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Microbial inactivation and cytotoxicity evaluation of UV irradiated coconut water in a novel continuous flow spiral reactor

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Abstract

A continuous-flow UV reactor operating at 254 nm wave-length was used to investigate inactivation of microorganisms including bacteriophage in coconut water, a highly opaque liquid food. UV-C inactivation kinetics of two surrogate viruses (MS2, T1UV) and three bacteria (*E. coli* ATCC 25922, *Salmonella* Typhimurium ATCC 13311, *Listeria monocytogenes* ATCC 19115) in buffer and coconut water were investigated (D_{10} values ranging from 2.82 to 4.54 $mJ \cdot cm^{-2}$). A series of known UV-C doses were delivered to the samples. Inactivation levels of all organisms were linearly proportional to UV-C dose (r^2 >0.97). At the highest dose of $30mJ \cdot cm^{-2}$, the three pathogenic organisms were inactivated by more than 5 $\log_{10}$ ($p < 0.05$). Results clearly demonstrated that UV-C irradiation effectively inactivated bacteriophage and pathogenic microbes in coconut water. The inactivation kinetics of microorganisms were best described by log linear model with a low root mean square error (RMSE) and high coefficient of determination (r^2>0.97). Models for predicting log reduction as a function of UV-C irradiation dose were found to be significant ($p < 0.05$) with RMSE and high r^2. The irradiated coconut water showed no cytotoxic effects on normal human intestinal cells or, and normal mouse liver cells. Overall, these results indicated that UV-C treatment did not generate cytotoxic compounds in the coconut water. This study clearly demonstrated that high levels of inactivation of pathogens can be achieved in coconut water, and suggested potential method for UV-C treatment of other liquid foods.

Keywords: UV-C irradiation, continuous-flow UV reactor, bio-dosimetry, microbial inactivation, bacteriophage, inactivation kinetics

Industrial Relevance

This research paper provides scientific evidence of the potential benefits of UV-C irradiation in inactivating bacterial and viral surrogates at commercially relevant doses of 0 - 120 $mJ \cdot cm^{-2}$. The irradiated coconut water showed no cytotoxic effects on normal intestinal and healthy mice liver cells. UV-C irradiation is an attractive food preservation technology and offers opportunities for horticultural and food processing industries to meet the growing demand from consumers for healthier and safe food products. This study would provide technical support for commercialization of UV-C treatment of beverages.

1. Introduction

There has been an increased interest in coconut water beverages in many parts of world due to rising consumer demands for food products with potential health benefits. Coconut water (CW; classified as a juice), is rapidly gaining popularity, with sales escalating over 300% since 2005 worldwide (Burkitt, 2009). Although the liquid endosperm remains sterile in an undamaged coconut (Awua et al., 2011), the compositional and physico-chemical properties of coconut water (pH of 4.2-6.0 and a_w of 0.995) make it susceptible to microbial growth and contamination (Walter et al., 2009). Unhygienic handling and processing may introduce spoilage and pathogenic microbes to the raw product, with contamination of microbes like *Salmonella enterica*, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*.

Although there have been no outbreaks reported in coconut water, there remains the probability of microbial growth and survival of disease-causing organisms in coconut water, with repercussions for human health. Recent occurrences of food borne illness traced to consumption of unpasteurized apple and other low and high acid fresh juices have resulted in declaration of regulations requiring further processing for reduction of pathogens. For example, the United States Food and Drug Administration (US-FDA) instituted the federal juice Hazard Analysis Critical Control Point (HACCP) to ensure food safety of all juice products. (US-FDA, 2000). This requires that manufacturers use adequate processing techniques, capable of achieving a 5-$\log_{10}$ reduction in the numbers of most resistant pathogens. (Goodrich et al., 2005).

The US-FDA states that fruit juice processing is required to be subjected to regulations of HACCP (Federal Register [FR], 2001) and related regulation (21 CFR 110). At present, thermal pasteurization is the dominant technology used to achieve these goals, with an accessible and

well-understood strategy for treatment. The US-FDA has approved thermal pasteurization as an established technology for rendering fruit juice products safe from pathogenic microbes and enhancing the shelf-life of refrigerated juice products. (Donahue et al, 2004, US-FDA, 2001). The High-Temperature Short-Time (HTST) pasteurization process is widely used in large-scale continuous mode juice production. (Rupasinghe et al., 2012). Although they are widely used, thermal processing techniques may bring about considerable changes in nutritional content of the juices (Caminiti et al., 2012). Because of these drawbacks, various non-thermal pasteurization techniques for achieving significant microbial inactivation are being evaluated. One of these novel non-thermal technologies to control pathogens is UV-C light.

UV light forms a part of the electromagnetic spectrum in between the wavelengths of X-rays and visible light. UV is a non-thermal, low temperature treatment, producing little or no known toxic or significant non-toxic by-products during treatment (Islam et al., 2016), with minimal loss of sensory attributes and low energy consumption. The wavelength of UV light ranges from 100 to 400 nm and is categorized as UV-A (320 – 400nm), UV-B (280 – 320nm), UV-C (200 – 280nm) and vacuum UV (100 – 200nm) (Koutchma et al., 2009). The UV wavelength of 253.7 nm is commonly used for disinfection of water, air and surfaces. UV-C light, in particular, has been shown to have lethality effects on bacteria, yeasts, molds and viruses. The ability of UV-C light to penetrate through the cell wall, blocking DNA transcription and replication results in restricting the microorganism's ability to grow and multiply (Azimi et al., 2010). For all these reasons, UV-C is a promising technology that could have advantages over thermal methods of pasteurization. (Koutchma et al., 2009).

Currently, UV technology has been used to treat liquids foods including fresh juices and nectars to inactivate microorganisms such as *E. coli, Salmonella, Shigella, Zygosaccharomyces*

*bailli, and Saccharomyces cerevisia*e (Donahue, Canitez, & Bushway, 2004; Gabriel & Nakano, 2009; López-Malo, Guerrero, Santiesteban, & Alzamora, 2005; Lu et al., 2010; Murakami, Jackson, Madsen, & Schickedanz, 2006), and protozoa such as *Cryptosporidium parvum* (Hanes et al., 2002); enzymes such as polyphenoloxidase, ATPase, acid phosphatase, carboxypeptidase A, and trypsin (Falguera, Pagán, & Ibarz, 2010; Guerrero-Beltrán & Barbosa-Cánovas, 2006; Ibarz, Garvin, Garza, & Pagan, 2009).

In a recent study, we showed that using a collimated beam (Islam et al., 2016a, 2016b) and a flow-through UV system, treated apple juice resulted in little to no impact on the concentration of individual polyphenols and *in-vitro*- antioxidant activity. Though powerful in its proof-of-principle, the implementation of such a system in a food industry setting is challenging. Typical UV irradiation research studies utilize batch reactors (i.e., collimated beam devices); however, continuous-flow reactors are significantly more desirable for industrial food processes. The effect of UV irradiation on microbial and viral inactivation in coconut water using a flow-through system has not been reported to date.

Most of the UV irradiation studies in liquid foods do not consider the optical absorbance of the fluid, while using a batch or a continuous flow-through system (Unluturk et al., 2010; Caminiti et al., 2012). A simple analogy is that the UV Dose is the number of photons absorbed per surface area by an irradiated object during a particular exposure time. While UV dose delivered by UV system is often expressed as the product of the average UV intensity within the UV system and the theoretical treatment time, the experimental set-up gives intensity gradients within UV systems and gives rise to a distribution of delivered doses as opposed to a fixed value. Without proper mixing, fluid further from the lamp will receive a lower dose than that closer to the lamp. In this study, the optics (absorption coefficients) of the fluid are accounted for, and

dose delivery is verified through bio-dosimetry, ensuring that target levels of disinfection are achieved, and allowing direct comparisons with other UV-C treatment studies. In this novel study, the UV fluence was quantified and verified using a MS2 (Single Stranded RNA virus). MS2 inactivation has a linear response to UV and hence can be used to quantify and confirm the UV fluence. This parameter is also known as RED. (Reduction Equivalent Dose). If the RED for a UV system is 40 $mJ \cdot cm^{-2}$, it means that the UV system is delivering 40 $mJ \cdot cm^{-2}$ as measured by the validation organism.

