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UV-C treatment on the safety of skim milk: effect on microbial inactivation and cytotoxicity evaluation

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Abstract

The efficacy of UV-C irradiation as a non-thermal processing method for skim milk (SM) was investigated. SM inoculated with two surrogate viruses (MS2 and T1UV), and three bacteria (*Escherichia coli* ATCC 25922, *Salmonella enterica* serovar Typhimurium ATCC 13311, *Listeria monocytogenes* ATCC 19115) was treated with UV-C irradiation. Biodosimetry techniques were used to calculate the reduction equivalent fluence (REF). SM was irradiated using a Dean-flow spiral reactor with the fluid pumped around a central low-pressure mercury UV lamp (40 W) emitting at 254 nm wave-length. A series of known UV doses (0 – 168.33 $mJ \cdot cm^{-2}$) were delivered to the samples. The microbial loads of MS2, T1UV, *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* were reduced by more than 5 $\log_{10}$. At the highest dose of 168.33 $mJ \cdot cm^{-2}$, the results showed that UV-C irradiation effectively inactivated surrogate viruses in SM. The inactivation kinetics of all microorganisms were best described by log linear models with a low root mean squared error (RMSE) and higher coefficient of determination (R^2>0.95). This study demonstrated that high levels of inactivation of bacterial cells and viral particles can be achieved in SM.

Key terms: UV-C irradiation, biodosimetry, inactivation, pathogen, bacteriophage, kinetics, milk

Practical Applications

This scientific study provides evidence based data on the advantages of UV-C light in achieving microbial reduction in skim milk. The irradiated skim milk did not show any toxicity on mice liver and intestinal cells. UV-C irradiation is an efficient food preservation technology and offers opportunities for dairy and food processing industries to meet the growing demand from consumers for safer foods. This exploration would provide methodological evidence for commercialization of UV-C processing of milk and dairy based beverages.

1. Introduction

Avoidance and elimination of microbial contamination in food processes is a crucial issue. The persistence of foodborne pathogens in milk has become a perilous public health concern as foodborne outbreaks consistently occur as a result of pathogenic contamination (CDC, 2017). Despite common implementation of Hazard Analysis and Critical Control Points (HACCP), foodborne illnesses still pose a great public health dilemma (United States Food and Drug Administration, 2015). Milk safety in the United States is generally very high, driven by the 1924 Pasteurized Milk Ordinance. Since this federally directed quality standard for milk processors was enforced, outbreaks from milk have declined from approximately 25% of foodborne outbreaks to < 1% (USPHS/USFDA, 2009).

Skim milk spoilage by many pathogenic and non-pathogenic bacteria is prevalent due to its abundant nutrient content (Bandla et al., 2012; Dega et al., 1972; Oliver et al., 2005). Investigations by Langer (2012) and Claeys (2013) groups revealed that the mainly associated milk borne pathogens are as follows: *Shiga toxin–producing Escherichia coli O157:H7, Brucella, Salmonella spp., Listeria monocytogenes, Bacillus cereus, Mycobacterium tuberculosis, Campylobacter jejuni, Clostridium perfringens, Yersinia enterocolitica, Aeromonas hydrophila, Serratia marcescens, Staphylococcus aureus, norovirus,* and *Coxiella burnetiid.*

The most widely used and approved method for the inactivation of milk is thermal pasteurization (Jayarao et al., 2006; Crook et al., 2015). Pasteurization, in particular high-temperature short-time in the United States, is the primary heat treatment applied to milk.

Whereas in current pasteurization process conditions (72 °C at 15 s; National Advisory Committee on Microbiological Criteria for Foods, 2006), along with sanitary standards aim to achieve safe products, the thermotolerant spoilage organisms will survive and restrict the shelf life of products, as evidenced by code dates of 14 to 18 d (Cullor, 2011). Thermal processing has a number of disadvantages as it causes nutrient damage and off flavors (Guneser and Yuceer, 2012). Therefore, an alternative to non-thermal methods for treating skim milk and other opaque liquid foods is of a growing interest. It is envisaged that other non-thermal processing methods are being investigated for milk treatment but are not limited to Pulsed Electric Field, Ultraviolet light, and High Pressure Processing (Soumitra and Shanker, 2017).

UV technology has started to emerge as a promising non-thermal preservation processes for beverage preservation. Ultraviolet light in the germicidal range (UV-C specifically) from 200 to 280 nm, is being investigated for inactivating pathogens in skim milk (Cappozzo et al., 2015; Bandla et al., 2012). Primary mode of UV-C microbial inactivation occurs when cross-linked pyrimidine dimers form a as result of the nucleic acids absorbing UV-C light. Significant presence of pyrimidine dimers formed in critical loci lethally inhibit DNA repair mechanisms and prevent the organism from replicating, subsequently inhibiting pathogenicity (Bintsis et al., 2000; Guerrero- Beltrán and Barbosa-Cánovas, 2004; Shama, 1999; Hamkalo and Swenson, 1969).

