Food Science

Summer Scholar Program 2014

Research Abstracts

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FOREWORD

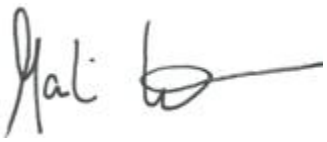
This booklet of abstracts provides a summary of the research conducted by the eight 2014 Food Science Summer Scholars. These students belong to the fifteenth class of the Summer Scholar Program and were a tremendous inspiration to us all. These research abstracts represent the results of ten weeks of hard work under the mentorship of faculty members at Cornell University.

The scholars had great opportunities to explore the world of food science through visits to Pepsi Cola Company in Valhalla, NY, Kraft Foods in Tarrytown, NY, the Culinary Institute of American in Hyde Park, NY and the Institute of Food Technologists (IFT) annual meeting held in New Orleans, LA. Scholars also had a chance to discuss ethics in food science, learning from research, applying for graduate school, preparing an abstract, and to hear about career opportunities in food science.

We acknowledge with gratitude the support and guidance from many people, including individuals who work hard to help fund the program including Marlene Quijano, Lisa Tirino, Marilyn Konopka as well as the students' faculty mentors, graduate students, and the administrative support provided by Andrea Elmore, Marin Cherry, and Louise Felker. A special thanks goes to Kristin Alongi who conducted mock interviews and a resume workshop.

Special recognition goes to Kirk Kealey for organizing the tour of the Pepsi R&D Center, Alexandra Bishop, Patricia Catauro and Katherine Meyers for organizing the tour of the Kraft Foods R&D Center and to Chris Loss for leading a discussion during the scholars visit to the Culinary Institute of America. We would also like to give special recognition to Carine, Arnold Nathan and Michael Feist and Gina Ravosa for hosting a wonderful dinner and guest speakers for the scholars. We thank Laura Pensiero and Lisa O'Sullivan for presenting at the dinner as well as Michael Inglis and Matthew and Delia Marks for attending the dinner and sharing experiences with the scholars.

It has been a pleasure to work with this year's Summer Scholars. We hope this program has been successful in conveying the excitement and opportunities associated with academic research in food science. We hope that all participants will go back with valuable new experiences and that some or all of them will move onto a career or graduate education in the field of food science and technology.



Martin Wiedmann
Professor
Program Co-Director

Carmen Moraru
Associate Professor
Program Co-Director

Janette Robbins
Program Coordinator

Scholar: Naa Ayikarkor Ankrah

University: Mount Holyoke College

Major: Chemistry

Faculty Mentor: Dr. Elad Tako

Assessing the effect of soybean isoflavones (daidzein and metabolites) on the intestinal brush border membrane functionality and beneficial bacteria populations.

Soybeans have been identified to have a high concentration of daidzein, an estrogen-like compound that has been shown to modulate menopausal symptoms and the risks of developing cancer. The gut bacteria have been shown to play an important role in transforming daidzein to its active metabolites, however there is a paucity of information in identifying specific interactions between the intestinal absorptive surface and daidzein metabolites. Our objectives were to assess the effects of various concentrations of daidzein on both the relative abundance of beneficial intestinal bacterial populations and brush border membrane functionality.

Cornish-cross fertile broiler eggs were obtained (n=100). On day 17 of incubation, eggs containing viable embryos were weighed and randomly divided into 5 groups (n=10). 1mL of a daidzein solution was injected into the amniotic fluid in concentrations of 0.5mg/mL, 2.5mg/mL and 5.0mg/mL. Two control groups: saline injected and non-injected were utilized to control for the effects of injection. Upon hatch, birds were euthanized, blood samples were collected for hemoglobin concentration, and tissues were harvested for liver ferritin and gene expression. Data were analyzed for significance among treatment groups and between both controls by a MANOVA test using the JMP software. Significance was determined when $P<0.05$.

Gene expression analysis, with results relative to 18S rRNA yielded significantly higher expressions of aminopeptidase (AP), Na+K+ATPase, DcytB, DMT-1, ferroportin and IL-6 ($P<0.05$). There were however, no significant differences in the population of beneficial bacteria among treatment groups and between both controls.

