Research reports from Honors undergraduate students receiving support from the Shackouls Honors College via the Summer Research Fellowship

Summer 2021

Program Director:
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Name: Riley Cutler

Faculty Mentor: Dr. Amy Dapper

Major: Biological Sciences

Department: Biological Sciences

Biological Illustration of *Caenorhabditis elegans*

Introduction

The Dapper Lab works with the model organism *Caenorhabditis elegans* to phenotypically measure recombination rates using inserted fluorescent markers. C. elegans make ideal model organisms due to their small size, low maintenance cost, short generation time, large quantity of produced offspring and cryopreservation (Corsi et al., 2015). That said, they are difficult to document: the nematode, only about 1mm in size, requires the help of a microscope to be seen. They also move quickly, which makes observation of anatomy, sexual mechanisms, and fluorescent patterns difficult. Biological Illustration enables me to circumvent these problems. It is defined as the intersection of art and science and communicates complex scientific concepts via visual explanations in ways that photography cannot.

Over the summer, I created several illustrations for the Dapper lab based off the research conducted in the lab. These projects included the following:

1. An illustration demonstrating the anatomical differences between male C. elegans and hermaphrodites.

2. A drawing that displays the GFP and td-Tomato fluorescent patterns seen on the C. elegans in the Dapper lab.

These drawings function both as instructional tools and additionally are used during outreach efforts by the lab.

Materials and Methods

The illustrations were created digitally via a Wacom Tablet and Photoshop. The first illustration involved extensive research of C. elegan sexual reproductive mechanisms utilizing papers, images, and videos. That, coupled with microscopy observation, ensured both anatomical accuracy and visual clarity.

Figure 1. C. Elegans male spicule inserted into hermaphrodite vulva (Source: Garcia, 2006)

The video image shown in Figure 1 was especially useful, as it showed how the male spicule is inserted into the hermaphrodite's vulva and the path of sperm ejaculation.

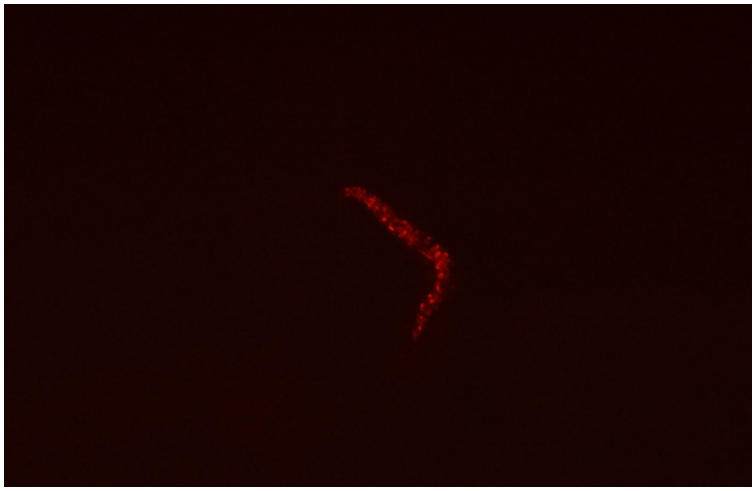

Figure 2. Fluorescent hermaphrodite with tdTomato marking

The second illustration was largely based on observation. It was aided by a fluorescent microscope photograph taken in the Brown lab (Figure 2).

Results

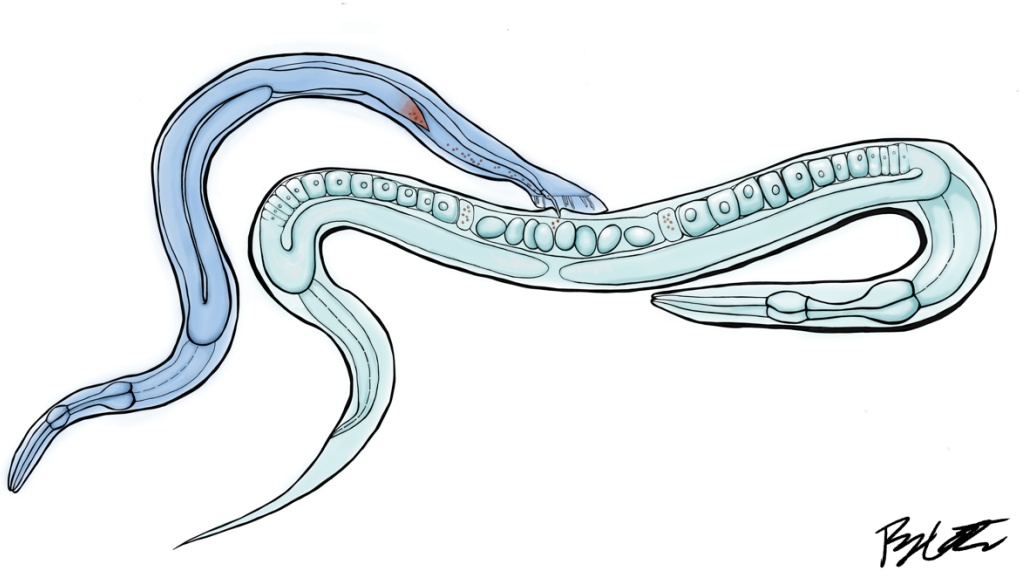

Figure 3. Reproduction in C. Elegans

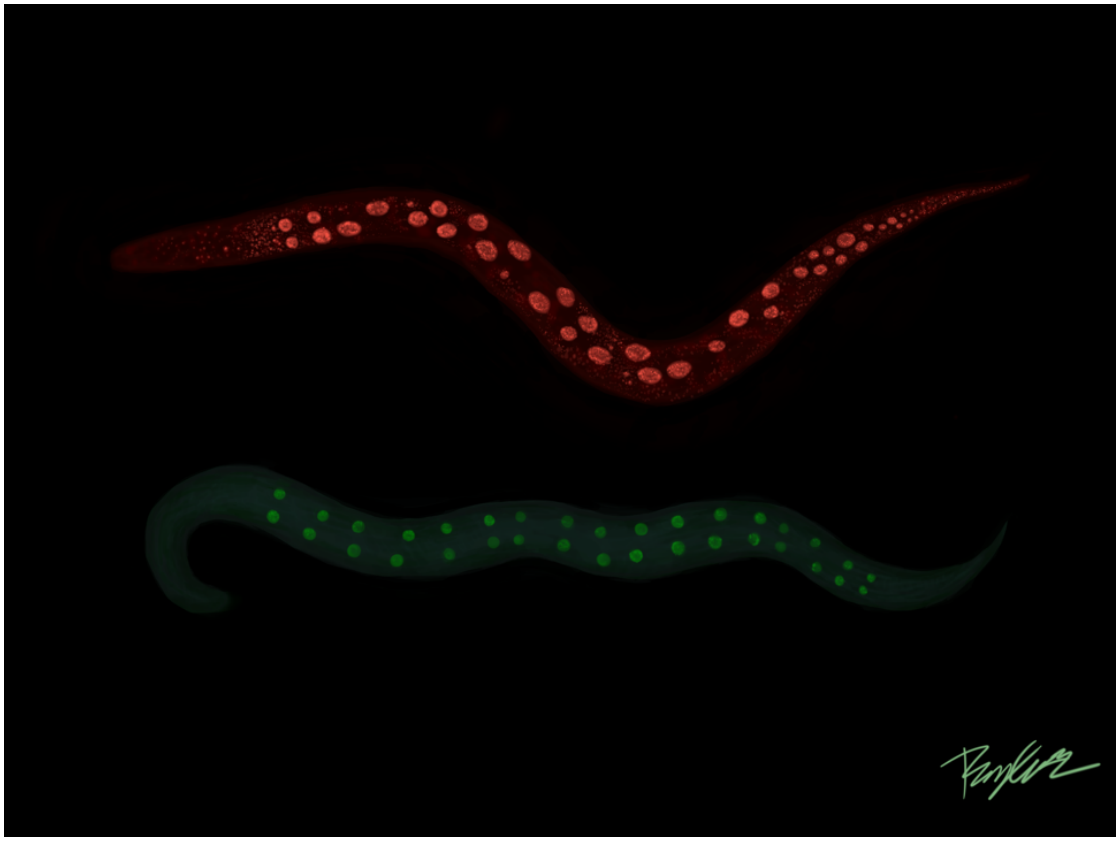

Figure 4. Dapper Lab td-Tomato & GFP fluorescent markers in C. elegans

Future Directions

A future project could entail drawing an illustration depicting m-Cherry, the third fluorescent marker used in the lab. Additionally, an illustration that depicts the phenotypes expressed before, during, and after a recombination event might be useful.

Works Cited:

Corsi A.K., Wightman B., and Chalfie M. A Transparent window into biology: A primer on *Caenorhabditis elegans* (June 18, 2015), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.177.1, http://www.wormbook.org.

Garcia, Rene. (2006). *C. elegans mating* [Video] Retrieved from: http://www.cco.caltech.edu/%7Ewormlab/movies/Rene_mating_movie.mpg

Name: Miller, Julie Anne

Faculty Advisor: Dr. E. Samuel Winer

Project Title: *Effects of grit on the relationship between depression and education*

This summer, I worked alongside Amanda Collins and Dr. Winer in the Emotional Processes and Experimental Psychopathology Laboratory. However, Dr. Winer began at a new school in July, so I switched mentors and began working with Dr. DeShong. Although the change was sudden, I was able to adapt well and successfully complete all my goals for this summer and more. This fellowship allowed me to strengthen my research skills, interests, and experience. Our laboratory held weekly meetings to discuss projects, plans, and articles of interest. I had the opportunity to work on many different projects which all serve a vital purpose to continuing my education in psychology.

Throughout the summer, I completed the virtual Psychological Networks Amsterdam workshop. This workshop advanced my knowledge about network analysis, an advanced statistical analysis that I will be able to utilize in future projects. Given that this workshop was moved to a virtual setting due to COVID-19, we redistributed my funds allocated for the workshop to a summer data collection with fellow lab members.

I worked with two other students to create an online study via Qualtrics to collect data via Amazon's MTurk. From this, I am interested in examining the relationship between perceived stress, loneliness, and depression. I am in the process of developing hypotheses for an abstract to submit to the Spring 2022 Undergraduate Research Symposium at MSU and to the annual convention of the Association of Psychological Science held in May 2022. I will use the research skills that I have developed from working on this project to create my Honors thesis project in the future.

