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FRI Newsletter

FOOD RESEARCH INSTITUTE UNIVERSITY OF WISCONSIN–MADISON

Research

Use of Plasma Technology for Decontamination

Cold plasma technology can be used to treat a variety of surfaces (e.g., oxidation, functionalization) or to deposit or graft thin layer macromolecular structures onto organic and inorganic substrates without altering the bulk properties of the materials. Applications include semiconductor chip fabrication, anticorrosion coatings, and improvement of barrier properties in packaging materials. Use of cold plasma technology to modify surfaces of materials used in food processing has been an ongoing interdisciplinary collaboration between the laboratories of Frank Denes, associate professor in the Department of Biological Systems Engineering and Center for Plasma-Aided Manufacturing, and Amy Wong at the FRI. One of our objectives is to create surfaces that can inhibit bacterial attachment and biofilm formation. Toward this end, we have used cold plasma to generate anti-fouling surfaces by depositing poly(ethylene glycol) (PEG) or synthesizing PEG-like structures on stainless steel and silicone rubber surfaces, which inhibited attachment and biofilm formation by *Listeria monocytogenes*, *Pseudomonas fluorescens*, *Salmonella enterica* sv. Typhimurium, and *Staphylococcus epidermidis*. In addition, we have deposited silver nanoparticles onto rubber surfaces and shown them to be bactericidal to *L. monocytogenes*.

Plasma can also be used to decontaminate and sterilize water, air, and surfaces. The majority of research related to plasma processes has been performed in low pressure environments because of the high efficiency of these discharges.

An advancement in the field is the development of atmospheric pressure plasma reactors, which eliminates the need for vacuum installations and provides for more flexibility. Many of these installations have application limitations due to low efficiencies, which only allow the surface treatment of bi-dimensional materials (e.g. films). These limitations are abrogated by two original (patented) atmospheric pressure-plasma reactors designed and developed by Dr. Denes. These reactors provide a novel and efficient method for both batch and continuous (on-line) disinfection of surfaces in contact with food products, air, and water.

The Dense Medium Plasma reactor allows an extremely efficient disinfection of contaminated water and other fluids. We have shown that 10^5 cfu/ml of a mixture of 16 different environmental isolates inoculated in water were completely inactivated in 20 seconds. This system has been used by the Navy for disinfection of grey water generated on ships, and a variety of companies have also shown an interest in its applications.

The second plasma generator, Array Electrode Reactor (AER), is composed of an electrode configuration that assures a uniform flow of plasma gases or gas mixtures. The AER allows plasma treatment of various static or moving substrate surfaces including metals, glass, paper, and polymers (e.g. polyethylene, Teflon, rubber). Depending on plasma conditions used, we were able to inactivate 3 to 5 log *Bacillus cereus* spores deposited on stainless steel in 2.5 minutes. Even when the surface was soiled with skim milk, a 5 log reduction was obtained in 3.5 minutes. Using this system, we have been able to inactivate *L. monocytogenes* biofilms developed on stainless steel. The AER can also be used for the disinfection of air (incoming or recycled), which opens up a novel route for the development of advanced technologies for the elimination of aerosol-based contamination in food processing locations.

—F. S. Denes, E. B. Somers, S. Manolache, and A. C. L. Wong

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Research In This Issue

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Metabiologics, Inc.

The Food Research Institute has a rich history of research with *Clostridium botulinum* and its neurotoxins. Beginning with Gail Dack in the 1940s and '50s, several professors and staff at the Food Research Institute have studied *C. botulinum*, botulinum neurotoxins, and food and infant botulism, including E. M. Foster, Hiroshi Sugiyama, Bibhuti R. DasGupta, Edward J. Schantz, Eric A. Johnson and others. The FRI has accumulated and developed an array of resources including many strains of *C. botulinum* and related clostridia, methods to produce high quality toxins, strategies to assess the risk of botulism transmitted in foods, and means to control growth of *C. botulinum* in foods. As a result of this expertise and resources, a spin-off commercial company was founded by Eric Johnson and colleagues from the University of Wisconsin. Metabiologics, Inc. began botulinum research at the UW–Madison in 1985, working with the late Edward Schantz. Johnson's laboratory has successfully purified the seven known serotypes of botulinum toxin.