Cytotoxicity of irradiated beverages is utmost important to make sure that a novel food processing technique such as UV irradiation does not produce toxic chemical compounds when treated at higher doses. In fact, none of the studies have evaluated the cytotoxicity of irradiated coconut water.

Through this study, using a novel continuous flow reactor the effectiveness of UV-C irradiation for the inactivation of *Salmonella* Typhimurium ATCC 13311, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 19115 and two bacteriophage (MS2 and T1UV) as model viruses in coconut water was investigated. In addition, this study also evaluated the cytotoxicity of UV-C irradiated coconut water on the mice liver cells and fibroblasts from normal colon cells (CCD-18Co).

2. Material and Methods

2.1 Preparation of coconut water

Fresh raw green coconuts (n =50) were procured from a local market (Nashville, TN, USA). The coconut shell was pierced from top and clear water was pipetted out. The whole volume of raw coconut water (CW) was then filtered through a 20-25 µm Whatman filter paper

(Fisher Scientific, Pittsburgh, PA) and stored at -20 ^{o}C until further processing. The frozen CW was thawed to room temperature before it was inoculated with bacterial culture followed by UV-C treatment. Coconut water was examined for background microbial population. pH and brix of coconut water was 5.6 and 0.9% respectively.

2.2 Bacteriophage and cultural conditions

Two bacteriophages were used as surrogates for viral pathogens: MS2 (Single Stranded RNA virus) and T1UV-C (Double stranded RNA virus). The cultures were obtained from GAP EnviroMicrobial Services Limited (London, Ontario, Canada). Cultures were kept at -4 ºC until further use and were found to maintain viability for many months with little variation in measured titre.

2.3 Bacterial strains and cultural conditions

Three strains of bacteria were used in this study. *Escherichia coli* (ATCC 25922), *Salmonella* Typhimurium (ATCC 13311) and *Listeria monocytogenes* (ATCC 19115) were obtained from American Type Culture Collection (ATCC). The bacterial cultures were stored in 25% glycerol in cryovials at -80 ^{o}C. *E. coli* and *S.* Typhimurium strains were grown by two successive loop transfers of individual strains incubated at 37 °C for 18 h in 15 mL Tryptic soy broth (Oxoid Ltd., Basingstoke, UK). *L. monocytogenes* was also subjected to two successive transfers in tubes containing 15 mL Buffered listeria enrichment broth (Oxoid Ltd., Basingstoke, UK) and incubated for 24 h at 37 °C. These cultures were used as the adapted inoculum. After incubation, *E. coli* and *S.* Typhimurium cultures were transferred into 60 mL of TSB and incubated for 18 h at 37 °C to stationary phase. *L. monocytogenes* culture was also transferred to 60 mL Listeria enrichment broth (Oxoid Ltd., Basingstoke, UK) and incubated for 24 h at 37 °C.

The bacterial cells were harvested by centrifugation (3000 × g, 15 min). Cell pellets were washed twice in 0.1% (w/v) phosphate buffer saline (PBS, Becton Dickinson, New Jersey, US) and re-suspended in 100 mL of PBS. To enumerate the original population densities in each cell suspension, appropriate dilutions in peptone water (in 0.1% PW) were plated in duplicate onto Tryptic soy agar (Oxoid Ltd., Basingstoke, UK) plates for *E. coli* and *S.* Typhimurium suspensions and incubated for 24 hours at 37 °C. *L. monocytogenes* suspensions were plated on Listeria selective agar base (SR0141E) (Oxoid Ltd., Basingstoke, UK) plates with incubation for 48 h at 37 °C.

2.4 Coconut water inoculation

Aliquots of 1000 mL of coconut juice were inoculated individually with each of the three bacterial cultures (*E. coli, S.* Typhimurium, and *L. monocytogenes*) targeting a concentration of 10^8 CFU/ml. To determine the original *E. coli* and *S.* Typhimurium titres, inoculated coconut water was plated on Tryptic soy agar (Oxoid Ltd., Basingstoke, UK) plates and incubated for at 37 °C for 24 h. Coconut water inoculated with *L. monocytogenes* was plated on Listeria-selective agar base (Oxoid Ltd., Basingstoke, UK) and incubated at 37 °C for 48 h.

2.5 Optical properties

The absorption coefficient at 254 nm was determined based on transmittance measurements from a Cary 300 spectrophotometer with a six-inch integrating sphere (Agilent Technologies, CA, US). 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 measurements were done in triplicate to avoid the measurement error.

2.6 UV-C irradiation experiments

Coconut water was irradiated using a continuous-flow reactor (Figure 1) with the fluid pumped around a central low-pressure mercury UV lamp (40 W) emitting at 254 nm wave-length (Trojan Technologies, London ON Canada). The reactor system was designed to achieve good mixing and uniform fluence to the test fluid. For inactivation of bacterial microbes with higher UV sensitivity, a cylindrical insert around the UV lamp with 1.5 cm slit was used to reduce the UV irradiance incident on test fluid. This insert reduces the UV-C fluence by ≈ 90%, as higher UV-C fluence would kill all the microbial population making it impractical to study the microbial inactivation kinetics. To achieve the desired fluence, the coconut water was passed through reactor system at 30 - 800 $mL \cdot min^{-1}$. After discarding a volume of fluid equal to three UV system volumes, irradiated coconut water was collected for microbial analysis. The UV reactor delivered a fluence of approximately 5, 10, 15, 20, 30 $mJ \cdot cm^{-2}$ at flow-rates of 215, 108, 72, 54, 36 $mL \cdot min^{-1}$ respectively. The actual fluence delivered was verified using the procedure described in the UV fluence section. For cell culture, higher UV doses/fluence was delivered to coconut water to evaluate cell cytotoxicity. UV doses of 0, 100, 200, 300, 400 $mJ \cdot cm^{-2}$ were selected.

2.7 UV fluence

The fluence, quantified as reduction equivalent fluence (REF) or dose (RED), delivered to the coconut water was determined using a viral clearance test with the challenge organism, MS2, inoculated in the coconut water. MS2 is a well characterized bacteriophage and is used extensively to validate UV disinfection systems for drinking water (Islam et al., 2016a). The fluence was quantified using a similar experimental set-up, but with only one reactor and passed at five different flow rates of 58.62 (using an insert), 662, 331, and 221 $mL \cdot min^{-1}$ delivering UV-

C doses of 0, 20, 40, 80, and 120 mJ·cm^{-2}. The log reduction in MS2, which is used to calculate the fluence delivered by the reactor, was determined by GAP EnviroMicrobial Services (ON, Canada), who also provided the bacteriophage culture. A linear relationship between the reduction equivalent dose and target dose was established. These tests confirmed that UV-C doses ranging from 0 - 120 mJ·cm^{-2} can be applied to coconut water. This approach also assumes that the UV doses are additive, which is a good approximation for well-mixed reactors such as the one used in this research study.

2.8 Flow Mechanism in Continuous Spiral Flow UV Reactor

Flow regime plays an integral part in inactivating microorganisms using continuous flow UV reactors. A coiled tube UV reactor was used in this study. The flow pattern in a coiled tube reactor is accompanied by secondary flow vortices, called Dean Flow condition (Dean, 1927). Dean Flow induces superior mixing conditions, leading to better exposure of liquid food to UV-C in a continuous UV reactor (Koutchma et al., 2007). The Dean number (D_e) (Eq 1) is the similarity parameter governing the fluid motion in coiled tube flow configuration.

$$D_e = R_e \sqrt{D/Dc} \qquad \text{Equation 1}$$

$$R_e = (\rho/\mu) \times V \times D \qquad \text{Equation 2}$$

Where D is the tube diameter, D_c is the coil diameter, and R_e is the tube Reynolds number (Eq 2), ρ is density of fluid, μ is dynamic viscosity of fluid, D is diameter of coiled tube carrying the fluid, and V is velocity of flow. The flow pattern of liquid food in a coiled tube reactor may be accompanied by secondary flow vortices, called Dean flow condition. This occurs when the ratio (D/ D_c) in equation (1) is within $0.03< D/D_c <0.1$ (Dean, 1927). In the current study, reactor

design induced dean vortices in the test liquid and was quite effective in inducing high mixing thus allowing efficient inactivation of *Escherichia coli, Salmonella* Typhimurium and *Listeria monocytogenes.* For flow-rates of 36, 54, 72, 108, 215 $mL \cdot min^{-1}$, the R_e was 322, 483, 644, 966, 1922 respectively.