Furthermore, my fellowship allowed me to purchase the software EndNote 20 to aid in manuscript writing. I utilized EndNote to create and organize citations for two manuscripts. I am presenting a poster at the Association for Behavioral and Cognitive Therapies convention this November that examines the effects of a mood induction procedure (MIP) in a previous laboratory data collection. I worked alongside Amanda Collins and Michael Gallagher, two graduate students in the laboratory, to write a manuscript related to the poster's findings. Moreover, Amanda and I were also able to work on a manuscript examining the effects of grit on the relationship between depression and GPA. I was not able to conduct archival analyses for the relationship between grit, depression, and education; however, I was able to nurture my interest in these relationships through manuscript writing. Working on this manuscript strengthened my knowledge of factors that can influence the relationship between depression and education (e.g., GPA, SAT scores). Both manuscripts will be submitted to journals in the next few months for publication.

Name: Mary Katherine Miller

Faculty Mentor: Dr. Deborah Eakin

Major: Interdisciplinary Studies

Department: Psychology

Metamemory and Aging: Analyzing Age Invariance in Semantic and Episodic Memory for Older and Younger Adults Through Feeling-of-Knowing Statements

Note: The focus of my summer fellowship research shifted somewhat as I worked with Dr. Eakin to develop the protocol. Rather than focus on emotional valence, I am focusing on the type of material being tested, as will be described.

Introduction: Memory is the ability to retain information while metamemory is the memory one has regarding one own's memory. Metamemory consists of the beliefs and thoughts one has about their own memory and the processes and mechanisms one uses to control these metacognition memory processes. The two types of memory if broken into two broad groups are semantic and episodic memory. Semantic memory is memory for general knowledge such as facts or historical events. Episodic memory is more personal being the memory that is not general that all know, but it is specific to oneself such as one's life events, personal conversations, and friends' names. Metamemory for semantic and episodic memory are two unique processes just as memory for semantic and episodic events are different. These two unique processes of semantic and episodic metamemory have been studied regarding the age of the participants to determine the impact of aging on metamemory. They are commonly studied using feeling-of-knowing (FOK) memory assessments. These assessments are predictions participants make about future recognition of information they learned in the

study. F Memory in younger adults and older adults does differ between age groups for episodic memory so the question has been asked is whether the same goes for metamemory. Some studies have provided evidence that metamemory does not have age differences in episodic memory(Eakin et al., 2014); however, other studies have reported age differences in metamemory (Morson et al., 2014). In fact, Morson et al. (2014) criticized Eakin et al., (2014) for failing to find age differences because they used pictures as their materials, citing the picture superiority effect (Paivio, 1969).

Proposed Research: The question as to whether age differences demonstrated in episodic metamemory when words versus pictures are used as materials is the focus for the proposed study that began over the summer as part of the Honors Summer Research Fellowship. The goal was to compare the effect of aging on metamemory for pictures versus words, and all combinations of them. Younger and older adults will study cue-target paired associations designed to test memory and metamemory under four conditions: image-image, word-word, word-image, or image-word pairs. This study was to be prepped for over the summer of 2021 through the Honors Summer Research Fellowship to then begin with data collection in the fall of 2021. The goal was to have all materials and recruitment prepared over the summer. In addition to preparing

Accomplishments/Results: The materials and recruitment for the study did occur over the summer through the Honors Summer Research Fellowship. Data from a study prior to this study in the lab was processed and analyzed over the summer to have this data ready to go for

comparison purposes. Furthermore, in terms of the older adult metamemory study, all materials for it were prepared from May-August. The basis of the study including the run time and protocol for it were completed, the lab area was cleaned and prepared, and Covid-19 precautions were thoroughly thought through. Because data collection would begin with adults 60-80 years, Covid-19 cleaning procedures were researched, protocols were written, and supplies was bought as to be prepared to the keep the older population safe and healthy. Additionally, articles and all necessary, relevant literature were read, studied, written about, and discussed to be familiar with all concepts and all current research for the proposed fall study. Lastly, recruitment for older adults was a main focus of the summer. Utilizing the summer for recruitment was very beneficial to be able to begin data collection as soon as possible in the Fall 2021 semester. Because this study cannot use participants from the student population and PRP website through Mississippi State University because the desired age group are older adults aged 60-80, outside heavy recruitment was necessary. Flyers were created and hung, phone calls were made with the details being logged, and other recruitment methods were researched to be ready to have a pool of participants in the fall. Overall, all proposed goals of both materials being prepped for the study and participants being recruited for the study were met.

Future Work: The study has continued into the fall semester with data collection already occurring at present day. All necessary and important materials being prepped during the summer gave additional time open to use the beginning fall months to inform the remainder of the lab assistants about the details of the study and then begin collection for the study. The

Howell older adult's lab is in current use on a weekly basis using the information and materials from the Honors Summer Research Fellowship to collect data effectively. The study, when collection and analysis are finalized, should accomplish answering the proposed research question of whether metamemory is or is not affected by age differences when studying semantic and episodic metamemory through feeling-of-knowing statements. Data collection should continue throughout the 2021-2022 school year.

Bibliography:

Eakin, D. K., Hertzog, C., & Harris, W. (2014). Age invariance in semantic and episodic metamemory: Both younger and older adults provide accurate feeling-of-knowing for names of faces. *Aging, Neuropsychology, and Cognition*, *21*(1), 27-51.

Morson, S. M., Moulin, C. J., Havelka, J., & Souchay, C. (2014). Parlez-vous français? Episodic and semantic feeling of knowing in aging. *International Journal of Psychological Studies*, *6*(2), 138.

Paivio, A. (1969). Mental imagery in associative learning and memory. *Psychological Review, 76,*

241-263.

Maggie Phillips

Faculty Mentor: Dr. Matthew K. Ross

Department: Comparative Biomedical Sciences

Lipid Activity of Human Carboxylesterase I Toward Oxidized Triacylglycerols

Introduction:

This summer, I joined the Ross Lab in the in the Department of Comparative Biomedical Sciences in the Mississippi State University College of Veterinary Medicine. Over the summer, I joined the lab to further its goal in understanding the interactions between particular immune cell enzymes and lipid molecules that activate cellular receptors related to inflammatory immune responses. Serine hydrolases are a family of enzymes recognized by their particular metabolic functions in immune cells (Ross et al.). The Ross Lab seeks to determine the specific role of Carboxylesterase 1 (CES1), a member of the serine hydrolase superfamily, in hydrolyzing oxidized lipid glycerol esters. Previous work showed that CES1 has hydrolyzes prostaglandin lipid esters, which are derivatives of 2-Arachidonylglycerol. My work in the lab focused on expanding the current understanding of CES1's substrate selectivity, regarding the oxidized derivatives, both the rate and preference of CES1 towards various oxidized triacylglycerols. To characterize the substrate specificity of CES1 towards oxidized di- and tri-acylglycerols, as well as the relative rates of reaction.

Methods and Materials

Samples of di- and tri-acylglycerols were incubated with $FeSO_4$ and triphenylphosphine to undergo oxidation. A portion of the oxidized samples was saponified to assess the level of

oxidation by KOH and heat. The non-saponified samples were incubated with CES1. The reactions were stopped with acetic acid, then an internal standard was added. The resultant lipids were extracted and analyzed by LC-MS. The results showed the level of hydrolysis that CES1 was able to achieve compared to the saponified samples, as well as *Pseudomonas lipase* as a positive control.

To compliment the study with CES1 and oxidized acylglycerols, human THP1 monocyte/macrophage cell lines were incubated with 15-S-HETE. The cell lines, control and CES1 Knockdown, were both incubated with 0.3 µM 15-HETE for 6 hours. Following the incubation, an assessment by LC-MS/MS compared the relative amounts taken up and utilized by the cells.

Results:

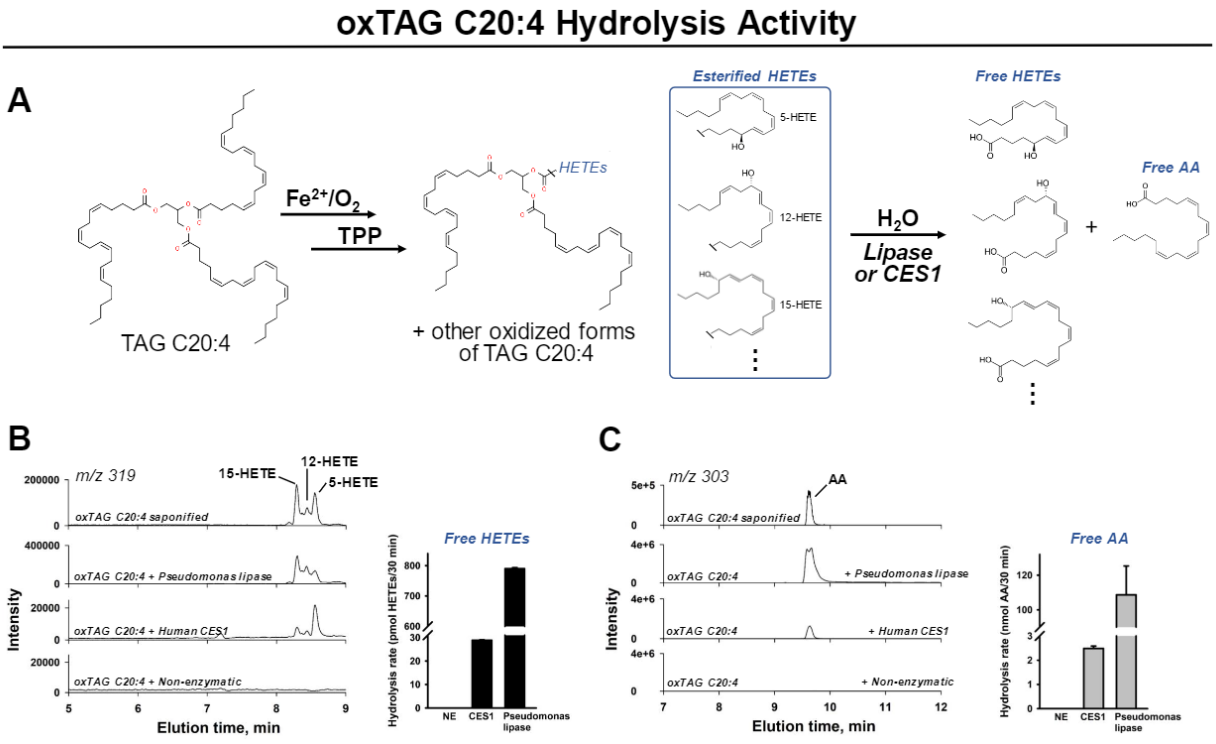

Figure 1. Chemical Scheme of CES1 Hydrolysis of oxTAG C20:4

Figure 1 shows the oxTAG C20:4 as it is partially oxidized by FeSO4 (0.5 mM; 1 h, room temp), followed by subsequent reduction of lipid hydroperoxides by triphenylphosphine (10 min, room temp) to yield esterified hydroxyeicosatetraenoic acids (HETEs) and other oxidized forms of TAG 20:4. The extent of oxidation of TAG 20:4 was <5% of the available arachidonoyl groups. Following its purification, oxTAG 20:4 (0.5 mM, final concentration) was incubated with the hydrolytic enzymes (60 min, 37°C) and the liberated HETEs and arachidonic acid (AA) were quantified by LC-MS using the internal standards 15-HETE-d8 (100 pmol) and AA-d8 (500 pmol). Mass chromatograms of HETEs (m/z 319) and AA (m/z 303) and their rates of formation are shown in (B) and (C), respectively. Data represent the mean ± SD of duplicate reactions.

