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Performance Assessment of an Ultraviolet Light Emitting Semi-Conductor Device in Treating Apple Juice: Microbial Inactivation and Biochemical Assessment Study

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Abstract

Inactivation of *Listeria monocytogenes* ATCC 19115 and *Salmonella enterica* serovar Muenchen ATCC BAA 1764 by a light emitting diodes (LED) operating at 279 nm was investigated. In addition, this investigation assessed the poly-phenolic and vitamin content of UV irradiated apple juice (AJ). Specific concentrations of bacteria were inoculated in AJ and irradiated at the designated UV doses of 0 to 10 mJ·cm^{-2} for *Salmonella* Muenchen and 0 to 12 mJ·cm^{-2} for *Listeria monocytogenes*. Results show that UV-C irradiation effectively inactivated pathogenic microbes in AJ. The log reduction kinetics of microorganisms followed log-linear and with higher R^2 (>0.95). The D_{10} values of 3.50 and 3.56 mJ·cm^{-2} were obtained from the inactivation of *Salmonella* Muenchen, and *Listeria monocytogenes* in apple juice. In addition, quantifiable UV-C doses ranging from 0 to 160 mJ·cm^{-2} were also delivered to AJ and polyphenols and vitamins were profiled. LC-MS/MS analysis was conducted to assess the stability of polyphenols or vitamins in UV-C exposed AJ. The polyphenol and vitamin results demonstrated that UV-C irradiation in AJ can cause significant reductions ($p<0.05$) if not properly delivered. Chlorogenic acid was reduced to 56%, at 80 mJ/cm^2 whereas 12% reduction was observed at 40 mJ/cm^2. Choline was observed to be relatively stable as a function of UV-C dosage. In contrast thiamine was significantly reduced at higher doses. In addition, Epicatechin was significantly reduced at high exposure doses. In

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contrast minor changes were observed at 40 mJ/cm^2. The results from this study imply that adequate log reduction of pathogens is achievable in AJ and suggest significant potential of using LED devices for UV-C treatment of highly turbid fluids.

1. Introduction

Apples and their respective products contain numerous antioxidant phytochemicals which exhibit bioactive properties. Phytochemicals are best defined as nutrient bioactive chemicals derived from plants present in fruits, vegetables, and grains; and provide advantageous benefits to overall health outside of basic nutrition to reduce major chronic disease risks (Jimenez-Garcia, et al., 2018; Liu, 2004). The bioactive compound categories include phytochemicals, vitamins, minerals, and dietary fibers. Apple juice is also composed of a variety of sugars, which primarily include, fructose, glucose, and sucrose (Pina-Pérez, Rodrigo & Martinez, 2015). In addition to these sugars, is also the presence of starches (oligosaccharides and polysaccharides), acids (malic, quinic, citramalic, organic, amino), tannins (polyphenols, phenols), amides and other nitrogenous compounds, soluble pectin, vitamin C, minerals, boron, and a vast array of esters (Pina-Pérez, Rodrigo & Martinez, 2015; Smeriglio, et al., 2017; Swamy, Muthukumarappan, &Asokapandian, 2018, Wojdyło, et al., 2021; Teleszko & Wojdyło, 2015).

Apple juice is heavily consumed because of the many nutritional benefits (Muñoz, et al., 2012), pleasant organoleptic qualities (Muñoz,et al., 2012; Włodarska, et al., 2019), prebiotics content (Ribeiro et al., 2021), probiotics (Dimitrovski, et al., 2015; Cousin, et al., 2017), polyphenols (Du, et al., 2019; Zhang, et al., 2021), vitamins A,C, E, K (Islam, et al., 2016b; Karasawa & Mohan, 2018; Akwu, et al., 2022), minerals and dietary fibers (Karasawa & Mohan, 2018), strong antioxidant activity, cancer cell proliferation inhibition, lipid oxidation decreases, and lowered cholesterol (Akwu, et al., 2022; Kidoń & Grabowska, 2021; Boyer & Liu, 2004) contents.

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Collectively, they function in providing great benefits to our bodies against diseases, ailments, clinical anti-allergic activity (Heinmaa, et al., 2016). Perhaps due to high sugar content and high vitamin levels, AJ can be contaminated with vegetative cells, spores and other micro-organisms.

Fruit juices can be spoiled due to the growth of microorganisms. Yeasts and molds, *Lactobacillus*, *Leuconostoc* and thermophilic *Bacillus* are common spoilage microorganisms. Perhaps the juices can contain pathogenic micro-organism at 1-2 $\log_{10}$ concentration level. Control measures for low acid and acidic beverages are critical, and are likely to involve multiple measures, for example, a combination of a process steps to destroy the nonproteolytic spores and ‘‘Keep Refrigerated’’ labeling if the juice does not receive a treatment sufficient to destroy the proteolytic spores (21 CFR Parts 113 and 114). Additionally, guidance from the FDA is now strictly recommending that processors subject to the pathogen reduction provisions of the juice Hazard Analysis and Critical Control Points (HACCP) regulation (USFDA, 2004) incorporate validated control measures for all *B. cereus* and *C. botulinum* spores into their HACCP plans (USFDA, 2007), to control their growth and toxin production. For acidic juice products, *Escherichia coli, Saccharomyces cerevisiae and Listeria innocua* are associated with food-borne pathogens (Basaran, Quintero-Ramos, Moake, Churey, &Worobo, 2004; Guerrero-Beltrán & Barbosa-Cánovas, 2005). A 1998 FDA ruling Code of Federal Register, (63 FR 37030), made it mandatory for fruit and juice processors to provide potential sickness product labels if consumed without a 5$\log_{10}$ reduction of pathogenic microflora. As a result of this ruling, processors began utilizing conventional heat methods such as pasteurization to reduce the microbial population by a 5-$\log_{10}$ reduction. In addition to this rule, the U.S. Food and Drug Administration suggested that retailers also implement the 5-$\log_{10}$ reduction in addition to their respective state laws and regulations to be in compliant. Heat severely impacted the quality of juice products. Several different authors

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reported the impact of heat on vitamins and polyphenols (Patras, et al., 2020; Patras, Tiwari, & Brunton, 2011; Akwu, et al., 2022). As a result of the heat impact on product quality, it is important to explore alternative methods of pasteurization and sterilization that will retain the quality and safety of the product.