2.9 Organism sensitivity test

To determine the UV-C sensitivity of the organisms, UV-C irradiations were performed in (0.1% w/v) peptone water using a collimated beam irradiation device. This approach, with high optical transparency, minimizes the intensity gradient in the fluid sample, reducing the mixing required to ensure uniform average dose delivery, reducing the uncertainty in the delivered RED. The following UV-C doses were delivered: 0, 10, 20, 30, and 40 $mJ \cdot cm^{-2}$ for *Escherichia coli* (25922), *Salmonella* Typhimurium (13311) and *Listeria monocytogenes* (19115); 0, 5, 10, 20, 30 $mJ \cdot cm^{-2}$ for T1 and 0, 20, 40, 80, 100 $mJ \cdot cm^{-2}$ for MS2. The UV-C dose per log inactivation, or the D_{10} values, are shown in Table 1.

2.10 Enumeration of pathogens in coconut water after UV-C treatments

After UV-C treatment, decimal dilutions of the treated samples and control were prepared in 0.1% buffered peptone water (Oxoid Ltd., Basingstoke, UK). The *E. coli*, *S.* Typhimurium and *L. monocytogenes* inoculated coconut water samples were diluted to between 10^0 and 10^{-6}. *E. coli* and *S.* Typhimurium viable cell counts were obtained by using plate count method on appropriate agar plates as described above. Plate counts within the range of 25-250 or 30-300 were considered for analysis. Bacteria colonies were counted and reported as log $CFU \cdot mL^{-1}$ of (undiluted) coconut water.

2.11 Cytotoxicity test

Fibroblasts from normal human colon (CCD-18Co; ATCC, Manassas, VA), and epithelial cells from normal mouse hepatocyte liver (AML12; ATCC) were maintained in DMEM supplemented with 10% FBS, at 37 °C with 5% carbon dioxide. Cells were routinely cultivated in Petri dish from Corning (Corning, USA). The cell culture medium was changed every other day, i.e., three times a week. Prior to cytotoxicity analysis, coconut water was extracted with ethyl acetate and was diluted with cell culture medium at different concentrations as compared to that of the original juice. Twenty-four hours after seeding in 96-well plates, cells were treated with coconut water extracts at different concentrations ranging from 50-fold dilution to 6.25-fold dilution for 3 days. After the indicated time periods, the cell viability was determined using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT). Cells in each well were incubated with 0.1 mL of culture medium containing 0.5 $mg \cdot mL^{-1}$ MTT at 37 °C for 1 h. MTT-containing media were removed prior to the solvation of reduced formazan dye using 0.1 mL of DMSO per well. The absorbance was then measured at 570 nm using a microplate reader (SpectraMax, Molecular Devices, Sunnyvale, CA, USA).

2.12 Statistical analysis

All log reductions from the UV-C inactivation treatments were recorded and log-linear models were fitted in JMP statistical software (SAS, 2016). A balanced designed with six replicates randomized in order were performed for each treatment. Model fit statistics including r^2, RMSE and rate constants were compared among the competing models. Independent sets of data were collected for three bacteria, and model performance was evaluated for each model. The magnitude of bias, precision and accuracy were assessed using independent dataset by generating

a suite of validation statistics such as average bias, relative error percent and model prediction efficiency (I^2).

2.13 Inactivation kinetics

Log-Linear model

Log-Linear model has been widely accepted and shown to describe the microbial inactivation resulting from application of both thermal and non-thermal processes. This model provides a good fit to data in which the inactivation follows the rule of first order kinetics. The model is given in the following equation (Van Boekel, 2002), where k_1 is first-order inactivation constant ($cm^2 \cdot mJ^{-1}$). Parameter k_1 is a property of the microbe under study. D is the UV dose received by the organism or fluid element.

$$Log_{10}\left(\frac{N}{N_0}\right) = -k_1 D \qquad \text{Equation 3}$$

Log reduction is calculated as $Log_{10}\left(\frac{N}{N_0}\right)$. Classical D_{10} value is calculated from the reciprocal of the first order rate constant (D_{10}=1/k, units in $mJ \cdot cm^{-2}$). Eq. (3) is also known as Chick Watson linear equation (Marugán et al., 2008).

3. Results and Discussion

3.1. *Bacterial and viral* inactivation

The optical and physico-chemical properties of coconut water are summarized in Table 2. It is apparent that UV light has very little transmission through coconut water due to the presence of colored compounds, organic solutes or suspended matter, and this may result in reduced efficiency of UV disinfection (Wright et al., 2000). Based on published results (UV sensitivity

of microbes), it was expected that the low UV doses (0 – 40 mJ·cm^{-2}) applied in this study could easily inactivate *E. coli, Salmonella* Typhimurium, *Listeria monocytogenes,* and T1UV. MS2 would require doses more than 100 mJ·cm^{-2}. Since UV inactivation kinetics are often first order, they can be characterized by a single parameter. UV sensitivity of bacteria and viruses is often characterized by the D_{10} value–the UV fluence required to reduce the microorganism population by one $\log_{10}$ CFU·mL^{-1}. For example, MS2, a non-enveloped bacteriophage often used to evaluate the potential for virus inactivation via UV irradiation, requires a fluence of approximately 23 mJ·cm^{-2} for one $\log_{10}$ reduction of the population (Islam et al., 2016). A single reactor set-up in this work was used to apply low and high fluences to the coconut water to test the limits of UV irradiation. Received UV-C fluence in coconut water measured by bioassay (MS2 bacteriophage). The reduction equivalent dose (RED) applied to coconut water was determined by well-characterized MS2 phage as the dose indicator. It was found that in the flow-through reactor the UV dose was directly proportional to average residence time, or inversely proportional to flow rate, indicating good dose uniformity. Reactors with poor dose delivery will show "tailing", where RED vs. residence time deviates from a straight line at high dose and high inactivation.

It is quite evident that inactivation kinetics for all microbes followed first order kinetics values unlike previous studies with collimated beam approach which have reported concavity and pronounced tailing at higher UV doses. (Koutchma, 2009; Schenk et al., 2008, USDA 2000; Unluturk et al., 2008). This may be attributed to the fact that the continuous reactor used in the present study induces adequate mixing in the fluid such that each fluid element received the same UV to provide uniform exposure.

Our results suggested that an excellent reduction of viable bacteria could be achieved when using a continuous flow UV reactor. This was despite the fact that the coconut water, being naturally clear, had a high absorption. Nevertheless, the results convincingly demonstrated the ability of this system to decrease pathogenic microorganisms including model viruses. Other investigators have suggested that in liquid foods with high UV absorptivity, the fluid must be subjected to UV in the form of a very thin-film, so that UV absorption by the liquid itself is low and bacteria are most likely to be subjected to lethal doses of UV-C light (Wright et al., 2000). By contrast, in our study, the UV reactor was not based on a thin-film design, but nonetheless bacteria could be inactivated to non-detectable levels in coconut water using flow rates between 36 and 215 $mL \cdot min^{-1}$ and pipe (Teflon) diameter of 0.5 cm. The UV reactor design induced Dean Vortices in the flowing liquid and was quite effective in circulating the bacteria and model viruses to proximity of the UV lamp and thus allowing efficient inactivation.

