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UV-C treatment of soymilk in coiled tube UV reactors for inactivation of *Escherichia coli* W 1485 and *Bacillus cereus* endospores

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Abstract

Coiled tube UV reactors were used to investigate the influence of tube diameter (1.6 mm ID, and 3.2 mm ID) and Reynolds number (Re) to inactivate *Escherichia coli* W1485 and *Bacillus cereus* spores in raw soymilk (RSM). Four levels of Re (343, 686, 1029 and 1372) were tested in RSM inoculated separately with each bacterium and treated in the UV reactors at a constant residence time of 11.3 s with UV-C dose of 11.187 mJ/cm^2 at 253.7 nm. Inactivation efficiency of both microorganisms increased with Re. Maximum reductions of 5.6 $\log_{10}$ CFU/ml of *E. coli* and 3.29 $\log_{10}$ CFU/ml of *B. cereus* spores were achieved in the 1.6 mm ID UV reactor. Inactivation efficiency was higher in the 1.6 mm ID UV reactor than the 3.2 mm ID UV reactor for both the organisms. Effect of UV-C light on lipid oxidation of untreated RSM, measured as malondialdehyde and other reactive substances (MORS) content, was much higher (95 nmol/ml) than the UV-treated (58 nmol/ml) and thermally pasteurized (55 nmol/ml) RSM during the storage period of 7 days. The UV-C treatment can be effectively used for reducing *E. coli* cells and *B. cereus* spores in soymilk without compromising its quality.

Keywords: Soymilk, UV-C, coiled tube UV reactor, *Bacillus cereus*, *Escherichia coli*, nonthermal processing.

1. Introduction

Soymilk is a popular beverage in Asian countries and is gaining popularity in America. It is prepared from ground soybeans, which contain major nutritional components such as protein, lipids, saccharides, and vitamins. Soymilk and its derivatives have considerable market potential for their nutritional and health benefits (Guo, Tomotada, & Masayuki, 1997). Soy protein is one of the commercially available vegetable proteins and is one of the least expensive sources of high nutritional quality protein. The United States Food and Drug Administration on 26 October 1999 authorized a soy protein health claim that the risk of heart disease may lowered by consumption of 25 g of soy protein a day (Fukushima, 2001; Hermansson, 1978). This health claim helped in increasing the consumption of soybean in the Western diets (Zhang, Lite, Tatsumi, & Isobe, 2005).

Heat treatment is currently used for pasteurization of soymilk to destroy potential pathogens and spoilage organisms. However, heat treatment may adversely affect color, flavor and nutrients of soymilk and soymilk products (Kwok & Niranjan, 1995). Nonthermal processing technologies are used to preserve freshness of foods and circumvent unnecessary changes and defects caused by thermal processing (Song et al., 2009). Nonthermal processing technology will benefit the soy food industry by providing shelf-stable soymilk and derivative products with superior organoleptic qualities (Zhang et al., 2005). Among the available nonthermal food processing technologies, ultraviolet (UV-C) technology is more economical (Koutchma, 2009). It can be used as an economical and easily adoptable alternative to thermal pasteurization for a range of liquid foods and ingredients (Sastry, Datta, & Worobo, 2000).

Voronov (2007) reported that within the UV-C region, the light band centered at 253.7 nm wavelength provides most effective germicidal effect. UV-C causes photochemical changes in proteins and nucleic acids and is responsible for inactivating microorganisms by mutations of nucleic acid sequences, disrupting DNA transcription and replication (Jay, 1996; Miller, Jeffery, Mitchell, & Elasri, 1999). UV-C radiation was found to be effective for treating apple cider (Hanes et al., 2002; Wright, Summer, Hackney, Pierson, & Zoecklein, 2000; Basaran, Quintero-Ramos, Moake, Churey, & Worobo, 2004) and inactivating microorganisms in fresh juices and liquid egg whites in continuous-flow UV-C reactors under laminar, turbulent and Dean flow conditions (Koutchma, Keller, Chirtel, & Parisi, 2004; Koutchma, Parisi, & Patazca, 2007; Franz, Specht, Cho, Graef, & Stahl, 2009; Unluturk, Atilgan, Baysaland, & Tari, 2008; Geveke, 2008). More than 5-$\log_{10}$ CFU/ml reductions of *Listeria monocytogenes* in goat milk were achieved by UV-C (Matak et al., 2005). Using a CiderSure 3500 UV apparatus, *E. coli* in skimmed cow milk were reduced by 2.3 $\log_{10}$ CFU/ml (Matak, 2004). Most recently, Choudhary, Bandla, Watson, Haddock, Bhattacharya, and Abughazaleh (2011) reported that using a coiled tube UV reactor, *E.coli* W1485 was reduced by 7.8 $\log_{10}$ CFU/ml in commercially processed skimmed cow milk (SCM) and by 4.1 $\log_{10}$ CFU/ml in raw cow milk (RCM). However they reported the endospores of *Bacillus cereus* were reduced by only 2.72 and 2.65 $\log_{10}$ CFU/ml in SCM and RCM, respectively (Choudhary et al., 2011).