A document titled 'Kinetics of Microbial Inactivation for Alternative Food Processing Technologies: Executive Summary; was published by Food and Drug Administration and the Center for Food Safety and Applied Nutrition. This report evaluates and elaborates on alternative technologies and addresses the knowledge gaps and research needs. (CFSAN, 2000). According to *Akwu, et al. 2022*; there is an urgent to investigate novel non-thermal processing technologies and generate microbial inactivation for a range of pathogens to demonstrate its effectives. UVLEDs are high-tech technologies that require validation via scientific research studies to demonstrate, "proof of principle;" which demonstrates the efficacy of them as a novel technology (Akwu, et al., 2022). UV technologies have shown considerable promise for pasteurization and sterilization of acidic and low acid fluids. (Akwu, et al., 2022; Patras, et al., 2020; Delorme, et al., 2020; Rahman, 2020; Koutchma, 2019; Islam, et al., 2016b). These alternative pasteurization methods have been registered for juices through the Code of Federal Register (CFR 179.39).

The electromagnetic spectrum contains three different wavelengths for UV-C irradiation that range from 100 nm to 400 nm. Between 320 nm and 400 nm is classified as the UV-A range, UVB is classified between 280 nm and 320 nm, and the UV-C range is classified between 200 nm and 280 nm (Akwu, et al., 2022; Centers for Disease Control and Prevention, 2022; Johns Hopkins Medicine, 2022; World Health Organization, 2016). Each of the wavelengths A, B, and C can pose challenges to our overall health, if we do effectively prepare ourselves. UV-C is the most effective antiseptic or disinfectant agent against many different microorganism types; as photons are

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consumed by DNA (Akwu, et al., 2022; Yang et al., 2019; Yin et al., 2013; Dai et al., 2012). As Patras, et al., 2020 mentioned, photochemical reactions that take place in DNA function by hindering the ability of microbial replication to occur. UV-C irradiation studies uses low pressure lamps as an optical source. There is an interest in implementing LED device for disinfection studies (Akwu, et al., 2022).

LED devices utilizing high UV intensity have been utilized by several authors (Kurup, et al., 2022; Akwu, et al., 2022; Prasad, et al., 2020; Kusuma, Pattison, & Bugbee, 2020). Though many studies have utilized ultraviolet (UV) light emitting diodes (LEDs); two major weaknesses are present to include, verification and calculation of the UV dosage (Heckman, et al., 2013; Beck et al., 2017) and the inadequacy of optical data utilized in the studies (Akwu, et al., 2022; Caminiti, et al., 2012; Unluturk, et al., 2010). LED spectral output is not a single wavelength but a collection of other wavelength (±10 nm), average fluence calculations need to account for all wavelengths. This study fills knowledge gaps in the literature on the efficacy of LED's and its ability to inactivate pathogens at relevant inactivation doses.

The main objective of this study was to develop standardized UV fluence response curves for *Listeria monocytogenes* ATCC 19115 and *Salmonella enterica* serovar Muenchen ATCC BAA 1764 suspended in apple juice under dynamic stirred conditions. A secondary aim was to assess the effect of UV-C irradiation on polyphenols and vitamins in apple juice.

2. Materials and methods

2.1 Chemicals

Phloridzin dihydrate, was bought from Sigma Aldrich, USA, Whereas, (-)-epicatechin was bought from Adooq Bioscience, CA, USA. Furthermore, analytical grade epicatechin, thiamine,

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choline chloride, B12 and Phlorizin were purchased from Sigma Aldrich, Missouri, USA. HPLC and LCMS grade water, methanol and acetonitrile were sourced from Fisher Scientific, USA.

2.2 Sample Preparation

Similar preparation was conducted as described by Akwu et al., 2022. Apples (*cv* Gala) were obtained from a local grocery store. All apples were thoroughly washed and juiced using a Brentwood 800W juicer. The juice was strained and then filtered using Whatman (28-30 µm, 2-3 µm). Optical data was obtained and recorded and 45mL per culture tube were stored at -20°C wrapped with aluminum foil until further processing, to avoid exposure to light. Two filtration steps were implemented to remove solid particulates. Prior to irradiation, AJ samples were randomly assigned to a treatment process and thawed at room temperature. Following the juicing process, baseline optical data was obtained, and apple were divided into 20 aliquots of 10 ml each stored at −20 ºC until used. Physicochemical characteristics of the AJ is shown in Table 1. A balanced design with three replicates randomized in experimental order was performed for each UV dose.

2.3 Bacterial strains and cultural conditions

Two non-pathogenic and non-outbreak strains of different bacteria were used in this study *Salmonella enterica* serovar Muenchen ATCC BAA (1764) and *Listeria monocytogenes* (19115). The bacterial strains were procured from American Type Culture Collection (ATCC). The bacterial cultures were stored in 25% glycerol in cryovials at -80 ˚C. Fresh bacterial suspensions were prepared for inoculation into apple juice for every treatment. Two loops of individual strains of *S.* Muenchen *and L. monocytogenes* were transferred to 15 mL Tryptic soy broth (Oxoid Ltd., Basingstoke, UK) and incubated at 37 °C for 18 h. *L. monocytogenes* was also subjected to two successive transfers in tubes containing 15 mL of Buffered Listeria enrichment broth (Oxoid Ltd.,

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Basingstoke, UK) and incubation was done for 24 h at 37 °C. These cultures were used as the adapted inoculum. After incubation, *S.* Muenchen culture was transferred into 15 mL of TSB and incubated for 18 h at 37 °C to reach the stationary growth phase. Similarly, *L. monocytogenes* culture was transferred to 15 mL *Listeria* enrichment broth (Oxoid Ltd., Basingstoke, UK) and incubated for 24 h at 37 °C. Centrifugation (3000 × g, 15 min) was done to harvest the bacterial cells. A solution of 0.1% (w/v) phosphate-buffered saline (PBS), Becton Dickinson, New Jersey, US) was used to wash the cell pellets and re-suspended in 50 mL of PBS. For determining the original cell population densities, appropriate dilutions of each cell suspension were made in 0.1% peptone water (PW) and plated in duplicate using Tryptic Soy Agar (Oxoid Ltd., Basingstoke, UK) plates for *S.* Muenchen suspensions and incubation was done at 37 °C for 24 h. *L. monocytogenes* suspensions were plated on Listeria selective agar base (SR0141E) (Oxoid Ltd., Basingstoke, UK) plates with incubation at 37 °C for 48 h.

2.4 Apple juice inoculation

Aliquots of 45 mL of AJ were inoculated individually with each of the two bacterial cultures (*L. monocytogenes, S.* Muenchen) targeting a concentration of 10^7 CFU/mL. The inoculated apple juice was plated using decimal dilutions on Tryptic soy agar (Oxoid Ltd., Basingstoke, UK) plates to determine the original *S.* Muenchen titers and incubation was done at 37 °C for 24 h. Apple juice inoculated with *L. monocytogenes* was plated on *Listeria* selective agar base (Oxoid Ltd., Basingstoke, UK) and plates were then incubated at 37 °C for 48 h.