Metabiologics, Inc. manufactures and sells all seven serotypes and botulinum toxin for nonhuman use by accredited researchers and companies worldwide. The company also produces toxoids and antibodies, along with ELISA standards and ELISA kits for detection of botulinum toxin. Metabiologics, Inc. manufactures toxin products to the highest industry standard for purity and specific activity, and the company is committed to the development of novel, rapid and sensitive botulinal toxin detection systems. It has provided products to more than 100 researchers and companies worldwide. Metabiologics, Inc. encourages research collaborations with individual scientists and business entities.

Botulinum toxins prepared at Metabiologics are not for human use.

—Eric A. Johnson

Development of Decision-making Strategies to Identify Safe Process Cheese Product Formulations

The 1986 "Tanaka model" or "FRI model" (reported in Tanaka et al. J. Food Prot. 49:526–531) has become the standard by which FDA and many manufacturers measure process cheese product safety. This comprehensive model defines safe process cheese spread formulations using the parameters of moisture, salts and pH to inhibit growth and toxin production by *Clostridium botulinum*. Although this model is very reliable in predicting safety under the conditions in which it was run, the effects of additional factors, such as emulsifier type, antimicrobials, or fat, sodium, or cheese solids reduction, are not considered. Hence, additional challenge studies and strategies are required to predict safety of nonstandard products.

One objective of this project funded by Dairy Management, Inc. was to determine the effect of substituting cheese with other dairy solids on the botulinal safety of nonstandard process cheese products. Over 75 formulations were manufactured using five levels of Cheddar Cheese (0, 15, 25, 35, 45%), three levels of moisture (55, 60, 65%), three pH levels (5.5, 5.75, 6.0) and three levels of total salts (NaCl plus disodium phosphate emulsifier; 3, 4, 5%). Cheese solids were substituted with a mixture of dry dairy ingredients (whey, whey protein concentrate, and nonfat dry milk) and fat levels standardized to ~20% with anhydrous milkfat. Formulated products were inoculated with 3-log *Clostridium botulinum* spores per g and assayed for botulinal toxin at designated sampling points during 6 months' incubation at 30C.

Data revealed that products formulated with all dry dairy ingredients (0% cheese) delayed botulinal toxin production and tended to have slightly lower water activity, compared with products manufactured with 25 to 45% cheese. Furthermore, products formulated with all dry dairy ingredients required more acid to achieve any given pH, implying greater buffering capacity associated with these formulations. These results suggest that substitution of cheese solids with whey, whey protein concentrate, or nonfat dry milk does not negatively affect the botulinal safety of model process cheese products compared with standard process cheese formulations.

In a separate phase of this project headed by Professor John Norback in the Food Science Department, data generated from this study, as well as from other process cheese safety studies completed at FRI, are being used to develop novel decision-making strategies for formulation adjustments. The logistic regression predictive models, such as used by Tanaka et al., are inadequate when considering the large number of formulation factors in non-standard process cheese products. Therefore, machine learning approaches are being developed to work concurrently with and supplement the statistical models in making reliable toxin production predictions.

Machine learning methods tested include Bayesian networks, decision tree, support vector machine and artificial neural networks. Bayesian network is used to estimate probabilities of botulinal toxin production and to compare with logistic regression outcomes. The decision tree approach evaluates formulation factors and provides a reasoning sequence for using their antibotulinal properties (e.g. *if water activity* $\leq$*0.945 go to Tier A, if* $\geq$*0.945 go to B*). Artificial neural networks and support vector machine each use approaches to separate potentially hazardous formulations from safe formulations. Using a combination of these methods, a user-friendly interface is being developed to discriminate safe environmental conditions from potentially hazardous conditions, and to predict the response by *Clostridium botulinum* in various environments. Rigorous testing of this program is required to verify its accuracy in predicting the safety of non-standard process cheese formulations before it will be made available for use by trained microbiologists and product developers.

—Kathy Glass, Wei Zhang, John Norback, and Eric Johnson

Upcoming Meetings

Immunology of Bovine Lactoferrin

Lactoferrin (Lf) is present in various bodily fluids and exhibits diverse biological activities that impact on host resistance and immunity. Lf binds to macrophages, eosinophils and all major lymphocyte subsets, suggesting possible functions in both innate and acquired immunity. Lf is known to interact with accessory molecules involved in the Toll-like receptor 4 (TLR4) pathway, including CD14 and LPS Binding Protein, suggesting that Lf may activate components of the TLR4 pathway.