There is regulatory guidance for applying new food safety technologies to low acid beverages. However, this guidance excludes milk. It has been concluded by the United States Department of Agriculture (USDA) and United States Food and Drug Administration (USFDA) that the application of UV-C light at 253.7 nm for food treatment is safe and has further approved the usage (CFR 179.39) as an alternative method to reduce pathogens and other microorganisms (USFDA, 2000). USFDA approved the use of UV-C light in the production, processing, and handling of food, detailed in Code21 CFR 179.41.

In recent years great effort has been made to improve the understanding of light propagation in turbid fluids. For optically thick fluids, such as skim milk, appropriate mathematical methods are required to separate the reduced scattering coefficient (μ_s) from the absorption coefficient (μ_a) to calculate the transmission of light. Highly opaque liquids, such as milk, are difficult to treat using the current UV reactors as these systems are unable to deliver uniform irradiation to the entire fluid volume, resulting in inadequate performance. It is difficult to obtain a uniform fluence in turbid fluids (e.g. skim milk) that have high optical scattering and absorption coefficients. When a liquid is turbid or opaque, such as skim milk, UV-C photon contact with microbes is minimal (only about 0.003 cm at 254nm) as penetration into the bulk liquid is shallow as opposed to deep (Koutchma, 2009). Thus, ensuring uniform UV dose to test fluids is key in adequate microbial inactivation. The residence time, flow pattern, and microorganismal position in specific regions of the irradiance UV light field are significant factors influencing uniform dose delivery.

Ultraviolet treatment has been found to have a major effect on total coliforms, *Escherichia coli*, and *Salmonella* and *Listeria monocytogenes* (Bhullar et al., 2017; Gunter-Ward et al., 2017). Recent technology developments involving continuous flow UV processing have enabled fundamental understanding of microbial inactivation. Continuous flow UV systems are more desirable in an industrial setting as compared to batch UV systems. For example, in laboratory studies conducted at the University of California-Davis (Rossitto et al., 2012) on milk with 3.5 and 2% fat at UV doses of 880 and 1,760 J/L, continuous turbulent flow UV processing has been shown to be effective against milk microflora and capable increasing milk shelf-life to 28 to 35 d. In a different study, a reduction in pathogenic organisms such as *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella senftenberg*, *Yersinia enterocolitica*, and *Staphylococcus aureus* has been achieved and help to ensure the safety of milk using UV processing (Cullor, 2011; Crook et al., 2015). The UV dose of 2,200 J/L, as reported by Crook et al. (2015), was sufficient to achieve a 5 $\log_{10}$ reduction of the most UV-resistant milk-borne pathogen (i.e., *Listeria monocytogenes*) using a turbulent flow SP-4 UV system (SurePure AG, Zug, Switzerland). However, in both studies, UV-C dose/fluence units were recorded in Joules/Liter, which are not applicable in parallel with microbial reduction. As an illustration, the Joules/Liter in a scattering fluid such as milk will not result in the equivalent Joules/Liter in transparent water due to differing optical properties for the two fluids resulting in varying penetration of UV light and subsequently unidentical microbial inactivation curves.

Our group quantified and verified UV fluence using bacteriophages MS2 and T1UV. According to United States Environmental Protection Agency (USEPA), MS2 bacteriophage inactivation response to UV is well-established and has been recommended for the purpose of confirming the UV fluence in a flow system (Pirnie et al., 2006). This process parameter is referred to as Reduction Equivalent Fluence (REF).

In literature, cytotoxicity was not investigated in prior explorations of UV treatment on milk. UV photons at 254 nm can disrupt chemical bonds, possibly resulting in modification of chemical compounds. Pyrimidine dimer formation between adjacent nucleic acids is the mechanism of action by which UV inactivates targeted bacteria and viruses. Cytotoxicity of UV exposed fluids must be investigated to confirm that this novel food processing method does not produce toxic by-products.

In this study, we provide a systematic experimental investigation of a continuous flow UV and its effectiveness against *Salmonella* Typhimurium ATCC 13311, *Escherichia coli* ATCC 25922 , and *Listeria monocytogenes* ATCC 19115 in skim milk. In addition, this study also evaluated the cytotoxicity of UV-C irradiated milk on the normal colon cells (CCD-18Co), and healthy mice liver (AML-12) cells.