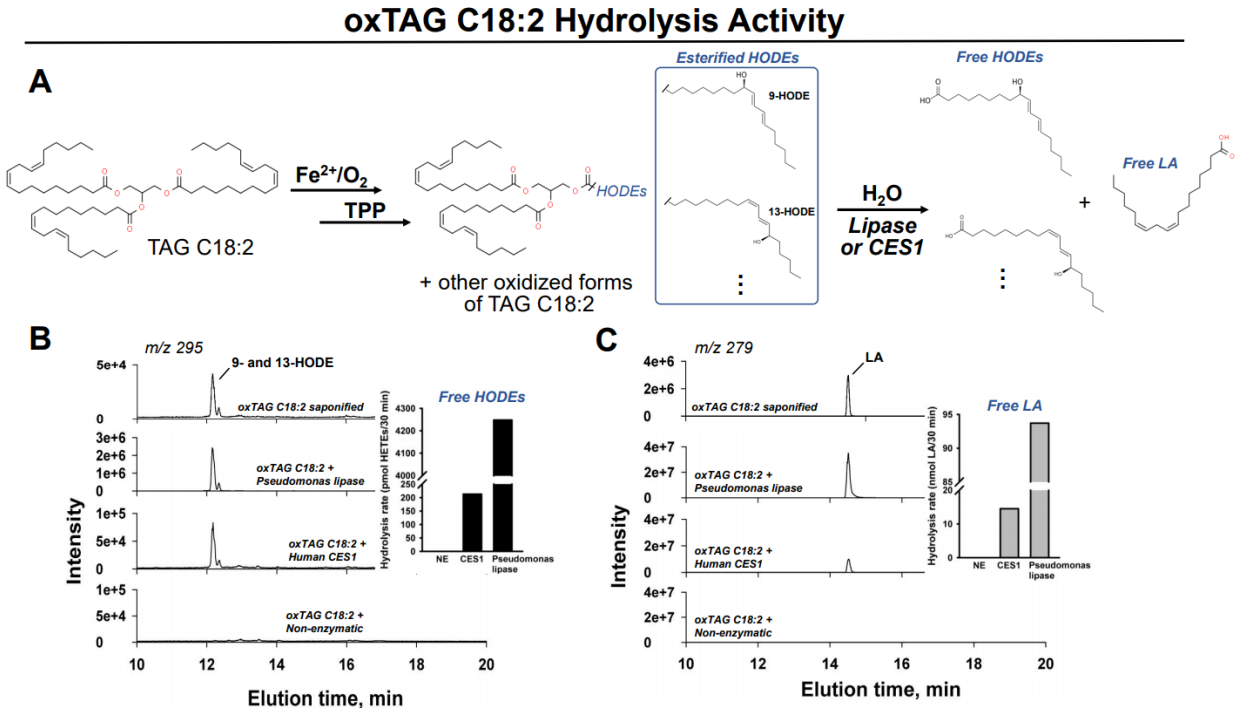

Figure 2. Chemical Scheme of CES1 Hydrolysis of oxTAG C18:2

Similarly, Figure 2 illustrates the Chemical scheme describing how TAG 18:2 (1 mM, final concentration) is partially oxidized by $FeSO_4$ (0.5 mM; 1 h, room temp), followed by subsequent

reduction of lipid hydroperoxides by triphenylphosphine (10 min, room temp) to yield esterified hydroxyoctadecadienoic acids (HODEs) and other oxidized forms of TAG 18:2. The extent of oxidation of TAG 18:2 was <5% of the available linoleoyl groups. Following its purification, oxTAG 18:2 (0.5 mM, final concentration) was incubated with the hydrolytic enzymes (60 min, 37°C) and the liberated HODEs and linoleic acid (LA) were quantified by LC-MS using the internal standards 15-HETE-d8 (100 pmol) and AA-d8 (500 pmol). Mass chromatograms of HODEs (m/z 295) and LA (m/z 279) and their rates of formation are shown in (B) and (C), respectively. Data represent the mean ± SD of duplicate reactions.

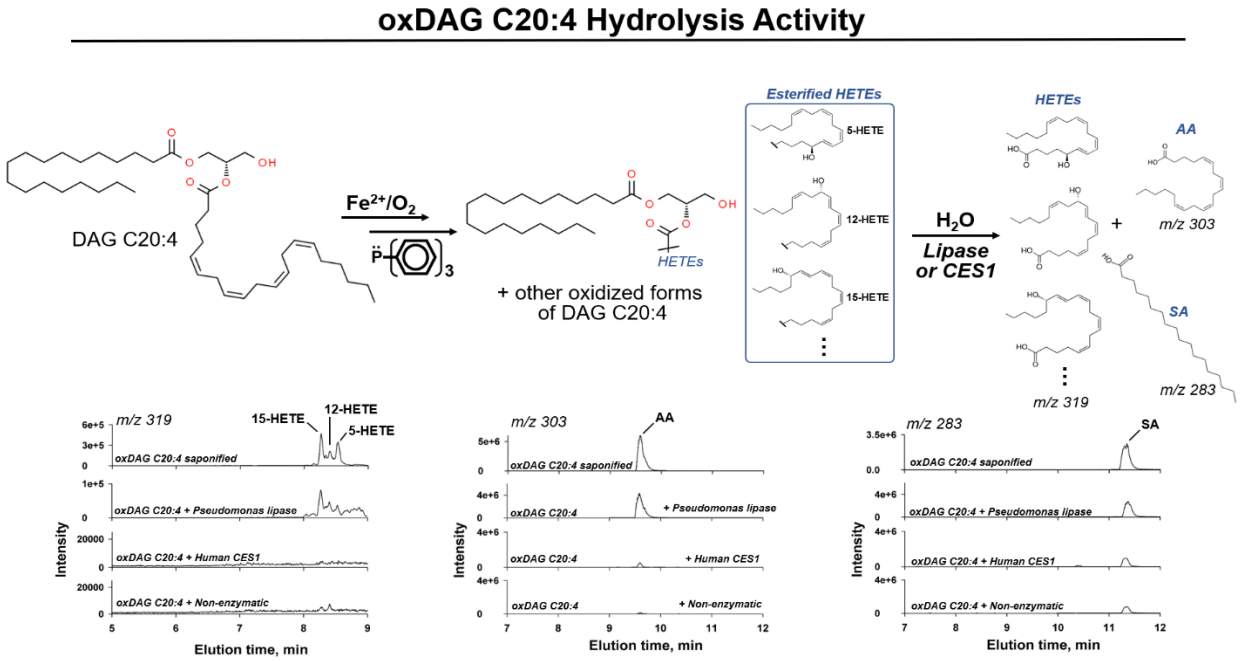

Figure 3. Chemical Scheme of CES1 Hydrolysis of oxDAG C20:4

Comparatively, Figure 3 demonstrates the ability of CES1 to hydrolyze diacylglycerols as opposed to triacylglycerols. DAG 18:0, 20:4 (1 mM, final concentration) is partially oxidized by $FeSO_4$ (0.5 mM; 1 h, room temp), followed by subsequent reduction of lipid hydroperoxides by triphenylphosphine (10 min, room temp) to yield esterified HETEs and other oxidized forms of DAG 18:0, 20:4. The extent of oxidation was <5% of the available arachidonoyl groups.

Following its purification, oxDAG 18:0, 20:4 (0.5 mM, final concentration) was incubated with hydrolytic enzymes (60 min, 37°C) and the liberated HETEs and arachidonic acid (AA) were quantified by LC-MS using the internal standards 15-HETE-d8 (100 pmol) and AA-d8 (500 pmol). Mass chromatograms of HETEs (m/z 319) and AA (m/z 303) and their rates of formation are shown in (B) and (C), respectively.

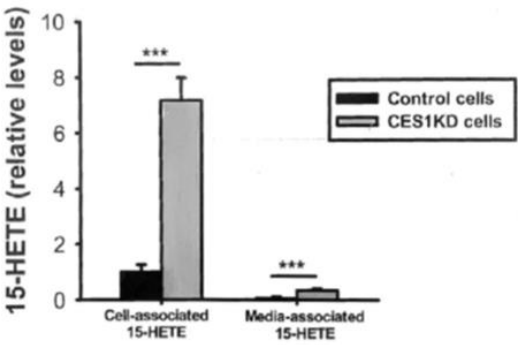

Figure 4 illustrates the relative efficiencies of control macrophages, which have and can utilize the CES1 enzyme, and CES1 knockdown cells. The amounts of 15-HETE remaining in each of the cells and the medias, respectively, are demonstrated in the graph.

Figure 4. Cell-associated and media-associated 15-HETE

Conclusions:

Oxidized trilinoleic acid is more efficiently hydrolyzed by carboxylesterase 1 than triarachidonic acid. This is demonstrated by the linoleic acid and oxylipins (HODEs) are released preferentially from tri-linolein than arachidonic acid and oxylipins (HETEs) released from tri-arachidonin. Furthermore, derivatives of the oxidized DAG are not as efficiently hydrolyzed by CES1 as the oxTAG derivatives. Considering the activity of CES1 towards oxidized triarachidonin (C20:4) specifically, 5-HETE is preferentially liberated compared to the 12- or 15- forms of HETEs.

In the comparison of the utilization of 15-HETE by control and CES1KD cells, control cells were observed to absorb 15-HETE from the environment and metabolize it significantly more efficiently than the CES1KD cells, which signifies that Carboxylesterase 1 plays an indirect role in the metabolism of 15-HETE.

The significance of these findings lies in the importance of determining the substrate specificity and efficiency of CES1, particularly regarding oxylipins such as HETEs and HODEs. These findings provide a deeper understanding of the inflammatory and anti-inflammatory regulation, which is significantly related to the pathways with which CES1 is involved. This knowledge may lead to novel approaches regarding lipid dysfunction and inflammatory disease.