The Mansfield lab in the Department of Bacteriology asked whether bovine Lf (bLf)-induced alterations in macrophage activation are TLR4-dependent. Both bLf and LPS stimulated IL–6 production and CD40 expression in the RAW 264.7 macrophage cell line and in BALB/cJ peritoneal exudate macrophages. However, in congenic BALB/cTLR4B/B macrophages and in C3H/HeJ macrophages with a TLR4 mutation, CD40 was not expressed while IL–6 secretion was increased relative to wild-type cells.

The signaling components NR-ÍB, p38, ERK and JNK were activated in RAW 264.7 cells and BALB/cJ macrophages after bLf or LPS stimulation, demonstrating that the TLR4-dependent bLf activation pathway utilizes signaling components common to LPS activation. In TLR4 deficient macrophages, bLf induced activation of NF-ÍB, p38, ERK and JNK whereas LPS-induced cell signaling was absent. The investigators conclude that bLf induces limited and defined macrophage activation and cell signaling events via TLR4-dependent and -independent mechanisms. bLf-induced CD40 expression was TLR4-dependent, whereas bLf-induced IL–6 secretion was TLR4-independent, indicating potentially separate pathways for bLF medicated macrophage activation events in innate immunity.

These studies were supported by an FRI grant, leading to a publication and a provisionally accepted patent application by WARF.

—John M. Mansfield

FRI Focus on Food Safety: Development and Production of Safe Process Cheese Formulations

With the April 26, 2005, meeting on "Development and Production of Safe Process Cheese Formulations," FRI launched its new *Focus on Food Safety* meeting series with in-depth presentations on specific topics of interest to food producers and processors. Speakers from FRI, other university departments, industry and government discussed pathogens of concern and practical and regulatory considerations for producing safe process cheese products. Over 60 persons, representing 27 companies, attended the meeting.

Pathogens of concern. Spore-forming bacteria (*Clostridium botulinum, C. perfringens, Bacillus cereus*) and fungi are resistant to heat inactivation, and toxins produced by these bacteria and spoilage caused by fungi are a concern for process cheese manufacturers. Preservation procedures, such as acidification, lowering water activity, refrigeration, and use of antimicrobials and sanitizers may all be used to ensure safety of process cheese.

In addition to pathogens that may survive heat treatment during process cheese manufacture, post-process contamination with some vegetative pathogens, such as *Staphylococcus aureus* and *Listeria monocytogenes*, must also be prevented or their growth inhibited. *S. aureus* can grow at a lower water activity than many other bacteria, and the toxin it produces is not destroyed by pasteurization. *L. monocytogenes* is ubiquitous in the environment and is infamous for growing at refrigeration temperatures.

Intervention strategies. Microbiological safety of process cheese and related foods has traditionally relied on heat to destroy vegetative pathogens, formulation to prevent growth of surviving heat resistant spore formers, and refrigeration to prevent growth of recontaminating bacteria. A predictive model developed by FRI defines safe formulations for standard process cheese products in terms of moisture, total salts, and pH. Recent trends in product development include non-standard process cheese products that contain higher levels of fat, particulate ingredients (such as vegetables), or dairy and non-dairy solids instead of cheese. Some new products are designed for storage at ambient temperatures rather than under refrigeration. These new products may be more susceptible to pathogen growth and toxin production. Each formulation and processing change must be evaluated to determine its effect on product safety.

Monitoring of cook times and temperatures, and analytical methods to determine purity and concentration of ingredients are essential parts of a HACCP program for process cheese products. Proper sanitary design of equipment and processes are also key components for producing safe products.

Regulations. Sanitary equipment design in the dairy industry, extended runs, record keeping and corrective actions were discussed by a representative of the Wisconsin Dept. of Agriculture, Trade and Consumer Protection. An FDA representative described electronic filing of reports by acidified and low acid food processors.

Our next *Focus on Food Safety* meeting will be held in September 2005 and present current information on "Strategies to Enhance Food Safety Using Antimicrobials and Sanitizers."

—M. Ellin Doyle, Ph.D.

Guangyun Lin, Ph.D.,
Assistant Scientist

I joined Dr. Eric Johnson's lab on April 1 this year as an assistant scientist. I will work on a few projects related to *Clostridium botulinum*, including micro sensors for the detection of botulinum neurotoxin (BoNT), development of vaccine against BoNT and HA antibody of *Clostridium botulinum*, in collaboration with Professor Johnson's lab and other research groups.