2. Material and Methods

2.1 Bacteriophage and cultural conditions

MS2 (a single stranded RNA virus) and T1UV (a double stranded RNA virus) were used as surrogate viral pathogens. The cultures were purchased from GAP EnviroMicrobial Services Limited (London, Ontario, Canada) and stored at -4 ºC until used (Bhullar et al., 2017). The host *E. coli*, *E. coli* HS (pFamp)R and *E. coli* CN13, were used in the plaque assay for MS2 and T1UV bacteriophage. Bacteriophages were cultured by a method described by the International Standards Organization (ISO, 1995). Counting range was 20-300 PFU/plate.

2.2 Bacterial strains and culture conditions

The three target strains of bacteria utilized in this study were *Escherichia coli* (ATCC 25922) (*E.coli*), *Salmonella enterica* serovar Typhimurium (ATCC 13311) (*S.* Typhimurium), and *Listeria monocytogenes* (ATCC 19115) (*L. monocytogenes*) which were prepared according to the methodology reported by Bhullar et al. (2017).

2.3 Skim Milk preparation and inoculation

The samples were obtained from a local Nashville, TN grocer. The commercially pasteurized skim milk samples were immediately placed in a refrigerator at 4 °C and stored for a period of no longer than 3 weeks. The skim milk was examined for background microbial populations of *E. coli*, *S.* Typhimurium, and *L. monocytogenes*.

After harvesting, the inoculum was plated to determine the bacterial concentrations (Baumann et al., 2005; Bhullar et al., 2017). Aliquots of 4000 mL of skim milk were individually inoculated with each of the three bacterial cultures. The initial titer inoculated for *E. coli*, *S.* Typhimurium, and *L. monocytogenes* was 10^8 CFU/mL. To determine the original *E. coli*, *S.*

Typhimurium concentrations in inoculated skim milk, untreated inoculated control samples were plated on TSA plates and incubated for at 37 °C for 24 h. Skim milk inoculated with *L. monocytogenes* was plated on LAB (Oxoid Ltd., Basingstoke, UK) and incubated at 37 °C for 48 h (Gunter-Ward et al., 2017; Bhullar et al., 2017).

2.4 Optical properties

Absorption and reduced scattering coefficients of the samples were measured in a double beam Cary 100 Spectrophotometer (Varian, USA) fitted with a 6-inch single Integrating Sphere (Labsphere, DRA-CA-30, USA) to include scattered light which measured at 254 nm wavelength (Prerana et al., 2008; Gunter-Ward et al., 2017). All measurements were done in thin quartz cuvettes (0.8mm, 1.0mm, and 1.2mm path-lengths) (H. Baumbach & Co Ltd, UK). The total transmitted and reflected (diffuse reflectance) light was collected by the integrating sphere when the sample was placed at the entrance and exit ports respectively. An eight-degree reflectance port was used to measure the total reflectance. The system scale was set by setting the 100% baseline using the blank provided with the NIST standards and then 0% baseline by blocking the beam. All scans were done in triplicate. Due to a potential impact of light losses in the quartz cuvettes, and depending on the wavelength of interest, the amount of light absorbed by the quartz cuvettes was also quantified as quartz optical depth. Ultraviolet transmittance (UVT %/cm), which is a measure of the fraction of incident light transmitted through a material over 1 cm path, was calculated as per equation 1.

$$\text{UVT (\%/cm)} = [10^{-A}] \times 100 \qquad (1)$$

where A represents the absorbance ($base_{10}$) of the test fluid at 254 nm for a 1cm path.

2.5 Fluence Verification and UV-C Treatment

Skim milk samples were treated with a Dean-flow spiral reactor detailed in the methodology by Bhullar et al. (2017). An insert was also used to control the UV exposure to the microbes. To achieve the desired fluence, the skim milk was passed through the reactor system at 30 - 800 $mL \cdot min^{-1}$. Length of the Teflon tube was 7.4 m and the distance of lamp from the reactor (tubing) was 3.0 cm. After discarding a volume of fluid equal to three UV system volumes, irradiated skim milk was collected for microbial analysis.

The Reduction Equivalent Fluence (REF) delivered to the skim milk was confirmed using a viral clearance test with the challenge organisms, MS2 and T1UV, inoculated in the skim milk. MS2 and T1UV are used extensively to validate UV disinfection systems for drinking water as it is a well characterized bacteriophage (Islam et al., 2016a; Bhullar et al., 2017). Flow rates utilized for T1UV were 0, 151, 81, 50, 38 (with insert), 605, 450, and 317 (without insert) $mL \cdot min^{-1}$ delivering REF doses of 0, 3.82, 6.05, 8.78, 16.86, 22.93, 27.12 $mJ \cdot cm^{-2}$. Flow rates utilized for MS2 were 0, 320, 215, 160, 100, 65 $mL \cdot min^{-1}$ delivering REF doses of 0, 43.32, 62.56, 79.84, 124.66, and 168.33 $mJ \cdot cm^{-2}$. The log reduction of both bacteriophages were used to calculate fluence delivered by the reactor. Log concentrations were determined by GAP EnviroMicrobial Services (ON, Canada), who also provided the bacteriophage cultures.