In order to test the effectiveness of UV-C on soymilk for food safety purposes, it will be worthwhile to demonstrate the effect of UV-C on lethality of a model Gram-negative organism namely *E. coli* W1485 and a Gram-positive endospore forming bacterium, namely *Bacilus cereus*. The *E.coli* strain W1485 (related to K-12) is more resistant to UV-C than the pathogenic strain O157:H7 (Murakami, Jackson, Madsen, & Schickedanz, 2005). *B. cereus,* a spore-forming psychrotroph, may pose serious challenges in meeting food safety objectives (Magnusson, Christiansson, & Svensson, 2007). Testing UV-C for inactivation of *B. cereus* spores in soymilk

would be a robust approach to assess the applicability of UV-C processing in soy industry (Donovan, 1958, Agata et al., 2002).

Key factors influencing efficiency of UV light disinfection include fluid turbulence and UV-C absorbance of liquid foods (Koutchama et al., 2004). Turbulent flow of liquid foods in continuous flow UV reactors increased inactivation of microorganisms in fresh juices, liquid egg whites and milk (Koutchma et al., 2007; Franz et al., 2009; Matak, 2004). Reynolds number (Re) is a measure of turbulence of liquid in a coiled tube reactor. It is expressed as:

$$Re = (\rho/\mu) \times vD \quad (1)$$

where, Re is Reynolds number, 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 (Geankoplis, 1993).

Laminar flow occurs when Re < 2100, whereas Re > 4000 indicates a turbulent flow condition. The Dean number D_e is a similarity parameter governing the fluid motion in coiled tube flow configuration.

$$D_e = Re \sqrt{(D/D_c)} \quad (2)$$

where D is the tube diameter, D_c is the coil diameter, and Re is the tube Reynolds number (Dean, 1927). 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 (2) is within $0.03 < D/D_c < 0.1$ (Dean, 1927). Dean flow causes superior mixing conditions, leading to better exposure of liquid food to UV-C in a UV reactor (Koutchma et al., 2007; Franz et al., 2009).

Matak (2004) reported off-flavor in UV-C treated milk. A thiobarbituric acid reactive substances (TBARS) test was used by Matak (2004) for measuring oxidized flavors in milk. We hypothesize that oxidation byproducts causing off flavors of milk may be reduced by minimal UV-C dose application using Dean flow reactor.

The first objective of this research was to determine the efficacy of UV light on inactivation of *E. coli* W1485 and *B. cereus* spores in raw soymilk (RSM) using two UV reactors with two different tube diameters, 1.6 mm ID and 3.2 mm ID, at four different levels of Reynolds number (Re). The second objective was to use RSM treated with the combination of factors from the first objective that yielded the highest inactivation of microorganisms and compare the quality aspect of lipid oxidation with thermally pasteurized and untreated soymilk after storage periods of 0, 1, 3 and 7 days.

2. Materials and Methods

2.1. UV reactors

Two UV reactors were used for this study as shown in Figure 1. The UV-C source for each was a 8.7 W, 110 V, UV-C germicidal lamp with peak emission at 253.7 nm, having a 505 mm arc length and 15 mm outside diameter (OD) (SBL325, American Ultraviolet Company, Lebanon, IN, USA). The construction details of these reactors are described in Choudhary et al. (2011). For both reactors, the UV lamps were enclosed within quartz glass sleeves (American Ultraviolet Company, Lebanon, IN, USA) of 22 mm OD with an air gap of 2.4 mm between UV lamps and sleeves. In the 1.6 mm ID UV reactor, perfluoroalkoxy polymer resin (PFA) transparent tubing of 1.6 mm ID by 3.2 mm OD was wrapped around the UV lamp sleeve. The 3.2 mm ID UV reactor was similarly constructed with a PFA tubing of 3.2 mm ID by 4.8 mm OD. Both reactors were clad with aluminum foil to prevent exposure of personnel to UV light.

