ABSTRACT

Title of Thesis:	STUDIES ON INTERACTIONS OF UV RADIATION WITH FOOD INGREDIENTS FOR IMPROVED QUALITY AND SAFETY
	Abraham Moses Montemayor, Master of Science, 2022
Thesis Directed By:	Dr. Rohan Tikekar, Associate Professor, University of Maryland – Department of Food Science and Nutrition

Ultraviolet (UV) radiation has been used in the food industry during processing, and with increased demands for safer and higher quality foods, UV-A and UV-B are being explored as antimicrobial treatments. This project consisted of three studies: the first study investigated the production of reactive oxidative species (ROS) by the action of UV radiation on fructose. The second study focused on evaluating the impact of UV-A irradiated chitosan-gallic acid (CH-GA) antimicrobial film on the quality of strawberries. The third study evaluated the effects of using UV-C individually or in combination with UV-A and UV-B to improve the fruit color and safety, respectively, of Honeycrisp apples.

It is known that fructose can generate ROS under thermal treatments and UV-C (254 nm) exposure. However, it is unknown whether UV-A or UV-B exposure can generate similar effects. For the first study, fluorescein, a fluorescent dye, was used as an indicator due to its known loss of fluorescence when exposed to ROS. Varying concentrations of fructose solutions combined with fluorescein were exposed to up to 1 J/cm^2 of UV-A or UV-B radiation. Ascorbic acid (AA), a known ROS scavenger, was added to the fructose-fluorescein solutions prior to UV exposure to verify ROS

generation. The fluorescence was measured at 485 nm (excitation) and 510 nm (emission), respectively. A storage study was done to determine whether ROS continued to generate following UV exposure. Fructose-fluorescein solutions were exposed to 0.1 J/cm^2 of UV-B radiation and stored at 4°C or 37°C. The UV-B exposure of fructose-fluorescein showed a dose-dependent fluorescence decay, whereas UV-A did not elicit this response. Fluorescein degradation followed first-order kinetics, as indicated by the rate constants. The rate constants in the presence of 10-, 50-, and 100- mM fructose were 0.7±0.01 J/cm^2, 4.3±0.6 J/cm^2, and 0.3±0.03 J/cm^2, respectively. However, in the presence of AA, fluorescein degradation deviated from first-order kinetics. The storage study indicated no significant difference between the UV-B exposed and control solutions, indicating ROS generation ceased after UV-B exposure. The results of the studies using control solutions were extrapolated to coconut water, a commonly consumed beverage. UV-B exposure did have a degradation effect on AA, but the ROS generated did not affect the AA. The ROS was produced only when fructose was exposed to UV-B. ROS can have adverse effects on the organoleptic properties of foods containing fructose, and the addition of AA can help quench ROS in a concentration-dependent manner.

The second study evaluated quality parameters such as color, texture, pH, total soluble solids, and titratable acidity of strawberries coated with an edible chitosan-gallic acid (CH-GA) coating. The strawberries were dipped in the CH-GA solution and allowed to dry. The coated strawberries were exposed to UV-A with appropriate, unexposed controls also being used for comparison. Previous studies have indicated that the coating can exhibit moderate antimicrobial activity when irradiated with UV-A at 360nm. A 180-minute exposure reduced *Escherichia coli (E. coli)* O157:H7 on CH-GA coated strawberries by ~2-3-log CFU/mL. However, when the quality parameters were evaluated, it was found that the UV irradiated strawberries may have been initially affected with respect to color and texture, but the loss in quality slowed down over a 14-day refrigerated storage period. It was also seen that no significant differences were observed in color and firmness between the control and experimental groups on day 14.

The third study (appendix 1) aims to evaluate UV-C radiation's efficacy on the inactivation of *Listeria monocytogenes (L. monocytogenes)* on apple surfaces. This study was performed within the broader aim of evaluating the effects of UV-A, UV-B, and UV-C and their combinations on the quality and safety of Honeycrisp apples. UV-C radiation can serve as an antimicrobial agent, while UV-A and UV-B radiations can affect the quality parameters such as color through the hormetic effect. Therefore, our goal was to identify optimum UV-A, UV-B, and UV-C radiation doses that can be applied to Honeycrisp apples to improve their coloration and microbial safety as the marketability of apples often depends on the redness of the fruit. The UV-C dose of 7.5 kJ/m^2 resulted in a 1.2±0.06 log CFU/sample inactivation of *L. monocytogenes* on the apple surface. Interestingly, the additional UV-C dose exposure did not result in additional inactivation. This observed lack of dose-dependence could be the result of a) UV-C penetration interference from previously inactivated microbial cells resulting in a shadowing effect, b) the formation of a biofilm during ambient air drying and 4°C incubation that provided some protection during treatment, or c) higher resistance of *L. monocytogenes* sub-population against UV-C inactivation. This data will allow for future exploration of a synergistic treatment that can improve the color and appearance of Honeycrisp apples and improve their safety at the same time.

UV radiation has shown promising antimicrobial activity and, through the studies carried out in this project, demonstrated potential beneficial or deleterious effects on food quality. The results from the first study showed the significance of understanding the interaction of food ingredients with UV radiation. The strawberry and apple studies show that UV radiation, when used at the correct dosage, can increase, or maintain the visual appearance of the fruits, making them more marketable. When used at the correct wavelength and for the appropriate duration, UV radiation can mitigate the prevalence of foodborne pathogens and contribute to food products' quality and shelf life.

STUDIES ON INTERACTIONS OF UV RADIATION WITH FOOD INGREDIENTS FOR IMPROVED QUALITY AND SAFETY

by

Abraham Moses Montemayor

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Advisory Committee:
Dr. Rohan Tikekar, Chair
Dr. Abani Pradhan
Dr. Cheng-I Wei

Dedication

This work is dedicated to my best friend, Dr. Forrest Craig, who has always shown me love, support, encouragement, and on occasion, a swift kick in the rear-end when needed. You have always motivated me by example to be a better man than I was yesterday. Thank you for always being authentically you.

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Chapter 1: Literature Review

UV in Food Processing

UV radiation, types, and specific wavelengths

Electromagnetic radiation extends across wavelengths, referred to as the electromagnetic spectrum. This spectrum includes gamma rays, which possess shorter wavelengths that are generally shorter than 0.1 nm [1], higher frequency, and higher energy, through radio waves with longer wavelengths that can range from thousands of meters to 30 cm, lower frequencies, and lower energy [2]. Sunlight striking the earth's surface ranges from 100 nm to 1 mm [3]. UV can be found in this spectrum from 100 nm to 400 nm, with three separate categories: UV-A, UV-B, and UV-C. In an indoor environment, we are still exposed to the electromagnetic spectrum by the light emissions from sources such as fluorescent or incandescent bulbs that can emit light that can range from 320 nm, which is considered UV-A, to 700 nm [4].

Differences between UV-A, UV-B, and UV-C radiation

The range for UV-A is 315 nm to 400 nm, UV-B is 280 nm to 315 nm, and UV-C is 200 nm to 280 nm [5] . UV-A is commonly associated with skin damage and its ability to increase skin pigmentation [6]. UV-B increases melanin production in human skin and increases the risk of skin cancer [6]. UV-C, by contrast, is best known for its germicidal properties since it can destroy bacteria and viruses [7].

Mechanisms of action of UV light

UV-C, in general, is believed to inflict damage to the nucleic acids, DNA, and RNA, ultimately preventing the transcription and replication functions of the cell, inactivating the

microorganism [8]. Further studies have shown that UV-C can affect pathogens differently depending on the wavelength. For instance, UV-C at 254 nm is known to affect DNA and RNA to prevent replication; while UV-C at the 207 nm to 222 nm wavelength range is believed to damage the proteins on the pathogen's surface, which is essential for the attachment of the pathogen to other cells [8]. It is believed that since UV-C is absorbed in the earth's atmosphere, pathogens have had millions of years to adapt to the effects of UV-A and UV-B but remain vulnerable to UV-C due to a lack of exposure [9]. UV-A and UV-B have little to no germicidal effect on pathogens. However, there is evidence that UV-A and UV-B can inflict oxidative stress, which can then cause damage to DNA or cause lipid peroxidation, eventually leading to the destruction of the cell [10]. Specific wavelengths of UV radiation can also have other applications, UV radiation below 230 nm can be used to disassociate chemical compounds, and 185 nm can produce ozone, making it optimal for environmental or air applications [5].

UV radiation sources

The sun is not the only source of UV light. UV radiation can be emitted from low (LPM) or medium pressure mercury (MPM) lamps, pulsed light lamps (PL lamps), excimer lamps (EL), and light-emitting diodes (LED) [5]. Each UV radiation source has its benefits and limitations that should be considered depending on its application and intended use. LPM lamps can emit wavelengths of 253.7 nm and 185 nm, making this bulb effective for germicidal and chemical compound disassociation applications [5].

Applications of UV radiation

Applications of UV radiation have included air, water, and surface disinfection. Disinfecting air using UV radiation involves 253.7 nm for its germicidal effect and 185 nm for generating ozone in the air [5]. When 185 nm is combined with 253.7 nm, it can inactivate

viruses, bacteria, molds, and airborne spores, making it a very effective photochemical air treatment alternative [5]. Proper application and engineering of UV radiation in air systems can also enable the oxidizing effect to remove offensive odors and break down grease build-up [5]. The application of UV in air systems can also be found in hospitals or food processing areas where air quality and sanitation are essential factors. The geometry of the air system and the intensity of the UV radiation selected will directly impact the UV light's ability to sanitize, sterilize, or break down substances [11]. In water, the application of UV-C radiation can include potable water systems, computer or microchip manufacturing centers, cooling towers, pharmaceutical laboratories, or any place where traditional chemical treatment of water is not allowed or feasible [11]. Surface treatment using UV-C is an excellent alternative when the material is incompatible with traditional heat sterilization techniques [11]. Examples of such items include bottle closures, cartons for food products, medical equipment, food processing equipment, or any previously cleaned surface free of debris.

Applications of UV radiation in food

UV light systems in food are still a novel concept, with new applications being explored every day. The appeal of UV radiation in food processing is primarily driven by its being non-thermal, dry, and relatively low in production and maintenance costs [11]. In liquid foods, UV-C can be used as a form of non-thermal pasteurization. In solid foods, such as fresh fruits and vegetables, UV-C radiation can be used to reduce the microbial load that might initially be found on the surface of raw products. It can also increase a product's resistance to a microorganism, called the hormetic effect [12]. This effect is thought to elicit the production of phenylalanine ammonia-lyase, which can stimulate the production of phenolic compounds [12]. These compounds have increased resistance to fungus in sweet potatoes, increased microbial resistance in citrus, and delayed the ripening of fruits [12]. Fresh fish, meat, and poultry can also be treated with UV-C radiation on the surface. Treatment before

refrigeration can have a 3-log reduction in surface microbiota; this reduction can positively affect the shelf life of the stored product [11].

Regulatory guidelines for the use of UV in food systems

The use of Ultraviolet radiation in food processing is regulated by the code of federal regulations (CFR) Title 2, part 179.39 – Ultraviolet radiation for the processing and treatment of food. This regulation specifies that the radiation must come from an LPM, with ninety percent of the wavelength emitted at 253.7 nm [13]. For food and food products, UV radiation can control microorganisms on the surface [13]. Limitations of this application in the absence of ozone require that high-fat foods are treated in a vacuum or in an inert atmosphere with a minimum radiation intensity of 1 W per 5 to 10 ft [2,13]. Water used in food production can also be sterilized using UV light. Water treatment without ozone production must have a coefficient of absorption ≥ 0.19 per cm, a flow rate of 100 gallons per hour per watt of 2,537 Å radiation, the water depth must be ≤ 1 cm, and the lamp temperature must be between 36°C and 46°C [13]. In juice, UV radiation can be used to reduce human pathogens and other microorganisms. Its use in juice processing requires a turbulent flow through tubes with a Reynolds number $\leq 2{,}200$ [13]. Under Title 21 CFR Part 120.24, juice processors must be able to demonstrate, at a minimum, that the control measures implemented in their HACCP plan can achieve a 5-log reduction in the pertinent microorganism[14]. Systems such as the CiderSure 3500 use UV radiation to meet or exceed these requirements set forth by the FDA. Food Processing Equipment, Inc. was established in 1998 by Phil Hartman; through his work with Cornell University, he created the CiderSure system, which was the first UV pasteurization system to be approved by the FDA for juice pasteurization [15]. The CiderSure 3500 System operates using a peak wavelength of 245 nm with an operating range of 4979 to 20,331 $\mu J/cm^2$ [16]. Studies have shown that to achieve a 5-log reduction of *E. coli*, a dose of 6,500 $\mu J/cm^2$ is sufficient [16]. The other pathogen of concern in apple juice is

Cryptosporidium Parvum (*C. Parvum*); the *C. Parvum* oocysts can be more resistant to UV radiation treatments than *E. coli* [17]. However, studies have shown that using the CiderSure 3500, a dose of 14.32 mJ/cm^2 for 1.2 to 1.9 seconds can achieve a reduction greater than 5-log of *C. parvum* oocysts [17].

Benefits

Consumer trends are moving in a direction forcing the food industry to explore more 'natural' and 'healthy' food production and preservation methods. UV radiation, specifically UV-C, has been studied extensively and has already proven to have several areas in which it has many advantages and benefits that align with consumer demands. In food processing, it is gaining popularity as a non-thermal processing technique that can reduce microbial loads on surfaces, extend shelf-life, and replace traditional thermal processing techniques such as pasteurization. Pasteurization has been the preferred method of reducing the microbial load of food products such as fluid dairy. However, the high temperatures and exposure time can result in undesirable changes to a product's flavor, aroma, texture, and color [18]. Thermal processing impacts other quality aspects of food that the consumer does not always notice. These include loss of vitamin content, protein denaturation, enzymatic inactivation induction, and bioactive compound reduction [19]. UV radiation treatments are also more energy-efficient and often require shorter processing times when compared to traditional thermal treatments resulting in lower energy consumption and increased product value [19]. UV radiation treatments can also increase the value of food products by enhancing their functional properties. As mentioned previously, the hormetic effect can elicit or strengthen natural resistance properties in foods. This effect can also elicit the formation of phenolic compounds, which can increase antioxidants within plant tissue and increase or enhance their nutritional or functional value [20]. Table 1 below contains examples of fresh fruits and vegetables, UV treatment parameters, and nutritional outcomes that resulted from the UV

Table 1 – Effects of UV radiation on functional properties of select food items

Product	UV-Dose	Results	Reference
Apples	UV-B – 0.20 W/m^2	+56% anthocyanins +12-15% quercetin glycosides +142% chlorogenic acid +6.5% ascorbic acid	[21], [22]
Blueberries	UV-C – 4.30 kJ/m^2	+54% anthocyanins +30-85% quercetin glycosides +11% chlorogenic acid +33.5% resveratrol	[23]
Peaches	UV-C – 2.47 kJ/m^2	+35% Putrescine +44% Spermidine +40% Spermine	[24]
Mushrooms	UV-C - 6.06 kJ/m^2	+173% Vitamin D_2	[25]
Mushrooms	UV-B – 4.93 kJ/m^2	+387% Vitamin D_2	[25]
Strawberries	UV-C – 2.15 kJ/m^2	+18.5% antioxidant capacity +30% phenolic content	[26]

treatment. While UV radiation has already been applied and used effectively in surface, water, and air sanitation for years, there is still a wide array of uses in the food processing

industry that remain to be fully explored. The dairy industry is one area where the product's organoleptic properties are crucial to success with consumers. Table 2 shows several food products being explored with UV radiation, their target pathogen, and the achieved results of UV-C application.