2.5 Optical properties and pH measurements

A method described by Akwu et al (2022) was adapted for optical property measurements. The absorption coefficient at 279 nm was determined based on transmittance measurements from a Cary 300 spectrophotometer with a six-inch integrating sphere (Agilent Technologies, CA, US).

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Baseline corrections, i.e., by zeroing (setting the full-scale reading of) the instrument using the blank and then blocking the beam with a black rectangular slide was carried out. All pH readings were measured using a standard pH meter (Jenway, Staffordshire, UK). All readings were taken in triplicate to lessen the measurement error. From this data, ultraviolet transmittance value was mathematically quantified.

2.6 UV-C irradiation treatments

A near collimated beam system that utilizes a light emitting diode device (Irtronix, Torrence, CA, USA) producing irradiation at 279 nm was used for the UV exposures. The system consisted of 3x3 configuration. Electro optical characteristics of UV-C LED has a maximum forward voltage of 35 Vdc, power consumption of 28 Watts with radiant power of 290 mW. To increase mixing, a 5-mL sample was stirred in a10-mL beakers (height: 3.2 cm; diameter: 1.83 cm) (Bolton &Linden, 2003; Chandra, et al., 2017). All optical parameters and UV dose calculations are described in our published studies (Stanley, et al., 2020; Islam, et al., 2016a; Islam, et al., 2016b). Using a high sensitivity sensor (QE Pro series, Ocean Optics, Dunedin, FL, USA), the central irradiance of the UV-C LED system on the surface of the test solution was measured. Based on the central irradiance of the lamp and optical properties (absorption coefficient) of apple juice, the average fluence was calculated. Four correction factors i.e. reflection (RF) factor, petri factor (PF), divergence factor (DF) and water factor (WF) were accounted for in the fluence calculations. UV-C dose was calculated as a product of average fluence and exposure time, equation 2. Correction factors and parameters for obtaining the average fluence rate are shown in Table 2.

The average UV fluence rate in the stirred sample can be calculated as (Eqn. 1):

$$E'avg = E_0 \times P_f \times (1 - R) \times \frac{d}{+L\,L \times Ad1ln - 1ln0 - (Ad10)}$$

Eqn. 1

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E_0 is the radiometer meter reading at the center of the beaker and at a vertical position so that the calibration plane of the detector.

$$Delivered\ UV\ dose\ (D) = E'avg \times t \qquad \text{Eqn. 2}$$

2.7 LC-MS Methods for Polyphenol Detection

This method was adapted from our previous studies (Akwu et al., 2022). A Shimadzu LCMS 8040 system (Shimadzu Scientific Instruments, Columbia, MD) which included two Shimadzu LC-20ADXR pumps, a SIL-20ACXR autosampler, a CTO-20A column oven, and an LCMS-8040 triple stage quadrupole mass spectrometer was used for LC-MS/MS analysis. Chromatographic separation was achieved on with a Phenomenex 1.6 µm Polar C18 100 Å column (100 × 2.1 mm) maintained at temperature of 40°C. The mobile phase consisted of 0.1% formic acid (FA) in water (A) and 0.1% FA in acetonitrile (B). The flow rate was 0.30 mL/min. Initially, solvent B concentration was 3% and increased linearly to 97% from 0.01 min to 2.0 min. Solvent B was held at 97% from 2.0 to 7.0 min, and then reduced to 3% until the end of the time program at 10.0 minutes. The injection volume was 2 µL. The LCMS analysis utilized an electrospray ionization source with the optimized source parameters DL temperature 250 °C, nebulizing gas flow, 3 L/min, heat block 450°C, drying gas flow, 20 L/min. The following transitions and collision energies (CE) were used to analyze compounds in the positive ion mode: pyridoxal m/z 168>150, CE -13; pyridoxamine m/z 168>152, CE -14; pyridoxine m/z 170>152, CE -15; thioamide m/z 264.9>122.05, CE -15; choline m/z 104.1>60, CE-22, Vitamin B12 m/z 678.2>147, CE -40, and chlorogenic acid m/z 354.9>163, CE -15. These compounds were analyzed in the negative ion mode, ascorbic acid m/z 175>114.85, CE 13; phlorizin m/z 535>273.2, CE 15; and epicatechin m/z 289>125, CE 20. A standard solution of 1000 ng/mL each compound was prepared calibration curves. A one-point calibration curve was used to determine concentrations of samples.

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Data were acquired and analyzed with Shimadzu LabSolutions software.

2.8 Statistics

The concentration of polyphenols and vitamins were evaluated for six UV doses levels. Microbial studies were conducted for 4 exposure levels. A balanced experimental design with three replicates were randomly assigned to each treatment, i.e. exposure to the selected UV-C irradiation. One-way ANOVA test (Tukey's HSD multiple comparison) was chosen to analyze the data to evaluate the effects of different UV doses on the concentration of polyphenols and vitamins using SAS computing environment. Data were reported as means ± one standard deviation from the mean and tests were statistically significant at 5% significance level.

Results and Discussion

From the optical attenuation data (Table 2), it may be seen that the AJ was a strong absorber (7.98 cm^{-1}) of UV-C light (Akwu, et al., 2022). Absorbance is a measurement of the amount of UV light that is absorbed by a substance at 279 nm over 1 cm path-length. Fluids exhibiting high absorbance values will create high UV gradients. On the contrary, some fluids have absorbance between value 0.1 to 22 (Patras, et al., 2020; Pendyala, et al., 2021; Vashisht, et al., 2022). Figures 2 and 3 illustrate the ultraviolet (UV) Spectra of apple juice as a function of wavelength. 279 nm wavelengths as demonstrated in Akwu, et al., (2022). Similarly, to, Akwu, et al., (2022), it clearly shows the absorbance at 279 nm is relatively lower in comparison to 254 nm wavelength that demonstrated a higher absorption, which is why 279 nm was the chosen wavelength (Akwu, et al., 2022). UVC exposure of beverages requires very specific requirements to be effective and efficient. One example of UV-C light treatment is a near collimated beam unit that assists in accurate delivery doses of UV-C light in water, wastewater (Gehr, 2007; Kuo, et al., 2003; Qualls, Flynn & Johnson, 1983, Patras et al., 2022). Similar principle can be applied in beverages.

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UV-C exposure has been well documented as it is notably changed in the presence of optical attenuation coefficient of the apple juice test fluid (Akwu, et al., 2022). UV- C doses must be uniformly delivered in the test fluid to achieve target log reductions of bacteria (Akwu, et al., 2022; Islam, et al., 2016 b; Patras, et al., 2020). This effectively illustrates that log reduction data demonstrated at various optical properties may only be examined in comparative studies if optical properties and UV dose delivered were both accurately accounted for (Patras et al., 2020) As described in Akwu, et al., 2022, all UV gradients in the system were considered in the UV dose/fluence calculations (Akwu, et al., 2022).