2.6 Buffer, Humic Acid Preparation and Inoculation

Buffered water (buffer) was prepared according to the protocol detailed by Gunter-Ward et al. (2017).

2.7 Organism sensitivity test

Organism sensitivity test was carried out in buffer by methodology described by (Gunter-Ward et al., 2017; Bhullar et al., 2017).

2.8 Enumeration of pathogens in test fluid after UV-C treatment

Following UV treatment, microbial loads in skim milk and buffer samples were determined via enumeration techniques as stated in (Bhullar et al., 2017).

2.9 Inactivation kinetics - Log-Linear model

Microbial inactivation, resulted from both thermal and non-thermal processes, has been described using the well-established Log-Linear model, an appropriate display of inactivation data that exhibits first order kinetics. The model is given as the Chick Watson linear equation (4) below (Van Boekel, 2002; Marugán et al., 2008):

$$\mathrm{Log}_{10}\left(\frac{N}{N0}\right) = -k_1 D \quad \text{Equation 4}$$

where, k_1 is the slope of the microbial inactivation and D is the UV-C dose in $mJ \cdot cm^{-2}$. Log reduction is calculated as $Log_{10}(N_0\text{-}N)$. Conventional D_{10} value is determined from the Escherichia coli reciprocal of the first order rate constant ($D_{10}=1/-k_1$, units in $mJ \cdot cm^{-2}$).

2.10 Cell Cytotoxicity

Cell cytotoxicity of irradiated milk was assessed by the method described by Bhullar et al. (2017). Normal human colon fibroblasts (CCD-18Co; ATCC, Manassas, VA), and normal mouse hepatocyte liver epithelial cells (AML12; ATCC) were used in this study.

2.11 Statistical analysis

Microbial log inactivation resulting from the UV-C irradiation treatments were recorded and log-linear models were fitted in Excel (Microsoft, 2013). Model fit statistics including r^2, RMSE, and rate constants were compared among the competing models. A balanced design with three replicates randomized in experimental order were performed for each UV dose. Cell viability and microbial inactivation data was analyzed using JMP statistical software (SAS, 2016).

3. Results and Discussion

3.1 UV fluence validation

UV fluence was verified using challenge organisms, MS2 and T1UV bacteriophages. This testing was modeled on the accepted practice for reactor validation as laid out by the USEPA (Ducoste et al., 2005; Bhullar et al., 2017). In brief, the UV sensitivity of the MS2 and T1UV was evaluated using a collimated beam apparatus. Irradiated stirred samples of MS2 and T1UV were inoculated in clear buffer in petri dishes. Survival curves ("Dose-response") were plotted and the log inactivation vs. UV fluence was determined for MS2 and T1UV. Skim

milk inoculated with MS2 or T1UV was passed through the reactor at various flow rates. The log inactivation of MS2 or T1UV at each flow rate was used to determine REF delivered to the fluid at each flow rate, using the dose-response relation described above. The calibration curves may be seen in Figure 1. Figure 1 displays REF versus 1/(abs ×flow) (approximately 8 to 50 $mJ \cdot cm^{-2}$) and (approximately 0 to 200 $mJ \cdot cm^{-2}$) delivered to skim milk samples inoculated with T1UV. The REF bias was also calculated for various flow-rates (data not shown). REF bias is the ratio of the calculated reduction equivalent dose for the bacteriophage (MS2 and T1UV). Since T1 is more sensitive to UV than MS2, it will be more strongly affected by poor dose distribution, and will yield a lower calculated REF. The ratio of these REF values is an approximate indication of the spread in the dose distribution. A perfectly mixed reactor would have a ratio of unity. For the system, the bias values increased at the lower flow rate. For flows higher than 50 $mL \cdot min^{-1}$, the REF bias was close to 1. The measured performance for the UV system was appropriate. These tests confirmed that UV-C fluence ranging from 0 – 168.33 $mJ \cdot cm^{-2}$ can be applied to skim milk. This verification of fluence approach also assumes that the REFs are additive, a significant indication of the efficient mixing capabilities of the reactors used in this research study (Bhullar et al., 2017).