Before I moved to Madison to join my husband, I worked with Invitrogen Corporation in San Diego, California, as a scientist for three years to develop Gateway adapted baculovirus expression systems. Before that, I spent almost four years as a postdoctoral research associate with Boyce Thompson Institute at Cornell University in Ithaca, New York. In those four years, I focused on the function of baculovirus late transcription factors and the generation of the stable cell lines expression baculovirus inhibitor of apoptosis (programmed cell death).

I came to the United States after I obtained my Ph.D. in molecular biology from Zhongshan University, P. R. China. My projects involved molecular biology research on baculovirus mutant and the expression of gold fish hormone using the baculovirus expression system. I also obtained my Masters degree from Zhongshan University, working on baculovirus. My undergraduate major was biochemistry. During the time I was in China, I worked as an assistant professor in Zhongshan University for three years.

I am married with two kids, one boy and one baby girl. Besides work, I like to play tennis, sing and travel. I also like to watch sports and *Seinfeld*. After I moved to Madison, I started to like gardening.

Colin Clancy, B.S.,
Associate Research Specialist

I completed my coursework in August of 2004 and received my Bachelor of Science degrees in Medical Microbiology and Immunology, and in Biology, from UW–Madison. I originally joined the Food Research Institute as a student hourly under Dr. Kathy Glass in the summer of 2003. While there I honed my microbiological skills, assisting in challenge studies involving *Clostridium botulinum*, *Escherichia coli*, *Listeria*, *Bacillus*, *Staphylococcus*, and other organisms in a variety of foods including process cheese, bratwurst, milk, and wiener slurry (sounds good, doesn't it?). In September of 2004, I defected to Dr. Eric Johnson's laboratory as an Associate Research Specialist.

My work includes cloning and expression and purification of a recombinant non-toxic form of the *C. botulinum* neurotoxin from an *E. coli* system as a possible vaccine candidate. This protein has undergone mutations at two amino acid sites to render it non-toxic. The plasmid was then isolated, cloned and transformed into *E. coli* cells. I plan to attend graduate school, hopefully in the fall of 2006, though my reluctance to study for and take the GRE may delay those plans.

I was born in Saudi Arabia and raised in the north suburbs of Chicago before joining the UW in 1999. My family includes two wonderful parents along with two sisters and two brothers-in-law. Being from Chicago, I'm a rabid fan of the Bears and Cubs, which has forced me to endure constant ridicule from Packer fans given their domination of the rivalry in recent years. My hobbies include watching various sports, including baseball, football, Aussie Rules Football, rugby, and Formula 1 racing, along with playing sports, including disc golf. I also enjoy reading novels and European and Asian history books.

Carlos Echavarri-Erasun,
Ph.D. candidate

It may seem funny that I was asked to write my bio for the FRI newsletter now that I'm so close to completing my Ph.D. degree and leaving the FRI. However, the timing couldn't be more perfect since I can use this opportunity to pay tribute to the Food Research Institute's family and especially to Professor Eric Johnson and his team.

I was born in Santander, a midsize town in North Spain in a very close family. The oldest of three brothers and a sister, I can assure you that I had a good time while growing up. It was probably all the time I spent on the ocean and outdoors that motivated me to pursue a B.S. degree in Biology at the University of

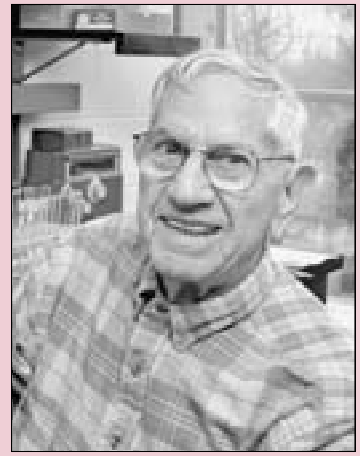
Navarra (Pamplona) where I did, academically speaking, quite well, avoided the bulls on the streets nicely, and excelled when I qualified to the National Olympic Sailing team in the laser class. However, academics and sports were way too much for me, and a couple of years later I had to quit the national team to focus on my studies. Shortly after, my grades benefitted.