UV-C inactivation of different pathogens has been extensively conducted by many researchers (Tosa & Hirata, 1999; Yaun, et al., 2003; Sommer, et al., 2000; Wilson, 1992; Wu, et al., 2011). A great example was demonstrated in McSharry et al, (2022), where *Listeria monocytogenes* T1093, illustrated an initial reduction, then, subsequently first-order kinetics up to 6-$\log_{10}$, with D_{10} in the log-linear region of about 1.8 $mJ \cdot cm^{-2}$ (McSharry et al., 2022). Presently, many microbial challenge studies in liquid foods and beverages have been carried out at 254nm (Vashisht, et al., 2022; Sauceda-Gálvez, et al., 2021; Bhullar, et al., 2019; Usaga, et al., 2017; Chandra, et al., 2017; Gunter-Ward, et al., 2017; Torkamani & Niakousari, 2011), however, there is very limited data that is available at the 279 nm wavelength in UV-C microbial challenge studies. We hypothesize that D_{10} value at 254 and 279nm will be very comparable but the optical properties of the suspensions will be significantly different. Kim, Kim, & Kang (2015), studied the effects of Ultraviolet light-emitting diodes (UV-LEDs) on the inactivation of Gram-positive and Gramnegative foodborne pathogenic bacteria and yeasts (Kim, Kim, & Kang, 2015). The bacterial and yeast cocktails were exposed to UVC-LED modules that were connected to electronic printed circuit boards that were clouded for four UV-LEDs with peak voltages at 266, 270, 275, and 279

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nm (Kim, Kim, & Kang, 2015). The average UVC-LED voltages applied ranged from 6.36 V to 6.92 V; while the nominal power consumptions were 0.16 W or 0.13 W. The authors observed a significantly higher Propidium Iodide (PI) uptake percentage in Gram-positive in comparison to Gram-negative and Yeasts (Y); which had a maximum of 8% Propidium Iodide uptake (Kim, Kim, & Kang, 2015). It seemed that the authors did not quantify and validate the delivered dose. The authors did not provide the data on fluid mixing.

In a different study, Nyhan et al., (2021), demonstrated that when UVA-LEDS and UVCLEDS could be paired by using the germicidal effect of UV-C and greater penetrating ability of UV-A. The researchers discovered that use of multiple wavelengths 254/270/365 nm demonstrated a reduction after each reduction at 5, 10, 20s, and 40s respectively (Nyhan, et al., 2021). This study investigated the use of UV-LEDs in bacterial inactivation in powdered food ingredients. Damage that occurs to the bacterial membranes is that when exposed to UV-A and UV-C wavelengths microbial inactivation was increased (Chevremont, et al., 2012). Xiang, et al., (2020), applied dosages between 200 - 1200 mJ/cm^2 and the populations of *Zygosaccharomyces rouxii* in apple juice samples were reduced by 4.86 and 5.46-log at 800 and 1200mJ/cm^2.

The authors irradiated the samples using a collimated beam apparatus consisted of a low-pressure mercury UV lamp with peak radiation in the 275 nm wavelength range as demonstrated in Fig.1. In our study, 10 mJ/cm^2 UV dose at 0.126537 mJ/cm2 average irradiance resulted in 3.50 $\log_{10}$ CFU/mL reduction of *S.* Muenchen at a depth of 1.5 cm using UV-LEDs emitting light at 279 nm. Fluid absorbance is a critical component in UV technology, as it determines the intensity of UV light and its ability to penetrate. This study rectifies this issue and accounts for UV intensity gradients in the dose calculations (Akwu et al., 2022). The current study examines the inactivation

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of *Salmonella* Muenchen and *Listeria monocytogenes* in natural apple juice, for which the authors found limited published literature regarding 279 nm wavelength or closer exposure wavelengths.

The populations of *Salmonella* Muenchen were reduced by 1.17, 1.32, 2.90, $\log_{10}$ respectively at a UV-C dose level of 4, 5, 10 $mJ \cdot cm^{-2}$. The microbial inactivation results demonstrate first order kinetics, as plotted in Fig. 4. Log inactivation was proportional to UV dosage. Based on the rate constant (cm^2/mJ) and D_{10} value of *Salmonella*, 17.5 $mJ.cm^{-2}$ dosage will be required to achieve 5 $\log_{10}$ reduction. Our experiments were conducted at bench scale and apple juice samples were stirred continuously throughout the irradiation treatment duration, to ensure uniform dose delivery to the homogeneous test fluid (Akwu et al., 2022). The log inactivation increased with increase in the UV-C exposure, as also evidenced in Fig 4. This can be concluded due to the fact that the presence of UV-C light functions in disrupting microbial population replication of DNA and altering gene functions. In some instances when a dose delivery is not uniform, the UV-C dose may exceed the threshold capacity and cellular damage result in rapid lethal inactivation of cells, and DNA repair mechanisms will fail to undo changes (Miller, et al., 1999; Akwu et al., 2022). Previous studies reported D values between 3.5 – 3.85 mJ/cm^2 for 3 and 4 $\log_{10}$ reductions (Gopisetty, et al., 2019; Sommer, et al., 2000); and <2 mJ/cm^2 through 29 mJ/cm^2 respectively representing between 1 and 6 total log reduction (Yaun, et al., 2003). All data is in accordance with the published studies except with incidents of high d values, which could be a result of poor mixing within the system. This poor mixing also creates false tailing effects. A conventional method of UV-C exposure is the use of a collimated beam apparatus, which is assumed to employ sufficient uniform mixing while samples undergo UV-C exposure doses. In UV-C dose-response data it is assumed that the overall fluid is subjected to uniform dose distribution, while vigorous mixing takes place to provide accurate calculations (Akwu, et al., 2022). This assumption may not be