3.2 Bactericidal effect of UV

Optical and physical properties (pH, UVT, absorbance, and scattering) of skim milk are illustrated in Table I. The optical data measured clearly indicates that the UV-C light was strongly absorbed and scattered through skim milk. Low penetration of UV light is due to the

abundance of soluble and suspended solids (i.e. micro-molecules (amino acids) compounds, organic solutes, or suspended matter) in skim milk (Koutchma et al., 2004). Scattering reduces the reactor performance and needs to be accounted for.

Other researchers have suggested that in liquid foods with high UV absorption and scattering, the test fluid must be exposed to UV, source being a Dean-Flow system, to ensure that the liquid absorbs UV negligently and the targeted bacteria are primarily subjected to lethal doses of UV-C light (Wright et al., 2000). Contrastingly, this exploration showed despite the fact that UV apparatus was not based on a thin-film design, bacteria could be inactivated to levels undetectable in milk. Our results provide evidence that 5 $\log_{10}$ reductions of bacteria are achieved utilizing Dean flow UV reactor. Dean Vortices were induced in the liquid that contributed to uniform dose distribution. It is quite evident that the laminar Poiseuille flow in the cross-section of curved pipes undergoes centrifugal forces. The parabolic pattern of the laminar/primary flow disrupts this external force and subsequently shifts the maximum point of velocity central distribution towards the concave wall of the channel. The pressure increases due to the sharp velocity gradient, and the velocity closest to the walls is not sufficient to maintain pressure gradient equilibrium (Dean, 1928; Barua, 1963; Berger et al., 1983; Dombrowski et al., 1993). This imbalance is known as Dean instability and leads to recirculation of fluid in the form of vortices directed from center of the channel towards the outer channel wall hence inducing high mixing.

For flow-rates of 151, 81, 50 (with insert), 605, 450, 320, 215, 160, 100, 65 (without insert) $mL \cdot min^{-1}$, the Re was 1351, 724, 447, 5411, 4025, 2862, 1923, 1431, 894, 581 respectively. Figure 2 displays *E. coli*, *S.* Typhimurium, and *L. monocytogenes* inactivation rates in skim milk. These microbes were not present in the skim milk prior to inoculation. The dose response curves display the log reduction $Log_{10}(N_0\text{-}N)$versus UV fluence. The UV-C sensitivity of *E. coli* in skim milk in our study is consistent with the literature. Reported D_{10} values for *E. coli* were approximately 2 -3 $mJ \cdot cm^{-2}$ (Sommer et al., 1998; Zimmer and Slawson, 2002; Chang et al., 1985). Our results exhibited a D_{10} of about 3.50 $mJ \cdot cm^{-2,}$ based on 6.23 log_{10} inactivation at a dose of 22.33 $mJ \cdot cm^{-2}$ for *E. coli* in skim milk. We also observed a D_{10} value of 2.72 $mJ \cdot cm^{-2}$ for *E. coli* in phosphate buffer was (Table II) (Gunter-Ward et al., 2017). *E. coli* was reduced by more than 5 log_{10} CFU/mL at a maximum UV-C dose of 22.33 $mJ \cdot cm^{-2}$. The dose levels used to inactivate *E. coli* were 3.82, 6.05, 16.86, and 22.93 $mJ \cdot cm^{-2}$ resulting in 1.62±0.09, 2.70±0.07, 4.79±0.18 and 6.23±0.26 log_{10}, reduction. The inactivation curve followed a log linear model with R^2 =0.95. These results are in alignment with the literature which states that the D_{10} values of *E. coli* O157:H7 cells ranged from 0.4 to 3.5 $mJ \cdot cm^{-2}$ (Sommer et al., 2000, Tosa and Hirata, 1999).

In general, both UV studies do not account for fluid absorbance. For example, Keyser et al. (2008) reported use of UV-C radiation via a continuous commercial UV system to inactivate *E. coli* K12 in apple juice by 7.42 log_{10} reductions using 1377 $mJ \cdot cm^{-2}$ (D_{10}~186 $mJ \cdot cm^{-2}$). Similarly, Guerrero- Beltrán and Barbosa-Canovas (2004) achieved log_{10} reductions of 1.34

± 0.35, 4.29 ± 2.34, and 5.10 ± 1.12 for *S. cerevisiae*, *L. innocua*, and *E. coli* respectively. The three bacteria were inoculated separately into apple juice and treated for 30 min of treatment with reported doses ranging from 75 to 450 $kJ \cdot m^{-2}$ (or 7.5 to 45 $mJ \cdot cm^{-2}$) at different juice flow rates (0.073–0.548 $L \cdot min^{-1}$). Those reported doses are relatively higher for *E. coli* inactivation. It is of great importance to note that the authors of the previously mentioned investigations did not consider the hydraulic flow path of the fluid or the opacity of the test fluid. This could have contributed to ineffective dose distributions, consequently resulting in poor inactivation. Also, the UV dose was calculated as a product of hydraulic retention or treatment time and surface fluence. Various other non-thermal technologies have been evaluated for milk treatment. For example, Miller, Sauer , and Moraru (2012) tested pulsed UV light against *E.coli* ATCC 25922 in milk. The author treated milk with a dosage of 14.9 J/cm^2 resulting in a 3.4 $\log_{10}$ reduction. In a different study, Li et al. (2006) utilized High Pressure Processing to inactivate *E. coli* 8739 in enriched soy milk. The authors recorded 5 $\log_{10}$ reduction at 345 MPa for 4 min at 30 °C.