In this study, *E. coli* was inactivated by more than 5 $\log_{10}$ $CFU \cdot mL^{-1}$ at a maximum UV-C dose of 12 $mJ \cdot cm^{-2}$. Four different doses levels of 3, 6, 9, and 12 $mJ \cdot cm^{-2}$ were used to inactivate *E. coli* by 1.79 ± 0.15, 2.94 ± 0.47, 4.27 ± 0.30 and 5.78 ± 0.32 log, respectively. The inactivation curve followed a log linear model with r^2 =0.97 and D_{10} value of 1.95 $mJ \cdot cm^{-2}$ (Figure 2), which is similar to the values reported in literature. *E. coli* O157:H7 cells were reported to have D_{10} values ranging from 0.4 to 3.5 $mJ \cdot cm^{-2}$ (Sommer et al., 2000, Tosa & Hirata, 1999; Yuan et al., 2003). The data is in good agreement with the literature values. The 5- log reduction demanded by the US Food and Drug Administration for refrigerated fruit juices thus was clearly achieved in this study.

Other studies have reported extremely high UV doses required for inactivating *E. coli*. However, these studies generally did not adequately account for optical absorbance. For example,

Guerrero-Beltran and Barbosa-Canovas (2005) reported that after 30 min of treatment with reported doses between 75 and 450 kJ·m^{-2} (7.5 and 45 mJ·cm^{-2}) at different juice flow rates (0.073–0.548 L·min^{-1}), log reductions of 1.34 ± 0.35 for *S. cerevisiae*, 4.29 ± 2.34 for *L. innocua* and 5.10 ± 1.12 for *E. coli* were achieved. Those reported doses are relatively higher for *E. coli* inactivation. In a different study, Keyset et al. (2008) reported use of UV-C radiation to inactivate *E. coli* K12 in apple juice by 7.42 log reductions using 1377 mJ·cm^{-2} (D_{10}~186 mJ·cm^{-2}) in a continuous commercial UV system. In another study, Guerrero-Beltran and Barbosa-Canovas (2005) observed a log reduction of 5.1 $\log_{10}$ CFU·mL^{-1} for *E. coli* in pasteurized juice using flow rate of 0.548 L·min^{-1} and UV dosage of 450 KJ·m^{-2} (45 mJ·cm^{-2}). It is important to note that the authors in the above studies calculated UV dose as a product of surface fluence and treatment time (hydraulic retention time), and didn't consider opacity of the fluid and the hydraulic flow path of the fluid which would have likely resulted in poor dose distributions and consequently poor inactivation. It is also possible that microbes might form clumps and could possibly protect other cells from the UV light during the inactivation, resulting in false tailing.

In this study, maximum UV dose of 30 mJ·cm^{-2} resulted in > 5 log reduction of *Salmonella* Typhimurium with linear inactivation kinetics (r^2=0.98) as shown in Figure 2. UV-C doses of 5, 10, 20 and 30 mJ·cm^{-2} were used to inactivate *Salmonella* Typhimurium by 1.02 ± 0.14, 2.07 ± 0.18, 4.44 ± 0.28 and 5.56 ± 0.12 log reductions respectively with D_{10} value of 4.9 mJ·cm^{-2}. It is reported that different strains of *S. enterica* including Typhimurium have D_{10} values in water ranging from <2 to 7.5 mJ·cm^{-2} (Tosa and Hirata, 1998), which fits well with the results of the study. It is apparent that system design of the continuous flow UV-C reactor provided adequate mixing that resulted in log linear inactivation of microbes even up to 5 log or more (Schmidt and Kauling, 2007).

A study by Barbosa-Canovas et al. (2009) reported 0.53 log reduction of *S. cerevisiae* in red grape juice using an annular flow continuous mode UV system at flow of 1.02 $L \cdot min^{-1}$ after 30 mins of treatment time. The authors did not report the dosage, nor did they verify the dose delivery. It is of fundamental importance to consider the optical attenuation coefficients of the test fluid (Camini et al. 2012, Unlurk et al. 2010) and verification of UV fluence is critical. (Islam et al., 2016b). In a separate study, Carlos et al. (2014) showed that coconut milk treated with at different flow rates and treatment times delivering a dose range of 0.342 to 1.026 $kJ \cdot m^{-2}$ under UV-C light resulted in log reduction of 4.1 ± 0.1 for *E. coli* and *Salmonella* Typhimurium under recirculation at different flow rates.

UV irradiation even at low dosages (≈ 25 $mJ \cdot cm^{-2}$) used in our study was successful in inactivating *Listeria monocytogenes* in naturally opaque coconut water. A maximum UV dose of 25 $mJ \cdot cm^{-2}$ resulted in > 5 log reduction of *Listeria monocytogenes* with first-order inactivation kinetics (r^2=0.98) as shown in Figure 2. *Listeria monocytogenes* showed almost linear inactivation with increase in the UV-C dose. (Figure 2). The UV doses of 5, 10, 20, 25 $mJ \cdot cm^{-2}$ resulted in inactivation of 0.85 ± 0.09, 2.70 ± 0.13, 4.30 ± 0.24 and 5.85 ± 0.26 logs with a high regression coefficient r^2 = 0.98. The D_{10} value determined in this experiment was computed as 4.63 $mJ \cdot cm^{-2}$. Kim (2002) reported the D_{90} value of *Listeria monocytogenes* to be 181 $J \cdot m^{-2}$ in water. This value is 4 times higher than reported in our study which could be due to the fact that the author didn't encompass the optical properties of fluid. The UV sensitivity found in our testing is somewhat lower than that of some other authors, but all results show that Listeria is relatively easy to inactivate with UV-C treatment. A study reported by Matak et al. (2005) demonstrated that UV-C irradiation can be used to inactivate *Listeria monocytogenes* by more

than 5 logs with a dose of 15.8 mJ·cm^{-2}. In a different study, Lu et al. (2010) reported a 4-log reduction in *L. brevis* in beer using UV-C light at maximum dosage of 9.7 mJ·cm^{-2}.

The results of this research demonstrated that under all tested conditions UV-C irradiation treatment was effective ($p<0.05$) in inactivation of all three micro-organisms inoculated in coconut water. The populations of *E. coli*, *S.* Typhimurium, *and L. monocytogenes* were reduced by >5 logs at a dose level of ≈30 mJ·cm^{-2} and thus comply with the dose threshold set by the FDA (40 mJ·cm^{-2}) for use of UV-C technology in food processing.

Bacteriophages MS2 and T1UV were selected as model viruses in this study. A study by Dore et al. (2000) showed that F+ RNA bacteriophage (which include MS2) worked successfully as an indicator organism for noroviruses in a study on oyster contamination. MS2 phage belongs to serotype group I of the RNA coliphages within the family Leviviridae (Calender, 1988). The bacterial host for MS2 is *Escherichia coli*, and therefore it is found most frequently in sewage and animal feces. Like noroviruses, MS2 is adapted to the intestinal tract, it is a positive sense single-stranded RNA virus with icosahedral symmetry and is in the same size range at 26 nm diameter.

MS2 and T1UV inactivation was tested at various UV-C doses. Higher UV doses induced greater levels of MS2 and T1UV inactivation in coconut water. As expected, the UV-resistant phage MS2 required approximately 120 mJ·cm^{-2} to achieve near 5 log inactivation. Inactivation of MS2 demonstrated effective dose delivery in this reactor and verifies the UV-C fluence in coconut water. The general trends of these data are depicted clearly in Figure 3. The populations of MS2 were reduced by 0.90 ± 0.03, 1.83 ± 0.02, 2.89 ± 0.04, 4.20 ± 0.04 logs respectively at a UV-C dose level of 20, 40, 80, 120 mJ·cm^{-2}. As expected, T1UV was less resistant to UV, and

was inactivated by 1.33 ± 0.54, 2.04 ± 0.31, 3.34 ± 0.09, 4.73 ± 0.035 logs at UV-C dosage of 5, 10, 20, 30 $mJ \cdot cm^{-2}$. Both viral surrogate concentrations decreased exponentially as UV-C exposure increased; there was no tailing. UV-C irradiation applied in this study was enough to reach the ≈5 log reductions for model viral surrogates.