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applicable in some instances where the test fluid and micro-organisms utilized are different than described above. UV-C irradiation effectively inactivated *L. monocytogenes* ATCC 19115 in apple juice as may be seen in Fig. 5. The populations of *L. monocytogenes* were reduced by 1.20, 2.26, 3.48 $\log_{10}$ respectively at a UV-C dose level of 4, 8, 12 $mJ \cdot cm^{-2}$. The D_{10} value obtained overall in the apple juice was 3.53 mJ/cm^2. The graph demonstrates that when the UV dosage is increased, the log reduction is increasing. Based on the rate constant (cm^2/mJ) and D_{10} value of *L. monocytogenes*, 17.7 $mJ.cm^{-2}$ dosage will be required to achieve 5 $\log_{10}$ reduction. Previously published literature data suggested that *L. monocytogenes* UV sensitivity lies between 3.24 $mJ.cm^{-2}$ (Akwu, et al., 2022; Gunter-Ward, et al., 2017), 4 $mJ.cm^{-2}$ log reduction (Akwu, et al., 2022; Lu, et al., 2010), and greater than 5 $mJ.cm^{-2}$ (Akwu, et al., 2022; Matak, et al., 2005). D values that were higher than normal are possibly from lack of uniform dose delivery, which can be from poor mixing, causing huge UV gradients and inaccurate data. The FDA set the regulated UV-C dose at 40 $mJ \cdot cm^{-2}$; which is predicted that inactivation treatments should result in a 5-$\log_{10}$ decrease of *S.* Muenchen, and *L. monocytogenes* in apple juice. To conclude, this system almost allowed accurate estimation of the delivered doses required to inactivate 5 $\log_{10}$ reductions of these pathogens taking into account the UV-C irradiance, absorption coefficient (1/cm) (Akwu, et al., 2022). Kinetic data shows that both pathogens are very sensitive to UV light at 279 nm wavelength. Overall, in this study, 20 mJ/cm^{-2} of exposure treatment is the optimized dosage for safer and higher quality product as also demonstrated in (Akwu, et al., 2022).

Figures 7 and 8 illustrates the effect of UV-C irradiation on the content of polyphenolic and vitamin compounds present in AJ. Figure 6 demonstrates chromatograms of chlorogenic acid and epicatechin respectively; chlorogenic acid has notably demonstrated as being the most abundant of polyphenolic compounds present in apple juice (Akwu, et al., 2022; Bender & Atalay, 2021; Islam,

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et al., 2016a; Islam, et al., 2016b; Eisele & Drake, 2005). Another abundant polyphenol in apple juice is epicatechin (Akwu, et al., 2022; Marcotte, et al., 2022; Bae, et al., 2020; Boyer & Liu, 2004). Concentration of epicatechin was observed to diminish as a function of UV dosage ($p < 0.05$) (Akwu, et al., 2022). At the maximum dosage level (160 $mJ.cm^{-2}$), epicatechin and chlorogenic acid were significantly reduced by 98% and 76% respectively, similarly demonstrated in (Akwu, et al., 2022). Results suggested that epicatechin was relatively sensitive to UV-C dosage as compared to chlorogenic; as similarly found in Akwu et al., (2022). Overall, the data demonstrates an approximate 30% reduction at 20 $mJ.cm^{-2}$ for both polyphenols (Akwu et al., 2022).

Three vitamins and three polyphenols were identified and quantified in apple juice by LCMS/MS; all of the vitamins identified were significantly reduced ($p < 0.05$) as UV dosage was increased. Chlorogenic Acid, Epicatechin, Phlorizin, Thiamine (B1), Choline (B7), and B12. Thiamine (Stawny, et al., 2020; Whitfield, et al., 2018), and B12 (Juzeniene & Nizauskaite, 2013) are both photosensitive vitamins and therefore can demonstrate behaviors as photosensitizers; which is mainly due to double bond presence within their chemical structures. Additionally, this behavior can speed up other B vitamins oxidation processes (De Arrivetti et al., 2013). In this study, epicatechin concentrations in the samples decreased by 37%, 31%, 71%, and 84% at UV doses at 10, 20, 40, and 80 $mJ.cm^{-2}$, respectively. Epicatechin was rapidly degraded by UV-C exposures. Similar findings were reported in three other studies (Akwu et al., 2022; (Islam, et al., 2016a; Islam, et al., 2016b; Akwu et al., 2022). Based on the observed concentration reductions, it demonstrated that all vitamins subjected to the UV-C exposure demonstrated sensitivity in apple juice and as a result they decreased significantly. The mechanism responsible for vitamin degradation during UV irradiation is possibly from a couple of different chemical-based reactions

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and mechanisms to include photo-oxidation, thermal degradation, (Islam, et al., 2016 b; Sheraz et al., 2014; Akwu et al., 2022). In contrast, chlorogenic acid retained very well in the UV exposed samples, and the thiamine was significantly reduced following exposure to UV-C. Our study demonstrated that UV-C irradiation caused significant changes in vitamins and polyphenols, and it is quite indicative that UV irradiation is capable of changing a number of chemical food constituents similarly demonstrated in Akwu et al., (2022). To the best of the authors' knowledge, this is the first paper that demonstrates the efficacy of UVC 279 nm inactivating *Salmonella* Muenchen, and *Listeria monocytogenes* in highly turbid apple juice and retaining the quality between 20 - 40 mJ.cm^{-2} dosage.

Conclusion

UV-C irradiation was successfully applied to inactivate the microbial populations in apple juice using a near collimated beam device operating at 279 nm wavelength. This study found that UVC irradiation treatment at low doses (≈25 mJ·cm^{-2}) could be used to achieve 5-log$_{10}$ inactivation of *Salmonella* Muenchen, and *Listeria monocytogenes* strains. In addition, the D_{10} values of 4.12 and 3.56 mJ·cm^{-2} were obtained for *Salmonella enterica* serovar Muenchen ATCC BAA 1764, and *Listeria monocytogenes* ATCC 19115. The inactivation kinetics of these tested microorganisms were best described by log linear kinetics. UV-C irradiation induced minor reductions in the concentration of vitamins and polyphenols in apple juice at the FDArecommended fluency of 40 mJ cm^{-2} of pasteurized equivalent dose. Chlorogenic acid was reduced to 56%, at 80 mJ/cm2 whereas 12% reduction was observed at 40 mJ/cm^2. The decrease in the concentration of antioxidants is dependent on various intrinsic factors such as pH, presence of light sensitive aromatic chemical compounds and formation of non-specific reactive oxygen species Epicatechin was observed to be relatively stable as a function of UV dosage. Scale-up of the UV-C LED device

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(flow-through system), spore inactivation studies, and sensory evaluation of UV-C treated apple juice will be subject of further investigations. The lab is developing a scaleup model and its efficacy in inactivating microorganisms and other spores in apple juice on a larger scale will be subject to future investigation.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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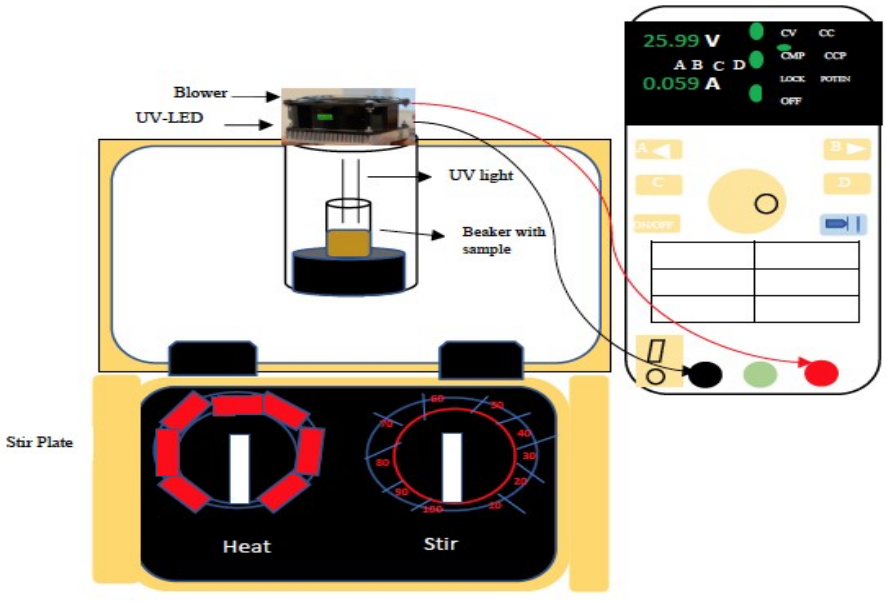

Figure 1.

