for scientists to know exactly how toxins are produced before they can develop effective ways to prevent them.

In 1988, an important new toxin called fumonisin was reported. The toxin is deadly to horses and swine and is a potential carcinogen in humans. Again Peoria researchers promptly carried out needed toxin research. Within months of the first report, they became the first scientists in North America to isolate the new toxin and, what is even more important, they were able to develop a sensitive analytical method to detect fumonisin in corn and corn products. Unusually large numbers of horse deaths and swine illness occurred at several places in the Midwest as the 1989 corn crop was used. Fumonisin concentrations in corn screenings linked to the livestock deaths were measured using the Peoria method.

***Botulism.*** The food poisoning most often fatal to human beings is botulism. Fortunately, because it has been recognized for many years and effective controls have been developed for food processing and preparation, its incidence today is low. Botulism is caused by a toxin formed in the absence of oxygen by *Clostridium botulinum,* a bacterium found in the soil. Long the bane of home canners, it has been found all too often in jars of low-acid foods like green beans that have undergone insufficient heat processing. Several cases in New York City were once traced to a single jar of mushrooms, preserved by a restaurant owner. Symptoms of botulism are weakness, headache, disturbed vision, and paralysis. The only treatment is an antitoxin that is available from health departments.

Canned tomatoes were long considered safe from botulism by virtue of their high acidity, and at home and commercially, they were processed only briefly. In the 1970's, however, several cases of botulism were traced by health officials to home canning of low-acid tomatoes, varieties widely available to home gardeners. As a result, Eastern center scientists identified several tomato varieties with an acid content low enough to constitute a hazard with routine canning. They also found that many presumably safe varieties of tomatoes were risky for canning if overripe or decayed.

The result was publication of a brand new set of procedures for safe home canning of tomatoes and tomato sauces, juices, and blends with other vegetables. One important ERRC recommendation was to increase the acidity of tomatoes with citric acid or lemon juice before canning to prevent the growth of the botulism bacterium. The new guidelines were disseminated by the media and extension workers. A few years later, a computerized data bank at the Eastern lab contained 450 safe canning recipes, mostly for tomato-based products.

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Botulism struck again in the United States and the USSR in canned tomato juice. In tests by mystified health officials, the products appeared to be acid enough to prevent formation of the poison. Again, ERRC scientists found the answer. The *botulinum* organism may thrive, even in a high acid product, they found, if certain molds are growing on the surface. The molds decrease the acidity of the tomato juice near the top, at the same time using up the oxygen that prevents the botulism from forming. Samples analyzed by local officials had been shaken, disguising the low acidity of the topmost layer of juice by mixing it with the rest of the juice in the can. Careful handling at the Philadelphia lab led to discovery of the hiding place for the toxin and to recommended procedures for preventing the problem.

A third discovery about botulism at the ERRC has since been widely disseminated by pediatricians and nutritionists. Infant botulism is the result of a *botulinum* infection of the intestinal tract in babies less than a year old. The source of the microorganism in several cases, researchers found, was honey. The most likely explanation, according to ERRC scientists, is that bees sometimes pick up spores of *C. botulinum* from contaminated water and carry them to the hive, where they adulterate the honey. Normal processing and packaging of the honey does not kill the spores. The studies resulted in a recommendation that honey not be fed to infants under 1 year in age. Children older than that do not contract the ailment.

*Salmonella*, *Listeria*, etc. Research to protect food from other types of bacterial food poisoning has been a major project at the ERRC since the mid-1970's. One of the first concerns was the presence of *Salmonella* bacteria in poultry products—still a problem today. ERRC scientists have come up with equations for poultry processors that enable them to predict the growth of *Salmonella* at various levels of saltiness, acidity, temperature, and oxygen. The model systems have been applied to the safe processing of poultry frankfurters and other products.
This work has recently been refined and expanded in a computer program that predicts the growth in commercially processed foods of several food-poisoning pathogens. Functioning as a kind of early warning system for processors, the program can reduce by 75 percent the number of tests for food poisoning bacteria now performed by the food industry.

To predict the growth rate of various harmful bacteria, ERRC scientists know that five key pieces of information are necessary: levels of oxygen, acidity, salt, storage temperature, and sodium nitrite concentration. Using information about these five factors, which interact to control the growth of microorganisms, ERRC scientists developed equations that enable them to predict the growth of pathogens. They first predicted growth patterns in foods of two bacteria that can be found in meat and dairy products, *Salmonella* and *Listeria*. Salmonellosis is estimated to cost nearly $1 billion a year in medical expenses; listeriosis, about $250 million. The growth curves in the computer program for *Salmonella* are based on research conducted by a team of British scientists; the *Listeria* curves are the result of 700 experiments performed at the Eastern center.