3.2. Modeling inactivation kinetics

Log-Linear model has been widely accepted and used to describe the microbial inactivation resulting from application of heat and non-thermal based processes. The inactivation curves of microorganisms in coconut water exposed to UV-C irradiation exhibited log linear behavior in all cases (Figure 2). No tailing was observed and it can be accredited to relative high mixing in the UV-C reactor used in this study. Tailing usually occurs from suspended material in the medium showing high turbidity that shields the bacteria during irradiation (Unluturk et al., 2008). Tailing also occurs when the UV is applied non-uniformly, so that poorly irradiated fluid dominates the survival at high log inactivation.

Applicability of linear model to experimental data was tested by plotting the $\log_{10}(N/N_0)$ against UV-C dosage. The data adequately fit the model as depicted in Figure 2. Parameter estimates and goodness of fit for the models are listed in Tables 3 and 4. Log linear models for all microbes had coefficient of determination (r^2) higher than 0.96. The independent set of data was used to calculate model validation statistics (Eq 4-6) for each model. Model prediction errors for each bacterium were estimated by calculating the difference between the observed and predicted values. Figure 4 shows the predicted and actual (experimental values) for microbial log inactivation *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 13311 and *Listeria monocytogenes* ATCC 19115 in coconut water.

The developed models for inactivation curves of pathogens describing the effect of lethal UV dose on log reduction in coconut water were validated using independent set of data. The model performance indices such as accuracy factor (AF) and bias factor (BF) were calculated for mathematical predictive model assessments. (Gunter-ward et al., 2017; Wei, Fang & Chen, 2001; Carrasco et al., 2006; Jaykus & Foegeding, 2000).

$$AF = 10^{\frac{\sum \log\left|V_p/V_E\right|}{n_e}} \qquad \text{Equation 4}$$

$$BF = 10^{\frac{\sum \log\left(V_p/V_E\right)}{n_e}} \qquad \text{Equation 5}$$

The average mean deviation (E) and multiple correlation coefficients (I^2) were used to determine the fitting accuracy of data (Gunter-ward et al., 2017; Tiwari et al., 2008).

$$E(\%) = \frac{1}{n_e}\sum_{i=1}^{n}\left\|\frac{V_E - V_P}{V_E}\right\| \times 100 \qquad \text{Equation 6}$$

Where, n_e is the number of experimental data, V_E is the experimental value and V_P is the predicted value.

To confirm the adequacy of the fitted models, studentized residuals versus run order were tested and the residuals were observed to be scattered randomly, suggesting that the variance of the original observations were constant for all responses. Further, the normality assumption was satisfied as the residual plot approximated to a straight line for all responses. The applicability of the models was also quantitatively evaluated by comparing the bias and accuracy factors for each of the parameters (Table 4). Overall, the accuracy factor values for the predicted model were

1.11 (*Escherichia coli* & *Listeria monocytogenes*) and 1.085 for *Salmonella* Typhimurium. In contrast, the bias factor values for the predicted models were close to unity, ranging from 0.98 to 1.019 for all the parameters. These values indicate that there was a good agreement between predicted and observed values. Ross, Dalgaard, and Tienungoon (2000) reported that predictive models should ideally have an AF = 1.00, indicating a perfect model fit where the predicted and actual response values are equal.

It is indicated from table 4, figure 4 that predicted values were in close agreement with the experimental values. The predicted values were found to be within the range of experimental values and were not significant at $p < 0.05$ using paired t-test. The error percentage (E%) for these models were calculated as 9.14, 8.04 and 11.01 %. Consequently, based on the validation statistics obtained from using independent set of experimental data, the predictive performance of the established model may be considered acceptable.

3.3 Cell culture

To ensure that UV irradiation does not produce toxic chemical compounds in coconut water, two healthy cell lines were incubated in a complete cell culture medium supplemented with coconut water extracts equivalent to a dilution series of original coconut water (i.e., 6.25- to 50-fold dilution). Our results showed that over the entire dilution range, untreated coconut water extract did not cause a significant inhibition of the viability of human normal intestinal CCD-18Co cells, as well as the viability of mouse normal hepatocyte liver AML12 cells. Figure 5 shows the effects of coconut water extracts irradiated with different UV doses (100, 200, 300, 400 $mJ \cdot cm^{-2}$) at different concentrations on the viability of CCD-18Co and AML12 cells. None of the UV dosages caused increased inhibition with respect to the viability of the cells in

comparison to that of untreated coconut water. These results suggest that UV irradiation at 100 to 400 $mJ \cdot cm^{-2}$ did not lead to the production of compounds cytotoxic compounds that are toxic to both either CCD-18Co and or AML12 cells.

4. Conclusions

UV-C irradiation was successfully applied to inactivate the microbial and viral populations in coconut water using a flow-through UV reactor. This study found that UV-C irradiation treatment at low doses (≈ 30 $mJ \cdot cm^{-2}$) could be used to achieve 5-log inactivation of several important pathogens. UV disinfection was demonstrated using pathogenic and non-pathogenic microorganisms including bacteriophages. The inactivation kinetics of these tested microorganisms were best described by log linear kinetics. In the cytotoxicity evaluation studies, coconut water extract showed no cytotoxic effects on normal intestinal and healthy mice liver cells. UV-C treatment did not change the cellular responses of both cell types to the coconut water extract. These results suggest that UV-C treatment didn't generate any cytotoxic compounds in the coconut water. Scale-up of the UV-C device, spore inactivation studies, and sensory evaluation of UV-C treated coconut water will be subject of further investigations. Scale up equipment has already been developed by the research team and its efficacy in inactivating microorganisms and other spores in juice on a larger scale will be subject to future investigation.

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List of Figures:

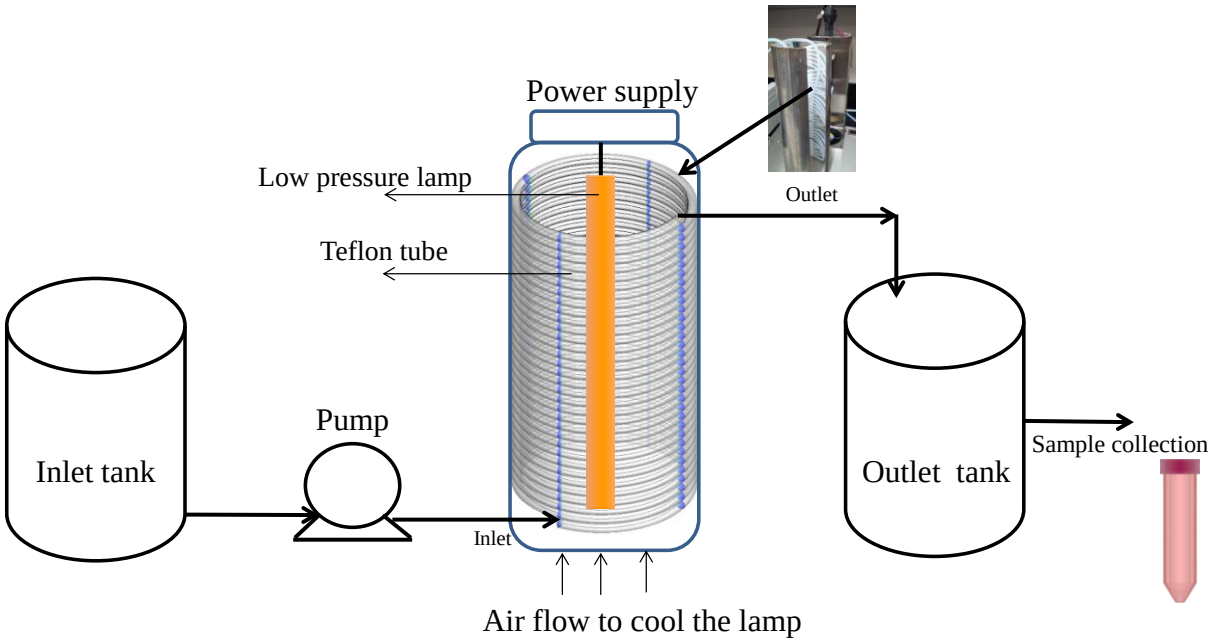


Figure 1. Schematic view of UV flow-through system

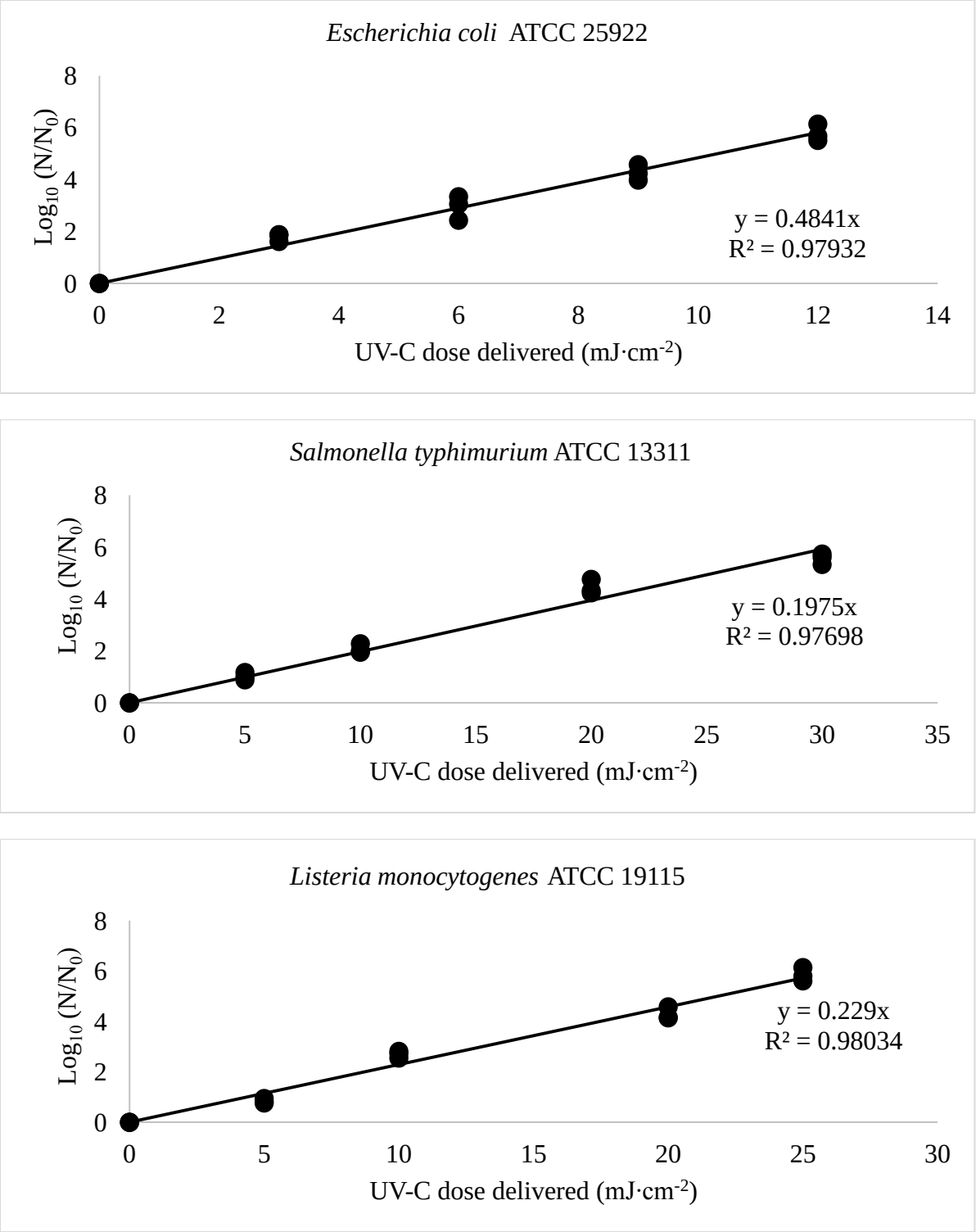
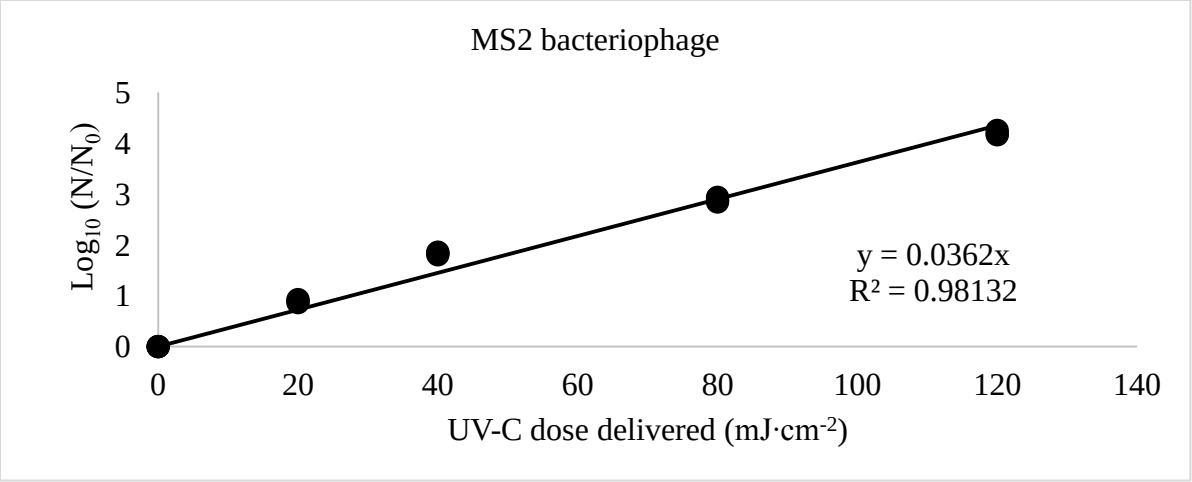


Figure 2. Inactivation of *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 13311 and *Listeria monocytogenes* ATCC 19115 in coconut water.