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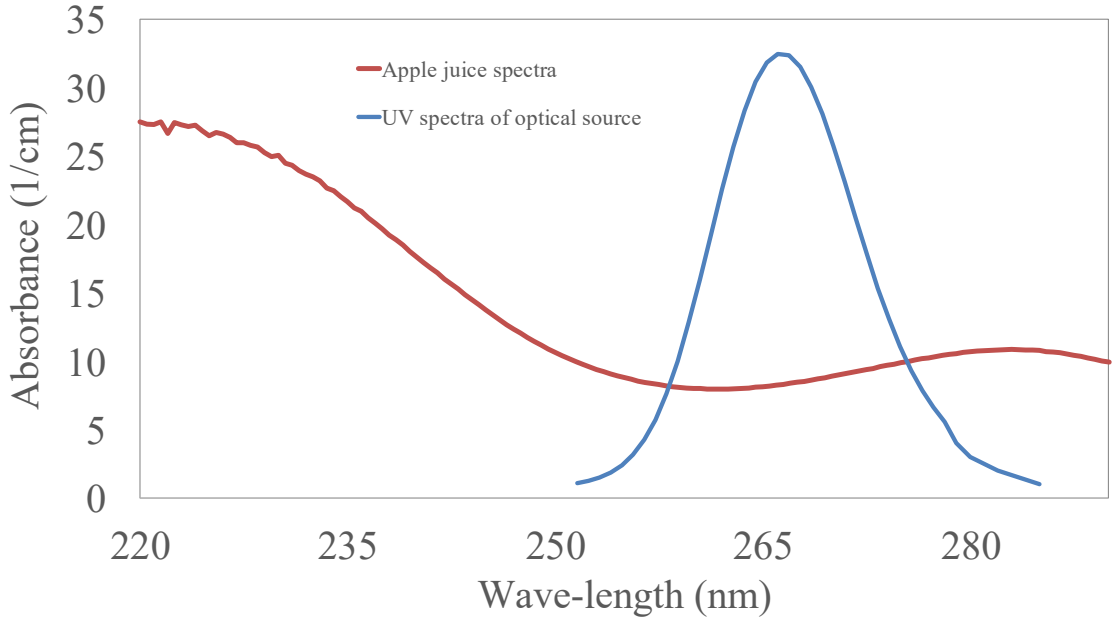

Figure 2.

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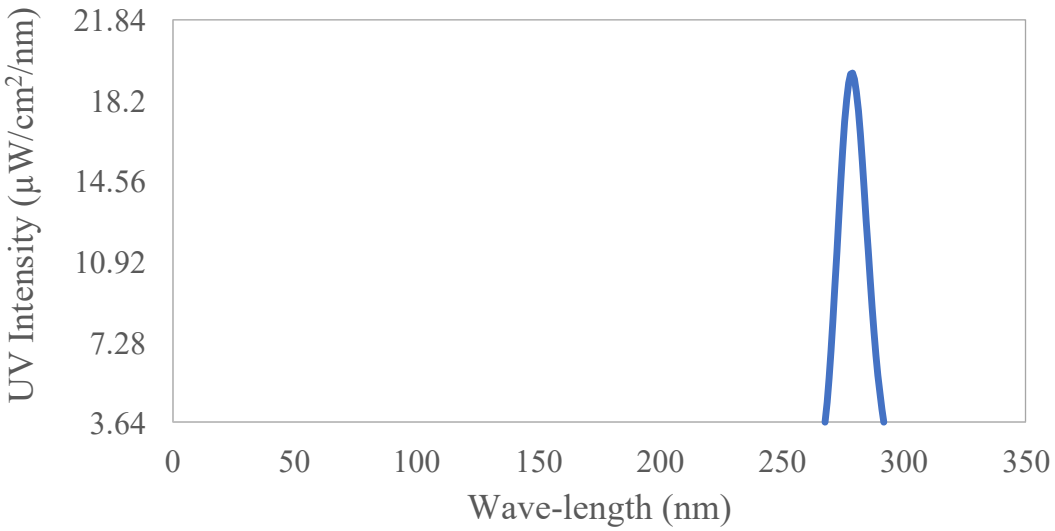

Figure 3.

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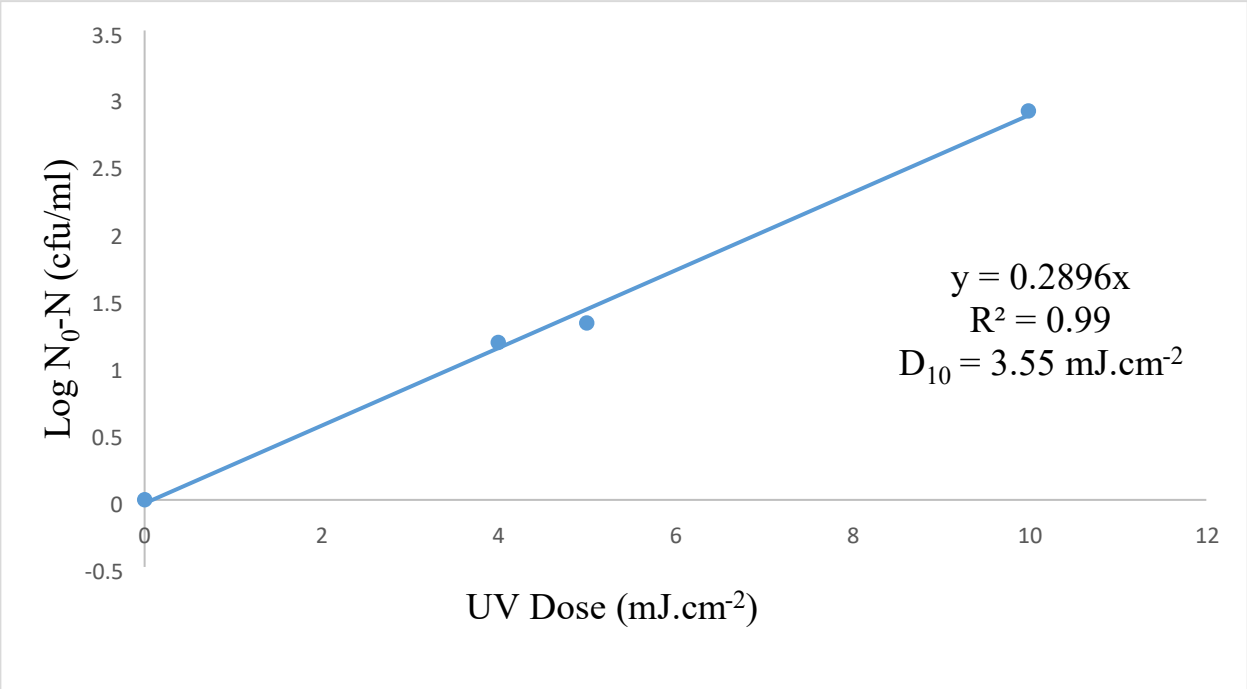