Table 2 – Effects of UV radiation on microbiological inactivation of food products

Product	**Pathogen**	**Survivability**	**Reference**
Cow Milk (Raw)	*Staphylococcus aureus*	0.55 – 7.26 log CFU/mL reduction	[27]
Goat's Milk	*Listeria monocytogenes*	>5 log CFU/mL reduction	[28]
Cottage Cheese	*Pseudomonas spp.*	1.5 log CFU/mL reduction	[19]
Mozzarella Cheese	*Pseudomonas spp., Enterobacteriaceae*	1 log CFU/mL reduction	[29]
White Wine (grape must)	*Metschnikowia pulcherrima*	4 log CFU/mL reduction	[30]
White Wine (grape must)	*Hanseniaspora uvarum*	6 log CFU/mL reduction	[30]
Sliced Ham (RTE)	*E. coli* O157:H7	1.52 log CFU/g reduction	[31]
Sliced Ham (RTE)	*Salmonella Typhimurium*	1.73 CFU/g reduction	[31]
Sliced Ham (RTE)	*Listeria monocytogenes*	1.55 CFU/g reduction	[31]

UV-C radiation has been the most widely studied in the UV spectrum because of its direct inactivation capabilities. However, there is still much to be explored with UV-A and UV-B. As discussed earlier, most UV-C emanating from the sun is blocked, reflected, or absorbed by our atmosphere. This means that the remaining UV radiation that makes it through our atmosphere is approximately 95% UV-A and 5% UV-B [32]. UV-A and UV-B have had much work on the physiological effects they can have related to skin damage and skin cancer in humans [32]. However, very little has been done to study the effects of these wavelengths in food compared to UV-C. Both wavelengths have significant effects via photosensitizing compounds and type 1 and type 2 mechanisms that produce ROS, which in turn can produce oxidative damage to proteins, lipids, and cell DNA [32]. UV-A has been used in combination with benzoic acid to create a synergistic effect that resulted in >5 log reduction of *E. coli* O157:H7 in the simulated washing of spinach [33]. UV-A has also been used in combination with gallic acid and lactic acid to achieve a 4.7±0.5 log reduction of *E. coli* O157:H7 [34]. In both studies, the combinations implementing UV-A's use increased membrane permeability, enhanced oxidative stress, and increased antimicrobial activity [33,34]. UV-B radiation has extensive plant and human sciences research for its effects on the cells. However, very little has been done to examine its effects on foods outside of plant science. However, the effects of UV-B radiation warrant further study as its mechanics could have significant implications for food production. A study on UV-B radiation during the postharvest processing of tomatoes showed the ability of UV-B to increase levels of ascorbic acid (AA) and carotenoids [35]. However, the same UV-B treatment had an adverse effect on the firmness of the treated tomatoes, leaving room for further studies to explore the cause and mechanisms of this adverse effect [35].

Limitations

UV processing technology is not without its limitations. When evaluating this technology's applicability as an alternative to pasteurization, the product characteristics such as color, composition, method of exposure, and exposure time must be carefully examined. One of the most important things that must first be considered is how the light moves through the fluid being pasteurized. Clear products like water have high light transmissivity, making it an easy and excellent candidate for treatment with UV-C light. However, products like juice contain significant amounts of organic compounds and other particulates that can drastically lower the transmissivity [36]. In most cases, UV radiation penetration in juices is limited to a depth of 1 mm [37]. When the product's turbidity increases and the UV-C light's transmissivity is lowered, the penetration is reduced and desired germicidal effect is negatively impacted [38]. In a study of patulin degradation in a CiderSure UV system, the lower degradation rate between apple cider and apple juice was attributed to the increased scattering that occurred due to the suspended organic particles [39]. The higher turbidity in the unfiltered apple cider reduced the effectiveness to such a degree that it was deemed impractical in the study [39]. The need for relatively low turbidity can limit what products are optimal for this pasteurization method or require alterations to flow rate, volume, and intensity to overcome challenges associated with penetration. In some cases, these adjustments could negatively affect the product's organoleptic properties [40]. Another challenge associated with treatments that utilize UV radiation is a cell's ability to reverse the germicidal effects of UV radiation. As mentioned before, UV-C radiation damages the cell's DNA to prevent its reproduction. Depending on the organism, inherent repair enzymes are enhanced by specific wavelengths of blue light in the visible light spectrum; this is called photo-reactivation [41]. Similar to how stressful environments can strengthen bacteria, photo-reactivated cells can build a resistance to UV-C treatments and make it harder to achieve the

desired reduction in the microbial load of the product if they are being exposed to a UV-C for a second time [41]. UV radiation can also cause changes in chemical composition, initiate free radical oxidation, and induce carbohydrate crosslinking and protein fragmentation when applied at high doses, requiring extensive study and analysis to prevent quality loss and ensure effective microbial reduction [42]. When configuring UV application systems, it must be taken into account that most LPM bulbs have a life expectancy between 6000 and 8000 hours, which equates to about one year of continuous use [43]. Over the course of the bulb's life span, the emission efficiency of the bulb can drop as much as 30% [43]. The most efficient and practical UV lamps or bulbs use mercury as one of their main components [44]. Mercury, however, is a heavy metal and a highly toxic substance [45]. Each lamp can contain several milligrams of mercury, meaning that these lamps' production and disposal require great care to avoid damage to the environment and risk to human health [45]. Photooxidation is another factor that must be considered and understood as the components of each product can have varying interactions when exposed to UV radiation. UV radiation can induce lipid oxidation directly with the dissociation of *bis*-allylic hydrogen, creating alkyl radical L* or induce lipid oxidation through the generation of ROS [46]. In red meats, hemoproteins, such as myoglobin, contribute to lipid oxidation when exposed to UV radiation by serving as a photosensitizer in the food or product [46]. When ROS is generated, nutritive compounds such as vitamin D or riboflavin can react, leading to photodestruction [46]. Table 3 below shows the adverse effects varying UV treatments can have on some food products' physical, sensory, and nutritional properties.

Table 3 – Effects of UV exposure on selected quality parameters in food products.

Product	**UV radiation source and dose**	**Results**	**References**
Milk pasteurized (3% milk fat)	LPM lamp - 234 minutes, 19 seconds	Vitamin C mg/L – −35.13±1.56	[18]
Fructose (30% w/v)	LPM lamp - 4 minutes, 56 seconds	pH - −1.94±0.10 Color a* - −0.99±0.06 b* - −6.34±0.28	[18]
Apple Juice	LPM lamp - 140 minutes, 38 seconds	Vitamin C mg/L - −1.30±0.07 Color a* - −5.68±0.31	[18]
Goat milk	LPM lamp - 18 seconds	UV treated goat milk had an aroma similar to manure	[28]

However, the adverse effects of UV exposure can also be unintentional and the result of storage conditions. One study on UV exposure in milk reported up to a 30% loss of riboflavin in milk after 30 minutes of exposure to sunlight and 80 – 100% of vitamin C after 60 minutes of exposure to sunlight [47].

Fructose

Basic and structural properties of fructose

Fructose was discovered in 1847 by French chemist Augustin-Pierre Dubrunfaut 1847 [48]. However, English chemist William Allen Miller gave the actual name fructose in 1857 [49]. In its dry form, fructose is a white crystalline solid with a sweet flavor, odorless, and

water-soluble [50]. Fructose belongs to a class of carbohydrates called monosaccharides and is one of the two monosaccharides of the disaccharide sucrose [51]. Fructose is often found in fruits or plants, so it is sometimes referred to as "fruit sugar."

Table 4 – Fructose content of foods

Food (raw)	**Fructose (free fructose % by fresh weight)**
Apples	7.6% by fresh weight [50]
Apricots	0.7% by fresh weight [50]
Bananas	2.7% by fresh weight [50]
Cherries, sweet	6.2% by fresh weight [50]
Coconut juice, young	2.29 g/100 mL [52]
Figs	2.8% by fresh weight [50]
Grapes, American	6.9% by fresh weight [50]
Peaches	1.3% by fresh weight [50]

To understand the potential interactions with fructose, we must closely look at the chemical structure. Fructose is a ketose since it contains one ketone group per molecule and is also known as a hexoses, since it is a monosaccharide with six carbons, and the carbonyl group is located on the second carbon atom [53].

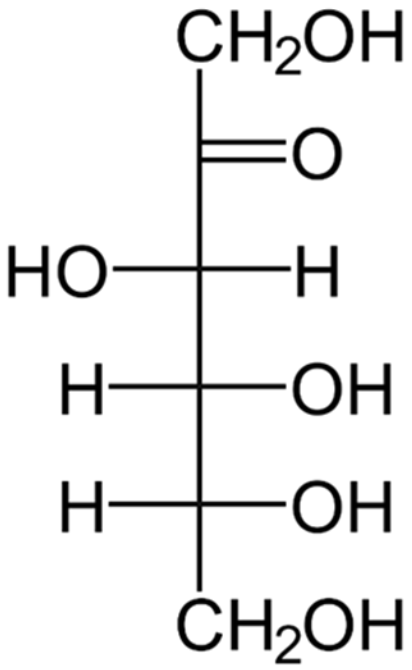

Illustration 1: Open chain form of fructose [54]

The carbonyl functional group in fructose is a ketone group responsible for its

chemical reactions [54]. The carbonyl group is a carbon atom with a double bond to an oxygen atom [55].

Reduction and oxidation reactions of fructose

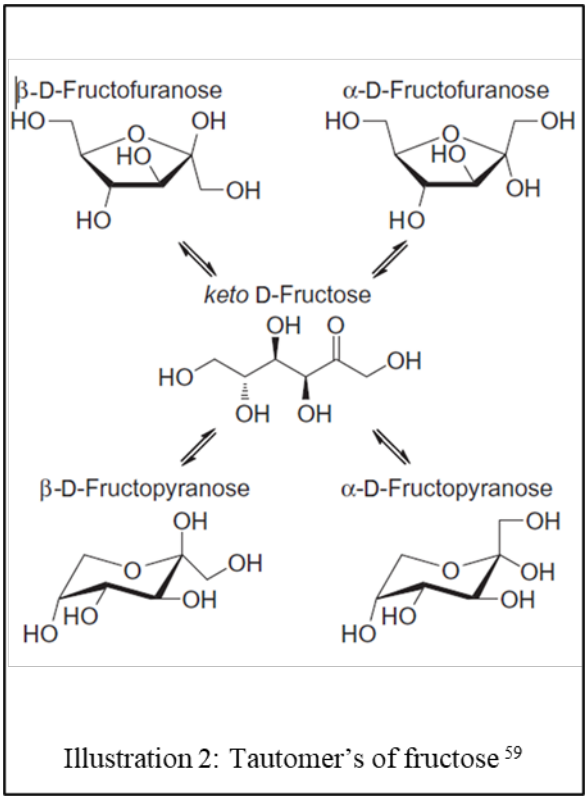

Illustration 2: Tautomer's of fructose [59]

The aldehyde or ketone groups are the functional groups responsible for the reducing ability of sugars, including fructose [56]. However, before fructose can act as a reducing sugar, it must first tautomerize to an aldose [57]. Tautomerization is when chemical compounds readily interchange; typically, this interchange is caused by the movement of a hydrogen atom within the compound [58]. In solution, fructose has five tautomers: ß-D-fructopyranose, α-D-fructopyranose, ß-D-fructofuranose, α-D-fructofuranose, and *keto* D-fructose [59]. Pyranose indicates that the molecule has a structure that consists of a six-member ring: 5 carbons and one oxygen [57]. Furanose indicates that the molecule has a structure that consists of a five-member ring: 5 carbons and one oxygen [57]. While in its open-chain form, after a series of tautomeric shifts, fructose can produce an aldehyde group when exposed to UV radiation [57]. The newly formed aldehyde group can then undergo a redox (reduction-oxidation) reaction and be oxidized [57]. The oxidative nature of fructose by the ROS generated when exposed to UV-C radiation was demonstrated in a study using d-fructose and fluorescein sodium salt and AA [60]. This study compared the ROS potential of sucrose and glucose alongside fructose. Sucrose and glucose did not show any degradation in fluorescein after 12 minutes of

exposure to UV-C [60]. Fructose, however, demonstrated a 90% degradation in fluorescein after only 2 minutes of exposure [60]. This same study also showed a concentration-dependent decay of fluorescein when exposed to UV-C radiation [60]. The rate of fluorescein degradation when exposed to UV-C followed first-order kinetics at concentrations of 10-, 20-, 100-, 300-, and 500- mM of fructose [61]. The increase in fructose concentration increased the decay rate until the concentration reached 300 mM of fructose; results at this concentration indicate an excess and no longer showed a statistically significant increase in decay [60].

Interactions of fructose and UV radiation

Fructose absorbs the UV-C photons in the open-chain form and cleaves the carbonyl group in its excited state, leading to hydroxyalkyl and acyl radicals [61]. These free radicals can then undergo additional reactions producing additional free radicals such as peroxyl radicals, superoxide radicals, and hydrogen peroxide (HP) [61]. In juice products containing AA, the free radicals formed reacted with AA, a known antioxidant, resulting in its degradation [62]. One study examining AA degradation in UV-C apple juice processing revealed that when compared to juice with no sugar added, 50% degradation of AA after 7.14±0.13 J/cm^2, sucrose, 50% degradation of AA after 6.97±0.56 J/cm^2, did not have a significant effect on AA degradation [62]. Glucose, in turn, produced a slight protective effect with its 50% reduction in AA occurring after 8.86±0.37 J/cm^2 [62]. However, the most significant finding in this study was that the presence of fructose resulted in a significant increase in concentration-dependent AA degradation[62]. The UV-C dose needed in this study for AA to achieve a 50% reduction in the presence of 0-, 2-, 5-, and 10% fructose was 7.14±0.13, 4.77±0.24, 2.91±0.08, and 1.46±0.09 J/cm^2, respectively [62]. In juice sweetened with high fructose corn syrup, which contains fructose, furan formation was elevated when the juice was exposed to UV-C radiation [63]. Elevation in furan levels was also demonstrated in apple cider and plain fructose solution exposed to UV-C [64]. However, it is essential to note that minimal furan

formation (<1 ppb) occurred when used to achieve the required 5-log reduction of *E. coli* O157:H7 [64]. Meaning that furan formation occurred only when the apple cider was overtreated with significant amounts of UV-C radiation did furan formation occur[64]. In another study, hydroxymethylfurfural (5-HMF), a furan derivative considered potentially carcinogenic, can be formed in sugar products like high fructose corn syrup (HFCS). This furan derivative proved to have a different interaction with UV-C light [65]. When HFCS was treated with UV-C radiation, it not only had an antimicrobial effect, but the UV-C radiation reduced 5-HMF by 43 – 62% that was generated by prior thermal treatment, as well as had a restorative effect on quality attributes that may have been lost to thermal treatments such as color and aroma [65].

Fluorescein

Structure and properties of fluorescein

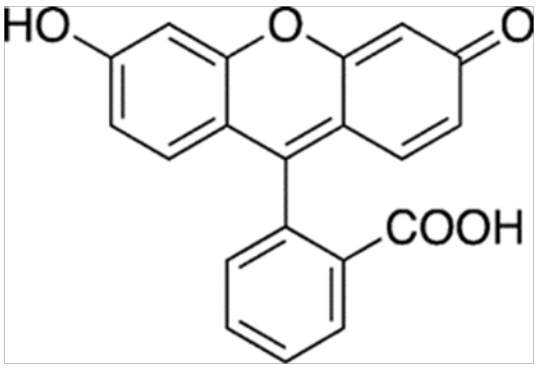

Illustration 3: Chemical structure of fluorescein [67]

Fluorescein is a fluorophore used in microscopy that absorbs light at a set wavelength and emits a longer wavelength called fluorescence [66]. As fluorescein absorbs light energy, it enters an excited state, and as this absorbed energy decays, the fluorescein enters an emission state; both the excitation and emission state are a function of wavelength [67]. Fluorescein has a broad emission spectrum with a peak of 490 nm and an emission peak of 525 nm [67].

Fluorescein and its use to detect antioxidant activity

Fluorescein is sensitive to oxidation and can be used to detect ROS, including hydroxyl and peroxyl radicals [68]. The oxidized fluorescein reaction mechanism follows a classic hydrogen atom transfer (HAT) mechanism [69]. When fluorescein is used as an indicator, the loss of fluorescence indicates damage or decay that has occurred by ROS [70]. Thus, the area under the loss curve can indicate 'antioxidants' protective effect [70]. Compared to other indicators such as B-phycoerythrin and R-phycoerythrin in studies, Fluoresceinas proved to be less interactive, has greater photostability, and is a more cost-efficient testing standard when measuring chain-breaking antioxidant activity [70]. The use of fluorescein to measure antioxidant activity has proven to be quite versatile as it has also been used to measure hydrophobic and lipophilic antioxidants [71]. This allows for the total antioxidant capacity of a sample to be measured using similar conditions and standards [71].

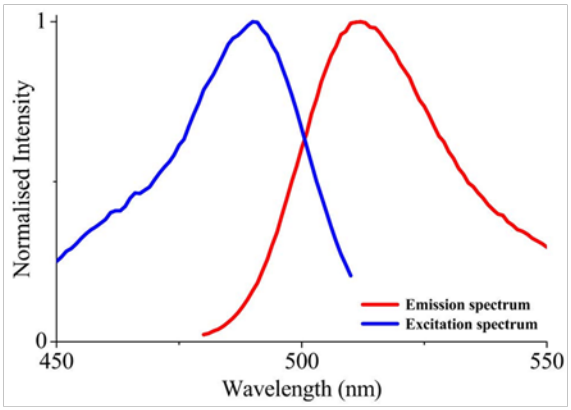

Illustration 4: Excitation and emission spectrum of fluorescein [67]

Ascorbic Acid

Structure and properties of ascorbic acid

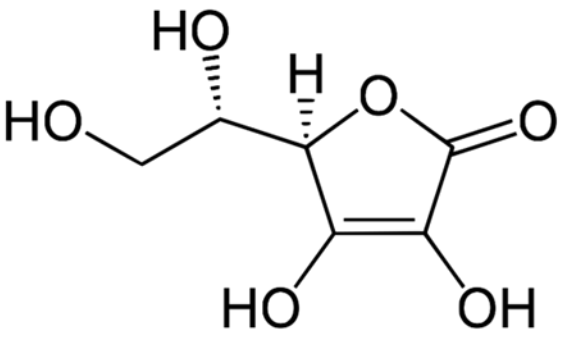

Illustration 4: Chemical structure of ascorbic acid [72]

Ascorbic acid (AA), also known as vitamin C is a naturally occurring water-soluble vitamin [72]. It was discovered in 1912, later isolated in 1928, and finally synthesized in 1933 [73]. AA is a white to light yellow, odorless, sharp, or sour tasting crystalline powder [72]. It is a potent antioxidant and reducing agent used in the food, cosmetic, nutritional, and medical industries [72].

