

***2022 Food Science Graduate Student Research
Accomplishments***

Kansas State University

Food Science Institute

Fall 2022

Dear Food Science Enthusiasts,

This remarkable compilation of accomplishments by K-State Food Science Institute (FSI) on-campus graduate students (M.S. and PhD) demonstrates the superb quality of the work and experience gained by our students during their academic path to their graduate degree. The pages within also demonstrate the value of our faculty's contributions in delivering graduate education and training. Our students' research is making a difference in all our lives. Our food science students, guided by K-State faculty, are working across food science disciplines to make food safer, tastier, more nutritious, and an overall higher quality for a growing global population.

This is the first time we have compiled FSI graduate research abstracts presented during summertime annual professional meetings. The 2022 compilation includes presentations at the following summertime professional meetings: the Institute of Food Technologists, International Association of Food Protection, American Dairy Science Association, American Oil Chemists' Society, and the Reciprocal Meat Conference. The following pages demonstrate our students' research and findings and, in some cases, professional society awards for their research and presentation excellence.

I am incredibly proud of our students' careful and excellent work and equally proud of our FSI faculty who mentor them. I know you will agree that this is an impressive collection of research.

Please reach out to me if you have any questions about the work described in this document or about ongoing efforts within the Institute.

Happy Reading!



Jeanette Thurston
Professor and Director
Food Science Institute
College of Agriculture
K-State University
JThurston@ksu.edu

Jimeng Bai

M.S. Food Science

Began graduate degree program: Fall 2021

Major Professor: Dr. Sara Gragg (saragragg@ksu.edu)

***Salmonella* Quantification (SalQuant™) Utilizing the BAX® System for Pork Primary Production Boot Covers Samples**

International Association for Food Protection 2022

Pittsburgh, PA

July 31-August 3, 2022

Poster presentation

***Salmonella* Quantification (SalQuant™) Utilizing the BAX® System for Pork Primary Production Boot Covers Samples**

Jimeng Bai, Sara E. Gragg, Erin Fashenpour, Tyler P. Stephens, Savannah F. Applegate

Introduction: Personnel involved in the rearing of swine routinely wear boot covers, which can be used for quantifying *Salmonella* in a swine herd, as *Salmonella* is a natural inhabitant of the swine gastrointestinal tract and can be found in the environment of swine farms.

Purpose: This study was conducted to develop and validate SalQuant™ as a rapid PCR quantification for *Salmonella* in boot cover samples from the pork primary production setting.

Methods: Boot shoe covers were worn in a pork primary production environment and used as samples to represent background flora and debris. Samples were screened for *Salmonella* and negative samples were inoculated with *Salmonella Typhimurium* (ATCC 14028) at concentrations of 0.00 – 4.00 log₁₀ CFU/mL. Samples were enriched with BAX® MP with Quant solution for 6, 8, or 10h at 42°C and then quantified using the BAX® System Real-Time Assay for *Salmonella*. A 3 × 5 MPN was also conducted, and the two quantification procedures were compared using JMP® v. 15.

Results: Incubating the boot cover samples for 8 hours at 42°C resulted in a linear fit equation with an R² of 0.91 and a log RMSE of 0.46, which was the best linear fit equation produced by the three incubation times investigated. A statistical difference was not observed for the MPN and SalQuant™ methods.

Significance: According to the results of this experiment BAX® System SalQuant™ can accurately quantify *Salmonella* from pork primary production boot covers after an 8-hour incubation at 42°C. This provides the pork industry with a rapid approach to quantifying *Salmonella* populations in the primary pork production environment.

Janae Brown

M.S. Food Science

Began graduate degree program: Fall 2020

Major Professor: Dr. Kelly Getty (kgetty@ksu.edu)

Development and Evaluation of a Baking Hands-on Exercise with Soy Ingredients for an Undergraduate Bakery Science Laboratory Course

Institute of Food Technologists (IFT) 2022

Chicago, Illinois

July 10-14, 2022

Poster presentation

Award:

1st place-Education Extension & Outreach division poster competition

Development and Evaluation of a Baking Hands-on Exercise with Soy Ingredients for an Undergraduate Bakery Science Laboratory Course

Janae Brown and Kelly Getty

Hands-on learning in science laboratory courses improves understanding and encourages the development of soft skills desired by employers, including the ability to apply knowledge and communicate effectively. Ingredients made from soy improve bakery formulations by increasing the protein or binding, and impact physical and organoleptic properties of baked goods. A laboratory exercise featuring the inclusion of soy ingredients in baked foods provides an opportunity for students to observe alternative formulations of common products. The objective of this study was to evaluate student perception of a hands-on laboratory exercise utilizing soy ingredients in a bakery science course. Students in Baking Science II baked muffins with soy flour (0%, 15%, 30%) to observe the effect on physical and organoleptic properties. Additionally, students baked yellow layer cakes with soy lecithin and soy milk as an egg replacement. Students evaluated physical (volume, color, cell structure) and sensory (flavor, texture, appearance) properties of each product and wrote a laboratory report to communicate their results. After completing the baking laboratory, students (n=9) participated in an online survey with a five-point Likert scale to evaluate their perception of the laboratory exercises. The survey included questions on demographics, understanding of physical and sensory properties of baked goods, research and writing skills, and food processing knowledge. All the respondents were juniors or seniors majoring in Bakery Science or Food Science. Over 88% of respondents somewhat or strongly agreed that participation in the laboratory exercise improved their understanding of the effect of soy ingredients on sensory and physical properties. All respondents somewhat or strongly agreed that the exercise reinforced their ability to accurately communicate scientific data. Additionally, over 88% of students somewhat or strongly agreed that participation in the exercises encouraged application of principles of food and baking science to practical issues. Overall, the exercise was successful in improving understanding of the effects of baking with soy ingredients and improving soft skills such as the ability to communicate data and apply knowledge. Due to the strong positive responses from the perception survey, the laboratory exercise is a good model for the development of future hands-on laboratory activities.