The work has recently been refined and expanded in a computer program that predicts the growth in commercially processed foods of several food-poisoning pathogens that can occur in meat, dairy products, and seafood. These include *Shigella, Aeromonas, Staphylococcus, Escherichia coli 0157:H7,* and *Bacillus cereus.* So far, some 300 companies have expressed an interest in using the computer model. *Salmonella* in fresh poultry meat can be sharply reduced or eliminated by treatment with ionizing radiation at pasteurizing doses that do not lower the nutritive value of the meat in any appreciable way. The process has been approved by the Food and Drug Administration and USDA's Food Safety and Inspection Service, making it permissible for industry to use ionizing radiation to provide fresh poultry to the consumer that is free of *Salmonella.*

A different approach to reducing the incidence of food poisoning is presently being sought by another ERRC researcher. It is a bacterial "hit man" that kills *Salmonella* and other food pathogens. Three species of the rod-shaped *Bdellovibrio,* all of them parasitic bacteria, are common inhabitants of soil and water. Harmless to people, they feed on Gram-negative bacteria, a broad class of microorganisms that includes *Salmonella.* A *Bdellovibrio* bacterium can kill a *Salmonella* bacterium in half an hour. Hopefully, the bacterium can be sprayed on meat, eggs, and milk during processing and packaging to help control food-poisoning pathogens. If *Salmonella* started growing in the food, the *Bdellovibrio* could gobble them up. "Even if the idea works," comments a scientist, "it would be no substitute for adequate cooking and refrigeration to prevent food poisoning. It would simply provide extra insurance for the consumer."

In other food safety research, improved tests were developed at ERRC for detecting an agent of food poisoning, *Yersinnia enterocolitica,* that can grow at temperatures as low as 32°F. A purple dye binds to this bacterium but not to harmless ones, making it easier for industry and regulatory agencies to pinpoint virulent strains.

In another ERRC study, this time with *Listeria,* researchers found that once *L. monocytogenes* has contaminated a food processing plant, it can persist for long periods if the temperature is low enough and the bacteria are suspended in various foods. A more reassuring finding was that current methods of processing frankfurters can prevent growth of *Listeria.* Researchers demonstrated that a standard frankfurter process carried out in the ERRC smokehouse was sufficient to inactivate the *Listeria* likely to be encountered in the product prior to cooking.

***Chemical Residues.*** The USDA's responsibility for ensuring the safety of meat, poultry, and eggs requires analysis of more than 100,000 samples a year for residues of drugs and other chemicals. To assist in this task, ERRC researchers have devised new ways to speed handling of analyses and to screen samples for residues on the farm or in the plant. A robot, for example, performs a procedure that detects half a dozen drugs at once, automatically helping check for residues at levels as low as 100 parts per billion. In another instance, a simple, low-cost analysis signals analysts with a color change when a test chemical is exposed to meat extracts, milk, or urine containing certain chemical residues, including antibiotics. And a commercial test for residues, based on immuno-chemistry, was modified by ERRC chemists so that a farm or dairy worker without specialized technical training can carry it out. In this way, farmers and plant operators can make sure their products are residue-free.

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Similar investigations are conducted at the Western center, where there is a search for better ways to detect residues of a class of chemicals used as fungicides and to kill intestinal parasites in meat animals. Current methods of analysis are slow, requiring a check for one chemical at a time, and they are inconvenient for field use. WRRC scientists are developing antibodies that will react with whole groups of these chemicals in an effort to develop tests that will screen for several residues at a time.

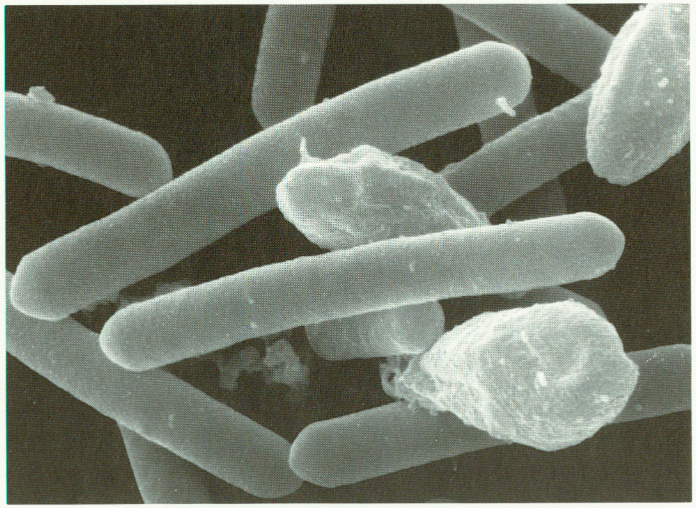
In a related area of concern with chemicals, ERRC played a major role in nitrite-nitrosamine research. (See chapter on "Meat and Meat Products," p. 75.) Researchers helped avert a ban of both nitrite and bacon advocated by consumer groups and changed the way bacon is produced by the meat processing industry. ERRC scientists developed analytical methods, elucidated the chemistry involved, studied processing techniques that affected the formation of nitrosamines, and developed means for their reduction in bacon. They also helped change relevant Federal regulations.