Our maximum UV dose of 22.93 $mJ \cdot cm^{-2}$ resulted in greater than 5 $\log_{10}$ reduction of *S.* Typhimurium with linear inactivation kinetics (R^2=0.99) as shown in Figure 2. UV-C doses of 3.82, 6.05, 16.86 and 22.93 $mJ \cdot cm^{-2}$ were used to inactivate *S.* Typhimurium by 0.56±0.13, 1.07±0.13, 3.89±0.23, and 5.60±0.43 $\log_{10}$ reductions respectively with D_{10} value of 4.24 $mJ \cdot cm^{-2}$. The results suggested that the design of the continuous flow UV-C reactor systems provided adequate mixing that resulted in linear correlation between microbial reduction of

up to 5 $\log_{10}$ or more and accurate UV-C dose exposure (Schmidt and Kauling, 2007). Tosa and Hirata (1999) reported that multiple strains of *S. enterica,* including Typhimurium, have D_{10} values in water ranging from less than 2 to 7.5 $mJ \cdot cm^{-2}$, which is comparable with the results of the study.

Ochoa-Velasco et al. (2014) demonstrated that coconut milk irradiated at different treatment times and flow rates under UV-C light at doses of 0.342 to 1.026 $kJ \cdot m^{-2}$ resulted in $\log_{10}$ reduction of 4.1 ± 0.1 for both *Salmonella* Typhimurium and *E. coli.* An investigation by Guerrero- Beltrán et al. (2008) reported that after 30 min of treatment time *S. cerevisiae* was reduced *by* 0.53 $\log_{10}$ in red grape juice when exposed to UV-C light via an annular flow continuous mode UV system at flow of 1.02 $L \cdot min^{-1}$. Dose verification was not conducted nor was dosage reported by the authors. Consideration of optical attenuation coefficients of the test fluid as wells as verification of UV fluence is fundamentally important to effective inactivation of food borne illnesses in opaque liquid foods. (Caminit et al. 2012, Unluturk et al., 2010) (Islam et al., 2016b). It is quite evident that system design of the continuous flow UV-C reactor provided adequate mixing that resulted in $\log_{10}$ linear inactivation of microbes even up to 5 $\log_{10}$ or more for *E. coli* and *S.* Typhymurium. The reactor also achieved good result with the more resistant MS2 challenge organism.

L. monocytogenes was exposed to a maximum UV dose of 16.86 $mJ \cdot cm^{-2}$ which resulted in more than 5 $\log_{10}$ *L. monocytogenes* with first-order inactivation kinetics (R^2=0.98) as shown in Figure 2. *L. monocytogenes* displayed linear inactivation with increase in the UV-C dose

(Figure 2). The UV doses of 3.83, 6.05, 8.78, 16.86 $mJ \cdot cm^{-2}$ resulted in inactivation of 0.81±0.10, 1.72±0.28, 3.01±0.19and 5.16±0.06 $logs_{10}$ with a high regression coefficient r^2 = 0.98. The D_{10} value determined in this experiment was calculated as 3.24 $mJ \cdot cm^{-2}$. Lu et al. (2010) reported after UV-C application on *L. brevis* in beer using a 4 log_{10} reduction in UV-C light at maximum dosage of 9.7 $mJ \cdot cm^{-2}$. A different study reported the D_{90} value of *L. monocytogenes* to be 181 $J \cdot m^{-2}$ in water (Kim et al., 2002). This value is 4 times higher than reported in our study which could be due to the fact that the author did not account for the optical properties of test fluid. The UV sensitivity determined in our testing is moderately lower than that of some other authors, but all results demonstrate that listeria is relatively simple to inactivate with UV-C treatment. An exploration by Matak et al. (2005) exhibited that UV-C irradiation can be used to inactivate *L. monocytogenes* by greater than 5 $logs_{10}$ with a dose of 15.8 $mJ \cdot cm^{-2}$. 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 skim milk. The populations *E. coli*, *S.* Typhimurium, and *L. monocytogenes* were reduced by >5 $logs_{10}$ at a dose level of $\approx$23 $mJ \cdot cm^{-2}$. To the best of our knowledge, no recommendations or guidelines are set-up by the USFDA in regard milk processing using UV light. However, it has been reported that UV-C irradiation for the decontamination of water, juices, and food surfaces, has been approved for use under specific conditions (USFDA, 2000; USFDA, 2015).