This preliminary study illustrates the stimulatory properties of daidzein on the functionality of the brush border membrane through increases in AP and ATPase expression. In addition, higher expressions of DcytB, DMT-1 and ferroportin demonstrate a potential to improve Fe status. Future studies are now warranted to assess the systemic effects of daidzein in vivo.

Scholar: Madeleine Bee

University: American University

Major: Chemistry

Faculty Mentor: Professor Gavin Sacks

Determining volatile acidity in wines through acetic acid gas detection tubes

Volatile acidity refers to the steam-distillable, water-soluble, short chain organic acids found in wine and other alcoholic beverages. In wine, acetic acid makes up over 95% of these acids, although formic, butyric, and propionic acids also fall into this category. Volatile acids enhance the flavor of fixed acids and tannins in wine, but can also produce an overpowering vinegar aroma and indicate spoilage by acetic acid producing microbes. As a result, volatile acidity is one of the few chemical properties of wine that is subject to legal regulation, with limits of 1.2 g/L in white and 1.4 g/L in red wines in the United States. Traditionally, a Cash still is used for steam distillation of the volatile acids, and acidity is then determined by titration. Neither accurate nor reproducible, the Cash still equipment is expensive, the procedure is time consuming, and it requires a skilled operator. Modern analytical methods such as HPLC, GC, and enzymatic methods are inaccessible for many modestly equipped wineries. Therefore, there is a need for an easily operated, inexpensive, and reproducible method for volatile acidity detection.

Colorimetric gas detection tubes were originally developed for the mining industry, and have successfully been adapted to measurement of SO2 and H2S in wines. A modified method was developed for acetic acid measurement. The setup is simple: a sample is pulled into a syringe, which is pulled further to create 100 mL of headspace. The tip of the syringe is fitted with a Luer two-way valve and connected to a gas detection tube. The system is closed and the headspace and liquid are allowed to equilibrate. Following equilibration, the valve is opened and the headspace expelled through the detection tube. The resulting color change on the gas detection tube is proportional to the acetic acid headspace concentration, which is in turn correlated to the acetic acid concentration in wine via Henry's coefficient.

Headspace-liquid equilibration could not be reached even after 24 hours of static conditions or 15 minutes of manual agitation, while agitation by stir plate achieved equilibrium within 5 minutes. Allowing for rest time between agitation and gas measurement resulted in a 5% higher relative standard deviation and a lower apparent headspace concentration of 0.36 uL/L, on average, compared to measurements taken without rest time. These results suggest that the plastic syringes had adsorbed/absorbed acetic acid while the glass syringes did not. To prevent this, plastic syringes were left overnight with 1% acetic acid solution and 100 mL headspace, to allow the acetic acid gas to equilibrate with the plastic before measurement. This lowered the relative standard deviation from 55% in plastic and 50% in glass to 40%, and maintained similar average values so precision was not lost.

The results observed so far are promising, though there are still several directions to pursue to ensure reliability of this method. To improve sensitivity, gentle heating methods (~40˚C), larger headspace volume, and addition of salt are several ways to potentially shift the equilibrium toward gaseous acetic acid to establish both an accurate and precise detection method. If successful, measuring headspace acetic acid content by gas detection tubes could replace the traditional Cash still method as an easier, cheaper, and more sensitive technique.

Scholar: Avery Becker

University: Ithaca College

Major: Biochemistry

Faculty Mentor: Professor Martin Wiedmann

Assessing antibiotic susceptibility of *L. monocytogenes* directed by alternative σ factor regulation