Once graduated, I worked in a clinical microbiology lab at the Hospital Montecelo in Pontevedra where I got involved in a *Streptococcus pyogenes*-hemolytic group A antibiotic resistance project that hooked me into research. At this stage, it was clear to me that continuing my academic preparation was a must. In searching the microbiology programs of U.S. academic institutions, the UW–Madison was repeatedly mentioned. I came to Madison in the summer of 1997 to pursue a M.S. degree in the UW–Madison Bacteriology Department. Soon after, I joined Professor Eric A. Johnson's laboratory where I continued the project regarding astaxanthin biosynthesis in the yeast *Xanthophyllomyces dendrorhous*. Hopefully at the time you're reading this my Ph.D. defense is past and my dissertation rests in the library.

Over the years I have learned to appreciate Madison. I like the diversity of its people, the outstanding academics, the city and also the little things like enjoying sailing on Lake Mendota, no matter if in a nice warm breeze while drinking a beer or in a chilly wind in an iceboat while spilling hot coffee with brandy all over (we call it defroster). It is a charming city, in many regards similar to my own hometown. I had some glorious moments around here that I won't forget, like having fun with my Hoofers friends, some unexpected lab results, the old 119 lab crowd and so on. Not even the distance to my loved ones was really a problem. I won't forget watching my brother entering the Athens Olympic stadium on TV last summer. I have never been so proud. Also, Eric's staff has been a pleasure to work with, and I will miss them sorely, including Eric's "problematic" computers. My plan for the future is to join Professor Jack Fell at the University of Miami as a postdoc in a project to develop rapid techniques to identify pathogens in recreational waters. You can be assured I will show up in Madison again. Hopefully soon. Cheers.

Edward J. Schantz

August 27, 1908 – April 28, 2005



Edward J. Schantz, biochemist and emeritus professor of food microbiology and toxicology at the University of Wisconsin–Madison, died April 28, 2005 in Madison. He was 96 years old.

Schantz was born Aug. 27, 1908 in Hartford, Wis. and grew up on a dairy farm near Sparta, Wis. He earned a bachelor's degree in biochemistry from the UW–Madison, master's degree in animal chemistry from Iowa State University, and a doctorate in biochemistry from the UW–Madison.

Schantz served in the Army in World War II and remained as an Army reserve officer for more than 20 years. From 1946 to 1972 he worked as a biochemist for the U.S. government. Schantz headed the chemistry department at the Biological Research Center, Fort Detrick, Md., and lectured in chemistry and biochemistry in the University of Maryland's extended program for Department of Defense personnel. During his tenure at Fort Detrick he developed methods for the isolation and purification of various toxins including botulinum toxin, staphylococcal enterotoxin, and paralytic shellfish poison.

In the 1960s Schantz was contacted by Dr. Alan Scott, a surgeon specializing in strabismus (commonly known as cross-eye), who was seeking a compound that could weaken overactive muscles pulling the eye out of alignment. Ed provided Scott with preparations of botulinum toxin, and it was demonstrated that this toxin could correct the induced strabismus in monkeys. After more than 10 years of animal research, Scott received approval by the National Institutes of Health to use Schantz's toxin in humans.

In 1972 Schantz joined the faculty at the UW–Madison, where he continued his research at the FRI as an emeritus professor through the 1990s. Schantz's work focused on isolation, purification and characterization of foodborne microbial toxins, including those produced by *Clostridium botulinum*, the enterotoxins produced by *Staphylococcus aureus* and *Bacillus cereus*, and the toxins produced by marine "red tide" microorganisms that cause shellfish to become poisonous. Although Ed was well recognized for the study of various foodborne toxins, he focused his studies in the 1980s and 1990s on botulinum neurotoxin.

In 1985, Eric Johnson joined the faculty and worked closely with Schantz in producing toxin for treatment of human disorders. They together produced several batches of toxin that were used for injection into humans for a variety of neuromuscular disorders. The Food and Drug Administration approved botulinum toxin in 1989 for the treatment of strabismus, blepharospasm, and hemifacial spasm. In the 1980s until the present, the utility of botulinum toxin as a therapeutic has increased dramatically, and is being used for treatment of dozens of maladies including dystonias, pain syndromes, tremors, and several other disorders.

Schantz is survived by his children, Mary (Frank) Krueger of Jay, N.Y.; Edward (Sue) Jr. of Champaign, Ill.; Katharine Fleisner of Pittsburgh, Pa.; Elizabeth (John) Burmeister of Madison; and Robert of Bend, Ore.; six grandchildren and two great-grandchildren; his sister Jane; and many nieces and nephews. He was preceded in death in 1998 by his wife of 58 years, Katharine Lee, and a daughter, Mary Jane.

A private funeral service was held May 3, 2005.