All four Re values used in this study (Table 1) were achieved by using different flow rates of RSM through the UV reactors and calculating the resulting Re, based on equation 1. The density (ρ) and viscosity (μ) of RSM was taken from literature based on the total solids of

soymilk. For similar total solids at 24–26°C, density (ρ) and dynamic viscosity (μ) of soymilk were reported as 1029 kg/m^3 and 1×10^{-3}Ns/m^2 respectively (Rouhana, Edler-Nissen, Cogan, & Frøkiær, 1996; Estrada-Girón, Guerrero-Beltrón, Swanson, & Barbosa-Cónovas, 2007).

In order to maintain a consistent residence time of product exposure to UV, different lengths of tubing were used for each flow rate (and corresponding Re). The FDA recommends bacterial reduction of more than 5 $\log_{10}$ CFU/ml for UV pasteurization of liquid foods (FDA, 2003). Based on our preliminary experiment (Bandla, 2010) on UV treatment of cow milk inoculated with *E.coli* W1485, more than 5 $\log_{10}$ CFU/ml reduction was obtained at 11.3 s residence time. As a result, the residence time of 11.3 s was selected for this study.

The calculated value of D/D_c was 0.06 for the 1.6 mm ID UV reactor and 0.11 for the 3.2 mm ID UV reactor. The UV-C dose was calculated using equation (3) by multiplying the residence time (11.3 s) with the irradiance intensity at the soymilk surface (Quintero-Ramos, Churey, Hartman, Barnard , & Worobo, 2004).

UV-C dose (mJ/cm^2) = Irradiance intensity (mW/cm^2) × Exposure time (s) (3)

Where, Irradiance intensity is the intensity of incident UV-C light on the surface being treated. The irradiance intensity was estimated by multiplying the UV-C intensity of the lamp at 5 mm from the lamp (1.375 mW/cm^2) with the transmittance of quartz glass sleeve (90%) and PFA tube (80% in germicide range). Using these values, the estimated UV-C dose for the designed UV reactors was 11.187 mJ/cm^2.

2.2. *E. coli* W1485 culture preparation

Stock cultures of *E. coli* W1485 obtained from Department of Microbiology, Southern Illinois University, Carbondale, IL was used to inoculate 120 ml tryptic soy broth (TSB) and incubated in a gyratory water bath shaker (Model 650D, New Brunswick Scientific Edison, NJ, USA) at 34°C for 18 - 24 hours (Marshall, 2004). The culture obtained from growth in TSB was centrifuged (Beckman J2-M1, Schaumburg, IL, USA) at 12,000 rpm (17,400 × g) for 15 min at 4°C and the supernatant was discarded (Krishnamurthy, Demirci, & Irudayaraj, 2007). The pellet was immediately mixed with soymilk as described in section 2.6.

2.3. *B. cereus* endospore preparation

B. cereus (ATCC Preceptrol® strain) was obtained from a -70°C glycerol stock supplied by the Department of Microbiology, Southern Illinois University, Carbondale, IL. Endospores of *B. cereus* were prepared according to Beuchat, Clavero, and Jaquette (1997). Briefly, nutrient broth (Difco Laboratories, Detroit, MI) was inoculated with a pure culture and incubated at 30°C. Three successive loop transfers at 24 h intervals were made before spreading 0.1 ml aliquots onto the sporulation medium (Nutrient agar, supplemented with 0.05 g of $MnSO_4$/L). The plates were incubated for 72 h at 30°C and the spores were harvested by depositing 5 ml of sterile distilled water on the surface of each plate and rubbing gently with a sterile bent glass rod. The harvesting step was repeated twice. The resulting spore suspension was centrifuged at 3,000 rpm (1,090 × g) for 20 min at 5°C (Beckman J2-M1, Schaumburg, IL, USA) and the supernatant liquid was discarded. The resulting pellet was suspended in 100 ml of sterile distilled water and centrifuged at 6,000 rpm (4,350 × g) for 10 min at 5°C (Beckman J2-M1, Schaumburg, IL, USA). This procedure was repeated twice and the final pellet was suspended in 5 ml sterile de-ionized water in a 15 ml sterile centrifuge tube (Fisher Scientific, Hanover, IL, USA). The centrifuge tubes were kept in a water bath (Fisher Scientific, Hanover, IL, USA) which was set at 80±5°C. Temperature of a blank centrifuge tube which contained only deinoized water, was monitored using a glass thermometer. Time was monitored with a stop clock (Fisher Scientific, Hanover, IL, USA). All centrifuge tubes were kept in the water bath at 80°C for 30 min to kill

vegetative cells of *Bacillus cereus*. Gram stains of the endospores were observed with a light microscope (Zeiss bright field microscope, Saint-Louis, MO, USA) for the presence of vegetative cells to ensure that all vegetative cells were killed by the heat treatment . Endospores (5ml) contained in 15 ml centrifuge tubes were stored in a cold room at 4°C before inoculation of soymilk.