Listeria monocytogenes is a Gram-positive, foodborne bacterium that is ubiquitous in the environment and the causative agent of listeriosis. This illness most commonly affects persons with compromised immune systems resulting in meningitis, encephalitis, as well as septicemia, while causing stillbirths or life-threatening infections of newborns for pregnant women. It has been estimated that 1600 cases of listeriosis occur annually within the United States, leading to 400 to 500 deaths [1]. Reasons for *L. monocytogenes* successful virulence include its ability to survive a wide range of temperatures, the presence or absence of oxygen, as well as numerous levels of osmotic or pH stress. An important mechanism for regulating the transcription of appropriate genes in response to dynamic environments is *L. monocytogenes's* alternative sigma (σ) factor network. These four factors- σB, σC, σH, and σL- can each associate with the core RNA polymerase, allowing for the enzyme's recognition of specific DNA sequences and thus providing a regulated response [2]. Understanding how this organism responds to and survives hardship is critical to providing solutions for both treatment and prevention in the food supply as well as clinical settings.

To allow for the study of a single σ factor's ability to regulate responses, strains of *L. monocytogenes* were previously constructed with triple σ factor deletions. Along with the four triple mutants, a single quadruple mutant was created as a control. The general experimentation this summer involved the exposure of the five mutant strains as well as the wildtype strain to a variety of stresses to assess differences in susceptibility. The hypothesis is that the five mutant strains and the wildtype will each respond differently to various antibiotic agents as a result of changes in the organism’s regulatory network.

The primary method for comparing the strains was completed via disk diffusion assay. Brain-heart infusion agar plates were inoculated with the six *L. monocytogenes* strains to produce bacterial lawns. Filter disks loaded with antibacterial agents were set onto the surface of the plates. The antibiotics used were selected based on their class of action. These include: membrane affecting agents- Nisin, Bile salts, Polymyxin B, Bacitracin, and detergents including Triton, SDS, and Tween; cell wall affecting agents- Lysozyme, Fosfomycin, Penicillin G, and Ampicillin; protein synthesis affecting agents- Erthromycin and Tetracycline; as well as the DNA replication affecting agent- Ciprofloxacin. After a night of incubation, approximately 14-18 hours, the circular zones of growth inhibition were measured and compared across the strains.

The results thus far have shown a consistent trend of the quadruple deletion mutant being most susceptible. This fits with the current understanding that this strain lacks sophisticated regulatory mechanisms. Also, certain strains did interact with particular stresses differently and will be addressed with future, rigorous investigation. The majority of the agents did not show drastically different zones for the strains which has been speculatively attributed to the diffusion properties of the antibacterial agent being predominant rather than each strain’s particular susceptibility. Further, the agents used are incredibly potent and were all set at 100mg/ml concentrations so it may have been unlikely that we could observe differences between the strains.

Scholar: Jie Cheng

University: Bryn Mawr College

Major: Chemistry

Faculty Mentor: Professor Martin Wiedmann

Identifying the cold growth of Paenibacillus and Bacillus in milk

Nearly 20% of fluid milk, purchased in the United States every year, may be discarded before consumption partly due to microbial spoilage, which limits the extending of the shelf-life of pasteurized diary products[1]. Even though the majority of bacteria in milk become inactive after pasteurization or stop growth under the refrigeration temperature, some psychrotolerant spore-forming bacteria can still survive and propagate. Two bacteria species, Bacillus and Paenibacillus, have been identified as the prominent genera of milk spoilage bacteria in dairy farm environments, processing facilities, and pasteurized milk[2]. Both Bacillus and Paenibacillus are psychrotolerant, Gram-positive, endospore-forming bacteria

The research is aimed to characterize the cold growth of representative Bacillus spp. and Paenibacillus spp. strains isolated from pasteurized milk, to observe their cold growth, and to understand how they affect the shelf-life of the pasteurized milk. The result will provide a better understanding of the cold growth of Bacillus and Paenibacillus subtypes and facilitate the development of approaches to reduce food microbial spoilage by psychrotolerant spore-forming bacteria.