Kavya Dileep

M.S. Food Science

Began graduate degree program: Spring 2021

Major Professor: Dr. Jay Amamcharla (jayendra@ksu.edu)

Preliminary studies on tailoring protein interactions to modify functionality of milk protein concentrate

American Dairy Science Association Annual Meeting 2022

Kansas City, MO

June 19-22, 2022

Poster presentation

Preliminary studies on tailoring protein interactions to modify functionality of milk protein concentrate

K. Dileep*, S. Beckman , H. Meletharayil, and J. K. Amamcharla

Milk protein concentrate (MPC) is widely used as a food ingredient due to its functional and nutritional benefits. Depending on the application, bringing desirable changes in its functional properties would further expand the possibilities for MPC as an ingredient. Functionality of MPC is dependent on the type of protein interactions (casein-whey protein vs whey protein whey protein). It was already established that whey proteins preferentially interact with other whey proteins when milk is heated at pH 6.6–6.9. On the other hand, whey proteins associate with casein micelles when milk is heated below pH 6.6. The objective of the study was to manufacture and evaluate the functionality of MPC made from skim milk (SM) preheated at different pH levels to influence the protein interactions. The pH of SM was adjusted to 6.5, 6.8, or 7.1 and subsequently divided into 2 parts. The first part was heated at 90°C for 15 min and the remaining part was used as control. After heating, the pH of SM was readjusted to 6.8. The SM was ultrafiltered (10 KDa) to 5x concentration with 120% diafiltration water to manufacture MPC. The MPC was subsequently spray dried in a pilot scale spray dryer (inlet temperature 184°C and outlet temperature 104°C). The experiment was conducted in duplicate from 2 independent lots of SM. The average protein content in the MPC was 84.4%. The MPC was reconstituted to 5% protein solution and characterized in terms of particle size and apparent viscosity. An increase in apparent viscosity was observed in preheated samples at all pH levels. At a shear rate of 50 s⁻¹ , the apparent viscosity of unheated and heated samples at pH 6.5 increased from 2.59 mPa.s to 4.65 mPa.s, respectively. Similarly, unheated and heated samples at pH 7.1 increased from 2.21 mPa.s to 3.34 mPa.s. However, the apparent viscosity (at 50s⁻¹) of heated samples at pH 6.5, 6.8, and 7.1 are not significantly (P > 0.05) different. Unheated and preheated at pH 6.5 MPC resulted in a mean particle size of 242.80 and 194.53 nm, respectively. The results show indication of casein-whey protein interaction or whey protein whey protein interaction depending on the preheating pH.

Parastou Farshi

PhD Food Science

Began graduate degree program: Fall 2019

Major Professor: Dr. J. Scott Smith (jsschem@ksu.edu)

Effect of whey protein isolate based edible films containing amino acids on the PhIP level and physicochemical properties of pan-fried chicken breasts

Institute of Food Technologists (IFT) 2022

Chicago, IL

July 10-14, 2022

Poster

Award

2nd place, Food Safety & Toxicology division poster competition

Effect of whey protein isolate based edible films containing amino acids on the PhIP level and physicochemical properties of pan-fried chicken breasts

Parastou Farshi, Jayendra Amamcharla, and J. Scott Smith

Heterocyclic amines are the mutagenic and carcinogenic compounds that are formed during cooking muscle foods at high temperatures. The 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) is the most abundant of these heterocyclic amines. In this study the effects of edible films containing amino acids (AAs) on PhIP in chicken breasts and physicochemical properties of the edible films and chicken breasts covered with these edible films, were evaluated.

Methods

Heated whey protein isolate (HWPI)-based casting solution (100 g) was made by heating 5 g whey protein isolate (WPI) solution at 90°C for 30 min in a water bath and subsequently mixing with 2.5 g glycerol (GLY), and L-tryptophane (Trp) or L-lysine (Lys) at 0.25%, 0.5%, and 0.75% concentrations. Unheated whey protein isolate (UHWPI)-based casting solution was prepared with the same method but without heating of WPI solution. Chicken breasts were cut at the same weights and were covered with the prepared edible films. Chicken breasts with no edible film and covered with edible film containing no AAs were used for comparison.

Results

PhIP level is significantly ($p < 0.05$) lower in chicken breasts covered with most of HWPI edible films, with Lys being more effective than Trp. Lower antioxidant activity and lower water solubility were observed in HWPI edible films. Color measurements after frying showed that the color difference between chicken breasts covered with edible films and the control (no edible film) was in the range of 2.81 to 7.10. Moreover, chicken breasts covered with HWPI edible films had lower cooking loss and improved tenderness compared to the chicken breasts with no edible film.

Significance

The prepared edible films containing Trp or Lys can be used on the surface of chicken breasts before cooking, to prevent the formation of PhIP, a carcinogenic compound.

Zilfa Irakoze

M.S. Food Science

Began graduate degree program: Fall 2020

Major Professor: Dr. Sara Gragg (saragragg@ksu.edu)

Evaluation of Peroxyacetic Acid and Chlorine as Treatments for Surface Water Used in Produce Post-harvest

International Association for Food Protection 2022

Pittsburgh, PA

July 31-August 3, 2022

Poster presentation

Evaluation of Peroxyacetic Acid and Chlorine as Treatments for Surface Water Used in Produce Post-harvest

Zilfa Irakoze, Londa Nwadike, Don Stoeckel, Manreet Bhullar, Patrick Byers, and Sara E. Gragg

Introduction: Produce contamination accounts for close to half of foodborne illness outbreaks, and water used both pre-harvest and post-harvest may contribute to contamination.

Purpose: Peroxyacetic acid and chlorine were evaluated as treatments for simulated surface water inoculated with generic *Escherichia coli* to determine efficacy at achieving 'no detectable generic *E. coli*' in 100 mL post-harvest agricultural water, as required by the FDA Produce Safety Rule.

Methods: Simulated surface (agricultural) water was prepared to turbidities of 2 and 100 NTU, pH was adjusted to 6.5 or 8.4, and then equilibrated to 12°C or 32°C, by following the EPA's protocol for development and registration of treatments for preharvest agricultural water, with modifications. Samples were inoculated with a generic *E. coli* cocktail (~5 log CFU/ml) and treated with 25±2 ppm free chlorine (C), 75±5 ppm peroxyacetic acid (P), or a water control (W). At 0, 5, 10, 60 min, 24 and 48hrs, samples were neutralized in Dey/Engley broth, enumerated on *E. coli*/coliform petrifilm®, and enriched in 2X Tryptic Soy Broth. Enrichments were streaked to MacConkey agar to confirm the absence of generic *E. coli*.