Figure 4.

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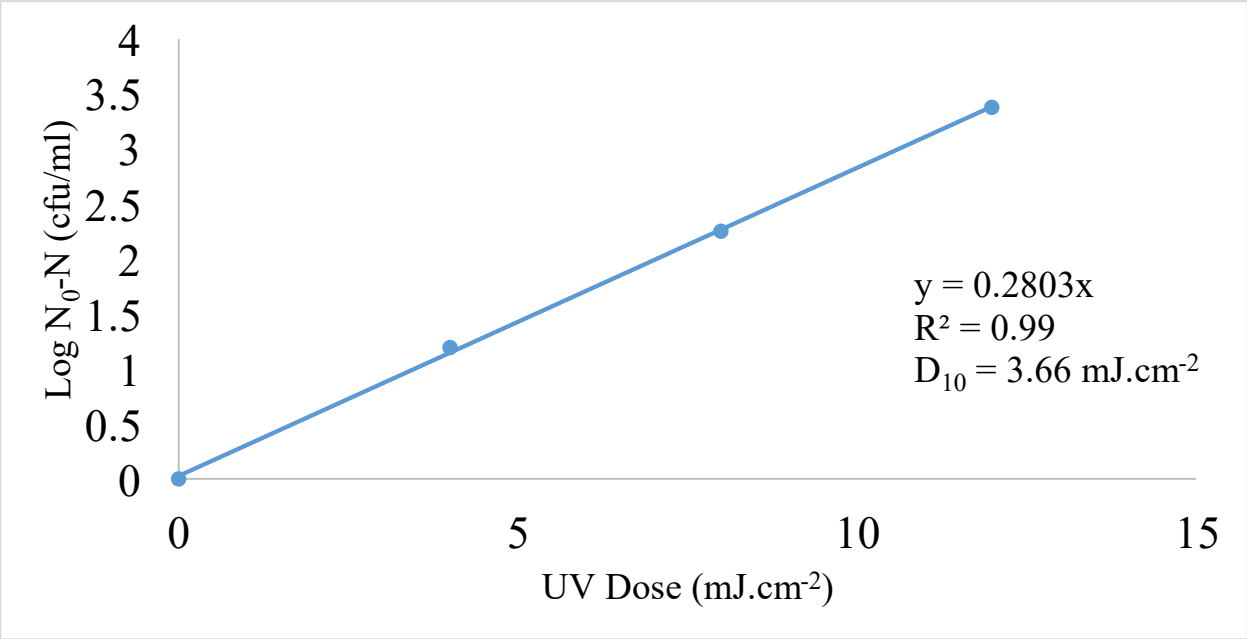

Figure 5.

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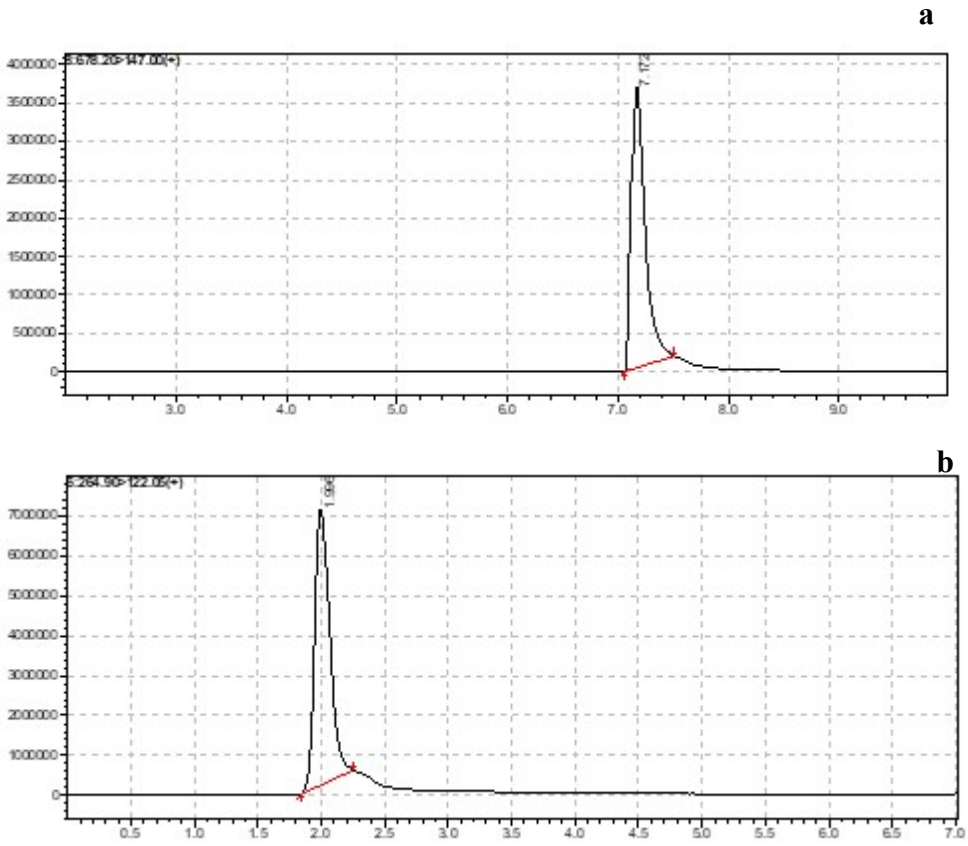

Figure 6.