***Radioactive Fallout.*** Concern over the effects on U.S. food supplies of radioactive fallout reached a peak in the 1950's, with atomic testing in the United States and the Soviet Union. In the early 1960's, the Northern and Eastern centers, in cooperation with other Federal agencies, developed ways to reduce or remove radioactivity in wheat and milk. Peoria scientists found that strontium-90 can be reduced to safe levels in wheat and wheat products by washing the wheat with dilute solutions of phosphoric or citric acid. ERRC scientists were able to remove radioactive strontium from milk by passing cold raw whole milk through a column containing a strong acid ion-exchange resin. The flavor of the resin-treated milk was satisfactory. A pilot plant was subsequently built that removed both the radiostrontium and radioiodine-131. Both the NRRC and ERRC research assumed new importance following atomic plant disasters at Three-Mile Island and Chernobyl.

*James L. Smith, ERRC microbiologist, checks a growth medium for evidence of bacteria that can cause food poisoning. Using the right medium, says Smith, is essential to getting an accurate bacterial count.*

***Natural Toxins.*** When grown under adverse conditions, some varieties of potatoes contain naturally occurring compounds called glycoalkaloids that are toxic to humans. In the early 1970's, to cite one instance, potato breeders developed a new variety with excellent pest resistance—always an important goal. Shortly before it was released, several graduate students became ill after eating some of the experimental potatoes, which had been grown in a greenhouse where they worked. The variety, needless to say, was never released.

The incident underscored the need for monitoring toxic compounds in the ever-growing number of new potato varieties. ERRC scientists developed several ways to analyze potatoes for content of glycoalkaloids so that breeders can find out prior to their release if their varieties would be toxic to people as well as to insect pests and nematodes. Fortunately, the research has identified several nontoxic potato species with good resistance to such problem pests as the Colorado potato beetle. With increasing restraints on the use of pesticides, built-in resistance is essential to maintain a satisfactory level of potato production.



*Magnified food spoilage microbe* Clostridium botulinum *produces a toxin in the absence of oxygen that is often fatal to humans. The sac-like dormant spores can survive conditions that are lethal for the rod-shaped cells.*

So-called anti-nutritional factors that block or inhibit trypsin, an enzyme the body needs to digest protein, have been isolated by Northern center researchers from raw and heat-processed soy flours. Simple, inexpensive techniques have been developed at

NRRC for inactivating these inhibitors, but food processors first need to know if they are present before they can take steps to eliminate them. In 1989, Western center researchers developed tests that make use of monoclonal antibodies to enable processors to check their products for traces of enzyme inhibitors. The tests are also useful to breeders seeking crop varieties low in both types of protease inhibitors. There is also interest by medical researchers in the inhibitors found in soybeans as possible cancer-fighters.

***Food Adulterants.*** The addition of most adulterants to food, if the consumer is not informed of their presence, is not unsafe as much as it is unethical. It is also likely to be illegal. The addition of vegetable proteins like soybean extenders to hamburger, for example, may even improve its nutritional qualities, but customers nevertheless have a right to know exactly what they are getting for their money. Regulations say that additives in food must conform to labeling requirements and meet product standards.

ERRC scientists have devised several chemical and immunological tests for identifying the source and amount of protein in sausages and frankfurters. This provides USDA's Food Safety and Inspection Service, as well as State agencies, with new tools for monitoring food products for the presence of undeclared adulterants. Particularly ingenious methods of analysis were invented at ERRC to detect adulteration of honey and orange and apple juice with enzyme-converted cane and corn syrups. By the mid-1970's, significant amounts of these syrups were being added illegally to juice and honey, and traditional analytical methods were inadequate to spot them. ERRC scientists found that the two juices and honey contained characteristic ratios of two carbon isotopes. The adulterants contained higher ratios and could be detected in mixtures of juice and syrups. A second method of analysis based on chemical ratios soon followed, and both are now officially sanctioned all over the world for monitoring for adulterants.