References:

Ross, Matthew K., Streit TM, Herring KL. Carboxylesterases: Dual roles in lipid and pesticide metabolism. J Pestic Sci. 2010;35(3):257-264. doi: 10.1584/jpestics.R10-07. PMID: 25018661; PMCID: PMC4087164.

Inactivation of the Antimicrobial Effects of Lemon Essential Oil and Carvacrol in a Lemon-Based Marinade

Benjamin Wheeler

Department of Food Science, Nutrition, and Health Promotion

Mississippi State University

Faculty mentor: Dr. Shecoya White

For their assistance with laboratory and cognitive activities, I thank Leah Brown, Jailyn Smith, Kyla Asher, Ainsley Jessup, Lauryn Heidelberg, Jordan Rogers, Maria Hidalgo, and Jacinda Leopard.

Introduction

Food waste is any food that is grown or produced for human consumption that is not eaten. Every year in America $218 billion, or 1.3% of the nation's GDP, is spent on the transport and production of food that is never eaten [1]. This is a huge waste of time, money, and nutrition. Both real food spoilage and consumer perception of good food being spoiled contribute greatly to such food waste. After purchase, at the household level, the most common reasons that people toss food is suspicion of food spoilage by length of time in storage or by actual food spoilage signs like odor or appearance [2]. As real or perceived food quality deteriorates, consumers are willing to pay less money for it. Food is discounted and then discarded if unsold. One strategy that grocers sometimes employ to heighten the value of their food is to marinade it.

Essential oils and their various applications make up a very popular and economically significant trend that is projected to grow in the coming years [3]. This trend, "Green Consumerism," is due to America's hesitance to consume foods with artificial preservatives, instead preferring companies to have a "Clean Label" [4]. Many consumers willingly pay more money just to purchase products that they think are more natural than others. Meanwhile, some essential oils or components of them, including lemon essential oil (LEO) and carvacrol, are known to exhibit bactericidal effects on many pathogenic bacteria species at low concentrations [5, 6]. The combination of the essential oil craze, the public's suspicion of artificial preservatives, and the established antimicrobial properties of LEO and carvacrol could create a perfect storm of added value and efficiency that benefits grocers and home cooks alike if essential oils can function as preservatives in marinades.

During the Spring of 2020, the efficacy of LEO added to a marinade on the spoilage microbiota of chicken tenders was investigated. It was hypothesized that the addition of LEO to

the marinade would result in an intensified bactericidal effect that would curtail bacterial growth. The results indicated that the marinade depressed bacterial growth. A decreased pH of the chicken substrate was observed and remained about the same regardless of whether or not the marinade had LEO. This Summer Research Fellowship grant was spent on continuing to investigate spoilage of chicken, the same marinade, and the antimicrobial efficacy of the marinade in the presence of LEO and carvacrol. Aside from spoilage, this summer's research additionally covered the marinade's effects on *Salmonella* because of its relevance to food safety specifically with poultry. The broad intent of this research was to make everyone wealthier and safer.

Chicken was chosen as the substrate for some of the experiments because it is the United States' most eaten meat [7]. Because it is so common among restaurants and home cooks, it was worth using as a substrate for experimentation so as to replicate a real-world spoilage or pathogen outbreak situation.

A lemon-based marinade was chosen because lemon flavored chicken is a common item that many restaurants and home cooks prepare for meals. Also, a marinade like this one could easily be added to fish or other types of food. Because it fit the flavor profile of the marinade, lemon essential oil (LEO) was chosen to be investigated for antimicrobial properties when in the marinade. LEO is "generally recognized as safe" (GRAS), meaning that the Food and Drug Administration has deemed that it technically isn't even an "additive" [8]. Also, there are no quantitative restrictions as to LEO's use in foods [8]. Carvacrol, a chemical found in thyme and oregano essential oils, was also chosen as an ingredient because it is known to have antimicrobial effects [6] and it was predicted to taste flavorsome with the chicken.

Salmonella enterica was chosen as an inoculant because of its notoriety as a gastrointestinal pathogen which plagues many sections of the food industry, especially poultry. *Salmonella*

enterica is estimated to be the leading culprit of deaths and hospitalizations from foodborne illness [9]. Some varieties of *Salmonella* are known to be hardy in acidic mediums [10] and can survive for several hours when suspended in lemon juice [11]. Salmonella is also known to be susceptible to lemon peel extract [12].

This research report was structured as a number of experiments all relevant to the broad goal while each testing different objectives.

Broadly Applied Methods

The following methods were used in many of the experiments.

We designed a marinade which is simple and could easily be made by home cooks. When a larger amount of marinade was needed, the recipe was scaled up with the same ratio of ingredients. The ingredients of the marinade were as follows:

- 30mL lemon juice, squeezed from store-bought lemons
- 45mL olive oil
- 13mL garlic, minced by hand
- 7.40mL ground black pepper
- For the marinade sans oil (0% essential oil), the recipe is complete
- For the 1% essential oil marinade, add 0.95mL essential oil
- For the 2% essential oil marinade, add 1.90mL essential oil
- For the 5% essential oil marinade, add 4.75mL essential oil

The ingredients were added to a jar and shaken vigorously to emulsify the hydrophilic and hydrophobic ingredients, and to mix the solid bits. For each of the experiments, the marinade was made no more than a day before its application to the substrate. When it was made a day before application, it was refrigerated overnight.

All experiments used the same marinade recipe.

The cut of chicken used for all experimentation, including that of last spring, was the "tenderloin," sourced from a local grocery store.

Butterfield's Phosphate Dilution Buffer (0.1%) was used for this experiment. The buffer was used for dilutions and to prepare inoculum of a desirable concentration.

When administering marinade to test tubes or to chicken, a 20mL pipette tip was used to draw out the mixed marinade. The ingredients of the marinade tended to settle out of emulsion over time, so the marinade was agitated or swirled vigorously between every few draws of the pipette.

The Objectives, Methods, Results, and Discussions of Each Experiment

Experiment 1: Time Kill Assay of *Salmonella*

Due to the results of a preliminary study, it was hypothesized that *S. enterica* would begin to rapidly die minutes after exposure to the marinade, and that the presence of LEO would hasten their death.

Two *S. enterica* serovars were used in Experiment 1: Heidelberg and Gaminara. *Salmonella* Heidelberg was the 12th most culpable serotype causing salmonellosis in the United States [10], and about three quarters of *S.* Heidelberg outbreaks were due to contaminated poultry or eggs [13]. *Salmonella* Gaminara, on the other hand, seems to be found more in fruits like papayas [14] or oranges [15]. The Gaminara serovar may be more acid-tolerant than Heidelberg. Before Gaminara and a couple of other serotypes were traced back to a citrus juice processor, it was generally believed that the acidity of citrus products prevented the transmission of *Salmonellae* [16]. For example, in a fruit juice with a pH around 3.0, *S.* Gaminara has been shown to mostly die after 2 days, but persister cells may remain viable [17]. As seen in Table 3, our marinade had a similar pH. Because the serotype Heidelberg is associated with poultry foods and the serotype Gaminara is associated with citrus and acid tolerance, the two serotypes together make a worthy cocktail upon which to test the marinade.

To test this, a time kill assay was conceived. Each variable was tested in triplicate. Two *S. enterica* serotypes were used: Heidelberg and Gaminara. These were grown up separately in tryptic soy broth (TSB) for 25 hours. To a 95mL bottle of buffer, 2.5mL of each *Salmonella* stock were added. From this bottle of mixed, buffered stocks, 1mL was transferred to the test tubes with the marinade. Those test tubes contained 4.5mL buffer and 4.5mL marinade. Two marinades were used: the marinade sans oil and the marinade with 5% LEO. At 5, 10, 30, 60, and 120 minutes after inoculation, some of the mixture was pipetted out into buffer to stop the reaction and begin to begin the serial dilutions. As a control, 1mL from the bottle of mixed, buffered stocks was added to a test tube containing 9mL buffer. This control was only diluted and plated 5 minutes after its preparation, rather than 5, 10, 30, 60, and 120 minutes like the samples containing marinade. Both this control sample and the other samples were diluted to the desired concentration and then plated onto xylose lysine deoxycholate (XLD) agar. The colonies were counted at 48 hours. The results can be found in Figure 1.