World Literature

Cycads and Cyanobacteria

Shortly after World War II, the incidence of neurological diseases (ALS-PDC) peaked among the native Chamorro people on Guam; today these diseases are rare. Initial hypotheses suggested numerous environmental factors including items present in or lacking in the local diet, as possible causes of ALS-PDC. One suspicious dietary item, used on Guam but in few other places, was flour made from cycad seeds. These seeds contain a neurotoxic amino acid, beta-methylamino l-alanine (BMAA), and a carcinogenic glycoside, cycasin. However, these compounds are washed out of the seeds by traditional methods of preparation. People would have to eat massive amounts of cycad flour on a daily basis to ingest a toxic dose. Two years ago an intriguing series of articles proposed that cycad toxins are indeed the cause of ALS-PDC but not when consumed directly in cycad flour. It was hypothesized that flying foxes (large fruit bats) eat cycad fruit-like structures and concentrate cycad toxins in their bodies. Analyses of flying fox skin revealed concentrations of 1287–7502 µg BMAA/g as compared to concentrations of 1–18 µg/g in cycad flour. Humans would then ingest toxic doses of BMAA when they consumed the flying foxes.

Now a new chapter in this story is emerging. Cycads are ancient plants that are now endangered in all their natural habitats. Many species can grow on relatively poor soils because of their association with cyanobacteria of the genus *Nostoc* that can fix atmospheric nitrogen to a form usable by plants. These bacteria live in special cycad roots, coralloid roots, that project above the surface of the soil. In addition to fixing nitrogen and carrying on photosynthesis, *Nostoc* provides its cycad partner with an antiherbivore compound, BMAA.

HPLC analyses of different tissues from cycads to quantify the presence of BMAA and of glutamic acid demonstrated that glutamic acid concentrations were distributed randomly in various tissues while BMAA was concentrated primarily in cycad reproductive organs, including the sarcotesta (fruit-like structure surrounding the seed).

BMAA occurs as a free amino acid, and also binds to proteins in *Nostoc*. This is also true in cycad tissues and brain tissues of Chamorro victims of ALSD-PDC. Since BMAA is polar and non-lipophilic, it would not be stored and biomagnified in fat as is the case with some other toxic compounds. But it may accumulate in a protein-bound form and serve as a neurotoxic reservoir. As proteins in the body are metabolized over time, BMAA may be released and damage the nervous system over several years. This may explain the apparent latency between consumption of the neurotoxin and the expression of symptoms of ALS-PDC.

BMAA is also produced by many other species of cyanobacteria from fresh water, marine and soil environments, and was detected in brain tissue of two Canadians who died of a progressive neurodegenerative disease (not ALS-PDC). It has been suggested that people may be exposed to this neurotoxic amino acid in other ways than by eating cycads and flying foxes, and that BMAA may play a role in other neurodegenerative diseases.

Cyanobacteria produce many toxins; these include hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritants. The March 15, 2005 issue of *Toxicology and Applied Pharmacology* included several review articles on risk management and health effects of these toxins, microcystins in drinking water and supplements, and hazards of cyanobacterial blooms. Cyanobacteria are often overlooked but are widely dispersed in fresh water and marine habitats and may have significant effects on human and animal health.

—M. Ellin Doyle

Raphides C-A New Cause of Foodborne Disease

Multiple cases of oral burning and facial edema occurred suddenly among employees eating at a cafeteria in a Chicago in 2003. One person experienced severe symptoms of facial and oral swelling, including possible airway obstruction, and was admitted to an intensive care unit. Another person was treated at an emergency room. Most frequently reported symptoms were stinging, burning, swelling, pain, numbness in the mouth and increased salivation. There were no reports of gastrointestinal symptoms, fever, or numbness in arms or legs. Several cases reported that symptoms persisted for several days to two weeks.

The rapid onset of illness pointed to a toxic substance rather than bacteria as the cause, and the lack of systemic symptoms (fever, numbness in the extremities) indicated that bacterial and seafood toxins were not the culprit. Epidemiological investigations indicated an entree called "Chinese braised vegetables." In fact, one subject had cut an ingredient from this dish in half before eating it and after experiencing symptoms upon consuming the first half, he brought the other half to the cafeteria staff. The piece of food appeared to be a mushroom and this dish contained straw mushrooms and cloud ear mushrooms. Microscopic examination of this piece of food revealed the presence of pieces of plants that differed from structures of fungi. Calcium oxalate crystals were also observed in this food.