2.4. Soymilk (RSM) preparation

Dried soybeans were obtained from Soybean Breeding Center, Department of Plant, Soil and Agricultural Systems, College of Agricultural Sciences, Southern Illinois University, Carbondale, IL. RSM was prepared from these soybeans using kitchen tools (Rouhana et al., 1996; Estrada-Girón et al., 2007). Soybeans were soaked in deionized water in the ratio of 1:10 (by weight) for 6 to 8 hours at room temperature. After completion of the soaking period, the soak water was discarded and the soaked beans were deskinned by rubbing between the palms. The resulting cotyledons were wet ground with 6 times water by weight using a kitchen grinder (Vortex 7 Waring Blender, Waring Products, Torrington, CT). The ground slurry was filtered through a cheese cloth to remove the okara (soy pulp). The resulting raw soymilk (RSM) was stored immediately in a cold room at 4°C (Rouhana et al., 1996; Estrada-Girón et al., 2007) until its use in the experiments within 5 h.

2.5. Quality of fresh soymilk

Total solids of RSM were determined by proximate analysis (Marshall, 2004). Standard plate count (SPC) was enumerated in the RSM as per Marshall (2004). In brief, RSM was serially diluted to yield plate counts between 25-250 colony forming units (cfu). 0.1 ml of diluted sample was dispensed and spread plated on agar plates and incubated at 32±1C for 48 hours. Colonies were manually counted. UV-C absorbance of RSM was measured at room temperature with a UV-visible spectrophotometer (model UV-1601, Shimadzu Scientific Instruments, Columbia, MD) at 254 nm wavelength by diluting each milk sample to 99% with deionized water. Disposable quartz cuvettes (Fisher Scientific, Hanover, IL, USA) with a path length of 10 mm were used to measure the absorbance. A pH meter (Corning 350 pH/ion meter, Corning, NY, USA) was calibrated using buffer solutions (pH 4 and 7) before measuring pH. The pH of soymilk at room temperature was measured three times and averaged.

2.6. Inoculation of soymilk with *E. coli* W1485 and *B. cereus* spores

A sterile glass bottle (2000 ml) with a magnetic stirrer was filled with 1500 - 1600 ml of the refrigerated RSM. An *E. coli* W1485 pellet obtained from a 120 ml culture was directly added to the RSM container and was slowly stirred for 30 min to warm the milk to room temperature prior to sampling for microbial enumeration and UV-C treatment. Samples for microbial counts were serially diluted and 0.1 ml was spread plated onto Petri plates containing violet red bile agar (VRBA) with 4-methylumbelliferyl-B-D-glucuronide (MUG) (Difco Laboratories, Detroit, MI). Colony forming units were manually counted after incubation overnight at 34°C for 24 hours.

A sterile glass bottle (2000 ml) was filled with 1500 – 1600 ml of RSM and inoculated with 5 ml of a *B. cereus* endospore preparation. The containers were slowly stirred on a magnetic stirrer for 15 - 20 min to suspend endospores uniformly in the soymilk and then used for enumeration and UV-C treatment. *B. cereus* endospores in the RSM were estimated using mannitol egg yolk polymyxin agar (MYPA) (Beuchat et al., 1997).

2.7. UV reactor operation and cleaning

The UV lamp was turned on three minutes before pumping soymilk through the reactors. The soymilk sample (500 ml) was pumped through the reactor at a preset flow rate

(corresponding to each Re) until all 500ml was exposed. Treated soymilk samples were collected in sterile bottles and immediately spread plated on agar plates and incubated for enumeration of survivor cells. The control samples were prepared by pumping the inoculated soymilk samples through the reactors while the UV lamp was turned off. Log reduction of bacteria were calculated by taking the difference between the bacterial count (log_{10} CFU/ml) in control samples and treated samples.

Both the UV reactors were cleaned immediately after each treatment by pumping deionized water at 70°C (500 ml) followed by 200 mg/kg hypochlorite (100 ml) circulation for 10 min, 95% ethyl alcohol (100 ml) recirculation for 4 min and a final rinse with sterile deionized water (500 ml) at room temperature (Bandla, 2010). The final rinse water was collected in a sterile test tube and spread plated directly onto TSA plates. It was ensured that the wash water had fewer survivors than the limit of detection (10 CFU/ml).