Oxidation and reduction properties of ascorbic acid

AA is often referred to as an antioxidant because of its capabilities as a reducing agent and can reduce oxidized species [74]. However, as AA donates its electrons to reduce metals such as copper or iron, superoxide, HP, and reactive oxygen species can be formed [76]. This indicates that AA can serve as an antioxidant as well as a pro-oxidant under specific conditions.

Ascorbic acid and UV radiation interactions

AA displays a strong ability to absorb UV radiation in the range of 220 nm to 300 nm, making it sensitive to most UV-C and UV-B wavelengths [75]. AA can oxidize when exposed to UV radiation to produce free radicals [76]. This process can be amplified or accelerated when in the presence of photosensitizers such as riboflavin [77]. Studies have

shown that with increased riboflavin content, AA degradation also increased [77]. For example, 6 ppm of riboflavin resulted in 100% degradation of AA within 12 minutes of exposure to light, but a sample without riboflavin only experienced 2% degradation of AA [77].

Conclusion

This study aims to fill in the gaps in the current understanding of UV light and products containing fructose. UV-C radiation, its interactions, and its applications in food products and systems have been thoroughly investigated. What has not been thoroughly explored are the mechanics, effects, and interactions that UV-A and UV-B can have in food systems and products, or can the current uses of UV-C radiation be paired with UV-A or UV-B to enhance both the quality and safety of food products and systems. Understanding the interactions of UV-A, UV-B, and UV-C will enhance and enable researchers and manufacturers to mitigate the potential adverse impacts of UV-A and UV-B. This information can also allow for novel applications or alternatives to current food processing techniques that may be associated with adverse effects on quality in exchange for safety outcomes.

Chapter 2: Elicitation of Reactive Oxidative Species (ROS) from Ultraviolet Irradiation of Fructose Solution

Abstract

It is known that fructose can generate ROS under thermal treatments and UV-C (254 nm) exposure. However, it is unknown whether UV-A or UV-B exposure can generate similar effects. Fluorescein, a fluorescent dye, was used as an indicator due to its known loss of fluorescence when exposed to ROS. Fructose solutions (0-, 10-, 50-, and 100- mM) combined with 2 μM of fluorescein were made in deionized (DI) water. A 75 mL sample was exposed to up to 1 J/cm^2 of UV-A or UV-B radiation. Periodically, a 100 μl sample was taken, and fluorescence was measured using an excitation and emission wavelength of 485 nm and 510 nm, respectively. Up to 10 μM of AA, a known ROS scavenger, was added to the treatment solutions prior to UV exposure to verify ROS generation. To determine whether ROS continued to generate following UV exposure, solutions were exposed to 0.1 J/cm^2 of UV-B radiation and stored at 4°C or 37°C. Samples were taken every 24 hours for 72 hours, and fluorescence was measured. Experiments were performed in triplicate. UV-B exposure of fructose showed a dose-dependent decay of fluorescence from fluorescein. UV-A did not elicit this response. Fluorescein degradation followed first-order kinetics, where the rate constants in the presence of 10-, 50-, and 100- mM fructose were 0.7±0.01 J/cm^2, 4.3±0.6 J/cm^2, and 0.3±0.03 J/cm^2 respectively. In the presence of AA, fluorescence decay deviated from first-order kinetics, and at 0.5 J/cm^2 UV-B exposure, relative fluorescence values reduced to 10.3±3.7%, 34.7±0.2%, 78.5±2.0% in the presence of 0-, 5-, and 10- μM AA, respectively. Concentration-dependent slowing of fluorescence decay by AA demonstrates its ability to quench ROS generated from UV-B exposure of fructose. The storage study revealed no difference in fluorescence decay between UV-B unexposed control

and treatment, indicating ROS generation ceased after UV-B exposure. The degradation of AA in UV-B exposed coconut water was studied to examine the behavior of UV exposed fructose in an actual consumer product. Results showed that exposure to UV-B caused AA degradation in coconut water. However, further addition of fructose to coconut water prior to UV-B exposure did not accelerate AA degradation ($P>0.05$), indicating a limited impact of fructose on the nutritional properties of UV-B exposed coconut water. Thus, despite its lower antimicrobial potential than UV-C, UV-B may be a better antimicrobial treatment for liquid foods containing fructose to reduce the impact on organoleptic properties.

Introduction

UV has emerged as a novel food processing technique that has a variety of applications. Applications include air, water, and surface disinfection. The appeal of UV radiation in food processing is primarily driven by it being non-thermal, dry, and relatively low in production and maintenance costs and its ability to inactivate bacteria [11]. UV can be found on the electromagnetic spectrum from 100 nm – 400 nm, with three separate categories: UV-A, UV-B, and UV-C. Specifically, UV-A is 315 nm – 400 nm, UV-B is 280 nm – 315 nm, and UV-C is 200 nm – 280 nm [5]. UV-C is the most explored range of UV radiation due to its ability to inflict damage to the nucleic acids, DNA, and RNA, ultimately preventing the transcription and replication functions of the cell, inactivating the microorganism [8] Previous studies have shown that the effectiveness and outcome of UV in food processing is heavily dependent on the type of UV being used, the intensity of the UV light, and characteristics of the product being treated such as turbidity, and flow [7,11,60,78]. Fructose is a carbohydrate type known as a monosaccharide and is one of two monosaccharides of the disaccharide sucrose [51]. Fructose is often found in fruits or plants, so it is sometimes referred to as "fruit sugar" and is quite common in everyday consumer products. For example, apples (7.6% by fresh weight), bananas (2.7% by fresh weight),

coconut juice (2.29% by fresh weight), and grapes (6.9% by fresh weight) all contain fructose [50]. The effect of UV in food processing systems has been shown to affect more than just pathogens in a food product. Studies have shown that UV-C can interact with fructose to produce a potential carcinogen like furan or degrade the concentration of patulin or AA in a juice UV processing system [39,40,63]. It has also been demonstrated that UV-C exposure to fructose can affect properties such as pH and color and even generate ROS under thermal and UV-C exposure [18,60,79]. These changes result from the products generated from fructose degradation that occurs due to the absorption of UV-C radiation or thermal breakdown. To the best of the author's knowledge, only a handful of studies have been performed studying the interaction of UV radiation and fructose reactivity. However, none specifically evaluated UV-A and UV-B radiation and if they have similar effects on UV-treated products containing fructose. In the studies performed with UV-C exposure, it was shown that, in its open-chain form, after a series of tautomeric shifts, fructose can photolyze to produce hydroxyalkyl and acyl radicals that can subsequently break down and react to produce a multitude of degradation products [61].

This study aims to demonstrate the ability of fructose to generate ROS under UV-A or UV-B radiation exposure using fluorescein as a model fluorescent indicator dye that loses its fluorescence intensity upon reaction with ROS. Factors such as the presence of antioxidant and storage conditions on ROS generation from fructose were also evaluated. Finally, the effect of UV-induced ROS generation from fructose on a model quality indicator, vitamin C (ascorbic acid), was evaluated in coconut water, a model liquid food system amenable to UV processing. This study will enhance our understanding of the impact ROS generation from UV-A or UV-B exposed fructose can have on food products and aid in developing strategies to minimize their effects.

Materials and Methods

Materials

D – Fructose (Spectrum Chemical MFG Corp., lot # 2JF0408), sodium salt of fluorescein (Fisher Science Education, lot # 4AK364132), L (+) - ascorbic acid, ACS (Acros Organics, lot # A0339362), hydrogen peroxide ACS reagent, 30% wt. in H_2O (Honeywell, lot # H0760) were obtained from respective vendors. 100% natural coconut water, not from concentrate, was obtained from ZICO Rising INC., lot # SEP2922R00:45. HPLC grade submicron filtered water (Fisher Chemical, lot # 203794), o—phosphoric acid, HPLC grade (Fisher Chemical, lot # 150310), and methanol, HPLC grade (Fisher Chemical, lot # 211792) were also obtained from respective vendors.

UV processing unit

A batch-UV processing unit was used for UV-A (model # XL-1000A), UV-B (model # XL-1000B), and UV-C (model # XL-1000) (Spectrolinker UV Crosslinker, Spectroline Laboratory, Farmingdale, NY) was used for all experiments. The UV chamber contains five UV bulbs emitting at either UV-A (365 nm, BLE-8T365), UV-B (312 nm, BLE-8T312), or UV-C (254 nm, BLE-8T354) within a 34.3 × 17.8 × 19.1 cm inner chamber. UV radiation doses were measured by an internal radiometer calibrated and programed by the manufacturer. Processing units were programmed to shut off after the prescribed dose level was achieved. Variation in incident intensity was minimized using the manufacturer's recommendation of a five-minute warm-up period from a cold start to allow the UV tubes to stabilize for more accurate operation. An intensity check was also performed to ensure that units were operating at the proper intensity each day.

Experimental design and fluorescence measurement

A 2 μM fluorescein solution was prepared in DI water (pH 7.2±0.4). The effect of fructose on fluorescence decay rate from fluorescein was investigated by dissolving various quantities of fructose individually in the fluorescein solution and exposing it to UV-A, UV-B, or UV-C light. Treatments were carried out by adding 75.0 mL of the respective solution into an uncovered glass petri dish and exposing it to various doses of UV radiation in the UV processing unit. The samples in petri-dish were stirred constantly during treatment using a magnetic stirrer to achieve uniform exposure of the sample to UV radiation. Whenever stored outside the UV chamber, samples were covered with aluminum foil to minimize interaction with ambient light. Ambient room temperature (19 – 22 °C) was maintained for all experiments. To measure the fluorescence intensity of the solution, after a 0.1 J/cm^2 dose interval, 100 μL of the sample was pipetted from the petri dish into a well of 96-well plate optimized for fluorescence measurement. Fluorescence was measured in a SpectraMax M5 fluorescence microplate reader (Molecular Devices, Sunnyvale, CA) with excitation and emission wavelengths of 485 nm and 510 nm, respectively. All fluorescence values obtained were normalized using:

Eq. (1): $Relative\ fluorescence\ intensity = \frac{100 \times I_d}{I_0}$

Where I_d = fluorescence intensity after "*d*" dose of UV radiation exposure, I_0 = fluorescence intensity with a "*0*" dose of radiation.

Comparison of UV-A, UV-B, and UV-C for their ability to inflict oxidative stress on fructose solution

To examine the effect of fructose on the fluorescence decay rate of fluorescein upon exposure to various UV radiation wavelengths, fructose was separately dissolved in 500 mL of a 2 μm fluorescein solution at 100 mM fructose. These solutions were subsequently

exposed to UV-A, UV-B, and UV-C radiation at 0.1 J/cm^2 for a total of 1 J/cm^2, with 100 µL samples taken at each 0.1 J/cm^2 dose interval.

Effect of fructose concentration when exposed to UV-B radiation

To investigate the role of concentration of fructose on fluorescence decay rate of fluorescein, 10-, 50-, and 100- mM of fructose was dissolved in 2 µM fluorescein solution and exposed to UV-B radiation at 0.1 J/cm^2 for a total of 1 J/cm^2, with 100 µL samples taken at each 0.1 J/cm^2 dose interval.

Effect of ROS scavenger

To investigate the effect of an added antioxidant on the fluorescence decay rate, AA was added at concentrations of 5 and 10 µM to 2 µM fluorescein solution containing 50 mM fructose prepared in DI water (pH 7.2). The sample was then exposed to UV-B radiation at 0.1 J/cm^2 for a total of 1 J/cm^2, with 100 µL samples taken at each 0.1 J/cm^2 dose interval.

Quantitative comparison of oxidative effect of UV-B exposed fructose with a known oxidizer

The oxidative effect of fructose on fluorescein was quantitatively compared with HP, a compound known to produce oxidative species upon exposure to UV radiation [60]. Hydrogen peroxide (HP) (30% wt. in H_2O) was added to a 2 µM fluorescein solution to obtain a final concentration of 500 mL of 1mM HP and 2 µM fluorescein solution. This solution was subsequently exposed to UV-B radiation at 0.1 J/cm^2 for a total of 1 J/cm^2, with 100 µL samples taken at each 0.1 J/cm^2 dose interval. Fructose was then added at a level of 50 mM to 2 µm fluorescein solution, and the experiment was performed similarly. The plot of % relative fluorescence against dose was plotted against the total dose of UV-B radiation exposure. The area under the curve (AUC) for each sample was calculated using the formula for the area of trapezium as shown in:

Eq. (2): $AUC = \Delta d \ \frac{f(d)+f(d+\Delta d)}{2}$

Where "*d*" is the dose in J/cm^2, and "*f*" is the relative fluorescence intensity. Relative oxidative potential (ROP) was calculated by comparing the AUC value for fructose and HP at the respective molarities of the solution, as shown in:

Eq. (3): $ROP = \frac{AUC_{Fructose} \times AUC_{Hydrogen\ peroxide}}{AUC_{Hydrogen\ Peroxide} \times M_{Fructose}}$

where "M" is the molarity of fructose in the solution.

Evaluation of continued generation of ROS following UV-B exposure of fructose

To investigate the effect of storage temperature on fluorescence decay rate, two 500 mL samples of 2 µM fluorescein solution containing 50 mM fructose were prepared in DI water (pH 7.2). The sample was then exposed to one dose of 0.1 J/cm^2 UV-B radiation and stored at 4°C and 37°C. A 100 µL sample was then taken at 24-hour intervals for a total of 72 hours, with 100 µL samples taken at each dose interval.

Effects of UV-B induced ROS from fructose on the degradation of ascorbic acid in coconut water

To investigate the effect of ROS generated from UV-B exposed fructose on AA, AA was added to 500 mL of coconut water at 300 mg/L. The AA fortified coconut water solution was then used to create 100 mM and 20 mM fructose solutions (pH 5.32) fortified with 300 mg/L of AA. Samples were filtered through a sterile 0.20 µm filter, and then 75.0 mL of the respective solution was added into an uncovered glass petri dish and exposed to UV-B radiation at 1 J/cm^2 doses for a total of 4 J/cm^2. The samples in the petri-dish were stirred constantly during treatment using a magnetic stirrer to achieve uniform exposure of the sample to UV radiation. To measure the AA concentration of the solution, 1 mL samples were taken at each 1 J/cm^2 dose interval and put into clear glass 12 × 32 mm screw thread vials compatible with the HPLC autosampler. Whenever stored outside the UV chamber, samples were covered with aluminum foil to minimize interaction with ambient light. Ambient room temperature (19–22ºC) was maintained for all experiments. High-

performance liquid chromatography (HPLC) was used to determine the concentration of AA in coconut water. Prominence HPLC (Shimadzu USA: degasser – DGU-20A5, liquid chromatography – LC-20AD, communications bus module – CBM-20A, UV/vis detector – SPD-20A, column oven – CTO-20AC, and autosampler – SIL-20ACHT) was used with a Kinetez® µm C18 LC column 250 x 4.6 mm. The UV-Vis detector was set at the wavelength of 245 nm. A 1% phosphoric acid (H_3PO_4) - 50% methanol (CH_4O) solution was used as the mobile phase. Figure 1 below is the standard curve generated for AA concentration comparison. The curve was generated under the same conditions as the experiment and settings above using 50-, 100-, 150-, 200-, 250-, and 300- mg/L AA solutions.