There has been additional non-thermal studies utilizing other processing methods for milk treatment. Liepa et al. (2018) investigated the inactivation of microorganisms in skim milk using high hydrostic pressure processing at pressures of 250/15 min, HP400/3 min, and HP 400/15 min resulting in reductions of 3.6 $\log_{10}$ CFU/mL, 3.48 log CFU/mL, and 2.56 $\log_{10}$ CFU/mL from original microbial load of 5.11 $\log_{10}$ CFU/mL. Ruan et al. (2010) inoculated separate test fluids of skim milk with mixed strains of *E. coli*, *Salmonella*, and *Listeria*, treated with CHIEF plasma technology with one pass through resuting in 2.74, 3.07, and 2.74 CFU/mL reduction respectively. Vochon et al. (2002) treated raw milk incoculated separately with *E. coli* O157:H7 [ATCC 35150] or *Listeria monocytogenes*, using dynamic high pressure processing resulting in 8.3 $\log_{10}$ reduction at 200 MPa/10 min after five passes and 5.8 $\log_{10}$ CFU/mL at to 300 MPa/10 min after five passes respectively. It is important to note that our results occurred after one pass through our continuous flow spiral reactor.

3.3 Viral inactivation

The model viruses selected for this study were bacteriophages T1UV (Beck et al., 2015; Stefan et al., 2007) and MS2 (Beck et al., 2015; Hijnen et al., 2006; Park et al., 2011). MS2 phage belongs to the serotype group I of the RNA coliphages classified in the family Leviviridae (Calender, 1988). *E. coli* is the bacterial host for MS2 is, and is found most frequently in animal feces and sewage. MS2, liken unto noroviruses, is adapted to the intestinal tract. This positive sense single-stranded RNA virus is in the same size range at 26 nm diameter and exhibits icosahedral symmetry. A study on oyster contamination

demonstrated that F+ RNA bacteriophage (including MS2) was a successful indicator organism for noroviruses in (Dore et al., 2000).

Approximately 168.33 $mJ \cdot cm^{-2}$ was required to inactivate MS2 by 5 log_{10} cycles. 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. 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 $mJ \cdot cm^{-2}$ for one log_{10} reduction of the population (Islam et al., 2016b). Figure 3 clearly displays linear first-order inactivation data trends. The populations of MS2 were reduced by 2.01±0.07, 2.65±0.14, 3.32±0.10, 4.82±0.07, 6.14±0.09 $logs_{10}$ respectively at a UV-C dose level of 43.32, 62.56, 79.84, 124.66, 168.33 $mJ \cdot cm^{-2}$ ($p>0.05$). T1UV was less resistant to UV. It was inactivated by 0.76±0.09, 1.20±0.08, 1.75±0.12, 1.81±0.09, 3.96±0.10, 5.39±0.13, and 6.37±1.51 $logs_{10}$ at UV-C dosage of 3.82, 6.05, 8.78, 9.08, 16.86, 22.93, and 27.12 $mJ \cdot cm^{-2}$ ($p<0.05$). No tailing was observed and the concentration of both viral surrogates decreased linearly as UV fluence was increased.

3.4 Modeling inactivation kinetics

Log-Linear model has been accepted and used to describe the bacterial and viral inactivation resulting from application of heat and non-thermal based processes. The inactivation curves

of microorganisms in skim milk exposed to UV-C irradiation exhibited log linear behavior in all cases (Figure 2 and 3). No tailing affect was observed and it can be accredited to relative high mixing in the UV-C reactor used in this research study. Figures 2 and 3 display REF (UV-C dosage) versus $Log_{10}(N_0\text{-}N)$ (microbial reduction) which yielded first order kinetics. Applicability of linear model to experimental data was tested by plotting the $Log_{10}(N_0\text{-}N)$ against REF. The data adequately fit the model as depicted in Figures 2 and 3. Table III lists the Goodness of fit for the models. Log linear model fit the experimental data for *S.* Typhimurium, and *L. monocytogenes*, MS2 and T1UV with the correlation coefficient (R^2) higher than 0.95, and R^2 for *E. coli* was similar at 0.95. Estimates of Root Mean Square Error (RMSE) for each microbe were calculated using the difference between the predicted and observed values.