2.8. Thermal pasteurization of soymilk

RSM was thermally pasteurized as a comparison for determining quality of UV treated RSM after different storage periods. Freshly prepared RSM (900 ml) was collected in a sterile Pyrex glass bottle (1600 ml) and from there 100 ml was distributed to each of 8 Nalgene HDPE sterile bottles (250 ml capacity). A water bath was prepared with water level slightly above the RSM level in the bottles and the bath temperature was set at 80°C. RSM was heated to 72°C while occasionally stirring it with a sterile glass rod and held for 30 s to inactivate harmful microorganisms. The temperature of RSM was monitored by a mercury glass thermometer. The containers were fitted with lids and stored in a dark cold room maintained at 4 °C for 18 hours before measurement of lipid oxidation.

2.9. Quality assessment of stored soymilk

The treatment level that yielded highest microbial inactivation during the first experiment (1.6 mm ID UV reactor with flow rate of 100 ml/ min) was selected for determining the effect of UV treatment on lipid oxidation of soymilk. UV treated samples were collected in 250 ml HDPE containers (Nalgene Nunc Int., Rochester, NY) fitted with lids and stored in a dark cold room maintained at 4 °C for 18 hours before measuring lipid oxidation.

Malondialdehyde and other reactive substances (MORS) in the RSM samples were measured using a TBARS test kit, according to the manufacturer's directions (Zeptometrix corporation, Oxitek, TBARS assay kit, Buffalo, New York, USA). Armstrong, Hiramitsu, and Ueda (1998) reported that this test kit was most widely used to measure the byproducts of lipid oxidation in food samples. The TBARS test kit provides the following reagents: Thiobarbituric acid (TBA) reagent containing 0.53 g TBA each vial, TBARS diuent 1 containing acetic acid, TBARS diluents 2 containing sodium hydroxide, SDS solution containing dodecyl sulphate, MDA standard containing Malondialdehyde Bis (dimethyl acetate), MDA diluents containing sterile deionized water. For each analysis fresh TBA/buffer reagent was prepared by mixing 50 ml each of TBARS diluent 1, TBARS diluent 2, and 1 vial of TBA. The mixture was stirred until the TBA was fully dissolved. MDA solutions with MDA content of 0, 12.5, 25, 50 and 100 nmol/ml were prepared by adding appropriate volume of MDA diluents to MDA standard for calibrating a spectrophotometer (model UV-1601, Shimadzu Scientific Instruments, Columbia, MD) absorbance reading at 532 nm with MDA concentration. The calibration curve for absorbance values at 532 nm vs MDA content was obtained. To determine MORS value of the soymilk samples, 100 µl sample was added to a glass centrifuge tube. One hundred µl of SDS solution was added to the sample in the test tube and swirled to mix. To this test tube, 2.5 ml TBA/buffer reagent was added and the tube was covered with a glass marble and incubated at 95

°C for 60 min. The test tube was then cooled in an ice bath for 10 min. The cooled test tubes were centrifuged at 1090 × g for 15 min. The supernatant was removed and its absorbance was read using the spectrophotometer at 532 nm. Based on the linear calibration curve between absorbance vs MDA concentration, MDA equivalent (MORS) values were determined. MORS in the RSM samples were measured with three replications each for UV treated, thermal pasteurized, and untreated soymilk after 0, 1, 3, and 7 days of refrigerated storage.

2.10. Experimental design and statistical analysis

A full factorial experimental design was used for the first objective of this study. UV reactor (tubing diameter) and Re were the main effects and log reduction of the bacteria was the dependent variable. Each experiment consisted of eight treatments (2 UV reactors by 4 Re levels), which were completed in a randomized order and replicated three times.

Results for *E. coli* and *B. cereus* were analyzed separately with factorial ANOVA ($\alpha = 0.05$) using proc GLM in SAS 9.2 software (SAS, 2008). If the interaction of the main effects was significant, one-way ANOVA was used to determine the simple effects of Re within each UV reactor and the simple effects of UV reactor within each Re. Tukey's studentized range test was used to determine differences among means of significant effects.

For the second objective, each storage period of 0, 1, 3, and 7 days was analyzed separately. A one-way ANOVA ($\alpha = 0.05$) using proc GLM in SAS 9.2 software (SAS, 2008) was used, with treatment type (UV, thermal pasteurization, and untreated) as the main effect. The result of the TBARS test was the dependent variable. Tukey's studentized range test was used to determine differences among means of significant effects.