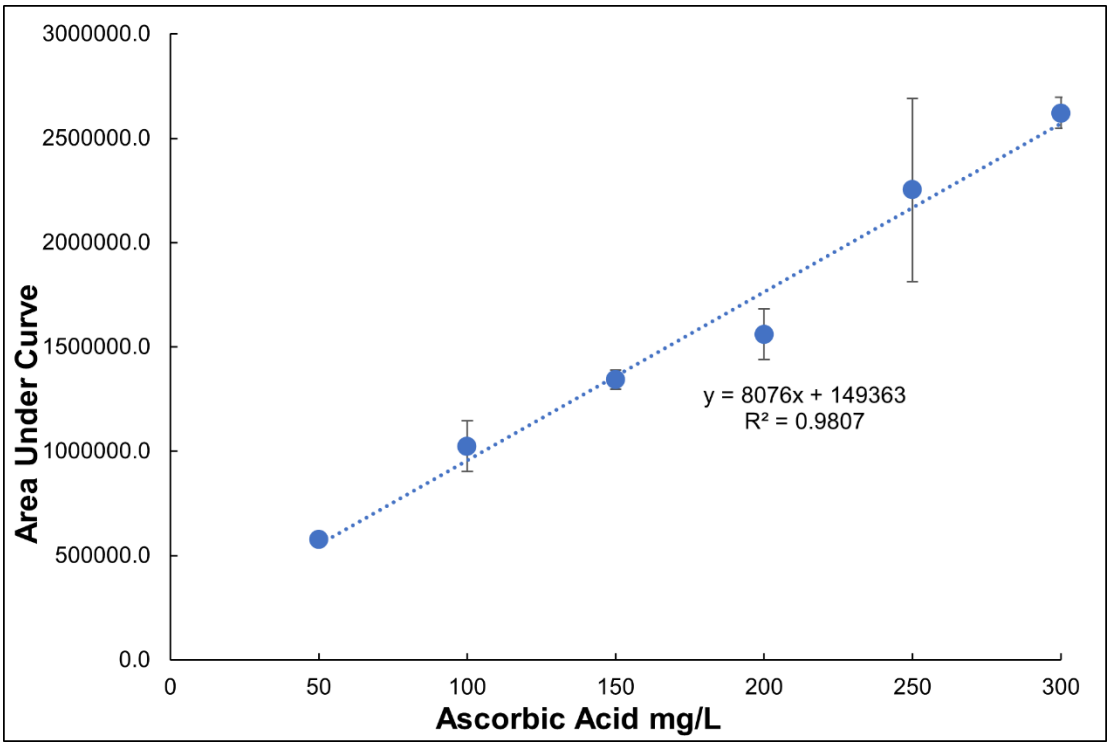

Figure 1. Ascorbic acid standard curve, each data point is an average of triplicate measurement ± standard deviation.

The concentration of AA in each sample was then calculated using:

Eq. (4): $concentration = \frac{(AUC - intercept)}{slope}$

Data analysis and statistics

All experiments were performed in triplicate. A statistical student's t-test ($\alpha=0.05$, $p<0.05$) from Microsoft® Excel® 365 was used to determine significant differences between treatments and concentrations.

Results and Discussion

Comparison of UV-A, UV-B, and UV-C ability to inflict oxidative stress on fructose solution

Fluorescein was used as a fluorescent indicator to measure the ability of UV exposed fructose to generate ROS since fluorescein is sensitive to oxidative reactions. When fluorescein comes into contact with ROS, it loses fluorescence and serves as a standard indicator used to measure antioxidant activity in food products [60,80]. Prior studies by Boxin Ou and Aachen Elsinghorst provided an established method for measuring fluorescence degradation as a result of UV exposed fructose [60,80]. Figure 2 below shows the comparison of fluorescein degradation of 2 µM fluorescein controls and 2 µM fluorescein with 100 mM fructose exposed to UV-A, UV-B, and UV-C radiation. Direct exposure of fluorescein solution to UV-A, UV-B, and UV-C showed no significant degradation, as seen in the controls (Fig. 1). The absence of degradation in fluorescence in the controls thus allowed for the measurement of fluorescein fluorescence decay in the presence of UV exposed fructose to be attributed to the interaction between fructose and UV radiation.

The ability of UV-A, UV-B, and UV-C radiation to elicit ROS generation from 100 mM of fructose is also shown in figure 2. UV-A radiation exposure did not result in loss of fluorescence in fluorescein after a total of 1 J/cm^2 of UV-A exposure, indicating that UV-A exposed fructose solution did not generate ROS. However, UV-B and UV-C radiation significantly affected the fluorescence of fluorescein, resulting in an 89% ± 3.7% loss of fluorescence after a total of 0.5 J/cm^2 and a 91% ± 0.5% loss in fluorescence after a total of

0.3 J/cm^2 exposure respectively. Fluorescence degradation data fitted well (R^2>0.96) into the first-order reaction kinetics equation, and the rate constants were compared for statistical significance. The ability of UV-C to elicit ROS generation has already been thoroughly explored in a study by Aachen Elsinghorst [60]. However, this study's data collected from UV-C exposure serves as a benchmark for comparing UV-B ability to generate ROS from fructose. The data illustrated in figure 2 indicates that while UV-B and UV-C radiation have an oxidative effect, based on a significantly higher rate constant (7.97 cm^2/J±0.8) compared to the rate constant for UV-B exposure (4.27 cm^2/J±3.2), UV-C is a statistically more potent (student's t-test, $p<0.05$) oxidizer of fructose.

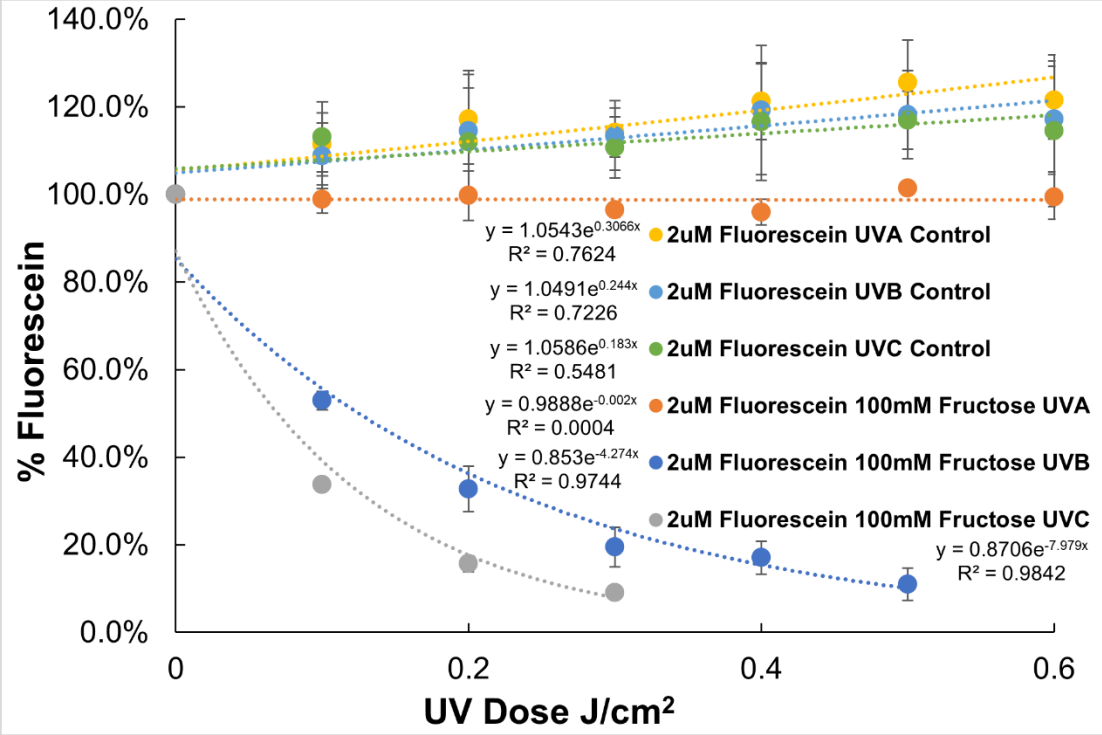

*Figure 2. Relative fluorescence decay of fluorescein in aqueous solutions containing no sugar (control) or fructose (100 mM) exposed to 0.6 J/cm^2 of UV-A, UV-B, and UV-C radiation. Each data point is an average of triplicate measurement ± standard deviation. *The reported dose is the dose measured by the radiometer located at the base of the chamber. The*

treatment solution sits raised on a stirring plate and therefore experiences higher intensity than reported.

Figure 3 below has been adapted from a study by Shaila Nayak [68]. The figure represents the absorbance spectrum of a 10% fructose solution. The absorption peak of fructose peaks at 278 nm, which is between the UV-C (254 nm) and UV-B (320 nm) radiation peaks [68]. The absorbance values indicate more absorption occurring in the UV-C spectrum (200 – 280 nm) than UV-B (280 – 360 nm). Since absorption of UV radiation is a requisite for triggering photochemical degradation of fructose (as described in the literature review section), lower absorbance in the UV-B region compared to the UV-C region by fructose serves as a reasonable explanation for why UV-B exposed fructose had a lower oxidizing effect than UV-C exposed fructose.

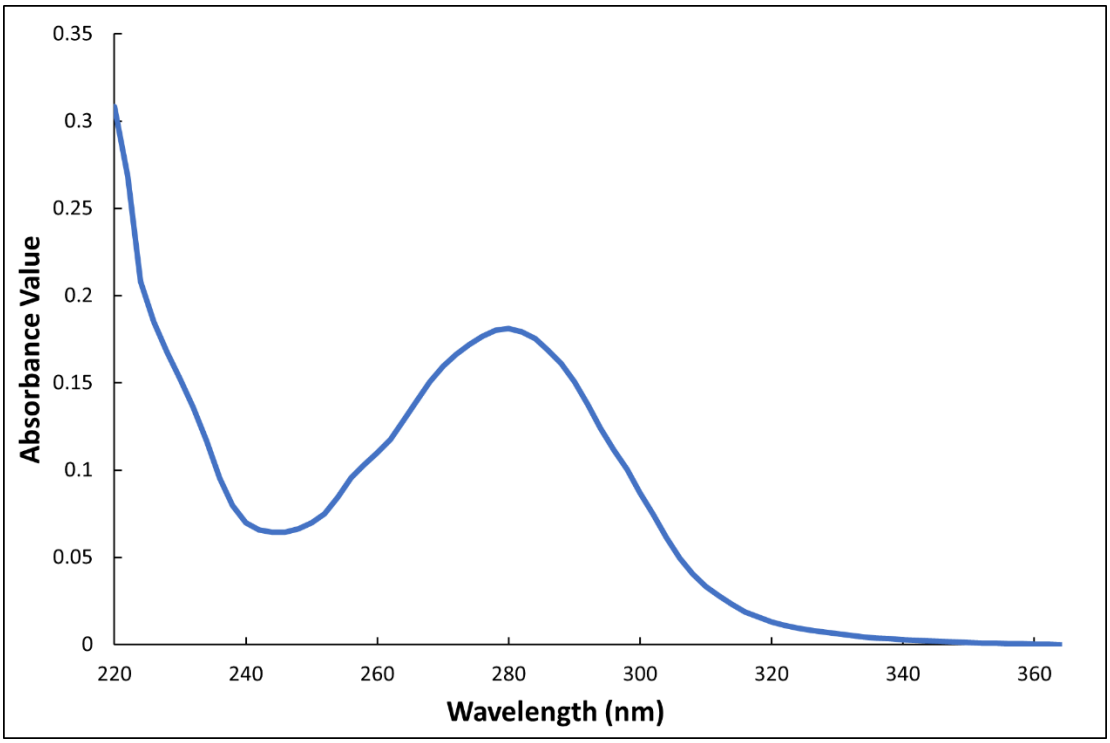

Figure 2 - UV Absorbance spectrum (220-360 nm) of fructose [68]

Indicating the need for further testing to determine the extent, effects, and similarities between UV-C and UV-B exposed fructose.

Dependence of fluorescence degradation on the concentration of UV-B exposed fructose

To explore this phenomenon further, we examined the concentration effect of UV-B exposed fructose. In figure 4 below, fluorescein fluorescence degradation followed first-order kinetics, where the rate constants in the presence of 10-, 50-, and 100- mM fructose were 0.7±0.01 J/cm^2, 4.3±0.6 J/cm^2, and 4.3±0.3 J/cm^2, respectively. Statistical analysis of the fluorescein degradation rate revealed that the degradation rate increased up to 50 mM of fructose (student's t-test, $p>0.05$). However, the decay rate between 50 and 100 mM of fructose did not show a statistically significant difference in the decay rate, indicating that fructose is in excess within the solution at these concentrations compared to the concentration of fluorescein. Nevertheless, this dose-dependence study further demonstrates that fluorescein degradation is specifically from products generated from UV-B exposed fructose that are likely to be oxidative in nature.

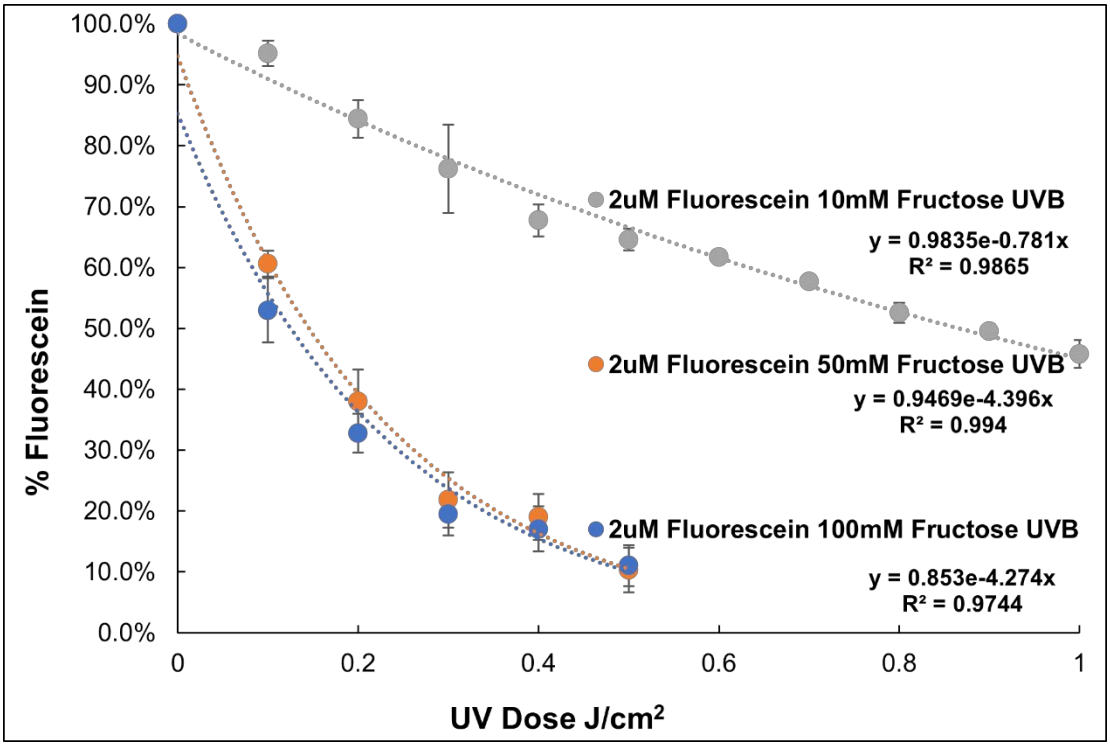

*Figure 4 - Relative fluorescence decay of fluorescein as a function of exposure duration to UV-B radiation in the presence of 10-, 50-, and 100- mM of fructose. Each data point is an average of triplicate measurement ± standard deviation. *The reported dose is the dose measured by the radiometer located at the base of the chamber. The treatment solution sits raised on a stirring plate and therefore experiences higher intensity than reported.*

In a study by Christian Triantaphylides, the author discovered that UV exposed fructose generated hydroxyalkyl and acyl radicals, which can further generate peroxyl, and superoxide radicals [61]. The generation of ROS could have significant adverse interactions with product components that are sensitive to oxidation. Components sensitive to oxidation include, but are not limited to, sensory properties like aroma, color, and taste or nutritional properties such as vitamins and antioxidants. In the study by Aachen Elsinghorst, the author demonstrated that the free radicals caused a loss in fluorescence from fluorescein due to the ROS generated from UV-C exposed fructose [60]. The results of this study with UV-B are consistent with the papers by Christian Triantaphylides and Aachen Elsinghorst, demonstrating that UV-B can elicit ROS generation in fructose [60,61]. In the study by Triantaphylides, the author noted that approximately 0.8% of fructose in solution is available in the open-chain form [62]. It is only when fructose is in the open-chain form that UV radiation can cleave the aldehyde group and allow the newly formed group to undergo a redox reaction and be oxidized upon exposure to UV light [62]. This means that a significant amount of fructose is needed to generate enough ROS to degrade the fluorescence of fluorescein. This observation can be seen in this study as well, where the degradation of fluorescence is observed at concentrations of fructose that are at a minimum 5000 – fold more concentrated than fluorescein.

Effect of ROS scavenger

In order to confirm that the products being generated by UV-B exposed fructose are oxidative in nature, 50 mM fructose and 2 μM fluoresceine was fortified with 0, 5, and 10 μM of AA to determine the potential impact on the rate of fluorescein fluorescence degradation. AA is a known antioxidant that has the ability to serve as a ROS scavenger [81] The results shown in figure 5 below demonstrate that the products generated by UV-B exposed fructose are, in fact, ROS. Fluorescence decay deviated from first-order kinetics, and at 0.5 J/cm^2 UV-B exposure, relative fluorescence values reduced to 10.3±3.7%, 34.7±0.2%, 78.5±2.0% in the presence of 0, 5, and 10 μM AA, respectively. Previous studies by Rohan Tikekar and Aachen Elsinghorst indicated that AA degradation increased when in the presence of UV-C exposed fructose [40,60,62]. The results of this study coincide with the previous studies' mentioned hypothesis that the products generated from UV-B exposed fructose are indeed ROS.