Representative isolates were selected from a large collection of dairy-associated spoilage bacteria isolates. One hundred isolates were initially picked out to represent different genera of dairy associated bacteria, majorly Bacillus and Paenibacillus, and to represent the predominant and most common rpoB allelic types. 4 Paenibacillus isolates, 3 Bacillus isolates, and 8 other milk spoilage bacteria isolates were firstly selected to assess cold growth. A single colony for each selected isolate was inoculated into BHI broth and grown for 18-24 h at 32°C, followed by serial dilution and inoculation into skim milk broth to reach a final inoculum level of 100 CFU/ml. The inoculated skim milk broth were stored at 6°C and samples were taken on days 0, 3, 7, 10, 14, 17, 21. Bacterial numbers were counted after spiral plating in appropriate dilutions on standard plate count agar and cultivation for 24 h at 32°C.

The preliminary result so far is that the 4 Paenibacillus isolates grow well as they generally increase by102.5 within 10 days. Regarding the research data, relatively Paenibacillus grow better than the 3 Bacillus isolates. This is consistent with the general understanding of the Paenibacillus and Bacillus and future studies and analysis will be conducted by involving more representative isolates.

Reference:

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2. Ivy, R.A., Ranieri, M.L., Martin, N.H., den Bakker, H. C., Xavier, B. M., Wiedmann, M., & Boor, K. J. 2012. Identification and Characterization of Psychrotolerant Sporeformers Associated with Fluid Milk Protection and Processing. Applied and environmental microbiology, 78(6), 1853-1864.

Scholar: Luis Fernando Paulatti Marostegan

University: University of Sao Paulo at Pirassununga

Major: Food Science

Faculty Mentor: Professor Carmen Moraru

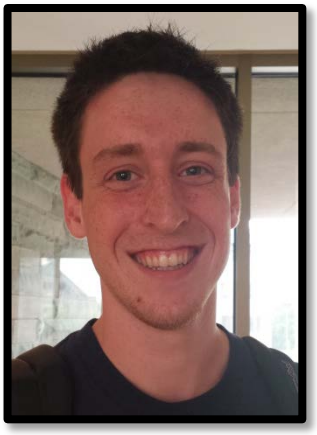
Effectiveness of Pulsed Light treatment combined with antimicrobials nisin and natamycin on the inactivation of *Listeria innocua* on cheese surface

The majority of foodborne outbreaks associated with cheese made from pasteurized milk involve *L. monocytogenes*, a psychrotolerant microorganism ubiquitous in the environment that can be introduced during slicing, cutting, packaging and handling. It was demonstrated recently that Pulsed light (PL) treatment, consisting of short, high intensity pulses of broad-spectrum light, can inactivate vegetative bacteria on cheese surface. This study examined whether the effectiveness of PL treatment against *L. innocua*, a surrogate for *L. monocytogenes*, can be enhanced when combined with antimicrobials nisin and natamycin. The effects of cheese type and order of treatments were investigated.

L. innocua FSL C2-008 was grown to early stationary phase at 37°C in brain heart infusion (BHI). Cheese substrates consisting of sharp white cheddar (Heluva Good!, NY) and processed cheese (Kraft Foods Inc., IL), chosen due to their different surface features, were cut into 2.5 x 5 cm slices. The cheese samples were spot-inoculated using ten droplets of 10 µL, resulting in an initial inoculum concentration of 7 log CFU/slice. For processed cheese, a sterile low-density polyethylene coupon was placed underneath each sample to maintain their integrity during manipulation. Cheese samples were exposed to PL doses of 1.1 to 13.2 J/cm2 and then treated with 3 mL of a 2.5 % nisin solution (Nisaplin®, DANISCO, Madison, WI) or a 50 ppm natamycin solution (Natamax®, DANISCO, WI). Treated cheese samples were then stomached for 2.5 min in a 1:10 dilution of Butterfield Phosphate Buffer (BPB). The extract was plated on Modified Oxford Agar (BD Difco, Radnor, PA) and survivors enumerated by standard plate counting (SPC). When survivor counts fell below the SPC detection limit, the most probable number (MPN) method was used. Experiments were performed in triplicate. One-way ANOVA was used to compare the data with results from previous work where the antimicrobial solutions were applied before PL.