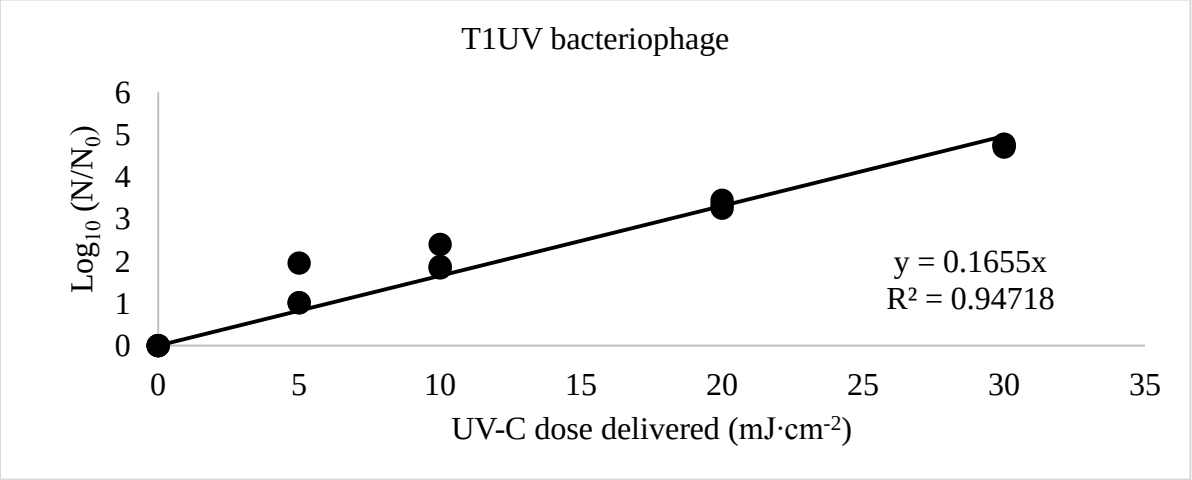
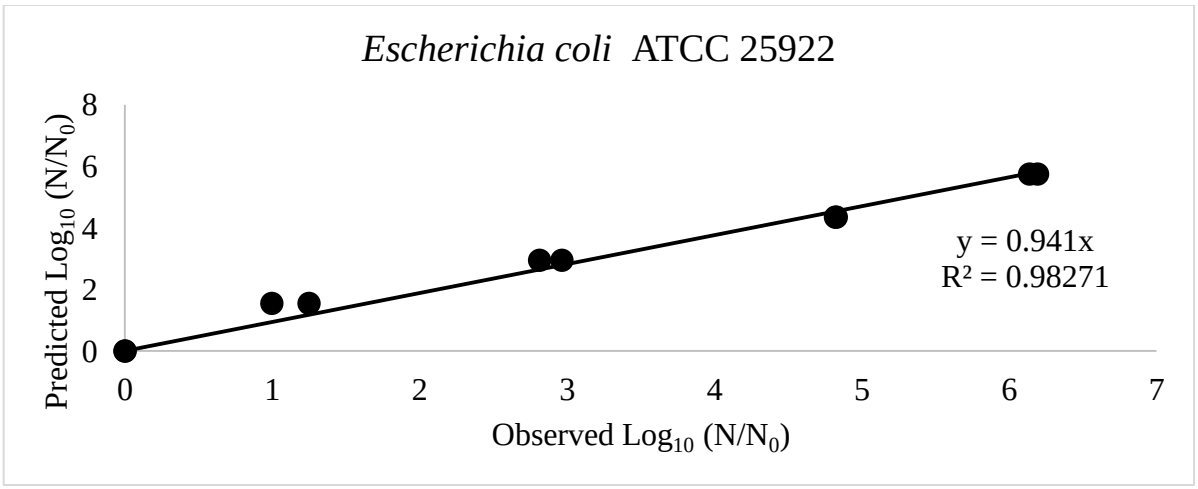
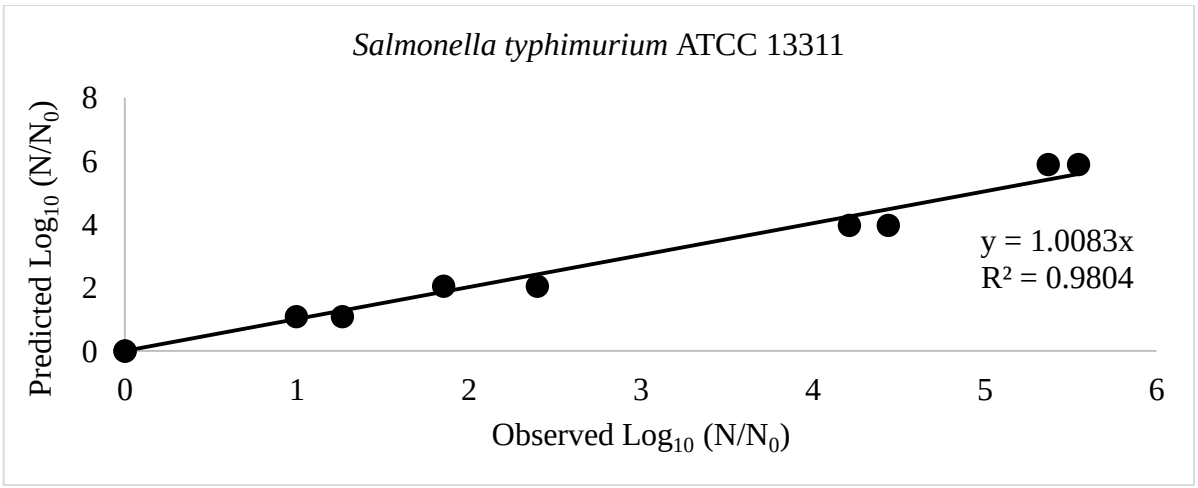


Figure 3. Inactivation rate results of MS2 and T1UV-C in coconut water





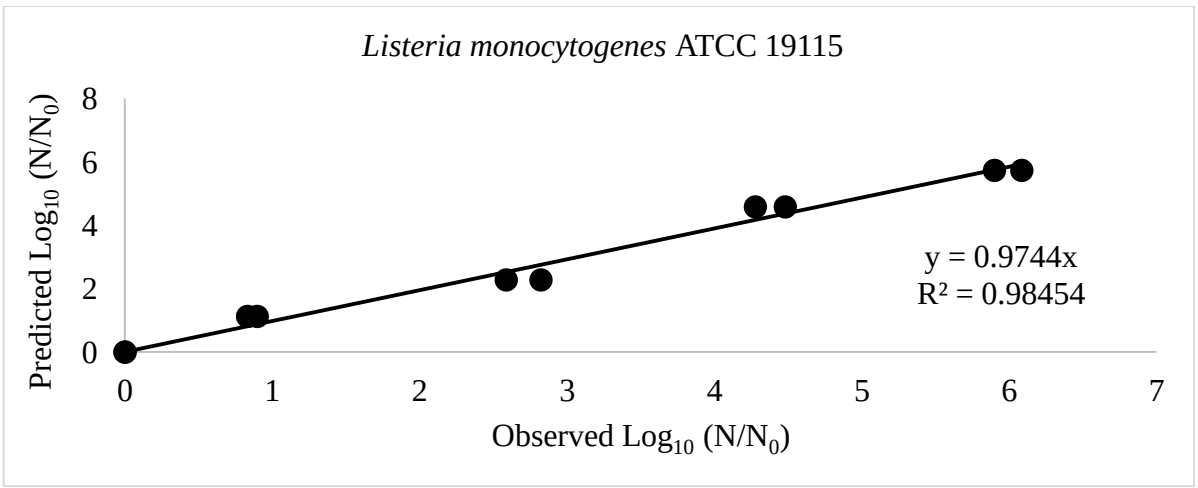


Figure 4. Predicted and actual (experimental values) for microbial log inactivation *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 13311 and *Listeria monocytogenes* ATCC 19115 in coconut water.