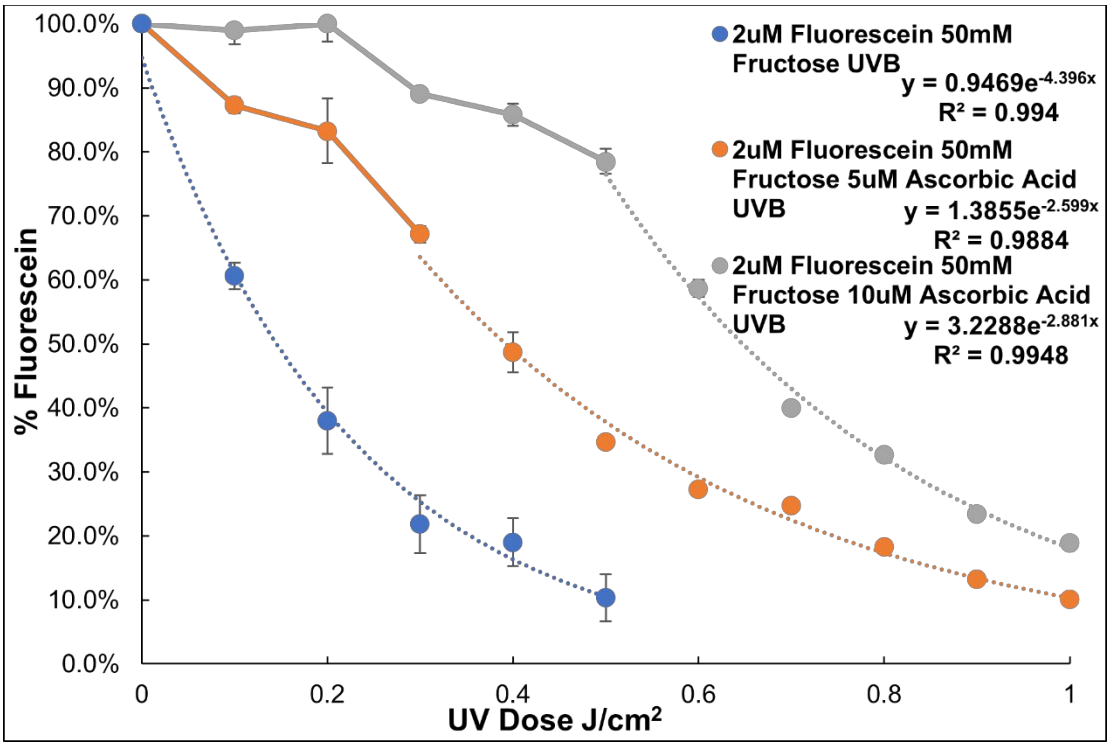

*Figure 5 - Relative fluorescence decay of fluorescein as a function of exposure duration to UV-B radiation in 2 µM fluorescein + 50 mM fructose solution containing 5 µM or 10 µM ascorbic acid. Each data point is an average of triplicate measurement ± standard deviation. *The reported dose is the dose measured by the radiometer located at the base of the chamber. The treatment solution sits raised on a stirring plate and therefore experiences higher intensity than reported.*

Quantitative comparison of the oxidative potential of UV exposed fructose with a known oxidizer

In order to examine the oxidizing capabilities of the ROS generated by UV-B exposed fructose, a 50 mM fructose and 2 µM fluorescein solution was compared to 1 mM of HP and 2 µM fluorescein. HP was selected as the known oxidizer because it has been demonstrated in previous studies that it generates ROS when exposed to UV-C [82]. The rate of degradation of fluorescein from HP compared to that from UV exposed fructose can be found in figure 6 below. The AUC was calculated using the equations outlined in the materials and

methods section to quantify the comparison of the two oxidizers. The calculations indicate that fructose's reactive oxidative potential (ROP) is 65.8% less than HP. This aligns with the Triantaphylides's study that shows that 0.8% of fructose is in the open-chain form and can generate ROS when exposed to UV-C [60,61].

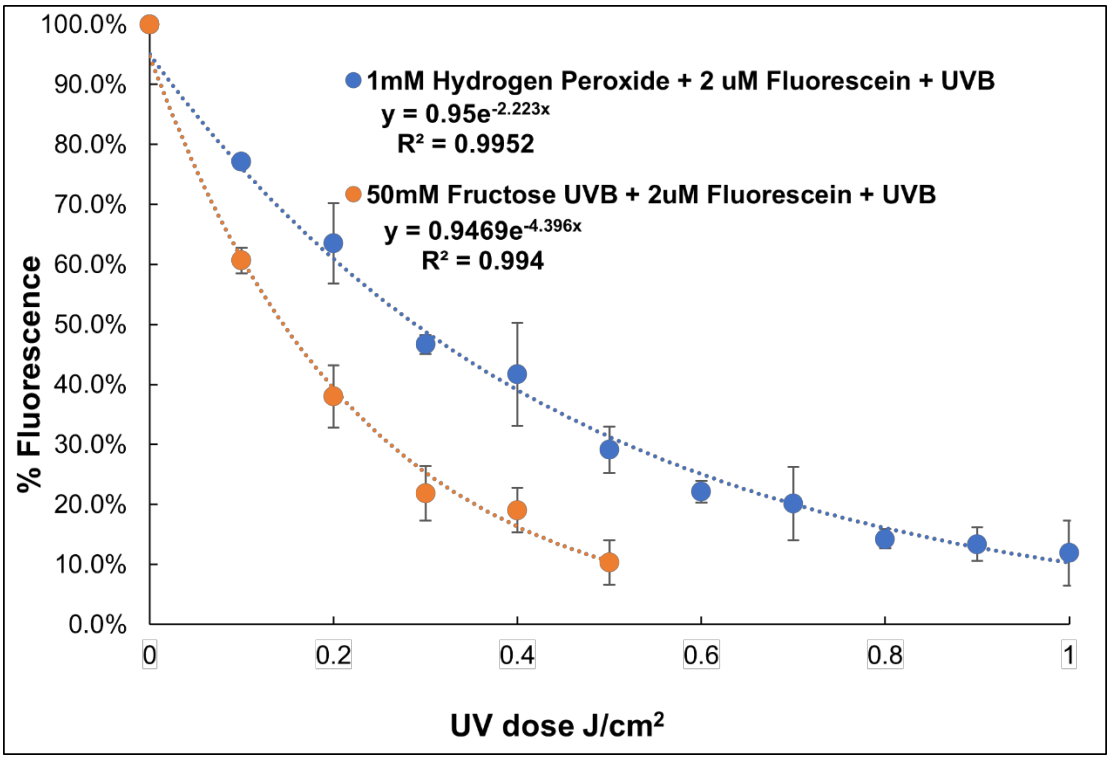

*Figure 6 - Relative fluorescence decay of fluorescein as a function of exposure duration to UV-B radiation in an aqueous solution of 50 mM fructose or 1 mM hydrogen peroxide. Each data point is an average of triplicate measurement ± standard deviation. *The reported dose is the dose measured by the radiometer located at the base of the chamber. The treatment solution sits raised on a stirring plate and therefore experiences higher intensity than reported.*

Evaluation of continued generation of ROS following UV-B exposure of fructose

Fluorescein fluorescence degradation in UV-B exposed fructose and unexposed fructose solutions were observed for 72 hours at 4°C and 37°C after an initial UV-B exposure

of 0.1 J/cm^2 (figure 7). Statistical analysis of the fluorescein degradation rate revealed that the UV-B exposed and unexposed sample's rates of degradation at 4 °C and 37°C were not statistically significant (student's t-test, $p>0.05$). However, the degradation rate between the UV-B exposed and unexposed samples at 4°C and 37°C was statistically significant (student's t-test, $p<0.05$). These results indicate that post-UV-B degradation of fluorescein was affected by storage temperature alone, and a significant amount of ROS was not generated during post-processing storage following the UV-B exposure.

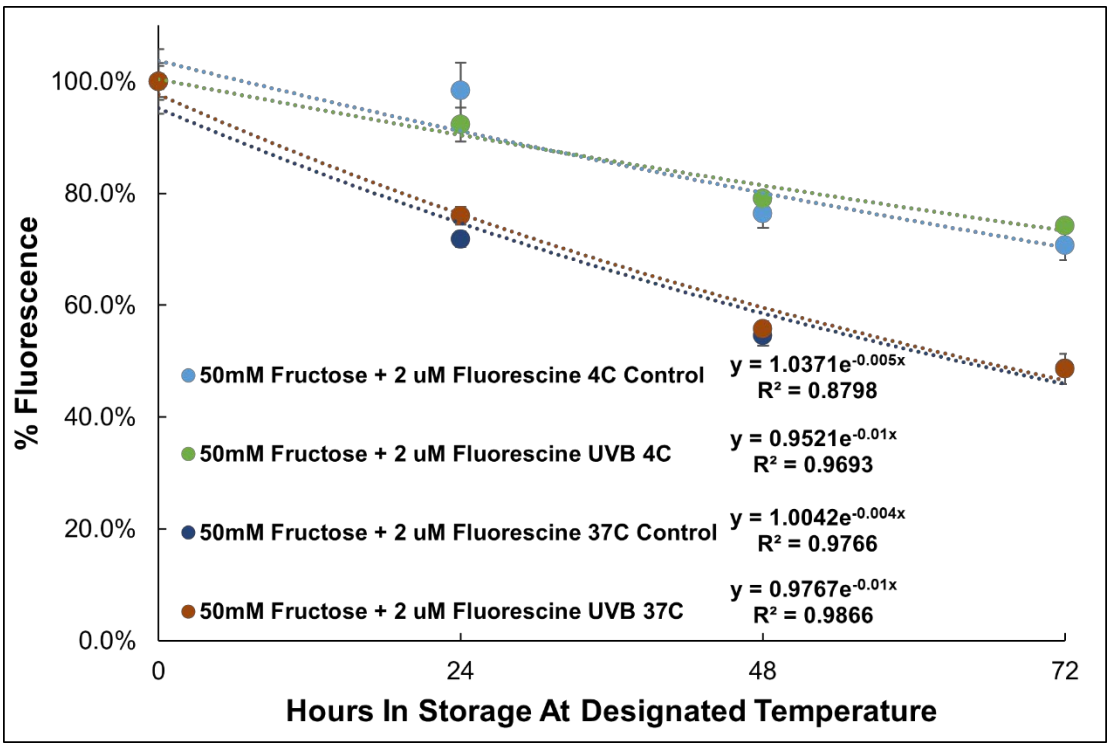

*Figure 7. Relative fluorescence decay of fluorescein as a function of initial UV-B dose (0.1 j/cm$^{2)}$ during post-treatment storage at 4°C or 37°C for 72 hours. Each data point is an average of triplicate measurement ± standard deviation. *The reported dose is the dose measured by the radiometer located at the base of the chamber. The treatment solution sits raised on a stirring plate and therefore experiences higher intensity than reported.*

Effects of UV-B exposed fructose on ascorbic acid (AA) in coconut water.

In an effort to bridge the gap between theory and practical application, the observations in the previous experiments performed were used to examine the behavior of an actual consumer product containing fructose. Coconut water was selected as it contains approximately 2.29 g/100 mL of fructose, has gained significant popularity among consumers for its suggested health benefits, and comes in various processing methods, packaging, and storage requirements [50]. In addition, being transparent and low in suspended solids, it is a promising candidate for UV processing. A study by Manreet Bhullar used a UV-C juice processing unit to achieve a 5 log inactivation of *E. coli, Salmonella Typhimurium,* and *L. monocytogenes* [83]. Another study by Sudheer Yannam examined the effect of UV-C treatment on sensory and nutritional components of coconut water [84]. This study showed that UV-C treatment could inactivate polyphenol oxidase and peroxidase enzymes, playing a significant role in quality degradation occurring during juice processing [84]. The study also showed that sensory characteristics of UV-C treated coconut water were rated higher than when compared to thermally treated coconut water [84]. ZICO® brand coconut water was selected for this experiment because the product does not have any advertised fortification or preservatives. According to the nutritional facts, there is approximately 37.53 g/L of total sugars. Using Duncan Burns's review on coconut water authenticity and portability, we can assume that of the 37.53 g/L of total sugar in the product selected, approximately 22.9 g/L is fructose [52]. In order to establish the existence of a concentration dependence of ROS generated from UV-B exposed fructose, concentrations of 50 mM and 10 mM of fructose were created using commercially purchased coconut water. This equates to 9 g/L and 1.80 g/L, respectively, with an assumed total of 31.9 g/L and 24.7 g/L with the natural fructose contained within the coconut water taken into account. The FDA's recommended dietary reference intake (DRI) of AA is 90 mg [85]. If a single serving of coconut water is 240 mL and

is assumed to have 100% of an individual's DRI, then it is also reasonable to assume that 1 L of coconut water would contain approximately 375 mg of AA. Under this assumption, 300 mg/L of AA was selected to verify and observe any degradation due to ROS generation from the UV-B exposed fructose.

HPLC was used to calculate the concentration of AA at intervals of 1 J/cm^2 for a total of 4 J/cm^2. The results in figure 8 revealed that when compared to the control (300 mg/L AA + 100 mM Fructose, no UV-B exposure), was statistically significant (student's t-test, $p<0.05$) from all other formulations that were exposed to UV-B radiation. However, 300 mg/L AA + UVB, 300 mg/L AA + 100 mM Fructose + UVB, and 300 mg/L AA + 20 mM Fructose + UVB, AA degradation was not statistically significant (student's t-test, $p>0.05$), indicating that even with the addition of fructose, ROS generation from fructose did not affect AA concentration. The AA degradation in this experiment can be attributed to AA's natural susceptibility to degradation when exposed to UV alone. This has been well established in studies by Rohan Tikekar [40,62]. A lack of dose-dependence may be attributed to (a) fructose being present in excess compared to AA even in coconut water not fortified with fructose, or (b) the relative amount of AA is much higher than the ROS being generated from UV-B exposed fructose that prevents observation of differing rates of AA degradation. Removing fructose from natural coconut water is a challenging process and was not explored in this study to identify which of these possibilities would most impact the observed phenomenon.

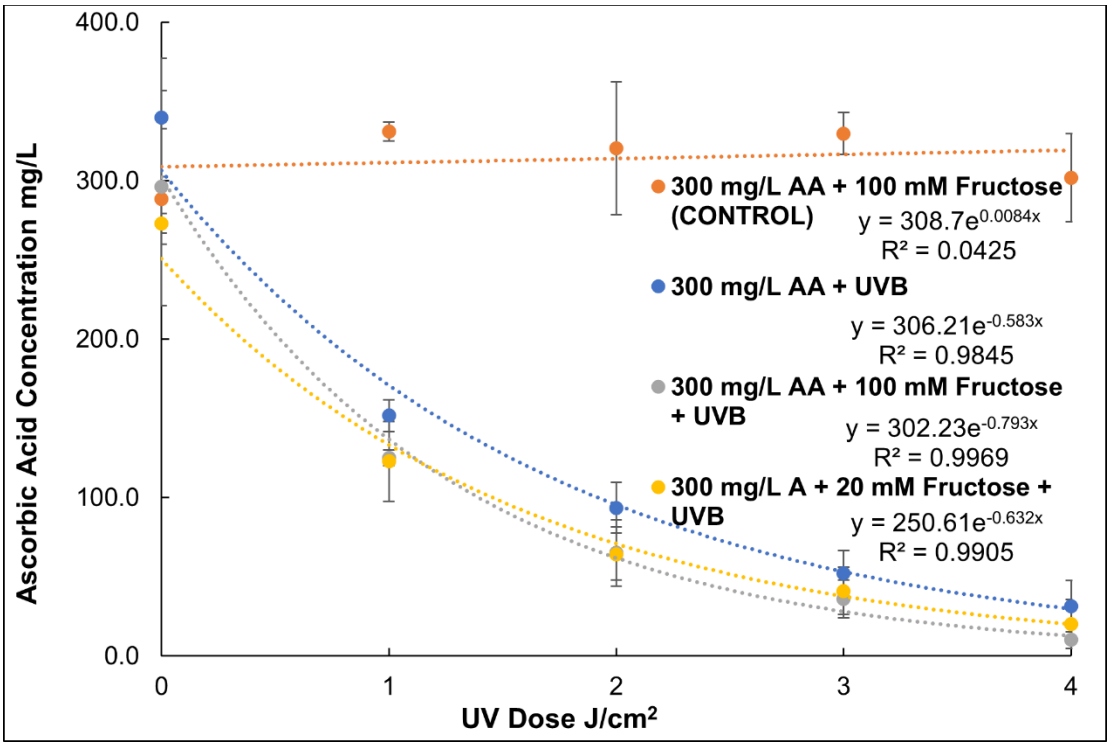

*Figure 8. Relative ascorbic acid (300 mg/L) concentration decay in coconut water solutions containing no added fructose, 100 mM, or 20 mM fructose (100 mM) exposed to 4.0 J/cm^2 of UV-B radiation. Each data point is an average of triplicate measurement ± standard deviation. *The reported dose is the dose measured by the radiometer located at the base of the chamber. The treatment solution sits raised on a stirring plate and therefore experiences higher intensity than reported.*

Conclusion

This study confirms that UV-B elicits the generation of ROS in fructose solution. The experiments performed suggest that UV-B has similar effects as UV-C on ROS generation in fructose. However, the data acquired in this study also confirms that UV-B is a weaker oxidizer than UV-C radiation. This aligns with previous studies that show the peak of the absorbance spectrum of fructose (278 nm) is at the threshold of the UV-C (200 – 280 nm) and UV-B (280 – 360 nm) radiation [68]. Considering the peak wavelengths of UV-C (254 nm)

and UV-B (320 nm), it can be interpreted that the absorption of UV-C radiation is approximately three times higher than UV-B radiation. It was also confirmed that post-UV-B degradation of fluorescein from UV-B exposed fructose was not observed during post-processing storage at 4°C and 37°C. Lastly, when observing the behavior of UV-B exposed fructose in a consumer product, it was determined that ROS generation from UV-B exposed fructose did not affect AA concentration in coconut water. Understanding the mechanics of UV-B interaction with fructose could lead to potential alternatives or improvements in juice processing, packaging, and storage, leading to better quality outcomes. Minimizing or controlling the amount of ROS generated can help protect color, flavor, and nutritional or functional compounds that are susceptible to oxidation.