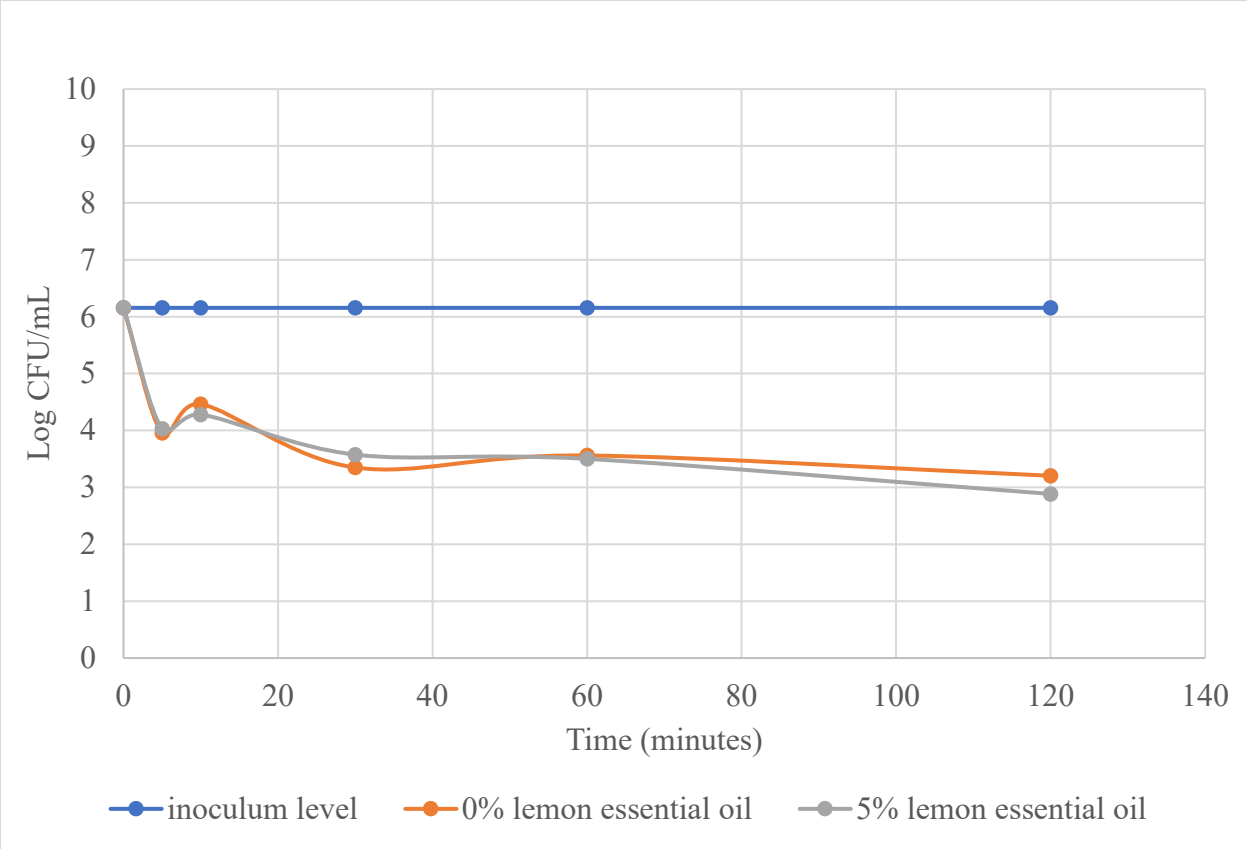

Figure 1: Time kill assay for the *Salmonella* cocktail exposed to the marinade sans oil and the marinade with 5% LEO. The inoculum level was measured only at the first 5-minute mark. The line representing the inoculum level does not represent actual data collected for the control beyond 5 minutes. It was extended over the whole graph to illustrate how a *Salmonella* population in buffer would remain alive and viable over the 2 hours and to highlight the number of *Salmonella* which died over the period.

Regardless of the presence of LEO, the marinade greatly affects *Salmonella* populations. Within the first 5 minutes after exposure to the marinade, the *Salmonella* population decreased by at least 90%, while about a tenth of a percent of them stayed alive through two hours. *Salmonella* survival did not differ appreciably between the marinade with LEO and the marinade sans LEO. The "inoculum level" line is an assumption that the bacterial concentration measured at the first few minutes in the buffer (sans marinade) would be the same. The inoculum level was really measured; the continuation of that line through two hours was assumed.

One may notice the significance of placing our highly acidic (see Table 3) marinade in the buffer, which has a neutralizing effect on pH. It is, in fact, appropriate to, if only a little bit, neutralize the pH of the marinade because chicken meat is known to have pH buffering properties, such that has a neutralizing effect on fluids it contacts [18].

Experiment 2: Assurance that Experiment 1 Was Not Flawed

One possible problem with this experiment was that the inoculation level was measured after only 5 minutes in the buffer. No other measurements were taken at 10, 30, 60, and 120 minutes after inoculation. Thus, this experiment was not controlled adequately. What if the *Salmonella* dies naturally over the course of 120 minutes when suspended in the buffer we used? If the buffer had some effect on salmonella, our results in Figure 1 were flawed because we don't know how much of the die-off was due to the salmonellicidal buffer and how much was due to the marinade. In order to ensure that buffer does not effect the survival of *Salmonella* over the course of 2 hours, we exposed the same serotype cocktail to buffer and tracked its survival over the time period. To do this, we prepared a mixed, buffered stock in the same way it was done for Experiment 1. An aliquot of 1mL was pipetted into 9mL test tubes, and then those were diluted and plated on XLD at the same timepoints as Experiment 1. This experiment was done in triplicate.

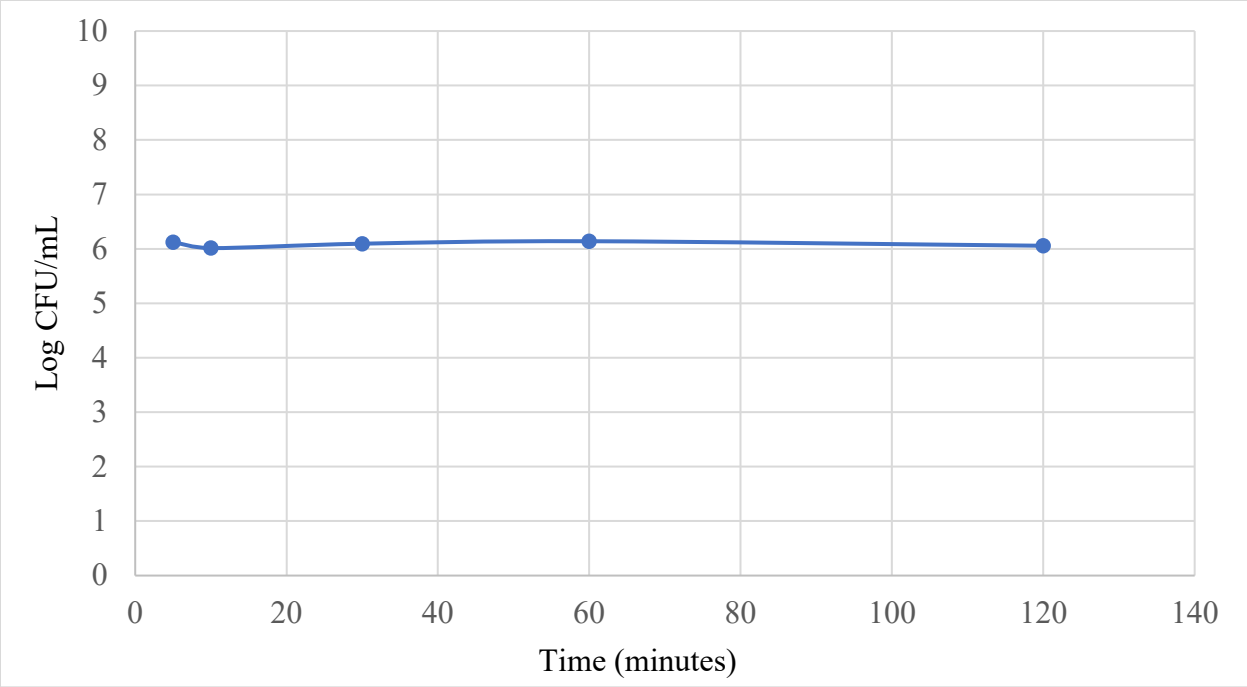

Figure 2: The survival of *Salmonella* in buffer over 2 hours.

Figure 2 demonstrates that salmonella's population will not naturally change throughout two hours in a 0.5% buffer solution. Thus, it was valid to assume that the buffer did not affect the survival of *Salmonella* in Experiment 1. Since the buffer does nothing to change the population of *Salmonella* over 120 minutes, the entire die-off can be attributed to the marinades and their properties. Therefore, Experiment 1 was not flawed.

Experiment 3: Effect of Marination With Carvacrol on Spoilage of a Chicken Tender

The purpose of Experiment 3 was to test the hypothesis that mesophilic and psychrotrophic spoilage bacteria would grow slower on chicken marinated with carvacrol than chicken marinated without carvacrol when stored at refrigerated and abusive temperatures. This hypothesis was tested by these methods:

Chicken tenderloins were cut into 10g chunks and placed into sterile stomaching bags. Marinades were prepared with 2% carvacrol and sans carvacrol. The chicken chunks were treated with 3mL of each marinade, pipetted into the bags. This amount amply coated the chunks. The

bags were then stored at either 4°C or 22°C. At certain times, bags were removed from storage, diluted with 90mL of buffer, and stomached to dislodge bacteria. Further serial dilutions were performed and then the dilutions were plated on Petrifilm™ to count. Petrifilm™ plates were incubated at 37°C or 4°C in order to grow and count mesophiles and psychrotrophs, respectively. The Petrifilm™ plates incubated at 37°C were counted after 48 hours while those stored at 4°C were counted after 7 days. The results can be found in Figures 3 and 4.

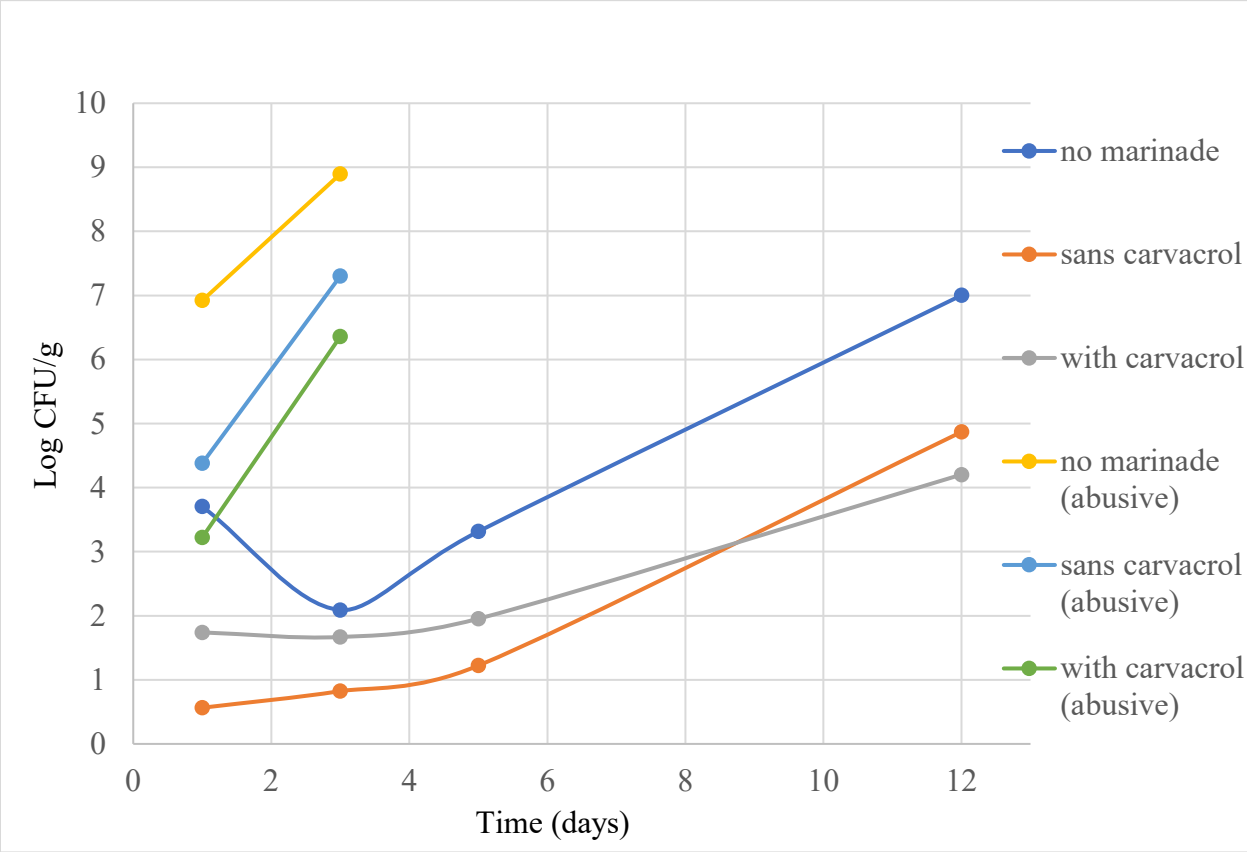

Figure 3: Growth of mesophilic bacteria over time in different marinade formulations, stored at refrigerated and abusive temperatures.