3. Results and discussion

3.1. Quality of soymilk

Microbial quality of RSM as indicated by standard plate count (SPC) was 1.5×10^5 CFU/ml for the first experiment of this study. In the second experiment (TBARS test), SPC of RSM was 1.15×10^7. The pH of RSM at 25ºC was in the range of 6.6–6.8, which was within the range of normal pH (6.6–6.8). Ultraviolet absorption coefficient of RSM at 254 nm wavelength was 1.59 ± 0.1 cm^{-1} at 99% dilution with deionized water. Total solids were 7.4 g/100g in RSM. The initial temperatures of RSM were in the range of 24 – 25°C. The average increase in the final temperature of milk at the outlet of UV reactors was 5°C with the 1.6 mm ID UV reactor and 2°C with 3.2 mm ID UV reactor. This increase in the milk temperature should not provide any measurable effect on inactivation of bacteria (Matak, 2004).

3.2. Microbial counts in control samples

The number of *E. coli* cells and *B. cereus* spores present in control samples (inoculated milk treated in UV reactor without the lamps on) were 7.30 ± 0.09 and 7.29 ± 0.05 $\log_{10}$ CFU/ml respectively. These counts indicate the potential maximum log reduction of bacteria in the UV-C treated milk.

3.3. *Microbial* inactivation

Each of the main effects of UV reactor and Re had a significant effect on log reduction of *E. coli* in RSM, while the interaction of the main effects was not significant. The 1.6 mm ID UV reactor had a significantly higher log reduction of 3.98 ($p = 0.0007$) compared with 1.64 for the 3.2 mm ID UV reactor (Table 2). The effect of Re was also significant ($p = 0.0097$) on inactivation of *E. coli* in RSM, with the highest Re (1,376) level providing significantly higher log reduction (4.28 $\log_{10}$ CFU/ml) than the lowest (349) Re (1.27 $\log_{10}$ CFU/ml). When Re effects were isolated for each UV reactor, there were no differences for the 1.6 mm ID UV

reactor, but for the 3.2 mm ID UV reactor the highest Re level (1,376) had a significantly higher log reduction of 2.95 $\log_{10}$ CFU/ml than the 0.72 $\log_{10}$ CFU/ml of the lowest Re level (349) (p = 0.0247) (Table 2).

The interaction of main effects UV reactor and Re upon log inactivation of *B. cereus* endospores in RSM was significant (p = 0.0034). The simple effect of Re was significant for the 1.6 mm ID UV reactor (p = 0.0016) with the highest level (1,376) of Re having a significantly higher log reduction of 3.22 $\log_{10}$ CFU/ml than the two lowest Re levels (349 and 694) with reductions of 2.09 and 2.43 $\log_{10}$ CFU/ml, respectively (Table 2). Similarly, the simple effect of Re was significant for the 3.2 mm ID UV reactor (p = 0.0057), with the highest Re level (1,376) yielding a significantly higher reduction of 1.66 $\log_{10}$ CFU/ml than the other Re levels (349, 694, and 1,047), with log reductions of 1.36, 1.69 and 1.44 $\log_{10}$ CFU/ml, respectively (Table 2). The simple effect of UV reactor was significant at all four levels of Re, with the 1.6 mm ID UV reactor resulting in greater reduction of endospores than the 3.2 mm ID UV reactor.

In general, the 1.6 mm ID UV reactor provided greater mean log reduction of bacteria than the 3.2 mm ID UV reactor at the same level of Re at constant UV-C dose of 11.187 mJ/cm^2. The 1.6 mm ID UV reactor benefitted from the combination of the smaller diameter tubing providing a thinner path length for the UV, and the Dean flow causing secondary vortices and better mixing. The 3.2 mm ID UV reactor with the near Dean flow was not able to overcome the disadvantage of the thicker path length of milk for the UV to penetrate.

As Re increased, the reductions of either bacterium increased in both reactors. As the Re is an indicator of the degree of turbulence in fluid, increasing the Re of soymilk resulted in better turbulence and mixing of soymilk molecules leading to higher exposure of UV-C radiation. Thus it can be safely concluded that Re plays a significant role in improving efficiency of UV-C reactors for nonthermal pasteurization of liquid foods. For the 1.6 mm ID UV reactor, inactivation (log reduction) of more than 5 logs of *E. coli* was observed at the highest level of Re (1376). With the 3.2 mm ID UV reactor, the maximum *E. coli* reduction was 2.95 logs. This can be attributed to the greater pathlength of milk in the 3.2 mm ID UV reactor compared to the 1.6 mm ID UV reactor.