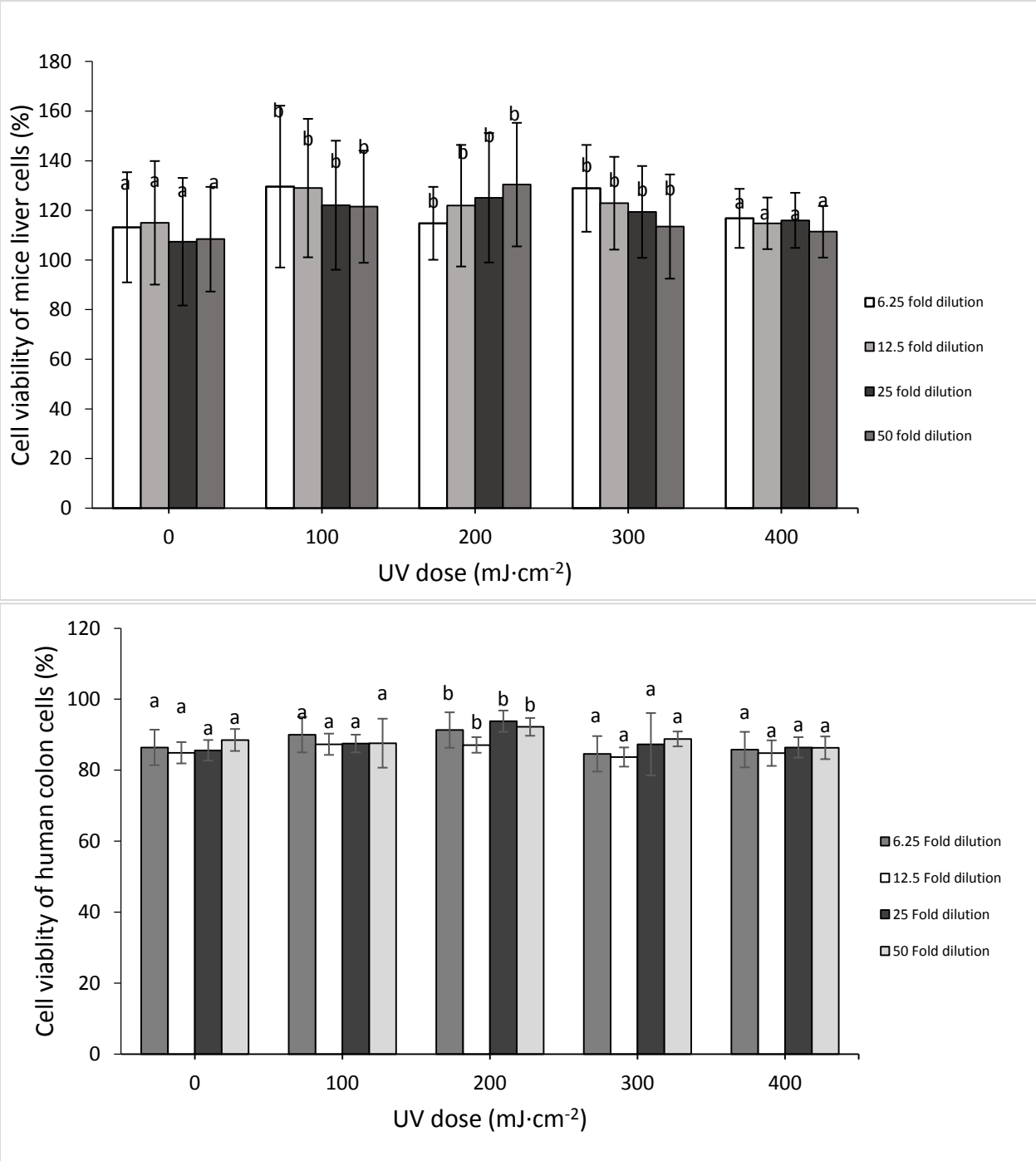


Figure 5. Effect of UV-C irradiation on cell viability of healthy mice liver cells and normal colon (CCD-18Co).

List of Tables:

Table 1. UV-C sensitivity or D_{10} values of *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 13311 and *Listeria monocytogenes* ATCC 19115.

Microbe	**D_{10} value**[a]
Escherichia coli ATCC 25922	2.82 ± 0.13
S. typhimurium ATCC 13311	3.06 ± 0.12
L. monocytogenes ATCC 19115	4.54 ± 0.10

[a]D_{10} value expressed as $mJ \cdot cm^{-2}$, Values expressed as mean±standard deviation

Table 2. Optical properties and pH values for Coconut water.

Parameters	**Values**
pH	4.88 ± 0.164
Absorbance (1/cm)	1.01 ± 0.018
Transmittance (%)	9.70 ± 0.406

Values expressed as mean±standard deviation

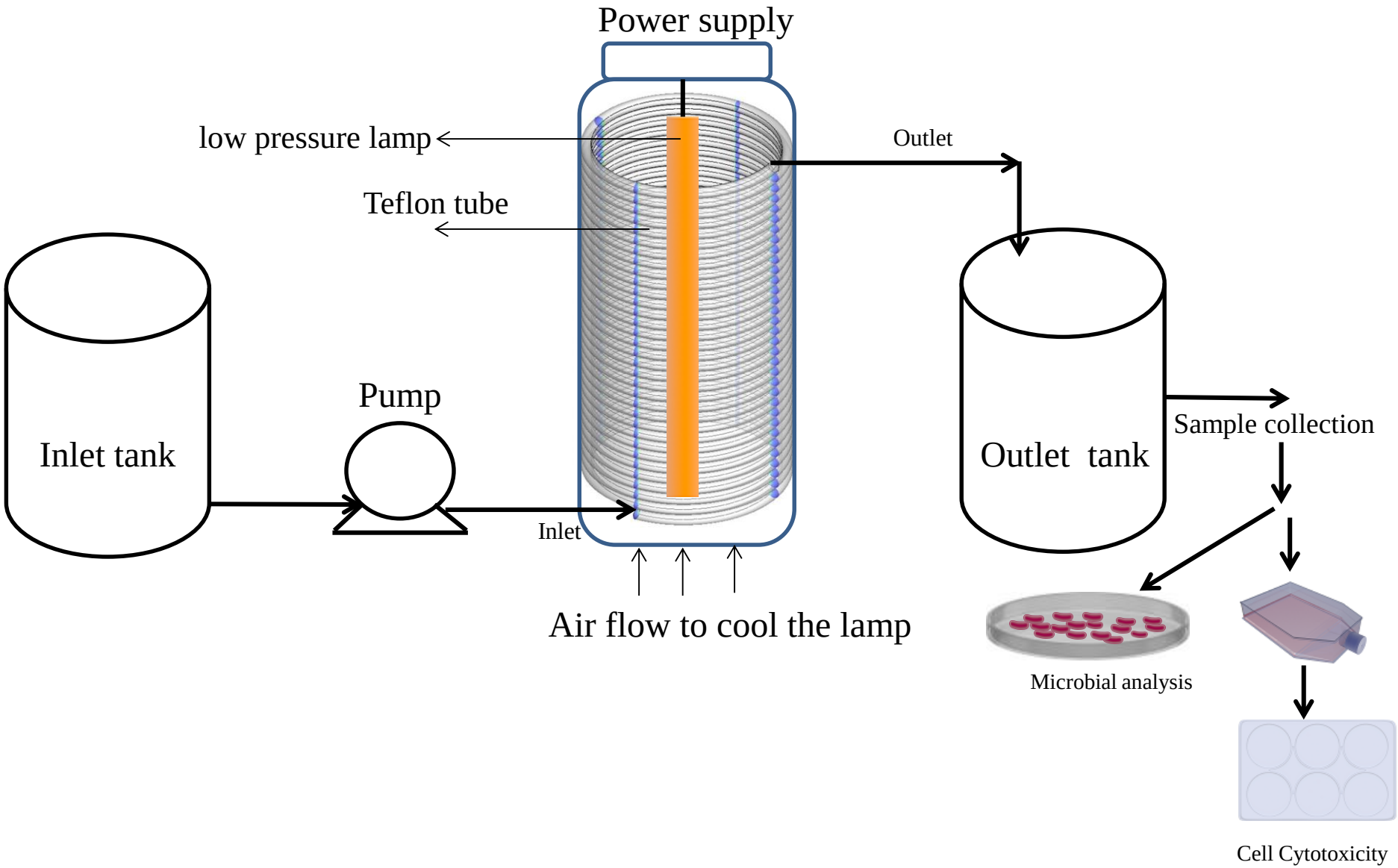
Table 3. Model fit statistics and rate parameter (k_1) estimate for each model (Model form – equation 1 was parameterized).

Microbes	Model fit statistics		Parameter estimates	
	r^2	RMSE	Rate Constant (k_1)	p-value
Escherichia coli ATCC 25922	0.979	0.295	0.484	<0.0001
Salmonella Typhimurium ATCC 13311	0.976	0.328	0.198	<0.0001
Listeria monocytogenes ATCC 19115	0.98	0.324	0.229	<0.0001
MS2	0.981	0.171	0.036	<0.0001
T1UV	0.947	0.335	0.165	<0.0001

Table 4. Validation statistics for model prediction using independent set of data for three bacteria – *E. coli*, *Salmonella* Typhimurium and *Listeria monocytogenes*.

Microbes	AF	BF	E%
Escherichia coli ATCC 25922	1.111	1.036	9.14
Salmonella typhimurium ATCC 13311	1.085	0.985	8.04
Listeria monocytogenes ATCC 19115	1.11	1.019	11.01

*Graphical Abstract



Continuous Flow UV Reactor