This demonstrates that both marinade formulations suppressed bacterial growth at both abusive and preservative temperatures. Furthermore, the results suggest that the marinade with carvacrol may serve to slow spoilage more effectively than the marinade sans carvacrol, on chicken

stored at abusive temperatures. However, in refrigeration, the marinade sans carvacrol seemed to perform better through at least the first several days. But by the twelfth day, it looked like the marinade with carvacrol had been a little more effective.

By the third day, the chicken stored at the abusive temperature offended all the lab's olfactory senses by exhibiting nasty odors unbecoming of fresh chicken. To protect the lab from malfeasant malodors, Experiment 3 was not continued past the third day for the room temperature samples.

One kerfuffle which almost certainly impacted the results seen in both Figure 3 and 4 is that an estimation error was made such that all samples for day 1 had to be replated. By the time new samples were taken from the stomaching bags, it is quite likely that the mesophiles and psychrotrophs had grown on the substrate, leading to very untrustworthy numbers for day 1. This may explain why the unmarinated chicken stored at 4°C appears to drop in microbial population between the first and third days, and then increase from there.

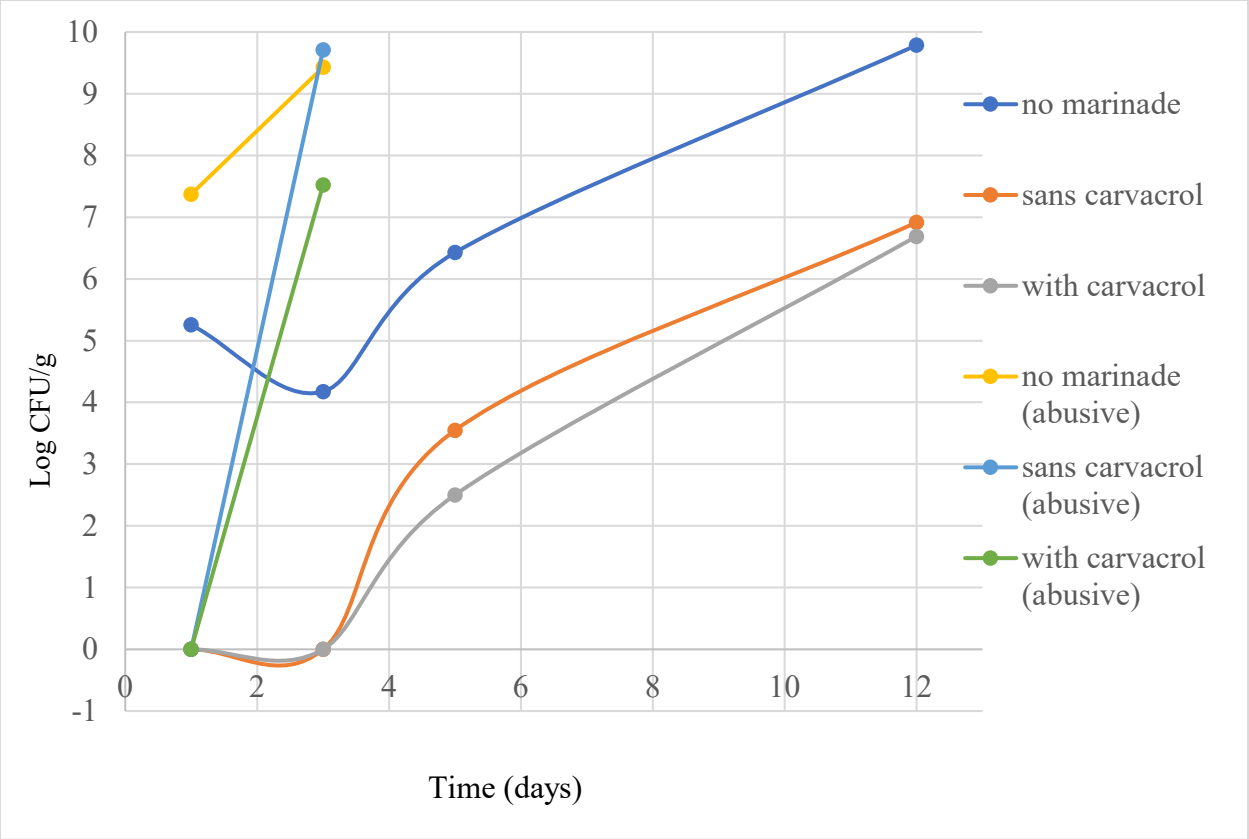

Figure 4: Growth of psychrotrophic bacteria over time in different marinade formulations, stored at refrigerated and abusive temperatures.

The data demonstrate that psychrotrophs grow faster at 22°C than in the refrigerator. This could be because although psychrotrophs can grow at refrigeration temperatures, many grow faster at more moderate temperatures [19]. The marinades seemed to stymie the growth of psychrotrophs at most data points except the chunks marinated sans carvacrol left at room temperature for three days. At 4°C, there is no appreciable difference between the microbial growth on the chicken marinated with carvacrol and the chicken marinated sans carvacrol. The dubiousness of the Day 1 counts can be seen in Figure 4 where the psychrotrophs on the unmarinated chicken seemingly experienced a die-off and then rebounded between the days 1 and 3.

All told, carvacrol did not work synergistically with the marinade against the spoilage bacteria, mesophilic nor psychrotrophic, preexistent on chicken tenders. However, both marinade formulations suppressed growth of both mesophiles and psychrotrophs over the 12 day period.

Experiment 4: Sensory Evaluation of Marinade With LEO, Carvacrol and Sans Additives

A sensory evaluation was performed on the marinade to figure out if the marinade formulations were delightful and delectable when applied to chicken and eaten. This is important because food treatments cannot significantly deteriorate the eating experience or else people will not want to eat the food. There's no sense in preserving a food in such a way that people will refuse to eat it. Two evaluators ate the chicken without marinade, with the marinade sans oil, and with the marinade containing 2% carvacrol. One of the two evaluators ate the chicken with the marinade containing 2% LEO on a prior date, from a different batch of chicken tenders and a different batch of marinade. They scored their experiences with each marinade on a scale from 0 to 10, with 0 being a loathsome eating experience and 10 being the best that a chicken tender can possibly be. Scaling "the best a chicken tender can possibly be" at 10 eliminates the possibility that the evaluator simply dislikes all chicken, and might rate even what is popularly accepted as the best chicken as a low number. The two evaluator's scores were averaged for the marinades graded by both of them.

No marinade	Marinade sans oil	Marinade with 2% carvacrol	Marinade with 2% LEO
5	7.5	3	8

Table 1: Sensory evaluation of the marinade formulation on chicken.

Figure 5: Cooked chicken tenders. From left to right: unmarinated, marinade sans oil, and marinade with 2% carvacrol.

The chicken without marinade served as a baseline by which to judge the marinade formulation. This demonstrates that marination sans oil yielded an improved sensory experience over unmarinated chicken. The marinade with 2% carvacrol was quite deleterious to the sensory experience because, when cooked, it smelled strongly of carvacrol and tasted just as pungent. Perhaps a lower concentration of the oil would have been more palatable. The marinade with 2% LEO improved the sensory experience. It tasted a little more of lemon than the marinade sans oil, but the experience was not appreciably better. The evaluator also noted that the chicken tender marinated with 2% carvacrol looked roughly equal to the tender sans carvacrol.

Experiment 5: Time Kill Assay of Spoilage Microbiota When Exposed to Differing Marinade Formulations in Vitro

To assess the survival of spoilage microorganisms exposed to the marinade with LEO and the marinade with carvacrol, Experiment 3 was set up like Experiment 1, such that tubes were prepared with 4.5mL buffer and 4.5mL marinade. This time, control tubes of 9mL buffer were diluted and plated at all sampling times that the others were. A 1mL aliquot of mixed, buffered stock was added to each tube to start the reaction. The mixed, buffered stock was prepared thus:

> The juices from some rotten chicken breasts from an unrelated university class demonstration were streaked onto tryptic soy agar (TSA), and three unique colony morphologies were identified. The three unique microbes were isolated and then grown separately in tubes of TSB, named A, B, and C. After 25 hours of growth, 3mL from each of the three TSB tubes was pipetted into a jar of 91mL buffer. A sample from each tube was also serially diluted and plated on Petrifilm™ to ascertain how much of each microbe was being added to the jar. This information is found in Table 2.

After the mixed, buffered stock was distributed to each tube, the tubes were diluted at certain times to halt the reaction. These dilutions were plated on Petrifilm™, incubated at 37°C, and then counted after 48 hours. The results were shown in Figure 6.

A	B	C
2.93×10^{7}	9.1×10^{8}	0×10^{1}

Table 2: Concentration in CFU/mL of A, B, and C stocks at the time of addition to the jar.

While the A and B TSB tubes were quite turbid, the C tube was very clear. The failure of any colonies to grow, even when the least dilute dilution was plated, demonstrates that no cells of microbe C were inoculated into the test tubes. However, large amounts of microbes A and B were inoculated into the test tubes of Experiment 5.