Chapter 3: Combination of UV-A light and Chitosan–Gallic Acid Coating to Improve the Safety and Quality of Fresh Strawberries

Publication status

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Abstract

Strawberries can be contaminated by foodborne pathogens or mold on-farm or postharvest handling. Photo-irradiation of edible chitosan coating containing gallic acid can be an innovative antimicrobial intervention to improve the safety and shelf-life of strawberries. Previously, a chitosan-gallic acid (CH-GA) coating that can exhibit moderate antimicrobial activities under 360 nm UV-A light was developed in our laboratory. After 180 minutes of exposure to UV-A, this coating resulted in a 2.3±0.4 log reduction of *E. coli* O157:H7 on CH-GA coated strawberries were achieved, which were significantly higher than CH, GA, and CH-GA without UV-A (average 0.3 – 1.0 log reductions); the resulted bacterial reduction was also significantly higher than GA + UV-A (1.2±0.4 log reductions), but not for CH + UV-A (1.8±1.0 log reductions). This study evaluated the impact of this coating on the quality parameters such as color, texture, pH, total soluble solids (TSS), and titratable acidity. The pH and TSS% values of different groups were generally stable. However, the results did

indicate that photo-irradiation may have an initial adverse effect on the color and texture of the strawberry, but the loss in quality slowed throughout the 14-day refrigerated storage. Ultimately no statistical differences ($p>0.05$) in color change or firmness remained between UV-A, CH-GA + UV-A, and the control groups on day 14.

Introduction

Strawberry is a delicate fruit typically not washed the same as other fresh produce before retail sales [86]. Pathogens can contaminate strawberries during many steps of growing, harvest, packaging, and transportation. These pathogenic bacteria can then survive throughout the expected shelf life of the fruit, posing a substantial food safety risk[87]. Consequently, there is a need to effectively develop alternative solutions to reduce microbial contaminations and quality losses in fresh strawberries.

Our lab previously explored chitosan combined with food-grade gallic acid as a photo-irradiated antimicrobial coating for fresh strawberries. Based on its mild antimicrobial capacity and good film-forming ability, chitosan coating alone can significantly improve the shelf-life quality of strawberries [88,89]. Therefore, we hypothesized that: 1) UV-A light can activate gallic acid within the chitosan coating, thus enabling the inactivation of pathogenic bacteria and native mold on strawberries' surface; and 2) coating will not show adverse effects on the quality of fresh strawberries after the treatments and during the subsequent storages. Results from the first objective showed that microbial inactivation was possible with a 2.3±0.4 log reduction of *E. coli* O157:H7 on CH-GA coated strawberries treated with UV-A was achieved, which was significantly ($P<0.05$) higher than CH, GA, and CH-GA without UV-A (average 0.3–1.0 log reductions) and UV-A alone. Therefore, I explored objective two as a part of this study.

Materials and Methods

Fresh strawberries were purchased in a local supermarket and used for the experiment within three days. Fruits in the same ripening stage were selected with a weight range from 15 to 30 g. Before the experiment, fruits with visible physical damage or fungal decay were removed. Gallic acid (98%) was purchased from Acros Organics (Fair Lawn, NJ). High molecular weight chitosan with a deacetylation degree of 76% was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals are chemical grade and produced by Sigma-Aldrich (St. Louis, MO).

Preparation of the chitosan-gallic acid (CH-GA) coating solution

The coating solution was prepared by dissolving gallic acid and chitosan in an aqueous solution of glacial acetic acid. The coating formation procedure and concentration of gallic acid were used in previous studies with some modifications [90,91]. Briefly, 15 mM gallic acid was prepared by dissolving in sterile DI water at ~50°C for 60 minutes. After cooling to room temperature (22±2°C), acetic acid (0.5 %, v/v) was added to the solution, followed by the high molecular weight chitosan (1%, w/v) and glycerol (0.3%, w/v). The mixed solution was magnetically stirred for approximately four hours, then centrifuged at 7,745×g for 10 minutes. Finally, the supernatant was separated and stored in the refrigerator before being used within two days. Similarly, a coating solution without gallic acid (only CH) or chitosan (only GA) was prepared before each experiment. Whole strawberries were dipped into the CH-GA coating solution for 30 s, drained, and air-dried for 30 minutes. Roughly half of the coated strawberries were used immediately (denoted as wet/unsolidified coating), and the rest were stored overnight in refrigerated condition (~4°C) to allow the solidification of coating (denoted as dry/solidified coating).

Color and texture analysis

Each package containing ~15 strawberries was used for the following measurements. First, the color of the strawberries was measured from the middle section of the fruit skin with two readings from the opposite side of the berry. Three berries from each package (n=9) were measured by a colorimeter model EZ-45/0 CX2405 (Hunter Associates Laboratory, Reston, VA, USA) calibrated with standard white and black tiles. Samples used for the color analysis were further used for texture (firmness) measurements by a texture analyzer TA-XT2i (Stable Micro Systems, Godalming, UK) calibrated with a 2-kg load cell according to the previous literature [92]. Each strawberry was cut in half, and a compression test (peak force, N) for each half strawberry was performed to obtain an average value of the force required to cause deformation of the fruit by 5 mm with a 2-inch flat probe occurred. The test speed of the probe was 5.0 mm/s with pre and post-test speeds of 10 and 5 mm/s, respectively. In total, nine replicates were analyzed from triplicated packages.

pH, total soluble solids (TSS), and titratable acidity

Strawberries (~ 20 g) were cut into 1.2 cm cubes and pureed by a high-speed homogenizer for 2 minutes in a 50 mL plastic tube. For pH measurements, calibrated pH probe was directly inserted into the strawberry slurry; the value was recorded when it reached a stable reading. 5 g of puree was weighed and diluted with 45 mL distilled water in a small beaker. The solution was titrated to pH 8.1 using 0.1 M NaOH. The total titratable acidity of the strawberry puree was calculated using:

Eq. (5) [93]: Titratable acidity $= \frac{V(NaOH)\times 0.1\times 0.064}{m(aliquate)} \times 100(\%)$

Where V(NaOH) is the volume of NaOH used for titration, 0.1 N is the molarity of the NaOH solution, 0.064 is the conversion factor for citric acid, and maliquot is the mass of the puree used for analysis. Finally, the TSS content of juice squeezed from these strawberries was measured using a refractometer.

Statistical analysis

All data had at least three independent replicates and were compared using one-way Analysis of Variance (ANOVA) between different groups using Duncan's method. Statistical analysis was performed by SPSS Statistics 22.0 (IBM Corporation, NY, USA) with a significance level of $p < 0.05$ for all comparisons. In certain specific conditions, student's t-test assuming unequal variance was performed with a significance level of $p < 0.05$

Results and Discussion

Effects of photo-irradiated CH-GA coating on quality indices of fresh strawberries such as color, total soluble solids, pH, titratable acidity, firmness, and water loss during storage are shown in Table 4 below. Immediately after the UV-A treatment, the redness (a) and yellowness (b) of strawberries were found to be lower than the value of the control, and

Table 5. Changes in quality parameters of strawberries treated by CH-GA, UV-A, and their combination for 14 days storage at 4°C - The values with different capitalized or non-capitalized superscripts indicate significance between each other, for those at different storage times or in different groups at the same time, respectively.

Storage time (day)	Treatment	L	a	b	TSS (%)	pH	Titratable acidity (g citric acid/100g)	Firmness (N)	Water loss (%)
0	Control	37.6 ± 2.5 Aab	40.5 ± 0.3 Aa	26.1 ± 2.6 Aa	9.0 ± 1.7 Aa	3.4 ± 0.1 Ab	0.9 ± 0.16 Ba	48.2 ± 6.0 Aab	N/A
	UV-A	36.6 ± 2.0 Ab	37.1 ± 2.0 Ab	25.1 ± 1.4 Aab	8.7 ± 0.6 Aa	3.5 ± 0.1 Aab	0.9 ± 0.03 Aa	43.5 ± 3.0 Ab	N/A
	CH-GA	39.4 ± 1.4 Aab	37.9 ± 0.4 Aab	25.4 ± 2.0 Aa	9.3 ± 1.2 Aa	3.7 ± 0.1 Aa	0.9 ± 0.08 Aa	50.1 ± 2.4 Aa	N/A
	CH-GA+UV-A	39.7 ± 2.0 Aa	35.4 ± 0.9 Ab	22.0 ± 1.0 Ab	9.7 ± 1.2 Aa	3.6 ± 0.1 Aa	0.9 ± 0.03 Aa	43.1 ± 3.9 Ab	N/A
7	Control	40.4 ± 3.2 Aa	41.8 ± 1.9 Aa	28.1 ± 3.0 Aa	8.3 ± 1.5 Aa	3.6 ± 0.2 Aa	0.9 ± 0.02 Ba	41.7 ± 1.3 Aa	1.1 ± 0.1 Bb
	UV-A	36.8 ± 2.6 Ab	38.6 ± 3.6 Ab	25.0 ± 3.3 Aab	8.0 ± 1.0 Aa	3.6 ± 0.1 Aa	1.0 ± 0.06 Aa	39.0 ± 7.1 Aa	1.3 ± 0.1 Bab
	CH-GA	37.8 ± 1.0 Aab	37.0 ± 0.7 ABb	23.4 ± 0.3 Abc	8.0 ± 1.0 Aa	3.7 ± 0.1 Aa	0.8 ± 0.04 Aa	36.8 ± 7.7 Ba	1.1 ± 0.1 Bb
	CH-GA+UV-A	35.8 ± 1.3 Bb	34.1 ± 1.4 Ac	20.6 ± 1.2 Ac	8.3 ± 1.5 Aa	3.6 ± 0.1 Aa	0.8 ± 0.10 Aa	40.0 ± 3.5 Aa	1.5 ± 0.2 Ba
14	Control	36.8 ± 3.6 Aa	35.2 ± 3.6 Ba	25.2 ± 2.9 Aa	6.7 ± 1.2 Aa	3.6 ± 0.1 Aa	1.2 ± 0.04 Aa	23.2 ± 5.6 Ba	2.2 ± 0.1 Aa
	UV-A	37.3 ± 2.0 Aa	34.4 ± 4.7 Aa	25.5 ± 4.1 Aa	8.0 ± 1.0 Aa	3.6 ± 0.1 Aa	1.0 ± 0.06 Ab	31.7 ± 13.1 Aa	2.4 ± 0.3 Aa
	CH-GA	39.0 ± 1.8 Aa	34.0 ± 3.4 Ba	25.3 ± 3.2 Aa	8.7 ± 0.1 Aa	3.6 ± 0.1 Aa	0.8 ± 0.06 Ac	32.8 ± 5.7 Ba	2.2 ± 0.1 Aa
	CH-GA+UV-A	37.6 ± 2.4 Aa	33.0 ± 3.4 Aa	23.1 ± 2.9 Aa	8.3 ± 0.1 Aa	3.6 ± 0.1 Aa	0.9 ± 0.06 Abc	36.7 ± 10.8 Aa	2.5 ± 0.2 Aa

CH-GA coated berries not exposed to light. On day 7, almost all the treated berries showed more color changes than the control group. For example, the redness of strawberries treated with CH-GA + UV-A (34.1±1.4) was significantly lower than CH-GA (37.0±0.7) and UV-A (38.6±3.6), both of which were even lower than the control (41.8±1.9) ($P<0.05$). However, these differences disappeared ($P>0.05$) on day 14, indicating that photoirradiation may have altered the strawberry surface color initially but also slowed down the rate of color losses during the storage. The texture of the strawberries generally turned soft during storage, and the firmness of UV-A (43.5±3.0 N) and CH-GA + UV-A (43.1±3.9 N) treated berries were slightly lower than CH-GA (50.1±2.4 N) and the control (48.2±6.0 N) at day 0, indicating the

impact from light treatment. In contrast, there were no statistical differences between UV-A, CH-GA + UV-A, and the control groups on days 7 and 14. Additionally, results showed that only on day seven, strawberries treated with CH-GA + UV-A reached a higher level of water loss (1.5±0.2 %) than the control (1.1±0.1 %) and CH-GA (1.1±0.1 %). UV-A light and the heat generated by the light bulb in the chamber during treatment might be the leading cause of the color and texture reduction in strawberries from day one to day seven since elevated temperature could accelerate the degradation of color and firmness of fresh strawberries [94]. During storage, the chitosan coating layer can provide a barrier against oxygen and water; hence the degradation slowed down [88]. Further, the water loss was lower than in some reported studies, perhaps due to the difference in storage temperature, humidity, and package sealing [88,93].

The pH and TSS (%) values of different groups were generally stable; only the control at day zero was lower than coated berries ($P<0.05$). For the titratable acidity, the control group increased from 0.9 to 1.2 on day 14 ($P<0.05$), at which point the value was also higher than other treated groups. Results indicated that the strawberries without treatments might have more chemical changes than the light treated or coated berries. The changing patterns of pH, TSS (%), and titratable acidity of the coated strawberries during storage generally fit with the previous studies [88,93]. Thus, the photo-irradiated coating had a limited impact on the chemical properties of strawberry fruits.

Conclusion

A novel UV-A light irradiated CH-GA coating was developed previously to improve fresh strawberries' safety and quality. This study shows that photo-irradiation may have altered the strawberry surface color initially but slowed down the rate of color losses during the storage, as evidenced during 14-day refrigerated storage. The same observation was seen regarding the firmness of the strawberries. The initial loss of color and firmness is most likely

the result of UV-A radiation and the heat generated by the light bulbs in the treatment chamber. These results suggest that the proposed approach can offer benefits in improving the safety and shelf-life quality of strawberries.

Appendix 1: Using Postharvest Ultraviolet Irradiation Treatments to Improve Red Skin Coloration and Decrease *Listeria Monocytogenes* Survival on Honeycrisp Apples

Abstract

The third study aims to evaluate UV-C radiation's efficacy in the inactivation of *Listeria monocytogenes (L. monocytogenes)* on apple surfaces. This study was performed with the broader aim of evaluating the effects of UV-A, UV-B, and UV-C and their combinations on the quality and safety of Honeycrisp apples. UV-C radiation can serve as an antimicrobial agent, while UV-A and UV-B radiations can affect the quality parameters such as color through the hormetic effect. Therefore, our goal was to identify optimum UV-A, UV-B, and UV-C radiation doses that can be applied to Honeycrisp apples to improve their coloration and microbial safety as the marketability of apples often depends on the redness of the fruit. The UV-C dose of 7.5 kJ/m^2 resulted in a 1.2±0.06 log CFU/sample inactivation of *L. monocytogenes* on the apple surface. Interestingly, the additional UV-C dose exposure did not result in additional inactivation. This observed lack of dose-dependence could be the result of a) UV-C penetration interference from previously inactivated microbial cells resulting in a shadowing effect, b) the formation of a biofilm during ambient air drying and 4°C incubation that provided some protection during treatment, or c) higher resistance of *L. monocytogenes* sub-population against UV-C inactivation. This data will allow for future exploration of a synergistic treatment that can improve the color and appearance of Honeycrisp apples and improve their safety at the same time.

Introduction

Poor red skin coloration at harvest is a significant factor limiting the production and marketability of the profitable apple cultivar Honeycrisp. Light wavelength and intensity, and temperature affect red skin coloration during ripening on the tree and throughout storage. Our goal was to evaluate and compare the effect of different postharvest UV irradiation treatments applied under different storage temperatures on Honeycrisp skin red coloration, surface blush percentage, and *L. monocytogenes* survival. To that end, this study focuses on identifying the UV-C dose needed to achieve *L. monocytogenes* reduction on the Honeycrisp apple surface.