Results: All C and P samples were below the detection limit (0.5CFU/ml) and negative for *E. coli* on MacConkey at all time points. The pH×treatment interaction was significant (P<0.0001), with W samples at pH 8.4, a mere 0.1 logs higher than 6.5. The time×temperature×treatment interaction was significant (P<0.0001), with *E. coli* increasing to 6 logs in W by 24hrs at 32°C.

Significance: Generic *E. coli* was not detected in simulated surface water following treatment with P and C, which suggests these interventions are effective at treating surface water (≤100 NTU) for post-harvest use in produce. Additional research should evaluate other turbidities and methods (enumeration and/or enrichment) to further explore the efficacy of these antimicrobials at treating surface water.

Eda Kaya

PhD Food Science

Began graduate degree program: Spring 2020

Major Professor: Dr. Umut Yucel (yucel@ksu.edu)

Response Surface Methodology Optimization of the Use of Acetyl triacylglycerol for Improving the Structure of Whey Protein Foams

and

Effect of high oleic acetyl triacylglycerol (acetyl-TAG) on functional properties of biodegradable sorghum DDGS packaging film

American Oil Chemists' Society (AOCS) 2022 Annual Meeting and Expo
Hyatt Regency, Atlanta, Georgia, USA
May 1-4, 2022

Poster presentation & Oral competition

Award:

1st Place Winner in Analytical Division Oral e-Pitch Student Poster Competition
3rd place-IFT Food Packaging Division

Response surface methodology optimization of the use of acetyl-triacylglycerol for improving the structure of whey protein foams

Eda Ceren Kaya, Dallas Johnson, Timothy Durrett, and Umut Yucel

Foam stability, capacity and viscoelastic properties are essential for foods such as whipped cream, meringue and other confectionary products. These properties can be controlled using novel surface-active ingredients. The 3-acetyl-1,2-diacyl-sn-glycerols (acetyl-TAG) obtained from transgenic *Camelina sativa* with controlled fatty acid composition and purity at high yield, can serve as a suitable compound to improve the foam properties for food applications. The study is aimed to decrease sucrose content in a model whipped cream formulated by whey protein isolate and incorporation of a novel ingredient: acetyl-TAG. The foam was prepared by mixing whey

protein isolate, sucrose and acetyl-TAG in deionized water (200 mL) at ratios determined by Box-Wilson design: whey protein isolate (WPI) (2, 4, 6, 8, 10% (w/v)), sucrose (10, 15, 20, 25, 30% (w/w)) and acetyl-TAG (ATC) (0, 0.25, 0.5, 0.75, 1% (w/v)). The mixture was whipped using a hand mixer (Hamilton Beach 6 speed hand mixer, Southern Pines, NC, USA) at the highest speed for 20 min with 5 min intervals. Software package of SAS Version 9.4 TS Level 1M5 was used for response surface methodology (RSM) (Statease, Minneapolis, MN, USA). The effect of process variables was analyzed by linear regression model fitting for each response. Backward elimination algorithm was performed to select significant variables. The foam was characterized for overrun (FO), stability against serum drainage (FD) and surface rheology with dynamic interfacial elasticity (E'), complex modulus ($|E|$) and viscosity (E'') using an optical tensiometer. Acetyl-TAG was composed of palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), α -linolenic acid (18:3) and eicosapentaenoic acid (EPA) (20:1) as analyzed by thin layer chromatography (TLC) and LC-MS. The models showed high correlation coefficients (R^2) ranging between 0.8- 0.96. Surface contour plots were created to find the optimum values for each response. Foam overrun was significantly affected ($P < 0.01$) by WPI and ATC, but not sucrose concentration. The highest overrun (1100%) was obtained at 10% (w/v) WPI and 0.5% (w/w) ATC. Drainage and viscoelastic properties were significantly ($P < 0.01$) affected by all process variables. The highest $|E|$ and E' (80 and 75 mN/m, respectively) were recorded when acetyl-TAG was higher than 0.5% (w/w).

Significance: RSM approach is a suitable tool to provide a systematic understanding on the effect of a novel ingredient to improve protein foam properties. Our findings indicated that the acetyl-TAG obtained from transgenic camelina seeds can serve as a potential foam stabilizer in reduced-sugar foam formulations, such as whipped cream.

Effect of high oleic acetyl triacylglycerol (acetyl-TAG) on functional properties of biodegradable sorghum DDGS packaging film

Eda Ceren Kaya, Timothy Durrett, Scott Bean, Valentina Trinetta, and Umut Yucel

Biodegradable packaging films from biodiverse resources have gained popularity around the world in recent years due to environmental and sustainability concerns. Earlier, we showed that distiller's dried grains with solubles (DDGS), a byproduct of grain fermentation in distilleries and breweries, can be used as a renewable source for packaging films. A general challenge with biopolymer-based films is resistance against moisture and mechanical strength. We hypothesized that the functional properties of films from biopolymers can be improved by the presence of the sn-3 acetyl group of TAG, obtained from transgenic *Camelina sativa*. The aim of this study was to investigate the effect of acetyl-TAG as a plasticizer on the physicochemical properties of sorghum DDGS films. The acetyl-TAG with high oleic fatty acid composition (ca.70%) was extracted from transgenic camelina seeds using hexane. The composition of the purified oils was characterized by thin layer chromatography (TLC) and LC-MS. Sorghum DDGS were pretreated via wet (Colloid mill) and dry milling (Udy mill), and co-dissolved with gum plasticizers in a mixture of organic (i.e., ethanol, chloroform) and aqueous solvents. The acetyl-TAG was added to the mixture as a plasticizer (0, 0.1, 0.2% w/v). Films were prepared by following a dispersion pour-casting approach and were characterized for their thermal properties (DSC), tensile strength, elongation (texture analyzer), thickness (micro caliper) and color (Hunter LAB colorimeter). Addition of acetyl-TAG significantly affected the mechanical properties of the films. Tensile strength significantly ($p < 0.05$) increased to 39.54 ± 0.04 MPa when acetyl-TAG (0.2% w/v) was added as compared to 20.77 ± 0.03 MPa of films without acetyl-TAG. The plasticizing resistance increased with the highest enthalpy of melting (2.51 J) for the films with 0.2% (w/v) acetyl-TAG. The solvent type significantly ($p < 0.05$) affected tensile strength by improving it to a max of 52.89 ± 0.03 MPa when chloroform was used at 1:2 water:chloroform ratio. Addition of ethanol (50% v/v) to water:chloroform also had significant ($p < 0.05$) effect on the film strength and improved the water resistance of the films. Physical properties, such as color, were significantly improved by addition of acetyl-TAG (i.e., the maximum lightness L^* (66.7) obtained in the presence of acetyl-TAG at 0.2%).