3.5 Cell cytotoxicity

It is of utmost importance that novel non-thermal technologies are not inducing toxic radiolytic by-products in foods. The types and amounts of products generated by radiation-induced chemical reactions depend on both the chemical constituents of the food and on the specific conditions of irradiation. The principles of radiation chemistry also govern the extent of change of nutrients and the microbiological profile of the treated foods. To address the safety of radiolytic byproducts, specific test needs to be conducted to determine consumer exposure to those compounds. Cell viability studies were conducted in order to confirm that UV exposure does not result in the formation of toxic chemical compounds or by-products in

skim milk. Two healthy cell lines, human normal intestinal CCD-18Co cells and normal hepatocyte liver AML12 cells, were incubated in a complete cell culture medium supplemented with skim milk extracts equivalent to a dilution series of original skim milk (i.e., 6.25- to 50-fold dilution). Different cell lines are used for different purposes. In general, both liver cells and colon cells are used in cytotoxicity tests. The liver is the major source of metabolism and biotransformation of compounds, thus being used in a toxicological test, while colon cells are better representatives for seeing an effect in the gastrointestinal tract which has direct contact with food. These cell lines were selected to model critical detoxication processes in the body as the liver and colon are key toxin metabolizing and nutrient absorbing/ waste excretory organs respectively (Grant, 1991; Cummings, 1975; NIH, 2017). Our findings exhibited that over the comprehensive dilution range, untreated skim milk extract did not cause a significant inhibition of the viability of both CCD-18Co and AML12 cell lines. Figure 4 displays the effects of skim milk extracts irradiated with different REF's (0, 6.05, 8.78, 43.32,62.56, 79.84, 124.66, and 168.33 $mJ \cdot cm^{-2}$) at varied concentrations on the viability of CCD-18Co and AML12 cells. UV treatments did not inflict toxicity on the cells in comparison to that of untreated skim milk ($p>0.05$). These findings suggest that UV irradiation at 6.05 to 168.33 $mJ \cdot cm^{-2}$ did not lead to the production of cytotoxic compounds that are toxic to neither CCD-18Co nor AML12 cells.

3.6 Economics of UV systems

The evaluation of the treatment costs is the one of the aspects which needs more attention in the food industry. There are a number of important factors in selecting a treatment technology, including Economics, economy of scale, regulations etc. In UV treatment, the Electrical Energy per Order (E_{EO}) is the most common criteria to evaluate electrical energy efficiency and to compare the performance of various UV flow through reactors. The E_{EO} is a specific parameter for a system design and can largely differ from one system to another. This parameter can be also adapted for UV systems for low UVT food fluids and beverages in order to understand the consumption of energy per unit of volume of treated fluid and unit of log of bacteria inactivated. E_{EO} is defined as the number of kilowatt-hours of electrical energy required to reduce the concentration of a contaminant or target bacteria by one order of magnitude (90% inactivation) in one m^3 of fluid (Bolton and Stefan, 2002). Most factors that affect E_{EO} (UV lamp output, lamp efficiency, path length/geometry) can be scaled up from laboratory unit to full-scale systems without much difficulty. A lower E_{EO} value signifies lower energy consumption. However, E_{EO} cannot be used to predict the hydraulic or mixing efficiency of a flow through reactor. E_{EO} value [kWh/m^3 /log] is calculated using the Equation 5.

$$E_{EO} = P_{UV}/(F \times Log\left(\frac{C_0}{C_t}\right)) \qquad \text{Eq. 5}$$

Where P_{uv} is the electric lamp power or total power of the lamp and power supply, kW, F is a volumetric flow rate, m^3/h, C_0 is the initial bacterial concentration, CFU/mL, C_t is the final bacterial concentration, CFU/mL.

The E_{EO} values for three microbes are shown in Table IV. For inactivation of *E. coli* from milk, it would require 0.172 kWh energy per m^3/log; *S.* Typhimurium 0.21 kWh energy per m^3/log and *Listeria monocytogenes* 0.196 kWh energy per m^3/log. The energy values for the three microbes are several magnitudes lower then heat pasteurization.

4. Conclusions

The dean- flow reactor effectively inactivated microbial and viral populations in skim milk. This study found that UV-C irradiation treatment at low doses (~ 23 $mJ \cdot cm^{-2}$) could be used to achieve 5-log_{10} inactivation of prevalent pathogens, particularly *S.* Typhimurium, *E. coli, Listeria monocytogenes*, and viruses (T1UV). First-order kinetics best described the microbial and viral inactivation. Skim milk extract showed no cytotoxic effects on healthy mice liver and normal intestinal cells. UV-C treatment did not alter the normalcy of cellular metabolism of both cell types to the skim milk extract, suggesting that UV-C treatment did not generate any cytotoxic compounds in the skim. Scale-up equipment has already been developed by the research team and its efficacy in inactivating bacterial spores in skim milk on a larger scale will be subject to future investigation.

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