Materials and methods

Materials

Honeycrisp apples were harvested at their green stage from a commercial orchard in Maryland, USA. Difco tryptic soy agar (Becton Dickinson – BD, lot # 1153602), Bacto tryptic soy agar (Becton Dickinson – BD, lot # 7324570), buffered peptone water (ThermoFisher Scientific – OXOID, lot # 3172913), Tween 20 (ThermoFisher, lot # 200514), spiral plater (IUL micro – Eddy Jet 2, model # 90003800, Barcelona
Spain) were obtained from respective vendors.

UV processing unit

A batch-UV processing unit (UV-C radiation, model # XL-1000, Spectrolinker UV crosslinker by Spectroline Laboratory, Farmingdale, NY) was used for all experiments. The UV chamber contains five UV bulbs (BLE-8T354) emitting UV-C (254 nm) radiation within a 34.3 × 17.8 × 19.1 cm inner chamber. UV-C radiation doses were measured by an internal radiometer calibrated and programed by the manufacturer. The processing unit was programmed to shut off after the prescribed dose level was achieved. Variation in incident

intensity was minimized using the manufactures recommendation of a five-minute warm-up period from a cold start to allow the UV tubes to stabilize for more accurate operation. An intensity check was also performed to ensure that units were operating at the proper intensity each day.

Preparation of apple surface

Honeycrisp apples were brought to the laboratory at the University of Maryland, College Park, MD, USA. The apples were carefully selected to ensure the absence of scarring or bruising. The apples were then sanitized with 70% ethanol, tween 20 (to remove wax), and rinsed again with deionized water. The apples (whole) were then placed inside a biosafety cabinet with laminar airflow to dry for 120 minutes at the ambient room temperature (approximately 22°C) to remove any remaining moisture from the apple's surface. Apples were then sliced along the sagittal plane into 6 even wedges using a food-grade stainless steel knife, sterilized with 70% ethanol. A circular impression with a 1-inch diameter was then made on the surface of each wedge using USDA standards for grades area gauge, IA #30 G, sterilized with 70% ethanol. Sterile plastic toothpicks were then inserted into the flesh of each edge in order to allow the wedge to sit upright with the outer surface (skin) of the apple facing directly upward and as level as possible on a sterile petri dish.

Inoculum preparation

Listeria monocytogenes (ATCC #43256) has been used in other studies that involved microbiological responses on the surface of fruits and vegetables and was selected as the pathogen of choice for this experiment. The *L. monocytogenes* strain was stored in 30% glycerol (w/w), 20% water (v/v), tryptic soy broth (TSB) in a -80°C freezer. Frozen *L. monocytogenes* was sub-cultured in TSB and incubated for 24 hours at 37°C three consecutive times. After the third sub-culture step, the final strain grown was incubated at

37°C for 24 hours, allowing the bacteria to reach a stationary phase (approximately 7.8 log CFU/mL). This is the initial inoculum population-level used for the experiment.

Inoculation of apple surface

The *L. monocytogenes* inoculum was vortexed for 20 seconds before each inoculation. The 1-inch circle marked on each apple slice was then spot inoculated with a total of 200 µL of the inoculum in a bead-like fashion. The inoculated apples were then allowed to dry in a laminar airflow biosafety cabinet for 120 minutes. Once dry, apple slices were incubated at 4°C for 24 hours to simulate commercial storage conditions.

Exposure to UV-C radiation and microbiological sample preparation

Upon completion of the 24-hour incubation period at 4°C, the apples were placed directly in the center of the UV processing unit and exposed to UV-C (254 nm) radiation for a total dose of 0-, 7.5-, 15- and 30- kJ/m^2. Each treatment dose was measured by the internal radiometer calibrated and programed by the manufacturer. Once the internal radiometer has reached the dose programmed into the system, the UV processing unit automatically shuts off.

Microbiological analysis of apple samples

Immediately after each treatment, the 1-inch marked surface area previously inoculated with *L. monocytogenes* was carefully cut away from the apple flesh using a sterile stainless steel disposable scalpel. The inoculated apple discs were then transferred into a sterile stomacher bag (Nasco sampling WHIRL-PAK®, 7 oz filter bag) containing 5 mL of 0.1% buffered peptone water (BPW). Samples were hand massaged for 2 minutes and stomached (Steward Medical London, Stomacher 80 lab blender) at the high-speed setting for 5 minutes. The supernatant collected after stomaching was then serial diluted to 0-, 10-, and 100× for each sample. 50 µL of each sample was then spiral plated on Tryptic Soy Agar

(TSA) plates using a spiral plater. The plates were then incubated at 37°C for 24 hours, and the colonies were counted using a colony counter (IUL Instruments, Flash – N' – Go, Barcelona, Spain).

Data analysis and statistics

All experiments were performed in triplicate. A statistical student's t-test (α=0.05, $p<0.05$) from Microsoft® Excel® 365 was used to determine significant differences in UV-C treatments on the inoculated surface of the Honeycrisp apple.

Results and Discussion

The initial population of *L. monocytogenes* used to inoculate the surface of each apple was approximately 7.8 log CFU/mL. However, after surface inoculation, drying for 120 minutes at an ambient temperature of 22°C, and a 24-hour incubation at 4°C, the average population on the apple surface before treatment (control) was measured to be 4.2±0.03 log/CFU sample. This initial reduction in the population of *L. monocytogenes* could be attributed to the stress of the ambient air drying and the 4°C incubation period. Table 6 displays the results of 0- (control), 7.5-, 15-, and 30- kJ/m^2 exposure to UV-C radiation. The average reduction of each individual UV-C treatments resulted in a 1.1±0.3 log CFU/sample inactivation of *L. monocytogenes* on the apple surface. Interestingly, the additional UV-C dose exposure did

*Table 6. Average logarithmic reduction levels of Listeria Monocytogenes on Honeycrisp apple surface. Each data point is an average of triplicate measurement ± standard deviation. *The reported dose is the dose measured by the radiometer located at the base of the chamber. The treatment solution sits raised on a stirring plate and therefore experiences higher intensity than reported.*

UV-C Dose (kJ/m^2)	**log CFU/sample**
0 (control)	4.2±0.03
UV-C Dose (kJ/m^2)	***L. monocytogenes* reduction - log CFU/sample**
7.5	1.2±0.06
15	0.8±0.06
30	1.4±0.17

not result in additional inactivation. This observed lack of dose-dependence could be the result of a) UV-C penetration interference from previously inactivated microbial cells resulting in a shadowing effect, b) the formation of a biofilm during ambient air drying and 4°C incubation that provided some protection during treatment, or c) higher resistance of *L. monocytogenes* sub-population against UV-C inactivation. Further studies should be performed in order to understand the resistance mechanisms displayed in this experiment.

Conclusion

This experiment is part of a larger objective to identify optimum UV-A, UV-B, and UV-C radiation doses that can be applied to Honeycrisp apples to improve their coloration and microbial safety. The UV-C dose of 7.5 kJ/m^2 was the minimum dosage needed to achieve a 1.2±0.06 log CFU/sample inactivation of *L. monocytogenes* on the apple surface, and the additional UV-C dose did not result in additional inactivation. This data will allow for future exploration of a synergistic treatment that can improve the color and appearance of Honeycrisp apples and improve their safety at the same time.

Glossary

AA – Ascorbic acid

AUC – Area under the curve

CFR – Code of Federal Regulations

CH-GA – Chitosan gallic acid coating

DI – deionized water

DNA – Deoxyribonucleic acid

DRI – Daily reference intake

EL – excimer lamp

FDA – United States Food and Drug Administration

HAT – Hydrogen atom transfer mechanism

HP – Hydrogen peroxide

HPLC – High-performance liquid chromatography

LED – Light-emitting diode

LPM – Low-pressure mercury lamp

MPM – Medium pressure mercury lamp

PL – Pulsed light

RNA – Ribonucleic acid

ROP – Reactive oxidative potential

ROS – Reactive oxidative species

RTE – Ready to eat

TSS – Total soluble solids

UV – Ultraviolet radiation

UV-A – Ultraviolet radiation (315 – 400 nm)

UV-B – Ultraviolet radiation (280 – 315 nm)

UV-C – Ultraviolet radiation (200 – 280 nm)

Bibliography

(1) Stark, G. *Gamma Ray*. Encyclopedia Britannica. https://www.britannica.com/science/gamma-ray (accessed 2022-04-11).

(2) Editors of Encyclopedia. *Radio Wave*. Encyclopedia Britannica. https://www.britannica.com/science/radio-wave (accessed 2022-04-11).

(3) Mattson, B.; Gibb, M. *Electromagnetic Spectrum*. NASA's Imagine the Universe.

(4) McColl, S. L.; Veitch, J. A. Full-Spectrum Fluorescent Lighting: A Review of Its Effects on Physiology and Health. *Psychological Medicine*. 2001, pp 949–964. https://doi.org/10.1017/S0033291701004251.

(5) Koutchma, T. *Food Plant Safety : UV Applications for Food and Non-Food Surfaces*; Elsevier Science & Technology: San Diego, UNITED STATES, 2014.

(6) Wolber, R.; Schlenz, K.; Wakamatsu, K.; Smuda, C.; Nakanishi, Y.; Hearing, V. J.; Ito, S. Pigmentation Effects of Solar-Simulated Radiation as Compared with UVA and UVB Radiation. *Pigment Cell and Melanoma Research* 2008, *21* (4), 487–491. https://doi.org/10.1111/j.1755-148X.2008.00470.x.

(7) Koutchma, T. *Preservation and Shelf Life Extension : UV Applications for Fluid Foods*; Elsevier Science & Technology: San Diego, UNITED STATES, 2014.

(8) Beck, S. E.; Rodriguez, R. A.; Hawkins, M. A.; Hargy, T. M.; Larason, T. C.; Linden, K. G. Comparison of UV-Induced Inactivation and RNA Damage in MS2 Phage across the Germicidal UV Spectrum. *Applied and Environmental Microbiology* 2016, *82* (5), 1468–1474. https://doi.org/10.1128/AEM.02773-15.

(9) Mackenzie, D. Ultraviolet Light Fights New Virus. *Engineering* 2020, *6* (8), 851–853. https://doi.org/10.1016/j.eng.2020.06.009.

(10) de Jager, T. L.; Cockrell, A. E.; du Plessis, S. S. Ultraviolet Light Induced Generation of Reactive Oxygen Species. *Adv Exp Med Biol* 2017, *996*, 15–23.

(11) Guerrero-Beltrán, J. A.; Barbosa-Cánovas, G. v. Review: Advantages and Limitations on Processing Foods by UV Light. *Food Science and Technology International*. June 2004, pp 137–147. https://doi.org/10.1177/1082013204044359.

(12) Stevens, C.; Liu, J.; Khan, V. A.; Lu, J. Y.; Wilson, C. L.; Igwegbe, E. C. K.; Kabwe, M. K.; Chalutz, E.; Droby, S. Application of Hormetic UV-C for Delayed Ripening and Reduction of Rhizopus Soft Rot in Tomatoes: The Effect of Tomatine on Storage Rot Development. *Journal of Phytopathology* 1998, *146* (5–6), 211–221. https://doi.org/https://doi.org/10.1111/j.1439-0434.1998.tb04682.x.

(13) U.S. Food and Drug Administration. CFR 2021 Title 21 Vol 3 Part 179.39 Ultraviolet Radiation for the processing and Treatment of Food.

(14) U.S Food and Drug Administration. *CFR 2021 Title 21 Vol 2 Sec 120.24 HACCP Systems - Process Controls*.

(15) Food Processing Equipment, Inc. *COMPANY — FPE*. https://headwaterfpe.com/company (accessed 2022-04-12).
(16) Quintero-Ramos, A.; Churey, J. J.; Hartman, P.; Barnard, J.; Worobo, A. R. W. *Modeling of Escherichia Coli Inactivation by UV Irradiation at Different pH Values in Apple Cider*; 2004; Vol. 67.
(17) Hanes, D. E.; Worobo, R. W.; Orlandi, P. A.; Burr, D. H.; Miliotis, M. D.; Robl, M. G.; Bier, J. W.; Arrowood, M. J.; Churey, J. J.; Jackson, G. J. Inactivation of Cryptosporidium Parvum Oocysts in Fresh Apple Cider by UV Irradiation. *Applied and Environmental Microbiology* 2002, *68* (8), 4168–4172. https://doi.org/10.1128/AEM.68.8.4168-4172.2002.
(18) Orlowska, M.; Koutchma, T.; Grapperhaus, M.; Gallagher, J.; Schaefer, R.; Defelice, C. Continuous and Pulsed Ultraviolet Light for Nonthermal Treatment of Liquid Foods. Part 1: Effects on Quality of Fructose Solution, Apple Juice, and Milk. *Food and Bioprocess Technology* 2013, *6* (6), 1580–1592. https://doi.org/10.1007/s11947-012-0779-8.
(19) Delorme, M. M.; Guimarães, J. T.; Coutinho, N. M.; Balthazar, C. F.; Rocha, R. S.; Silva, R.; Margalho, L. P.; Pimentel, T. C.; Silva, M. C.; Freitas, M. Q.; Granato, D.; Sant'Ana, A. S.; Duart, M. C. K. H.; Cruz, A. G. Ultraviolet Radiation: An Interesting Technology to Preserve Quality and Safety of Milk and Dairy Foods. *Trends in Food Science and Technology*. Elsevier Ltd August 1, 2020, pp 146–154. https://doi.org/10.1016/j.tifs.2020.06.001.
(20) Koutchma, T.; Orlowska, M.; Zhu, Y. *UV LIGHT FOR FRUITS AND FRUIT PRODUCTS*.
(21) Hagen, S. F.; Borge, G. I. A.; Bengtsson, G. B.; Bilger, W.; Berge, A.; Haffner, K.; Solhaug, K. A. Phenolic Contents and Other Health and Sensory Related Properties of Apple Fruit (Malus Domestica Borkh., Cv. Aroma): Effect of Postharvest UV-B Irradiation. *Postharvest Biology and Technology* 2007, *45* (1), 1–10. https://doi.org/10.1016/j.postharvbio.2007.02.002.
(22) Konczak, I.; Zhang, W. *Anthocyanins-More Than Nature's Colours*; 2004.
(23) Wang, C. Y.; Chen, C. T.; Wang, S. Y. Changes of Flavonoid Content and Antioxidant Capacity in Blueberries after Illumination with UV-C. *Food Chemistry* 2009, *117* (3), 426–431. https://doi.org/10.1016/j.foodchem.2009.04.037.
(24) Gonzalez-Aguilar, G.; Wang, C. Y.; Buta, G. J. UV-C Irradiation Reduces Breakdown and Chilling Injury of Peaches during Cold Storage. *Journal of the Science of Food and Agriculture* 2004, *84* (5), 415–422. https://doi.org/10.1002/jsfa.1675.
(25) Mau, J. L.; Chen, P. R.; Yang, J. H. Ultraviolet Irradiation Increased Vitamin D2 Content in Edible Mushrooms. *Journal of Agricultural and Food Chemistry* 1998, *46* (12), 5269–5272. https://doi.org/10.1021/jf980602q.
(26) Erkan, M.; Wang, S. Y.; Wang, C. Y. Effect of UV Treatment on Antioxidant Capacity, Antioxidant Enzyme Activity and Decay in Strawberry Fruit. *Postharvest Biology and Technology* 2008, *48* (2), 163–171. https://doi.org/10.1016/j.postharvbio.2007.09.028.
(27) Krishnamurthy, K.; Demirci, A.; Irudayaraj, J. M. Inactivation of Staphylococcus Aureus in Milk Using Flow-through Pulsed UV-Light

Treatment System. *Journal of Food Science* 2007, *72* (7). https://doi.org/10.1111/j.1750-3841.2007.00438.x.
(28) Matak, K. E.; Churey, J. J.; Worobo, R. W.; Sumner, S. S.; Hovingh, E.; Hackney, C. R.; Pierson, M. D. Efficacy of UV Light for the Reduction of *Listeria monocytogenes* in Goat's Milk. *Journal of Food Protection* 2005, *68* (10), 2212–2216.
(29) Lacivita, V.; Conte, A.; Lyng, J. G.; Arroyo, C.; Zambrini, V. A.; Nobile, M. A. del. High Intensity Light Pulses to Reduce Microbial Load in Fresh Cheese. 2018. https://doi.org/10.1017/S0022029918000134.
(30) Diesler, K.; Golombek, P.; Kromm, L.; Scharfenberger-Schmeer, M.; Durner, D.; Schmarr, H. G.; Stahl, M. R.; Briviba, K.; Fischer, U. UV-C Treatment of Grape Must: Microbial Inactivation, Toxicological Considerations and Influence on Chemical and Sensory Properties of White Wine. *Innovative Food Science and Emerging Technologies* 2019, *52*, 291–304. https://doi.org/10.1016/j.ifset.2019.01.005.
(31) Ha, J. W.; Kang, D. H. Enhanced Inactivation of Food-Borne Pathogens in Ready-to-Eat Sliced Ham by near-Infrared Heating Combined with UV-C Irradiation and Mechanism of the Synergistic Bactericidal Action. *Applied and Environmental Microbiology* 2015, *81* (1), 2–8. https://doi.org/10.1128/AEM.01862-14.
(32) Bignold Birkhäuser, L. P.; Basel, V.; Boston, ·; Berlin, ·. *Cancer: Cell Structures, Carcinogens and Genomic Instability*.
(33) Ding, Q.; Alborzi, S.; Bastarrachea, L. J.; Tikekar, R. v. Novel Sanitization Approach Based on Synergistic Action of UV-A Light and Benzoic Acid: Inactivation Mechanism and a Potential Application in Washing Fresh Produce. *Food Microbiology* 2018, *72*, 39–54. https://doi.org/10.1016/j.fm.2017.11.004.
(34) de Oliveira, E. F.; Cossu, A.; Tikekar, R. v; Nitin, N.; Björkroth, J. Enhanced Antimicrobial Activity Based on a Synergistic Combination of Sublethal Levels of Stresses Induced by UV-A Light and Organic Acids. *aem.asm.org 1 Applied and Environmental Microbiology Downloaded* 2017, *83*, 383–400. https://doi.org/10.1128/AEM.
(35) Castagna, A.; Chiavaro, E.; Dall'Asta, C.; Rinaldi, M.; Galaverna, G.; Ranieri, A. Effect of Postharvest UV-B Irradiation on Nutraceutical Quality and Physical Properties of Tomato Fruits. *Food Chemistry* 2013, *137* (1–4), 151–158. https://doi.org/10.1016/j.foodchem.2012.09.095.
(36) Shamma, G. Ultraviolet Irradiation. In *Encyclopedia of Food Microbiology*; Robinson, C., Patel, B., Patel, P., Eds.; Academic Press: London, 1999; Vol. 3, pp 2208–2214.
(37) Sizer, C. E.; Balasubramaniam, V. M. New Intervention Processes for Minimally Processed Juices. *Food Technology* 1999, 64–67.
(38) Wright, J. R.; Sumner, S. S.; Hackney, C. R.; Pierson, M. D.; Zoecklein, B. W. *Efficacy of Ultraviolet Light for Reducing Escherichia Coli O157:H7 in Unpasteurized Apple Cider*; 2000; Vol. 63.
(39) Tikekar, R. v.; Anantheswaran, R. C.; Laborde, L. F. Patulin Degradation in a Model Apple Juice System and in Apple Juice during Ultraviolet Processing.