Significance: High oleic acetyl-TAG from engineered *Camelina* seeds can be used to improve the physicochemical properties of sorghum DDGS films as a novel and high-yield plasticizer.

Erin Manville

M.S. Food Science

Began graduate degree program: Spring 2021

Major Professor: Dr. Valentina Trinetta (vtrinetta@ksu.edu)

**Evaluation of *Listeria monocytogenes* Biofilms Attachment and
Formation on
Different Surfaces Using a CDC Biofilm Reactor**

and

**Characterization of *Escherichia coli* Isolates from Produce Irrigation
Water in Kansas and Missouri by Whole-Genome Sequencing**

International Association of Food Protection (IAFP) 2022
July 31-Aug 3, 2022
Pittsburgh, PA

Oral & Poster presentation

Evaluation of *Listeria monocytogenes* Biofilms Attachment and Formation on Different Surfaces Using a CDC Biofilm Reactor

E. Manville, E. C. Kaya, D. Boyle, U. Yucel, V. Trinetta

Introduction: Bacterial attachment to surfaces and biofilm formation can be influenced by the physicochemical properties of the environment, surface characteristics and microbial motility. *Listeria monocytogenes* can adapt, persist, and form biofilms in a variety of environments, therefore representing a challenge for food safety.

Purpose: The purpose of this study was to evaluate the ability of *L. monocytogenes* to attach and form biofilms on different food-contact surfaces.

Methods: Multi-strain *L. monocytogenes* biofilms were grown in a CDC reactor up to 96-hrs on wood, nylon, and polycarbonate coupons at $20 \pm 2^\circ\text{C}$. Initial attachment was measured determining surface properties. Wettability and hydrophobicity were characterized by static contact angle and interfacial tension measurements using the standard sessile drop technique with deionized water and formamide. Biofilms structures and degree of growth were evaluated by Laser Scanning Confocal Microscopy (LSCM) after 2, 24 and 96-hrs. All experiments were done in triplicate for each material, respectively, and results analyzed for statistical significance.

Results: Coupon material, incubation time, and type of solvent significantly affected the hydrophobicity, interfacial tension, and wetting properties of biofilms ($P < 0.05$). Biofilm growth and incubation time resulted in increased hydrophobicity and contact angle, and in decreased surface energy and interfacial tension. The highest and lowest interfacial tensions were recorded as 16.65 N/m for the polycarbonate coupon (96-hr incubation) and 3.87 N/m for the wood coupon (2-hr incubation). Wood coupons presented a significantly strong biofilm attachment after 24-hrs, as compared to nylon and polycarbonate coupons ($P < 0.05$). Overall nylon had the highest microbial count, followed by wood and polycarbonate ($P < 0.05$). Material surface characteristics, eg. cervices and cracks, influenced biofilm structure and formation on the different materials as highlighted by the different images obtained.

Significance: Understanding biofilms attachment properties and growth abilities on different surfaces may enhance sanitation strategies in food processing environments.

Characterization of *Escherichia coli* Isolates from Produce Irrigation Water in Kansas and Missouri by Whole-Genome Sequencing

E. Manville, Y. Fu, E. G. Dudley, M. Bhullar, L. Nwadike and V. Trinetta

Introduction: Irrigation water has been found to be a major source of pathogens that can grow and persist on produce. Contaminated water has been the reported source of pathogenic *Escherichia coli* in recent produce-related outbreaks. It can subsist for weeks depending on temperature and other environmental conditions, posing an increased risk for produce safety.

Purpose: The purpose of this study was to characterize *E. coli* isolates from produce irrigation water sources using Whole-Genome Sequencing (WGS).

Methods: Ground and surface water samples were collected quarterly from five farms in Missouri and Kansas over a one-year period. Each sample was filtered, and *E. coli* was isolated using EPA Method 1603. Samples were then confirmed by Polymerase Chain Reaction (PCR). WGS was carried out using an Illumina MiSeq system. De novo genome assemblies were obtained with the Shovill pipeline version 0.9. NCBI Pathogen Detection was used to detect antimicrobial resistant (AMR) genes and single nucleotide polymorphism (SNP) clusters.

Results: A total of 238 PCR confirmed *E. coli* samples were collected in this study. *E. coli* levels in irrigation water were affected by ambient temperatures. Confirmed *E. coli* numbers were higher during spring and summer compared to winter ($P < 0.05$). An ample amount of diverse serotypes were observed from the collected samples. Isolates were divided across SNP clusters and using the NCBI database, strains were matched with other environmental and clinical isolates. AMR analysis showed that 40% of the strains carried at least one antimicrobial resistant gene.

Significance: Agricultural water source and climate conditions should be considered by growers when assessing produce safety. Recognizing the diversity of *E. coli* serotypes may be employed in developing guidance on agricultural water assessments as proposed in the new agricultural water rule.

Ellen Mendez Sosa

PhD Food Science

Began graduate degree program: Fall 2021

Major Professor: Dr. Jessie Vipham, jessiev@ksu.edu

Prevalence of *Escherichia coli* and coliform bacteria in lettuce and soil samples as a result of the use of organic fertilizers in Cambodia

International Association for Food Protection (IAFP) 2022

Pittsburgh, PA

July 30 – Aug 3, 2022

Poster presentation

Prevalence of *Escherichia coli* and coliform bacteria in lettuce and soil samples as a result of the use of organic fertilizers in Cambodia

Ellen A. Mendez, Carla L. Schwan, Jessie L. Vipham

Introduction: Fresh manure as well as animal waste “compost” are commonly used for vegetable cultivation by Cambodian farmers (33% and 11%, respectively). While organic fertilizers such as manure and compost offer many advantages, they can also be sources of bacterial contamination such as coliforms and *E. coli*.

Purpose: This study evaluated the level of contamination of coliforms and *E. coli* in soil and lettuce samples when using manure or compost as organic fertilizers in Cambodia.