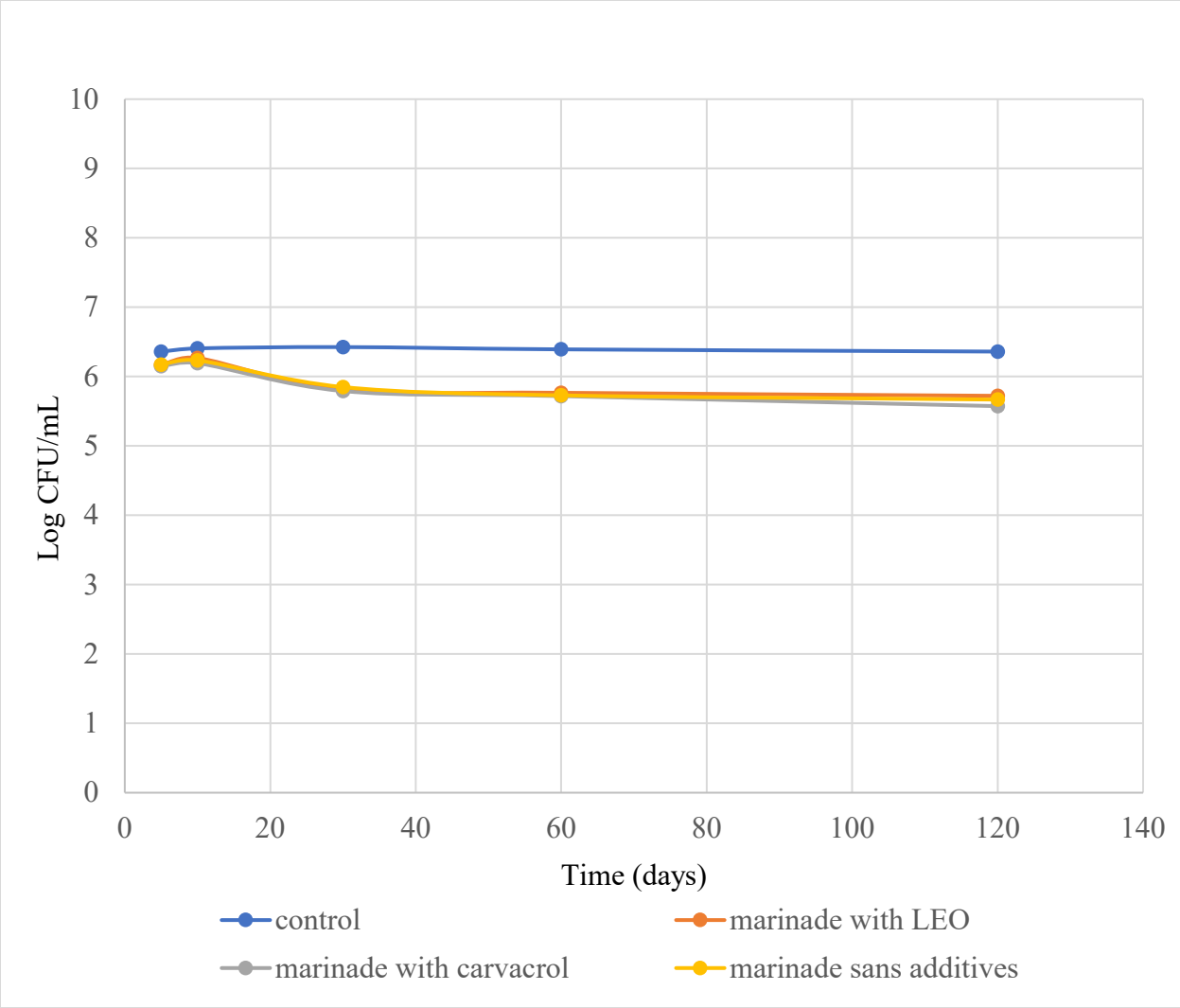

Figure 6. Time kill assay for the spoilage bacteria cocktail exposed to the marinade sans oil and the marinade with 5% LEO.

The results demonstrate very high bacterial survival but definitely a slow decrease in population continuing through the second hour. There was also no appreciable difference between the microbicidal effects of marinades with and without the additives.

Experiment 6: Identification of Survivors of Experiment 5

It was conceivable that the marinade may have affected one inoculant more than the other. To ascertain the ratio of survival between microbes A and B, the bacteria which grew on the Petrifilm™ had to be identified. A Petrifilm™ plate was selected for sampling on which grew

colonies which survived the marinade sans additives for 2 hours. Colonies were picked out of the Petrifilm™ gel with a pin and then streaked and poked onto TSA plates next to a streak from isolated stock of A and B as a control.

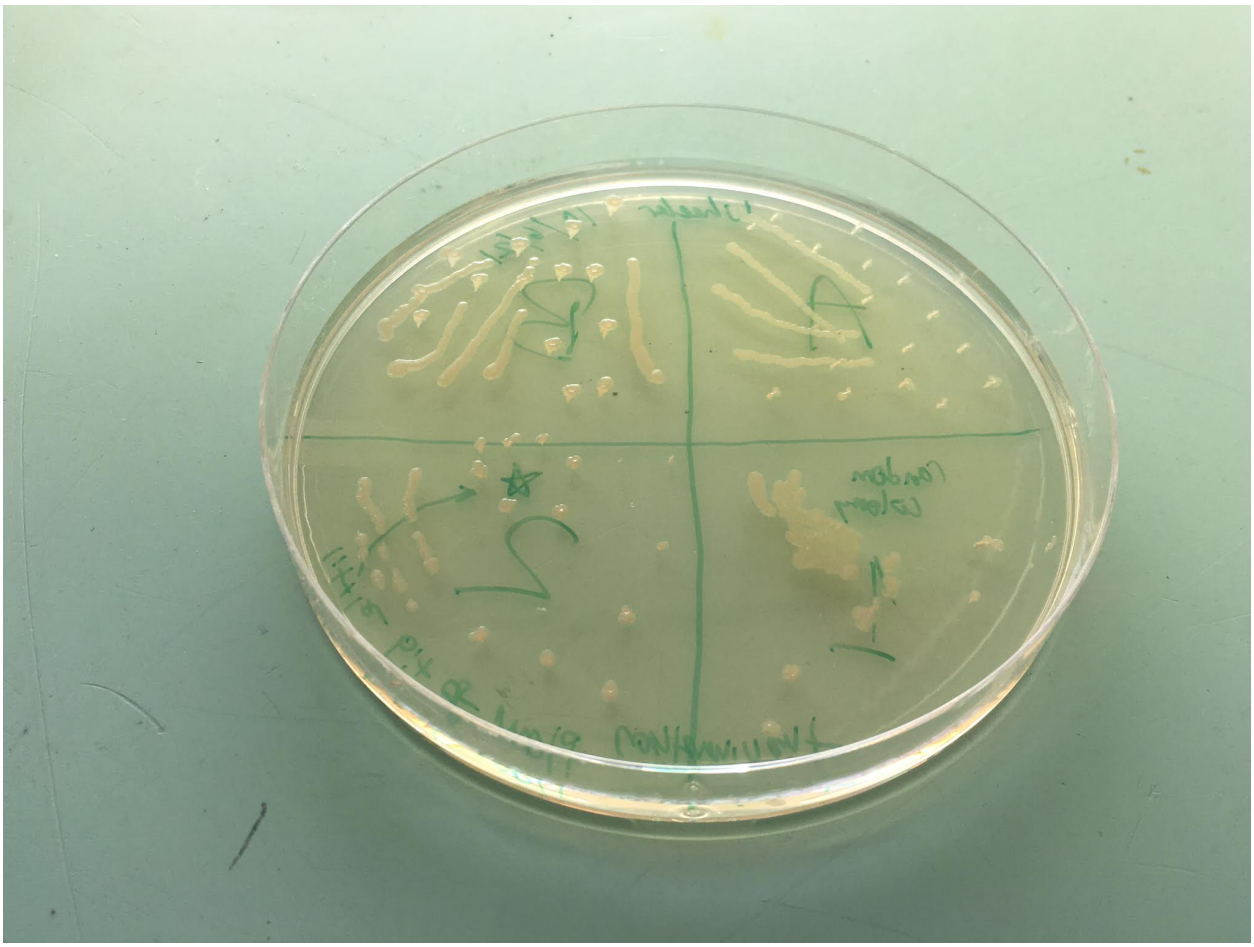

Figure 7: The first and second samples from the Petrifilm™, plated next to known A and B as a comparison and control.

Of the 10 colonies selected, 9 colonies were B, and 1 colony didn't grow. This meant at least that there was not a significant die-off of B throughout 2 hours in the marinade sans additives. Nothing else can be known because it is reasonable to find no A colonies in just nine random colonies, based on the ratio at which A and B were inoculated. It was possible that all of the A microbes were killed by the marinade or that none of the A colonies were killed. By the initial

inoculation levels, it was conceivable either way that no A colonies would have been selected, with a sample size of just 9.

Experiment 7: Acidity of the Marinade

Marinade formulations leftover from Experiment 6 were measured for their pH.

Marinade with 5% LEO	Marinade with 2% carvacrol	Marinade sans oil
3.07	3.03	3.03

Table 3: pH of the 3 marinades prepared for Experiment 5.

Despite the differences in composition, they varied little in their acidity. This demonstrates that the additions of particular oils throughout these experiments did not considerably affect the pH, which is worth noting because pH considerably affects bacterial survival and reproduction [20]. Had the pH varied appreciably between the marinades with or without the EOs, conclusions could not have been drawn regarding the efficacy of the oils. The pH of the marinade used in other experiments may have been slightly different due to the use of different lemons, which may have ripened differently.

Conclusion

Neither carvacrol nor LEO exhibited synergistic antimicrobial effects when in our lemon-based marinade. However, our marinade consistently acted to slow growth or to kill bacteria. Our marinade killed a large part of a *Salmonella* cocktail as well as a relatively smaller portion of a spoilage microbe cocktail. It successfully slowed the growth of psychrotrophic and mesophilic bacteria on a chicken substrate. Given that chicken marinated with LEO did not taste better than chicken marinated sans additives, and that chicken marinated with carvacrol tasted worse even than unmarinated chicken, neither LEO nor carvacrol should be added to the marinade. This research found no benefit conferred by the extra cost and effort to include those ingredients.

At most, the research displayed in this report was performed as one replication with triplicate samples. At least, some data comes from 1 replication of 1 number for 1 sample. This is below the standards of what would be acceptable to publish in a reputable journal. Therefore, all this research must be understood as preliminary research without maximum confidence in its veracity.

Further research could look into different additives and their antimicrobial effects. For example, *Thymus vulgaris* oil contains carvacrol, which this research found does not work synergistically with the marinade to effect antimicrobial destruction. However, *Thymus vulgaris* oil is effective against *Salmonella* on its own [21]. Perhaps it should be researched more in the marinade. Some other research could be done to find what it is about the marinade, or if it is indeed the marinade's responsibility, which overshadows or limits the effectiveness of the LEO and carvacrol's antimicrobial effects.

Poetry

These experiments used bacteria

And it cost money to grow 'em.

The grant was mostly spent on stuff

Other than this poem.

Poem 1: This poem is a reflection upon the generous Summer Research Fellowship Grant provided by the Shackouls Honors College and how it helped pay for research during this summer and autumn. Benjamin Wheeler is thankful for the grant and promises he didn't excessively use the funds to pay for his poetry.