Journal of Food Processing and Preservation 2014, *38* (3), 924–934. https://doi.org/10.1111/jfpp.12047.
(40) Tikekar, R. v.; Anantheswaran, R. C.; Elias, R. J.; Laborde, L. F. Ultraviolet-Induced Oxidation of Ascorbic Acid in a Model Juice System: Identification of Degradation Products. *Journal of Agricultural and Food Chemistry* 2011, *59* (15), 8244–8248. https://doi.org/10.1021/jf201000x.
(41) Hoyer, O. Testing Performance and Monitoring of UV Systems for Drinking Water Disinfection. *Water Supply* 1998, *16*, 424–429.
(42) Sikorski, Z. E.; Kolakowska, A. *Chemical and Functional Properties of Food Lipids*, 1st ed.; CRC Press: Boca, 2002.
(43) Summerfelt, S. T. Ozonation and UV Irradiation - An Introduction and Examples of Current Applications. In *Aquacultural Engineering*; 2003; Vol. 28, pp 21–36. https://doi.org/10.1016/S0144-8609(02)00069-9.
(44) Bergman, R. S. Germicidal UV Sources and Systems†. *Photochemistry and Photobiology*. Blackwell Publishing Inc. May 1, 2021, pp 466–470. https://doi.org/10.1111/php.13387.
(45) Tan And, Q.; Li, J. Potential Mercury Emissions from Fluorescent Lamps Production and Obsolescence in Mainland China. *Waste Management and Research* 2016, *34* (1), 67–74. https://doi.org/10.1177/0734242X15616473.
(46) Sikorski, Z. E.; Kolakowska, A. *Chemical, Biological, and Functional Aspects of Food Lipids*, 2nd ed.; CRC Press: Boca Raton, 2011.
(47) Min, D. B.; Boff, J. M. *Chemistry and Reaction of Singlet Oxygen in Foods Materials and Methods Discoveries of Oxygen and Its Properties*; 2001; Vol. 1.
(48) Rödel, W. J. S. Fruton: Molecules and Life – Historical Essays on the Interplay of Chemistry and Biology. 579 Seiten. Wiley-Interscience, New York, London, Sydney, Toronto 1972. Preis: 8,95 £. *Food / Nahrung* 1974, *18* (4), 471–472. https://doi.org/10.1002/FOOD.19740180423.
(49) Miller, W. A. *Elements of Chemistry; Theoretical and Practical*; London, John W. Parker & Son, 1857.
(50) Johnson, J. M.; Conforti, F. D. *FRUCTOSE*; 2003.
(51) Damodaran, S.; Parkin, K. L. . Fennema's Food Chemistry (5th Edition) - 5.5.2 Solubility. *CRC Press* 2017.
(52) Burns, D. T.; Johnston, E. L.; Walker, M. J. Authenticity and the Potability of Coconut Water-A Critical Review. *J AOAC Int* 2020, *103* (3), 800–806. https://doi.org/10.1093/jaocint/qsz008.
(53) Lindhorst, T. K. *Essentials of Carbohydrate Chemistry and Biochemistry, 3rd Completely Revised and Enlarged Edition | Wiley*, 3rd ed.; Wiley-VCH, 2007.
(54) Muller, P. *INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY ORGANIC CHEMISTRY DIVISION COMMISSION ON PHYSICAL ORGANIC CHEMISTRY* GLOSSARY OF TERMS USED IN PHYSICAL ORGANIC CHEMISTRY*; 1994; Vol. 66.
(55) Patai, Saul.; Zabicky, Jacob. *The Chemistry of the Carbonyl Group.*; Interscience Publishers, 1966.
(56) McMurry, J. C. *Organic Chemistry with Biological Applications*, 3rd ed.; Cengage Unlimited: Stanford, 2015.

(57) Campbell, M. K.; Farrell, S. O.; McDougal, O. M. *Biochemisty*, 9th ed.; Cengage Unlimited: Boston , 2016.
(58) Antonov, L. *Tautomerism : Methods and Theories*; Wiley-VCH: Weinheim, 2014.
(59) Barclay, T.; Ginic-Markovic, M.; Johnston, M. R.; Cooper, P.; Petrovsky, N. Observation of the Keto Tautomer of D-Fructose in D 2O Using 1H NMR Spectroscopy. *Carbohydrate Research* 2012, *347* (1), 136–141. https://doi.org/10.1016/j.carres.2011.11.003.
(60) Elsinghorst, A.; Tikekar, R. v. Generation of Oxidative Species from Ultraviolet Light Induced Photolysis of Fructose. *Food Chemistry* 2014, *154*, 276–281. https://doi.org/10.1016/j.foodchem.2014.01.008.
(61) Triantaphylides, C.; Schuchmann, H.-P.; Sonntag, C. von. Photolysis of D-Fructose in Aqueous Solution. *Carbohydrate Research* 1982, *100* (1), 131–141.
(62) Tikekar, R. v.; Anantheswaran, R. C.; LaBorde, L. F. Ascorbic Acid Degradation in a Model Apple Juice System and in Apple Juice during Ultraviolet Processing and Storage. *Journal of Food Science* 2011, *76* (2), H62–H71. https://doi.org/10.1111/j.1750-3841.2010.02015.x.
(63) Bule, M. v.; Desai, K. M.; Parisi, B.; Parulekar, S. J.; Slade, P.; Singhal, R. S.; Rodriguez, A. Furan Formation during UV-Treatment of Fruit Juices. *Food Chemistry* 2010, *122* (4), 937–942. https://doi.org/10.1016/j.foodchem.2010.03.116.
(64) Fan, X.; Geveke, D. J. Furan Formation in Sugar Solution and Apple Cider upon Ultraviolet Treatment. *Journal of Agricultural and Food Chemistry* 2007, *55* (19), 7816–7821. https://doi.org/10.1021/jf071366z.
(65) Ros-Polski, V.; Popović, V.; Koutchma, T. Effect of Ultraviolet-C Light Treatment on Hydroxymethylfurfural (5-HMF) Content in High Fructose Corn Syrup (HFCS) and Model Syrups. *Journal of Food Engineering* 2016, *179*, 78–87. https://doi.org/10.1016/j.jfoodeng.2016.01.027.
(66) Stockert, J. Carlos.; Blázquez-Castro, Alfonso. *Fluorescence Microscopy In Life Sciences.*; Bentham Science Publishers, 2017.
(67) Bennet, M. MULTI-PARAMETER QUANTITATIVE MAPPING OF MICROFLUIDIC DEVICES, 2011.
(68) Nayak, S.; Muniz, J.; Sales, C. M.; Tikekar, R. v. Fructose as a Novel Photosensitizer: Characterization of Reactive Oxygen Species and an Application in Degradation of Diuron and Chlorpyrifos. *Chemosphere* 2016, *144*, 1690–1697. https://doi.org/10.1016/j.chemosphere.2015.10.074.
(69) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe. *Journal of Agricultural and Food Chemistry* 2001, *49* (10), 4619–4626. https://doi.org/10.1021/jf010586o.
(70) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe. *Journal of Agricultural and Food Chemistry* 2001, *49* (10), 4619–4626. https://doi.org/10.1021/jf010586o.

(71) Prior, R. L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampsch-Woodill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for Hydrophilic and Lipophilic Antioxidant Capacity (Oxygen Radical Absorbance Capacity (ORACFL)) of Plasma and Other Biological and Food Samples. *Journal of Agricultural and Food Chemistry* 2003, *51* (11), 3273–3279. https://doi.org/10.1021/jf0262256.

(72) National Center for Biotechnology Information. *Ascorbic acid*. PubChem. https://pubchem.ncbi.nlm.nih.gov/compound/Ascorbic-acid (accessed 2022-04-06).

(73) *The Role of Food, Agriculture, Forestry and Fisheries in Human Nutrition*; Victor, S. R., Ed.; Encyclopedia of Life Support Systems: Oxford, 2009; Vol. 4.

(74) Parrow, N. L.; Leshin, J. A.; Levine, M. Parenteral Ascorbate as a Cancer Therapeutic: A Reassessment Based on Pharmacokinetics. *Antioxidants and Redox Signaling*. December 10, 2013, pp 2141–2156. https://doi.org/10.1089/ars.2013.5372.

(75) Koutchma, T. Advances in Ultraviolet Light Technology for Non-Thermal Processing of Liquid Foods. *Food and Bioprocess Technology*. 2009, pp 138–155. https://doi.org/10.1007/s11947-008-0178-3.

(76) Jurkiewicz, B. A.; Buettner, G. R. EPR Detection of Free Radicals in UV-Lrradiated Skin: Mouse Versus Human. *Photochemistry and Photobiology* 1996, *64* (6), 918–922. https://doi.org/10.1111/j.1751-1097.1996.tb01856.x.

(77) Jung, M. Y.; Kim, S. K. Riboflavin-Sensitized Photooxidation of Ascorbic Acid: Kinetics and Amino Acid Effects. *Food Chemistry* 1995, 397–403.

(78) Donahue, D. W.; Canitez, N.; Bushway, A. A. *UV INACTIVATION OF E. COLI O157:H7 IN APPLE CIDER: QUALITY, SENSORY AND SHELF-LIFE ANALYSIS*; 2004; Vol. 28.

(79) Wang, Q.; Durand, E.; Elias, R. J.; Tikekar, R. v. Generation of Reactive Oxidative Species from Thermal Treatment of Sugar Solutions. *Food Chemistry* 2016, *196*, 301–308. https://doi.org/10.1016/j.foodchem.2015.09.044.

(80) Ou, B.; Hampsch-Woodill, M.; Flanagan, J.; Deemer, E. K.; Prior, R. L.; Huang, D. Novel Fluorometric Assay for Hydroxyl Radical Prevention Capacity Using Fluorescein as the Probe. *Journal of Agricultural and Food Chemistry* 2002, *50* (10), 2772–2777. https://doi.org/10.1021/jf011480w.

(81) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT Food Science and Technology* 1995, *28* (1), 25–30.

(82) Nienow, A. M.; Bezares-Cruz, J. C.; Poyer, I. C.; Hua, I.; Jafvert, C. T. Hydrogen Peroxide-Assisted UV Photodegradation of Lindane. *Chemosphere* 2008, *72* (11), 1700–1705. https://doi.org/10.1016/j.chemosphere.2008.04.080.

(83) Bhullar, M. S.; Patras, A.; Kilanzo-Nthenge, A.; Pokharel, B.; Yannam, S. K.; Rakariyatham, K.; Pan, C.; Xiao, H.; Sasges, M. Microbial Inactivation and Cytotoxicity Evaluation of UV Irradiated Coconut Water in a Novel Continuous Flow Spiral Reactor. *Food Research International* 2018, *103*, 59–67. https://doi.org/10.1016/j.foodres.2017.10.004.

(84) Yannam, S. K.; Patras, A.; Pendyala, B.; Vergne, M.; Ravi, R.; Gopisetty, V. V. S.; Sasges, M. Effect of UV-C Irradiation on the Inactivation Kinetics of Oxidative Enzymes, Essential Amino Acids and Sensory Properties of Coconut Water. *Journal of Food Science and Technology* 2020, *57* (10), 3564–3572. https://doi.org/10.1007/s13197-020-04388-4.

(85) National Research Council (U.S.); Institute of Medicine (U.S.). *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids : A Report of the Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine.*; National Academy Press: Washington, D.C, 2000.

(86) Yu, K.; Newman, M. C.; Archbold, D. D.; Hamilton-Kemp, T. R. *Survival of Escherichia Coli O157:H7 on Strawberry Fruit and Reduction of the Pathogen Population by Chemical Agents*; 2001; Vol. 64.

(87) Gómez-Aldapa, C. A.; Portillo-Torres, L. A.; Villagómez-Ibarra, J. R.; Rangel-Vargas, E.; Téllez-Jurado, A.; Cruz-Gálvez, A. M.; Castro-Rosas, J. Survival of Foodborne Bacteria on Strawberries and Antibacterial Activities of Hibiscus Sabdariffa Extracts and Chemical Sanitizers on Strawberries. *Journal of Food Safety* 2018, *38* (1). https://doi.org/10.1111/jfs.12378.

(88) Jiang, Y.; Yu, L.; Hu, Y.; Zhu, Z.; Zhuang, C.; Zhao, Y.; Zhong, Y. The Preservation Performance of Chitosan Coating with Different Molecular Weight on Strawberry Using Electrostatic Spraying Technique. *International Journal of Biological Macromolecules* 2020, *151*, 278–285. https://doi.org/10.1016/j.ijbiomac.2020.02.169.

(89) Vu, K. D.; Hollingsworth, R. G.; Leroux, E.; Salmieri, S.; Lacroix, M. Development of Edible Bioactive Coating Based on Modified Chitosan for Increasing the Shelf Life of Strawberries. *Food Research International* 2011, *44* (1), 198–203. https://doi.org/10.1016/j.foodres.2010.10.037.

(90) Hernández-Muñoz, P.; Almenar, E.; Valle, V. del; Velez, D.; Gavara, R. Effect of Chitosan Coating Combined with Postharvest Calcium Treatment on Strawberry (Fragaria × Ananassa) Quality during Refrigerated Storage. *Food Chemistry* 2008, *110* (2), 428–435. https://doi.org/10.1016/j.foodchem.2008.02.020.

(91) Wang, Q.; de Oliveira, E. F.; Alborzi, S.; Bastarrachea, L. J.; Tikekar, R. v. On Mechanism behind UV-A Light Enhanced Antibacterial Activity of Gallic Acid and Propyl Gallate against Escherichia Coli O157:H7. *Scientific Reports* 2017, *7* (1). https://doi.org/10.1038/s41598-017-08449-1.

(92) Zhang, H.; Tsai, S.; Tikekar, R. v. Inactivation of Listeria Innocua on Blueberries by Novel Ultrasound Washing Processes and Their Impact on Quality during Storage. *Food Control* 2021, *121*. https://doi.org/10.1016/j.foodcont.2020.107580.

(93) Velickova, E.; Winkelhausen, E.; Kuzmanova, S.; Alves, V. D.; Moldão-Martins, M. Impact of Chitosan-Beeswax Edible Coatings on the Quality of Fresh Strawberries (Fragaria Ananassa Cv Camarosa) under Commercial

Storage Conditions. *LWT - Food Science and Technology* 2013, *52* (2), 80–92. https://doi.org/10.1016/j.lwt.2013.02.004.

(94) Alexandre, E. M. C.; Brandão, T. R. S.; Silva, C. L. M. Efficacy of Non-Thermal Technologies and Sanitizer Solutions on Microbial Load Reduction and Quality Retention of Strawberries. *Journal of Food Engineering* 2012, *108* (3), 417–426. https://doi.org/10.1016/j.jfoodeng.2011.09.002.