Methods: Research plots were randomly assigned treatments of organic fertilizers (manure and compost), and inorganic fertilizer (control). Organic fertilizer samples were collected on day 0 prior to placement on research plots, soil samples were collected from research plots on day 0 and day 30 and lettuce samples were collected on day 30. Samples were processed and methods for microbial enumeration of coliforms and *E. coli* were conducted using ECC petrifilms™. Results were analyzed using the GLM model in a randomized complete block design in Statistical Analysis System (SAS®) Studio with a level of significance of $P < 0.05$

Results: On day 0 there were higher counts of coliforms (5.78 log CFU/g) and *E. coli* (2.44 log CFU/g) in manure than in compost (4.31 and 0.12 log CFU/g, respectively). Soil samples treated with manure and compost showed statistically significant ($P < 0.05$) growth of *E. coli* after 30 days. Additionally, soil samples treated with organic and inorganic fertilizers showed statistically significant differences in coliforms and *E. coli* load after 30 days. Lettuces samples showed no statistical difference between organic and inorganic fertilizers on the load of coliforms and *E. coli*.

Significance: More research is needed to understand the transmission of bacteria between organic fertilizers, soil and produce, the present study represents an initial baseline for the impact of using organic vs. inorganic fertilizer in coliforms and *E. coli* loads in Cambodia.

Madelyn Mushrush

M.S. Food Science

Began graduate degree program: Fall 2020

Major Professor: Dr. Elizabeth Boyle, lboyle@ksu.edu

The impact of different production backgrounds on meat quality and sensory attributes of heritage and commercial bred turkey

Reciprocal Meat Conference

Des Moines, IA

June 12-15, 2022

Poster presentation

The impact of different production backgrounds on meat quality and sensory attributes of heritage and commercial bred turkey

M. Mushrush, E. Boyle, T. O'Quinn, E. Titgemeyer, C. Velasco Ayala

Objective: Evaluate meat quality attributes of slow growing, heritage turkeys, fast growing, free range commercial turkeys, and fast growing, conventionally produced commercial turkeys.

Materials and Methods: Heritage bred (HB), free range commercial (FR), and commercially bred (CM) turkeys (n=20 each), were obtained from retailers and a commercial processing facility and frozen (-40°C). Prior to fabrication, turkeys were thawed for 96 h in a 2-4°C walk-in cooler. Carcasses within treatments were processed in random order with CM turkeys processed one week before HB and FR turkeys. Before fabrication, whole carcass weights were obtained, and breast and thigh skin color were measured using a Hunter Mini-Scan colorimeter. Boneless breast and bone-in thigh weights were obtained, and then the skin was temporarily peeled to measure lean meat color. The thigh was deboned, reweighed, and pH was measured on breast and thighs. Breasts and thighs from the right side were allocated for trained sensory panel while the left side were used for proximate analysis. Breasts with skin and thighs with skin were individually pulverized in a food processor to form a paste, packaged in whirlpack bags and frozen (-80°C) until proximate analysis was measured within 3 weeks. For sensory, breasts and thighs were vacuum packaged without skin and frozen (-40°C) for 1 month before thawing at 4°C and then cooked in an 85°C water bath to a peak temperature of 74°C. Samples were analyzed for aroma, juiciness, tenderness, flavor intensity and off-flavors. Data was analyzed as a split plot design, with type of turkey serving as the whole plot and the part (breast or thigh) serving as the subplot.

Results: An interaction for pH was present within HB, CM, FR breast and thigh meat, with CM thigh having the highest ($P<0.05$) pH and HB breast having the lowest ($P<0.05$). All thigh pH, regardless of turkey type, had a higher pH than breast meat. An interaction between turkey type and part showed that fat content of HB and CM thighs was 3% higher than that of FR thighs. There was a main effect for turkey part protein content. The protein content of breast and thigh meat of FR and HB turkeys were higher ($P<0.05$) than CM. Breast and thigh meat of HB turkeys were darker ($P<0.05$) than that of CM and FR with CM being the lightest ($P<0.05$). Thigh meat of FR and HB had highest ($P<0.05$) redness amount and CM breast had the lowest ($P<0.05$) redness values. There were no differences ($P>0.05$) among breast treatments for overall juiciness, overall tenderness, or off flavor intensity. Turkey aroma was more intense ($P<0.05$) for HB than FR or CM regardless of part type. Turkey flavor intensity was the highest ($P<0.05$) among breasts for HB. For thighs, there were no differences ($P>0.05$) in off flavor intensity or turkey flavor intensity. Thigh meat from CM was juicier and more tender ($P<0.05$) than FR or HB thighs. While HB thighs had the lowest ($P<0.05$) overall tenderness, it had the highest ($P>0.05$) flavor intensity over all parts.

Conclusions: Heritage and free-range turkeys show advantages over commercial turkeys in protein content. Additionally, free-range had the lowest fat content. Differences in meat color were seen between breeds. For breast parts, heritage turkeys had more intense aroma and flavor. Commercial thighs were perceived as juicier and more tender than heritage or free-range thighs.

Jack Myers

M.S. Food Science

Began graduate degree program: Fall 2020

Major Professor: Dr. Karen Schmidt

Use of educational messages to influence dairy foods consumption in inadequate dairy consumers

American Dairy Science Association Annual Meeting

Kansas City, MO

June 19-22, 2022

Poster presentation

Use of educational messages to influence dairy foods consumption in inadequate dairy consumers

J.S. Myers, S. Clark, and K.A. Schmidt

Nearly 90% of the U.S. population consumes less than the 3-cup, equivalent of dairy foods/day. The objective of this study was to determine if social marketing theory, applied in the form of educational messages (EMs), could alter dairy foods consumption in inadequate dairy consumers (IDCs). IDCs attended a nominal modified focus group (MFG), in which researchers presented either (1) a primary (P) EM on reading nutrition facts panels or (2) the P EM plus a secondary EM on one of either three topics – lactose maldigestion (P+LM), nine essential nutrients (P+9), or prebiotics and probiotics (P+PP). Eighty IDCs completed the study, of which 15 received the P, 17 received the P+LM, 20 received the P+9, and 28 received the P+PP. To collect data on dairy foods consumption, IDCs completed surveys at the start of the MFG (MFG pre-survey) and one-month later (MFG follow-up survey) to self-report the amounts of cheese, ice cream, milk, and yogurt consumed. Answers were adjusted to estimate the number of dairy foods servings/week/person. To determine the effect of the EMs, Wilcoxon signed rank tests were performed on the difference in total dairy foods servings consumed/week. To determine significant changes in individual dairy foods, a paired t-test was used to compare servings consumed before the MFG to one month later. Panelists who received the P, P+LM, and P+PP messages reported a significant ($p < 0.05$) increase in dairy foods servings/week/person by 1.27, 2.02 and 2.06, respectively. Overall, this research helps to understand how social marketing can increase dairy foods consumption amongst IDCs, through EMs.