Note from Dr. White.

It truly has been a pleasure working with Mr. Benjamin Wheeler. With many of the experiments in this body of work they were created due to his curiosity. The basis for these small experiments

were to provide him with the understanding of food spoilage and how the food matrix (marinade and or chicken) can negate the impact of very promising antimicrobials. Ben has been able to create somewhat a story of knowledge with what he has gained from completing these studies. I look forward to seeing where his scientific creativity will go.

Dr. White

Resources

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Name: Peyton York

Faculty Mentor: Mahesh Gangishetty

Major: Biochemistry

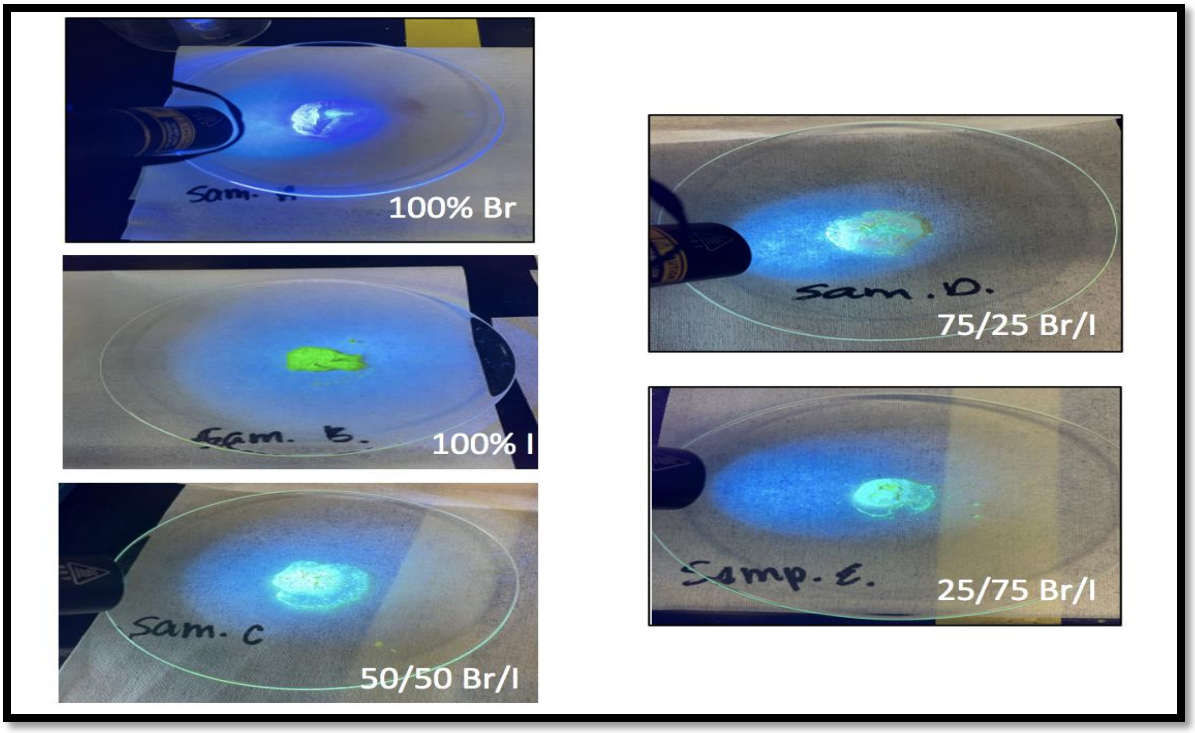
Department: Chemistry

Introduction:

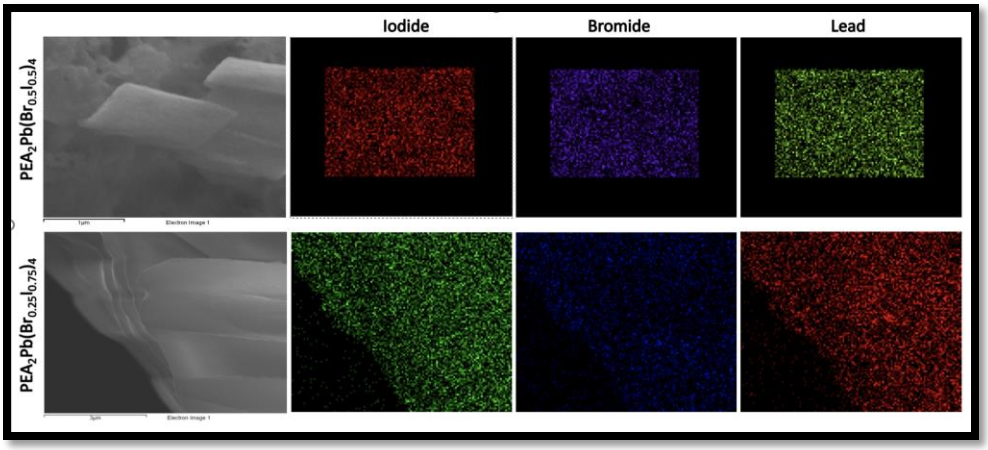
Recently, 2D Lead halide perovskites have been showing great promise as emissive layers in light-emitting diodes (LEDs). They have tunable optical properties with an ability of producing high color purity, and high photoluminescence quantum yields. The molecular composition for 2D-metal halide perovskites is $L_2[ABX_3]_{(n-1)}BX_4$, and L, A and X play crucial role in color tunability. "L" is a spacer molecule. The A is a small cation, which in our experiment is Cesium-bromide/iodide, and the B is a divalent Pb2+ ion, Neodymium ion or Manganese ion, and X is a halide ion. Specific to our research, the equation for perovskites is $[CsX]_2PbyNd_{(1-y)}X_4$. My long-term project is to optimize the production of 2D-halide perovskites, using bromine and iodine, to create environmentally stable, blue light-emitting diodes for their potential application into cellular and electronic devices. Using additional B-site dopants, like Manganese and Neodymium ions, we are attempting to tune the wavelength of emission towards 470nm, and optimize emission intensity of the perovskites. Additionally, I was working on a project to optimize the composition primarily by varying concentrations of bromine and iodine and developing methods to produce single crystals of lead halide perovskites. In this project, we are using P-XRD/single crystal XRD and SEM to observe the crystal structure, and UV spectroscopy to observe the emission of each perovskite composition.

Results:

In the figure below, five halide-metal perovskite crystals are shown of five different compositions. Starting at the top left, a composition of 100% Iodine ($[PEAI]_2PbI_4$), then 100% Bromine ($[PEABr]_2PbBr_4$) is shown. Following the 100% Br/I crystals, doping with both Bromine and Iodine concentrations was done in ratios of 50/50 and 75/25 for both elements. 100% Br perovskite emits a bright, strong blue color under UV light, and 100% I perovskite emits a bright green color under UV light. Changing the concentration ratios of bromine and iodine allows for the tuning of light absorption and emission of the perovskite, as pictured in the variation of concentrations below. Our goal is to produce a stable, long-lasting sky-blue emitting perovskite, with a wavelength around 470nm.

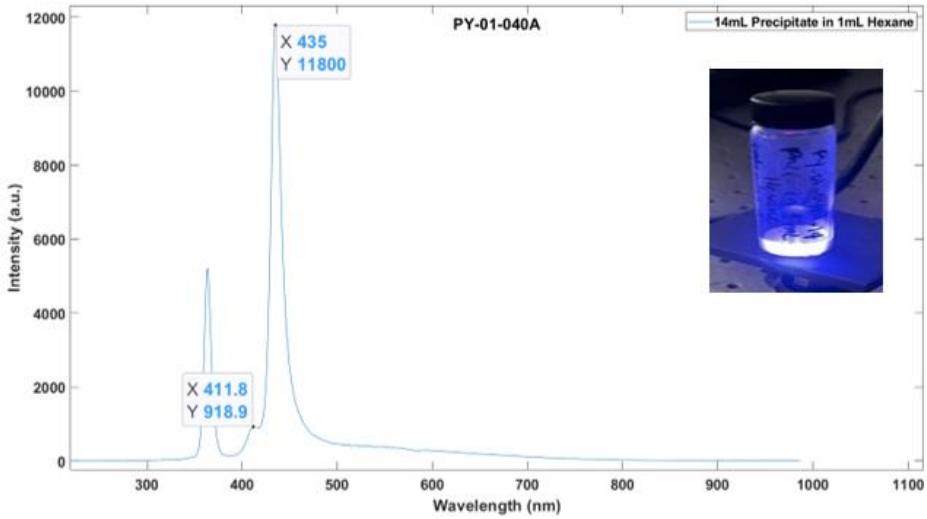


With these co-doped perovskites, the internal structure of how the dopants/halides are interacting with the crystal structure is a point of interest. Questions of homogeneity between the two halides were answered via a scanning electron microscope (SEM) analysis. Pictured below are the SEM images from the 50/50 and 25/75 (Bromine to Iodine) perovskite crystals. The images below show successful homogeneity between the Lead, Bromide and Iodide within the crystal. Thus, the crystallization of the perovskite solution is happening in equilibrium and the halide materials are not aggregating together, rather they are homogeneous.



Using Neodymium and Manganese, the B-site of the perovskite was additionally doped. Finding proper balance between the two dopants can potentially lead to heightened emission of light, as well as, more fine tuning of the emission wavelength. With Neodymium and Manganese, the goal is to target the 470nm wavelength for sky blue emission, with one intense emission peak on the PL spectra. Below, a

Manganese and Neodymium-doped perovskite solution is shown after precipitation and purification. There are two peaks shown, one at 411nm and one at 435 nm; the solution emission color is also shown in the top right of the graph. This result shows the potential for tuning of intensity and wavelength emission through the varying of Neodymium and Manganese-doping concentrations.



Future Work:

As halide-perovskites are ever-growing in commonality and importance in research and in light-applicative products, the research in understanding the physical structure and chemical interactions within the crystal is very important. With the SEM images, publication is currently being processed. However, continuation of research to further understand the structure and interactions of different dopants and elements within the perovskite will be done to fine tune perovskite composition and application.

This research helped to lay the groundwork for the translation of the Manganese and Neodymium-doping, along with other B-site dopants, with the Hot Injection Method. Getting a grasp on the underlying interactions between Manganese and Neodymium within the crystal structure, as well as, understanding how these dopants impact the emission of the solution in the PL spectra is important to larger applications, such as LED's. In the future, continuation of doping with various elements, and perfecting the process for spin-coating and LED fabrication must be done long term.

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