Needle-like, insoluble calcium oxalate crystals, known as raphides, are naturally present in a number of fruits and vegetables, including taro, kiwi, pineapple, spinach, figs, and persimmons (presumably they evolved to deter plant predators). There have been previous isolated reports of mechanical irritation caused by some of these foods but it appears that the number of raphides is generally small enough that most people do not experience a noticeable reaction to them. However, some people have reported a tickling or irritative sensation in the throat after eating kiwi. Microscopic examination of kiwifruits demonstrated the presence of long raphide crystals packed into tight bundles around the seeds.

Soluble oxalic acid and oxalates may contribute to the formation of kidney stones and are well known for their antinutritional effects caused by binding calcium and copper and preventing absorption of these minerals from the intestinal tract. Insoluble calcium oxalate, in the form of long raphide crystals pose a different hazard—mechanical irritation of the oral cavity when foods containing high concentrations of these crystals are ingested.

The source of the raphides in the Chicago outbreak is unknown. Mushrooms would not normally contain these crystals, but it has been hypothesized that some plant material containing raphides may have been inadvertently included in the package of cloud ear fungus. Another proposed hypothesis suggested that this may have been a case of intentional contamination.

—*M. Ellin Doyle*

Publications

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Presentations

Kathy Glass, Ph.D.
Invited speaker: "Controlling *Listeria* in Ready-to-Eat Foods," presented at the *Missouri Milk, Food and Environmental Health Association Annual Educational Conference*, Columbia, MO, April 7, 2005.

Annual progress report FDA-CFSAN Extramural Food Safety Research Program on the *Heat Treatment of Bacterial Spores in Dairy Products*, sponsored by FDA-CFSAN, College Park, MD, May 10, 2005.

Workshop speaker: "Potential Dairy Plant Microbiological Hazards" at the *Dairy HACCP Workshop*, sponsored by Wisconsin Center for Dairy Research, Madison, WI, May 11, 2005.

Invited Speaker: "Emerging Pathogens 101: What Lurks Around the Corner," presented at the *British Columbia Food Protection Association Speakers Evening*, Burnaby, British Columbia, May 18, 2005.

Professor **Amy Wong** spoke on "Post-process Contamination by Vegetative Pathogens" at the FRI Focus on Food Safety Series meeting *Development and Production of Safe Process Cheese Formulations*. April 26, 2005.

Professor **Jaehyuk Yu** was invited to give two talks at the *23rd Fungal Genetics Conference*, March 2005, Pacific Grove, Ca. His talks: "Growth and Development Mutants of *Aspergillus fumigatus*: Comparative Forward Genetics" and "Roles of G Protein Coupled Receptors (GPCRs), G Proteins and a Protein Kinase A in *Aspergillus nidulans*."

Food Research Institute
University of Wisconsin–Madison
1925 Willow Drive
Madison, WI 53706-1187

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Short Subjects

A FRESH Start!

Ann Larson and Jaehyuk Yu

The FRI is pleased to announce the start of a new seminar series named **FRESH** (**Food Research and Education Seminar Highlight**) starting this August. The seminar series will be held at 11:30 AM ~ 12:30 PM on Wednesdays twice a month. The series will include a wide variety of most up-to-date topics related to food safety/microbiology, including recent world-class research being conducted by FRI researchers and affiliate faculty.

FRESH is developed in accordance with the FRI mission: Maintain a leadership role by identifying and addressing food safety issues to meet community, industry, and government needs; interact with regulators, academia, industry and consumers on food safety issues and provide accurate information and expertise; facilitate and coordinate food safety research at the University of Wisconsin–Madison; deliver quality education and training in food safety.

The potential speakers include FRI faculty and affiliate faculty, students and alumni, as well as representatives from government, academic institutions and the food industry.

The FRESH committee is represented by two members from each laboratory of the five FRI core faculty and one from the Applied Food Lab, as well as Jean Johnson, Academic Department Supervisor. Professor Jaehyuk Yu and Ann Larson are leading the team. The titles and speakers will be posted on the FRI Web site (http://www.wisc.edu/fri/index.html) and in *Wisconsin Week*. The FRESH committee welcomes your input and ideas for the constant improvement of the seminar series. We also welcome volunteers and donations for refreshments.

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www.wisc.edu/fri/

Director FRI: Professor M.W. Pariza

Editor: Edwin Traisman

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