Treatments combining PL and nisin showed a synergistic effect on the inactivation of *L. innocua* on processed cheese, achieving a maximum log reduction of 3.7 ± 1.0 at a dose of 10.1 J/cm2. By comparison, PL alone achieved a maximum log reduction of 2.3 ± 0.1 at a dose of 10.1 J/ cm2; samples treated with antimicrobials before PL achieved a maximum log reduction of 2.5 ± 1.3 at a dose of 10.1 J/cm2. No synergistic effect was observed on cheddar cheese with any combination of PL and natamycin.

This study suggests that the order of treatment when combining PL with antimicrobials has a significant effect and that a hurdle strategy combining PL with nisin has strong potential for the decontamination of cheese surface.



Scholar: Maxwell Holle

University: Monmouth College

Major: Biochemistry

Faculty Mentor: Professor Olga Padilla-Zakour

Sensory evaluation and instrumental modeling as tools for evaluating consumer acceptability and processing behavior of food products

Reduction of Sodium in Ranch Dressing using NuTek 14510

Over consumption of sodium is known cause of hypertension which can lead to a multitude of health detriments. Potassium has been targeted for replacing sodium through substitution of KCl for NaCl. This is not a simple substitution because potassium chloride possesses a metallic bitter taste at certain concentrations. NuTek Salt Co. has developed a potassium chloride substitute (14510) in order to replace salt and/or MSG in ranch dressing. Ten formulations of varying sodium reduction were developed and evaluated while a sensory panel was performed using the best two (selected by NuTek). Sensory evaluations, a triangle and a forced paired preference test, were performed using a panel of 40 tasters between the ages of 20 and 60. The results indicated that there was no significant difference and no significant preference between a standard ranch dressing and the ranch dressing made with 14510. The 14510 can be used to reduce sodium and MSG in ranch dressing.

Evaluating Ascorbic Acid for Commercial UV Pasteurization Modeling

UV pasteurization has been of particular interest to small juice producers due to its cost, effectiveness, and minimal physical, chemical and nutritional changes in the product. The presence of UV absorbing chemicals interferes with the UV pasteurization. The UV absorbing chemical ascorbic acid is present in many juices. The absorption coefficients for varying ascorbic acid concentrations were determined as well as the correlating flow rate (determined using the UV CiderSure 3500). Using this relationship, pilot plant trials were conducted using 8 commercial juices of varying physiochemical characteristics in order to evaluate whether a juice's flow rate could be predicted using its absorptivity. In order to determine the safety validation of the method commercial juices were inoculated with E. coli ATCC 25922 in order to determine the log reduction after UV treatment. The model was able to predict flow rate, but 5 out of the 8 juices tested were not in compliance with the 5 log reduction required by the Federal Juice HACCP Regulation.

Scholar: Emily Mishina

University: University of Hawaii

Major: Microbiology

Faculty Mentor: Professor Randy Worobo

Characterizing antifungal mechanisms by assessing cellular viability

Today, while most bacterial pathogens are well understood, there are few available antimicrobials to combat fungal spoilage. Many of the antifungals used both in the food industry and in clinical settings have mechanisms that target the fungal membrane, but due to the similarities shared among all eukaryotic membranes, these antifungals may be toxic to the consumer as well. The widely used cheese preservative natamycin is an antifungal polyene, a category of compounds known for causing cell death by targeting the membrane. Recent studies suggest that membrane-permeability may be a secondary effect of this ergosterol targeting mechanism. Similarly, the broad-spectrum antifungal drug amphotericin B is a commonly used polyene in clinical applications. Studies have shown that higher concentrations of amphotericin B are required to induce alternative modes of action for fungal cell death such as membrane permeabilization.

These antimicrobials are well characterized in their respective applications. By gathering experimental results on their mechanisms of action, we can create a framework of reference data specific to each antifungal associated with its known mechanism. To characterize the mechanisms of these antimicrobials, the yeast *Candida albicans* was selected as a target organism. The minimum inhibitory concentration for both amphotericin B and natamycin were determined to be 3.9 µg/ml and15 µg/ml, respectively. Growth and survival dynamics were observed for *C. albicans* in the presence of both antimicrobials at the MIC, 0.5 x MIC, and 4 x MIC. Natamycin exhibited a more rapid antifungal effect than amphotericin B at equivalent concentrations, but both antifungals at concentrations at the MIC or above were able to inhibit fungal growth. A fungicidal effect was observed with treatment at 4 x MIC for both antifungals.