Inactivation of *B. cereus* spores was lower than that of the *E. coli* W1485 in RSM in both UV reactors. Similar results were observed for raw cow milk and commercially skimmed cow milk (Choudhary et al., 2011). The UV dose received by *B. cereus* spores in RSM was not sufficient to achieve a reduction of 5 $\log_{10}$ CFU/ml in both the UV reactors.

3.4. Lipid Oxidation

Based on the results of the first objective, the 1.6 mm ID UV reactor with the highest Re level was used to treat RSM for the second objective. Table 3 summarizes the results for this objective. At day 0, there was no difference in MORS values among the untreated, UV-C treated, and heat pasteurized soymilk samples. At day 1, there was a significant difference among treatments (p = 0.0062), with the MORS values of 70.8 nmol/ml for untreated RSM, 46.0 nmol/ml for UV treated RSM and 44.8 nmol/ml for heat pasteurized RSM. The significant differences were consistent on days 3 (p = 0.0005) and 7 (p = 0.0009) with the untreated RSM having a higher MORS value than both the UV-C treated and heat pasteurized RSM. On each of the days sampled, there was no difference between the MORS values of the UV treated and heat pasteurized RSM.

Higher malondialdehyde reactive substances content in untreated than in treated soymilk on days 1, 3, and 7 day were probably caused by active enzymes in untreated soymilk. Matak (2004) found a difference in oxidized flavor between UV-C treated and heat pasteurized goat

milk. Although this study used the TBARS test as a proxy for oxidized flavor, no difference was found between UV-C treated and heat pasteurized soymilk. More extensive tests should be conducted to determine if UV-C treated soymilk develops oxidized flavor sooner than heat-pasteurized soymilk.

4. Conclusion

The 1.6 mm ID UV reactor was more efficient than the 3.2 mm ID UV reactor on inactivating *E. coli* and *B. cereus* spores in RSM. Inactivation of both the bacteria increased with Reynolds number in both the reactors. *E.coli* W1485 was inactivated by more than 5 logs whereas *B. cereus* spores were inactivated to 3.3 logs at the highest level of Reynolds number (1,376) in RSM using the 1.6 mm ID UV reactor at an UV dose of 11.187 mJ/cm^2. Higher UV doses may improve the inactivation efficiency of the UV reactor. Malondialdehyde and other oxidative reactive substances content were statistically not different for the thermally pasteurized and UV-C treated soymilk right after treatment or during refrigerated storage for 7 days.

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Figure Captions

Figure 1: Coiled tube UV reactors used in this study (drawing not to scale). For the 1.6 mm ID UV reactor, the inside diameter of fluid carrying PFA tube was 1.6 mm, whereas for the 3.2 mm ID UV reactor, it was 3.2 mm.