Gunvantsinh Rathod

PhD Food Science

Began graduate degree program: Fall 2019

Major Professor: Dr. Jay Amamcharla, jayendra@ksu.edu

**Development & evaluation of spray-dried fibrillated model milk
protein concentrate**

and

**Process development for the manufacture of nonfat dry milk with
whey proteins as fibrils**

American Dairy Science Association Annual Meeting
Kansas City, MO
June 19-22, 2022

Oral presentation

**Flow properties of spray-dried functional milk protein concentrates
containing fibrillated whey proteins**

from the International Whey Conference, American Dairy Product Institute.
Chicago, IL
September 11-14, 2022

Award: 2nd Place: Young Scientist Award

Oral and Poster Presentation

Development and evaluation of spray-dried fibrillated model milk protein concentrate

G. Rathod, R. Kapoor, and J. Amamcharla

Recently, fibrillated model milk protein concentrate (F-MPC) as a liquid ingredient was developed. FMPC contained whey proteins as fibrils and consequently showed enhanced viscosity, consistency, and higher gel strength. However, F-MPC in powder form would expand its potential applications. The objective of this study was to understand the effect of spray drying process on the stability of whey protein fibrils in F-MPC powders. Control model MPC (C-MPC) was prepared by mixing solutions of micellar casein concentrate (MCC) and milk whey proteins isolate (mWPI) to maintain the same ratio of caseins to whey proteins as in milk. F-MPC was manufactured by converting whey proteins in mWPI solutions as fibrils and mixed with MCC dispersion to obtain F-MPC similar to C-MPC. C-MPC and F-MPC solutions were spray dried using a 2-stage spray dryer with Inlet and outlet air temperatures 193°C and 82°C, respectively. Moisture content for C-MPC and F-MPC was $3.26 \pm 0.15\%$ and $2.57 \pm 0.10\%$, respectively. Powders were collected in plastic bags and stored at -23°C until further analysis. C-MPC and F-MPC were reconstituted to 5% wt/wt on protein basis and evaluated in terms of functional properties. A significantly ($P < 0.05$) higher thioflavin T fluorescence value of F-MPC than C-MPC and presence of visible fibrils in transmission electron microscopy image of F-MPC confirmed the presence of fibrils in F-MPC. Further, F-MPC showed a significant ($P < 0.05$) increase in viscosity (49% at 100s^{-1}), emulsification capacity (8.8%), and foaming capacity (8.7%) than C-MPC. Foam produced using F-MPC was found to be more stable compared with the foam produced from C-MPC and further microscopic examination of the foam showed smaller air cells in the F-MPC. F-MPC showed significantly ($P < 0.05$) lower surface tension and interfacial tension than C-MPC. The above results confirm the survival of fibrils and retention of their functionality in spray drying process.

Process development for the manufacture of nonfat dry milk with whey proteins as fibrils

G. Rathod , S. Beckman, and J. Amamcharla

Nonfat dry milk (NDM) is used in a wide range of dairy and food products to increase the milk solids. NDM is classified based on the end product use as low heat, medium heat, or high heat depending on the level of whey protein (WP) denaturation. In the current work, whey proteins were selectively converted as fibrils in the manufacture of NDM and characterized in terms of viscosity, emulsification capacity, foaming capacity, and foam stability. Fresh skim milk (SM) was microfiltered (MF; 0.1 μm MWCO membrane) and subsequently the MF permeate was ultrafiltered (UF; 10 KDa membrane) to obtain 2% WP concentration in the UF retentate. The pH of UF retentate was adjusted to 2 and heated at 80°C for 14 h to convert WP into matured fibrils. After fibrillation process, the pH of UF retentate was readjusted back to 6.7. The UF retentate containing WP fibrils, UF permeate, and MF retentate streams were combined to produce fibrillated SM. Similarly, Control SM was also prepared without converting whey proteins into fibrils. Fibrillated and control SM were spray-dried using a 2-stage spray dryer (inlet and outlet temperatures: 190°C and 82°C, respectively) to manufacture fibrillated NDM (FNDM) and control NDM (C-NDM). The experiment was conducted using 2 independent lots of SM. Higher Thioflavin T fluorescence value and presence of visible fibrils in transmission electron microscopic image confirmed the presence of WP fibrils in F-NDM. The C-NDM and F-NDMs were reconstituted to assess functional properties. The reconstituted F-NDM (at 10%,15%,20% wt/wt TS) showed significantly ($P < 0.05$) higher apparent viscosity (at 100 s⁻¹) than C-NDM. Further, F-NDM showed a 10.25%, 11.70%, 18.88% increase in emulsification capacity, foaming capacity, and foam stability, respectively. F-NDM showed significantly ($P < 0.05$) lower surface tension (measured at air-liquid sample interface) and interfacial tension (measured at oil-liquid sample interface). Results of the study suggest that F-NDM can be used to improve the functionality of dairy products and reduce the use of synthetic thickeners, emulsifiers, and stabilizers.