These preliminary data will contribute to the characterization of mechanisms for new antifungal agents, specifically, the novel antifungal protein YvgO. There is potential for YvgO to be developed as a food antimicrobial, which may improve food quality and extend shelf life. Collectively, initiatives to accurately characterize antifungal mechanisms are important towards the development of food-safe antimicrobials. Future studies involving YvgO may lead to the development of antifungals for application to fruits, vegetables, juices, dairy, and other perishable products.

Scholar: Anissa Taylor

University: Alabama A&M University

Major: Food Science

Faculty Mentor: Professor Carmen Moraru

Bactericidal effect of a 405-nm light-emitting diode array on foodborne microorganisms *Escherichia coli* and *Pseudomonas fluorescens*

Food products that are cut and repackaged in retail environments such as cheese and deli meats are susceptible to surface cross-contamination. Blue light, or non-ionizing radiation of 405 nm wavelength, can potentially be used as a strategy to prevent safety and quality issues by inactivating contaminating microorganisms on food surfaces. The objective of this study was to evaluate the effectiveness of a 405-nm LED array system on the inactivation of foodborne microorganisms. Another objective was to establish the treatment dose at which blue light achieves comparable inactivation levels to Pulsed Light (PL), which is a broad spectrum light treatment. The two foodborne microorganisms used were *Escherichia coli* ATCC 25922, as a surrogate for *Escherichia coli* O157:H7 and *Pseudomonas fluorescens*, a microorganism commonly associated with food spoilage. The work was conducted in a liquid buffer.

E. coli ATCC 25922 and *P. fluorescens* 1150 were grown to early stationary phase in tryptic soy broth (TSB) for 22 ± 2 h and 24 ± 2 h, respectively. The initial culture was diluted ten-fold into Butterfield's Phosphate Buffer (BPB) to reduce possible interference from suspended media solids. One ml of the diluted inoculum was then transferred into five separate UV-transparent glass chamber slides. The glass chambers were covered with sterilized UV-transparent low density polyethylene (LDPE) coupons to prevent the thin-film inoculum from evaporating. The initial inoculum concentration was determined experimentally by plating the untreated, diluted inoculum for each replicate. The glass chambers were placed directly under the 405-nm LED lamp at 4 °C, at a distance of 25.4 cm. The bacterial suspension was treated at an irradiance of 0.5mW J/cm2 up to a cumulative dose of 97.2 J/cm2. The treated suspension was serially diluted in BPB and survivors were enumerated using the Standard Plate Counting (SPC) method. All tests were performed in triplicates and data was analyzed using a one-way ANOVA.

It was found out that *E. coli* was more sensitive to the 405-nm light than *P. fluorescens*, with a maximum log reduction of 7.5 ± 0.9 after a dose of 97.2 J/cm2. *P. fluorescens* only achieved a maximum log reduction of 1.2 ± 0.1 at a dose of 10.8 J/cm2 and showed a dosage-dependent inactivation pattern. A possible explanation for the observed difference is that *E. coli* ATCC 25922 does not benefit from the protection of siderophores that can absorb blue light like *P. fluorescens* does. When comparing the effectiveness of the 405-nm treatment with PL, *E. coli* ATCC 25922 has similar inactivation levels at 4 J/cm2 (or 1.3 s) for PL and 97.2 J/cm2 (or 54 h) for blue, 405 nm light. For *P. fluorescens*, blue light was not able to reach the inactivation levels achieved by PL at 1.2 J/cm2 or one single pulse, even at a cumulative dose of 97.2 J/cm2.

While the 405-nm LED system can potentially work as a prevention measure against cross-contamination with *E.coli*, it does not show the same promise for *P. fluorescens*. In both cases, more work is needed to assess the effectiveness of blue light on solid surfaces.