Figure 1

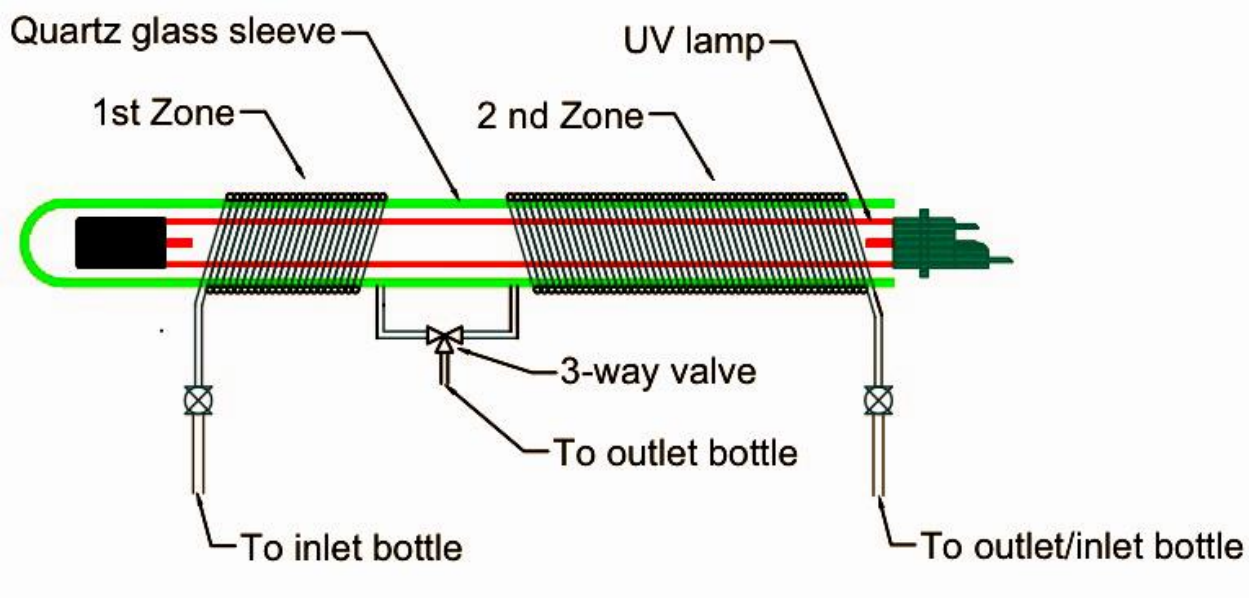

Figure 1: Coiled tube UV reactors used in this study (drawing not to scale). For the 1.6 mm ID UV reactor, the inside diameter of fluid carrying PFA tube was 1.6 mm, whereas for the 3.2 mm ID UV reactor, it was 3.2 mm.

Table 1. Levels of flow rate of raw soymilk (RSM), tubing length and Reynolds number (Re) for each UV reactor.

	1.6 mm ID UV reactor		3.2 mm ID UV reactor		
Re Level	Flow Rate (ml/min)	Tubing Length (cm)	Flow Rate (ml/min)	Tubing Length (cm)	Mean Re
1	25	240	50	120	349
2	50	480	100	240	694
3	75	720	150	360	1047
4	100	960	200	480	1376

Mean Re values were calculated from six observed flow rates of RSM during experiments. Re values at each Re level were same for both the reactors. The residence time of RSM in each reactor was 11.3 s and the UV dose was 11.187 mJ/cm^2.

Table 2. Simple effects of Re within each UV reactor upon inactivation of *E. coli* and *B. cereus* in RSM.

	Log Reduction (log_{10} CFU/ml)					
	1.6 mm ID UV Reactor		3.2 mm ID UV Reactor		Average	
Re	*E. coli**	*B. cereus**	*E. coli**	*B. cereus**	*E.coli*	*B. cereus*
349	1.81[a]	2.09[a]	0.72[a]	1.36[a]	1.27	1.73
694	3.64[a]	2.43[ab]	1.11[ab]	1.39[a]	2.38	1.91
1,047	4.87[a]	2.79 [bc]	1.79[ab]	1.44[a]	3.33	2.12
1,376	5.60[a]	3.22 [c]	2.95 [b]	1.66 [b]	4.28	2.44
average	3.98	2.63	1.64	1.46	2.82	2.05

* Within each combination of reactor and bacteria, means with the same superscript letters are not significantly different. Log reduction of bacteria were calculated by taking the difference between the bacterial count (log_{10} CFU/ml) in control samples and treated samples Means are based on three data points with each data point corresponding to a different replication.

Table 3. Malondialdehyde and other reactive substances (MORS) content, as measured by TBARS assay, in soymilk during storage periods of 0, 1, 3, and 7 days.

	MORS (nmol/ml)*			
Treatment	0 day	1 day	3 days	7 days
Untreated	59.5[a]	70.8[a]	90.4[a]	94.6[a]
UV treated	45.1[a]	46.0[b]	50.8[b]	58.1[b]
Heat pasteurized treated	44.9[a]	44.8[b]	49.7[b]	54.7[b]

* Within each day, means (n=2) with the same superscript letters are not significantly different.
MORS values indicate intensity of lipid oxidation. Higher values indicate higher oxidation.

Nomenclature

B.cereus	*Bacillus cereus*
CFU	colony forming unit
CFU/ml	colony forming unit per milliliter
D	diameter (ID) of tube
Dc	diameter of tube coil
De	Dean number
E.coli	*Escherichia coli*
ID	inside diameter
μ	dynamic viscosity of fluid (milk)
mJ/cm^2	milli joule per square centimeter
ml/min	milliliter per minute
mW/cm^2	milli watt per square centimeter
MYPA	mannitol egg yolk polymyxin agar
nm	nanometer
OD	outside diameter
PFA	per-fuoro-alkoxy
ρ	density of fluid (milk)
RCM	raw cow milk
R_e	Reynolds number
SCM	skimmed cow milk
SPC	standard plate count
TSA	tryptic soy agar
TSB	tryptic soy broth
UV	ultraviolet
UV-C	ultraviolet C
v	velocity of fluid flow
V	volt
W	Watt