Flow properties of spray-dried functional milk protein concentrates containing fibrillated whey proteins

Gunvantsinh Rathod^{1}, Kaliramesh Siliveru² and Jayendra Amamcharla¹*

¹ *Animal Science and Industry, Kansas State University, Manhattan, Kansas State, USA*

² *Grain Science and Industry, Kansas State University, Manhattan, Kansas State, USA*

Whey proteins deliver functional properties in dairy products such as gelling, emulsification, and foaming. Functionality of whey protein can be modified using various techniques including ultrasonication, micro-fluidization, and high-pressure processing. Recently, fibrillation is emerged as new technique that converts whey proteins into fibrils, which provide enhanced functionality such as thickening, gelling, emulsification, and foaming compared to native whey proteins. Considering the benefits of fibrillation, whey proteins were fractionated from skim milk using microfiltration and ultrafiltration, and subsequently converted into fibrils. Fibrillated whey proteins were then mixed with micellar casein to yield fibrillated milk protein concentrates (F-MPC). Similarly, control MPC (C-MPC) was manufactured by mixing whey proteins with micellar casein. Both MPC solutions were spray dried using a pilot scale spray dryer with Inlet and outlet air temperatures 193°C and 82°C, respectively and studied for powder flow properties. A similar microstructure was observed in scanning electron microscopic images for both powders, however, F-MPC has more fines than the C-MPC. Flow properties were measured with FT4 powder rheometer, where F-MPC showed a significantly ($P<0.05$) higher stability index, and flow rate index while significantly ($P<0.05$) lower specific energy. F-MPC showed lower compressibility and higher permeability at all applied normal stress (1-15 kPa). F-MPC showed significantly ($P<0.05$) higher bulk density and tapped bulk density while solubility was lower for F-MPC. Reconstituted F-MPC showed higher viscosity, emulsification, and foaming properties than the C-MPC. Above results suggest that whey proteins can be selectively converted into fibrils and used to manufacture F-MPC having better functionality. The developed F-MPC with fibrillated whey proteins can be used in the existing dairy product formulation to improve functionality without altering current industrial manufacturing process. Therefore, fibrillation of whey proteins can open doors for developing variety of functional dairy-based ingredients and reduce the need of non-dairy additives.

Karthik Sajith Babu

PhD Food Science

Began graduate degree program: Spring 2016

Major Professor: Dr. Jay Amamcharla, jayendra@ksu.edu

Effect of bulk nanobubbles during ultrafiltration on membrane performance

American Dairy Science Association Annual Meeting

Kansas City, MO

June 19-22, 2022

Poster presentation

Effect of bulk nanobubbles during ultrafiltration on membrane performance

Karthik Sajith Babu and Jayendra Amamcharla

Continuous acoustic/hydrodynamic cavitation technique was used to generate bulk nanobubbles (BNBs). The objective of this study was to evaluate the influence of BNB incorporation during the ultrafiltration (UF) process of skim milk. Three lots of non-fat dry milk powders were obtained from a commercial manufacturer. Powders were reconstituted to total solids of 25% and were incorporated with BNBs during the UF processing. Both lab and pilot-scale UF experiments were conducted to evaluate the effect of BNB incorporation on UF process by evaluating permeate flux, membrane microstructure, fouling resistance, energy consumption, and skim milk concentrate (SMC) characteristics. For both the lab/pilot-scale runs, after the initial water flux measurements, the membrane was fouled with the skim milk concentrate dispersions operated at 20°C under a constant transmembrane pressure 30 psi in constant concentration mode (the permeate was returned to the feed tank at regular 10-min intervals) and the total run time was fixed for 1 hour. UF experiments on the control SMC dispersions (C-SMC; no BNB treatment) and BNB-treated SMC dispersions (BNB-SMC; BNB-treated) were performed in duplicates. The results showed that BNB treatment had a significant effect on permeate flux in both the lab and pilot-scale runs. The permeate flux of the C-SMC was 8.50 and 6.03 L/m²·h for the lab and pilot-scale UF runs, respectively. The permeate flux significantly increased ($P < 0.05$) to 12.75 and 8.39 L/m²·h for the lab and pilot-scale UF runs, respectively after the BNB treatment. The confocal laser scanning microscopy was used to measure the thickness of fouled layer. It was observed that the measured thickness of C-SMC (45.17 μm) was notably different from the BNB-SMC (19.01 μm) fouled membrane. BNB incorporation also resulted in a significant decrease ($P < 0.05$) in apparent viscosity (C-SMC and BNB-SMC: 8.99 and 4.94 mPa·s at 100 s⁻¹, respectively). In conclusion, the BNB treatment helped to improve UF membrane performance and therefore this study suggests the potential of using BNB treatment for a more efficient UF processing.

Carolina Velasco Ayala

M.S. Food Science

Began graduate degree program: Spring 2021

Major Professor: Dr. Elizabeth Boyle, lboyle@ksu.edu

Effects of adding of egg powder from hens immunized against phospholipase α 2 on ground beef shelf life

Reciprocal Meat Conference
Des Moines, IA
June 12-15, 2022

Poster presentation

Effects of adding of egg powder from hens immunized against phospholipase $\alpha 2$ on ground beef shelf life

Carolina Velasco Ayala, Larissa A. Koulicoff, Colin Chun, Elizabeth Boyle, Travis O'Quinn, Mark Richards, Cassandra Jones, Michael D. Chao

Objectives: Lipid oxidation in beef may be enhanced by the hydrolysis of phospholipids by phospholipase $\alpha 2$ (PLA2) during postmortem storage and retail display. Anti-phospholipase $\alpha 2$ (aPLA2) is an antibody that may be able to prevent PLA2 activity. Past research has shown that aPLA2 can be mass-produced in eggs from hens immunized against PLA2, and the resulting egg can be spray -or freeze- dried in egg powder (EP) to preserve the antibody activity. Therefore, the present study investigated the effect of incorporating 3 different levels of dried EP containing aPLA2 for its potential to extend ground beef shelf-life.

Methods: Vacuum packaged USDA choice striploins from ten different beef carcasses were obtained from a USDA facility at 2 d postmortem. The next day, each loin was ground, divided into 4 equal batches, hand mixed with 0, 0.4, 0.8, or 1.6% dried EP containing aPLA2 (w/w), vacuum packaged and stored at 20°C for 14 d. After the storage period, each batch of ground beef was formed into four 114 g patties using a mold. Aerobically packaged patties were randomly assigned to one of three display times (d 0, 4, and 7), and displayed under fluorescent lighting at 0-4°C in coffin-style retail cases. Percent visual discoloration was determined using a trained panel (N=7). Additionally, L*, a*, and b* were measured using a colorimeter each day of display on the d 7 patties. At the end of each sample's designated display period, patties were removed from the overwrapped packaging, repackaged in vacuum packaging and stored at -80°C until analysis. Enzymatic activity of EP containing aPLA2 was assessed using an enzyme-linked immunosorbent assay (ELISA) from a crude antibody extraction using acidified PBS. Lipid oxidation status was measured on samples from all 3 retail display periods. The fatty acid (FA) profile was only determined on the d 0 samples.