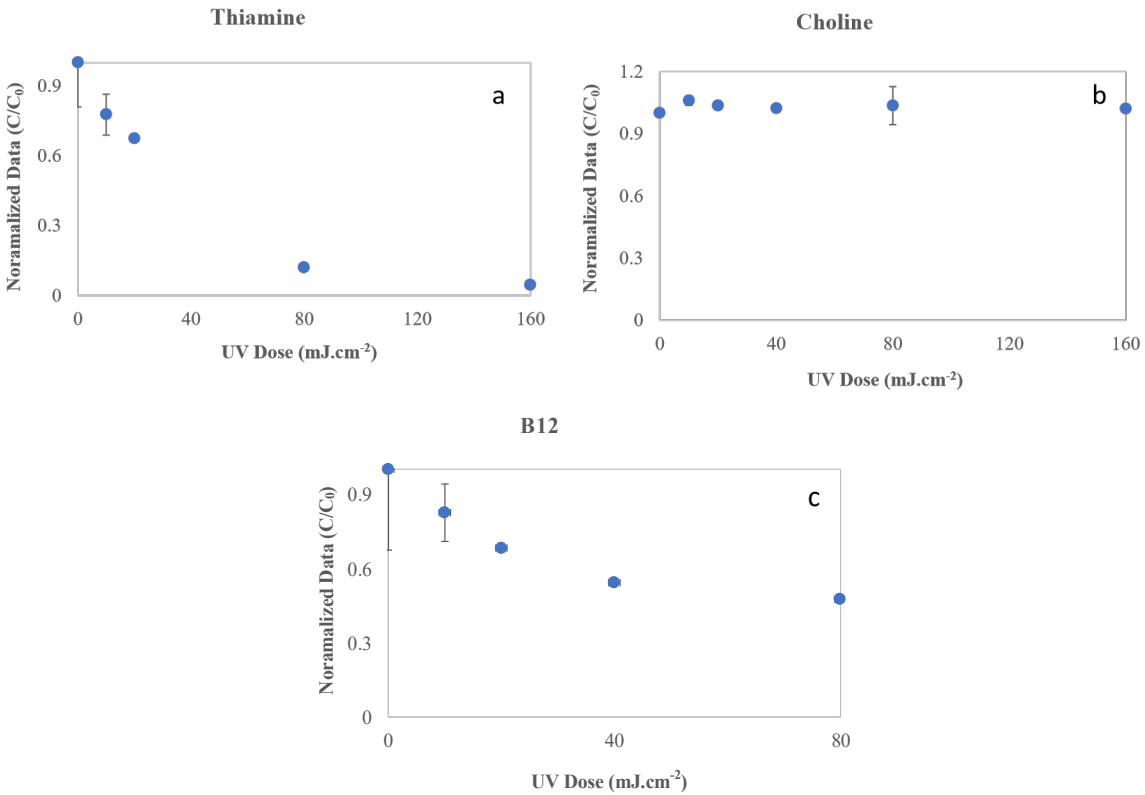
Thiamine
Noramalized Data (C/C0)
0.9
0.6
0.3
0
a
0
40
80
120
160
UV Dose (mJ.cm-2)
Choline
Noramalized Data (C/C0)
1.2
0.9
0.6
0.3
0
b
0
40
80
120
160
UV Dose (mJ.cm-2)
B12
Noramalized Data (C/C0)
0.9
0.6
0.3
0
c
0
40
80
UV Dose (mJ.cm-2)

Figure 7.

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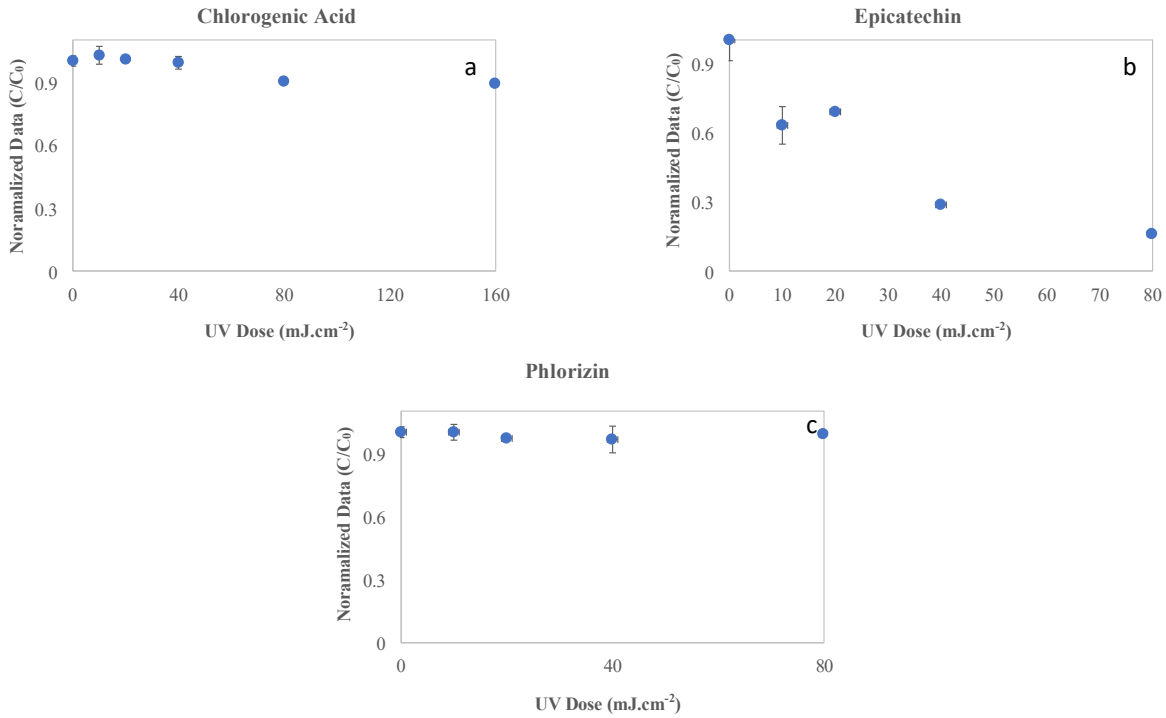

Figure 8.

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Table 1. Physiochemical characteristics of the Apple Juice.

Parameters	**Values**
pH	3.3 ± 0.164
Total soluble solids (%)	14.3 ± 0.018
Titratable acidity	5 ± 0.406

Data presented as mean±stdev

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Table 2. Correction factors and parameters for obtaining the average fluence rate.

Parameters	**Values**
Reflection factor	0.9750
Petri factor	1.0000
Water factor	0.3090
Divergence factor	0.99
Average fluence rate (mW/cm^{-2})	0.1265
UV transmittance (%/cm)	2.88E-08
Absorbance (cm^{-1})	9.54±0.005
Exposure doses ($mJ.cm^{-2}$)	0 – 160 $mJ.cm^{-2}$