Results: Throughout the 7 d of retail display, a* and b* values decreased ($P < 0.05$) and visual discoloration increased ($P < 0.05$). However, the inclusion of EP had no effect on beef patty visual discoloration, a*, or b* ($P > 0.05$). The L* value was not altered ($P > 0.05$, 0.2) due to EP concentration nor display day. Lipid oxidation increased ($P < 0.05$) for all treatments throughout the 7 d display periods. Beef patties containing 1.6% EP had higher ($P < 0.05$) lipid oxidation than the rest of the treatments. The addition of 1.6% EP to ground beef increased the relative percentage of C11-18:1 trans, C18:2, C18:3, C20:1, and C22:6, but decreased the relative percentage of C17:0, and C17:1 when compared to other treatments ($P < 0.05$).

Conclusions: Adding more than 0.8% of EP containing aPLA2 in ground beef altered the fatty acid profile by increasing the content of some polyunsaturated FA, particularly 18:2, which may likely lead to the enhanced lipid oxidation in ground beef patties. Although EP containing aPLA2

has been routinely used in other animal agricultural industries as a supplement to improve productivity, it did not demonstrate any effect to extend beef shelf life when incorporated into ground beef.

Wangyi Wei

M.S. Food Science

Began Graduate degree program: Fall 2020

Major Professor: Dr. Jay Amamcharla

Fabrication of nanofiber film via electrospinning of casein micelle and polyvinyl alcohol

American Dairy Science Association Annual Meeting

Kansas City, MO

June 19-22, 2022

Poster presentation

Fabrication of nanofiber film via electrospinning of casein micelle and polyvinyl alcohol

Wangyi Wei and J. Amamcharla

Electrospinning is a technique of nanofiber fabrication by utilizing high-voltage electric field to solidify or coagulate a polymer melt or solution. The casein micelle is challenge to independently fabricate to nanofiber through electrospinning, whereas casein micelle solutions are successfully electrospun with synthetic polymer (PVA). In this study, electrospun nanofiber with combination of micelle casein concentrate (MCC) and polyvinyl alcohol (PVA) was developed. The objective of this study is to investigate the effects of MCC and PVA on the spinnability of electrospun nanofiber.

The MCC solutions (8, 10, 12, 14, 16% wt.) were prepared one day before the experiment at 50°C, stirring for 1h, and store in refrigerator overnight for complete rehydration. The PVA powder was added to the MCC solution at concentration (1, 2, 3% wt.) at 95°C under 500 rpm for two hours. The mixture solution was placed in a 10ml plastic syringe with a blunt-tipped 18-gauge stainless steel needle, and a 13kV high voltage power with a flow rate of 1.3 ml/h flow were applied on the polymer solution. The nanofibers were collected on aluminum foil that has 10cm horizontal distance with the needle tip.

The spinnability of mixture solution of MCC and PVA has been compared. 14 % wt of MCC solution with 3%PVA is proved to be the maximum concentration of constantly forming nanofiber film while higher concentration solutions would block the spinneret in 1 minute. The concentration of the mixture solution with over 10% MCC but lower than 14% MCC at fixed 2%PVA were eligible to fabricate nanofiber. With increase either proportion of the MCC or PVA, the viscosity and conductivity of mixture increased, and directly affect the diameter of electrospun nanofiber.

Based on the scanning electron microscopy (SEM) images, the diameter of nanofiber with range of 344 ± 124 nm and the bead quantity are highly affected by the composition and concentration of both protein and polymer. With increasing of PVA concentrations, the morphological appearance of nanofiber contain beads were reduced. In addition, the higher proportion of MCC also contributes to the morphological appearance of nanofiber at fixed PVA concentration. The FTIR analysis indicates the presence of intramolecular structure MCC in both high-beads nanofiber and bead-free nanofiber.

The MCC: PVA blend nanofiber exhibit nanofiber quality improvement and the ability to form orientationally nonwovens nanofibers which can collect as edible film. Both MCC and PVA can increase the spinnability of mixture solution and the stability of the nanofiber. The 12%MCC with 2%PVA was considered as the optimal ration to fabricate to the nanofiber.

Baheeja Zaitoun

PhD Food Science

Began graduate degree program: Spring 2021

Major Professor: Dr. Jay Amamcharla, jayendra@ksu.edu

Preliminary studies on the influence of sodium hexametaphosphate chain length on the physiochemical properties of high protein

American Dairy Association Annual Meeting 2022

Kansas City, MO

June 19-22, 2022

Poster presentation

Preliminary studies on the influence of sodium hexametaphosphate chain length on the physicochemical properties of high protein

Baheeja Zaitoun and Jayendra Amamcharla

Protein-protein and protein-mineral interactions can lead to storage defects in high protein beverages. Cyclic polyphosphates such as sodium hexametaphosphate (SHMP) are used extensively in high protein beverages to improve stability. The objective of this study was to assess the effect of different chain lengths of SHMP and its concentrations (0, 0.15, and 0.25%) on the physicochemical properties of 8% protein dispersions. The average chain lengths of SHMP procured from a commercial supplier were 16.5, 13.3, and 9.9, and classified as long, medium, and short, respectively. Three independent lots of milk protein concentrate powder with 85% protein (MPC85) were obtained from a commercial manufacturer. MPC85 was reconstituted to 8% (w/w) protein and stored overnight for complete rehydration. The protein dispersion was divided into 7 equal parts and SHMP was added as per the experimental design. Sample containing no SHMP was used as a control. The samples were further divided into two parts and the first part was heated to 140°C for 15 s. Unheated and heated solutions were characterized in terms of viscosity, particle size, heat coagulation time (HCT), color, and turbidity. The HCT of the control sample was found to be 15.61 min. The addition of short-chain SHMP (0.15 and 0.25%) resulted in a significant increase ($P < 0.05$) difference in viscosity of control and all types of SHMP at 0.15% concentration. However, the viscosity of protein dispersions containing all types of SHMP at 0.25% was found to be significantly different ($P < 0.05$) effect on the viscosity. In conclusion, the type and concentration of added SHMP had primarily affected the HCT of 8% protein dispersions. Keywords: Physicochemical, SHMP, protein