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Nonthermal pasteurization of tender coconut water using a continuous flow coiled UV reactor

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ABSTRACT

A non-thermal pasteurization technology is desirable to naturally preserve nutrient and organoleptic properties of packaged tender coconut water (TCW) distributed under refrigeration. The goal of this research was to assess the antimicrobial effectiveness of ultraviolet light C (UVC) as a non-thermal pasteurization of TCW. A dean flow ultraviolet reactor was used with wavelength of 254 nm at the residence time of 14.0 s. The experimental variables were three Reynold numbers (R_e = 198.8, 397.7 and 596.4) and two diameters of transparent PFA tubes (3.2 mm and 1.6 mm). TCW was inoculated with cultures of *Escherichia coli* W1485 and *Listeria monocytogenes* separately before passing through the UV reactor. UVC treatment yielded 5.27 and 4.74 $\log_{10}$ CFU/mL *E. coli* count reductions for 1.6 mm and 3.2 mm ID reactors, respectively, whereas the reduction of *Listeria monocytogenes* were 4.18 and 2.96 $\log_{10}$ CFU/mL, respectively at Re = 596.4. Furthermore, as the Reynold number increased, microbial reduction also increased. The physico-chemical changes of cold pasteurized TCW were not significantly different compared to the fresh TCW.

1. Introduction

Green coconut water is regarded as a healthy drink (Nanda Kumar, 1995; Rethinam & Nanda Kumar, 2001). The heavy weight (0.8–2 kg) and short shelf life of whole tender coconut makes it desirable to distribute the tender coconut water (TCW) in convenient and long shelf life retail packaging (Chowdhury, Rahman, Islam, Islam, & Islam, 2009). Since thermal pasteurization of TCW causes undesirable color and flavor changes, suitable non-thermal pasteurization technology for TCW such as membrane filtration, high power ultrasound, high pressure processing and super critical CO_2 have been evaluated (Cappelletti et al., 2015; Damar, Balaban, & Sims, 2009; Prades, Dornier, Diop, & Pain, 2012; Reddy, Das, & Das, 2005). Ultraviolet light, a low cost nonthermal processing option for liquid foods successfully used in disinfecting drinking water, has been approved by the Food and Drug Administration (FDA) for pasteurizing fruits and vegetable juices (Choi & Nielsen, 2005; Food & Administration, 2001). UVC, 200–280 nm, is considered germicidal due to the formation of thymine dimers in the DNA of pathogens eventually terminating the replication, transcription and translation of bacterial gene and resulting in the inactivation of the organism (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). Low pressure UV lamps emit 95% of light at 253.7 nm and are very effective on microbial inactivation as it is so close to the DNA absorption peak of almost all bacteria (Kowalski, 2010). Basaran, Quintero-Ramos, Moake, Churey, and Worobo (2004) treated filtered apple cider inoculated with five strains of *E. coli* with UVC of 61–94 mJ/cm^2 and reported 5.0 log reduction of inoculated *E. coli* cells. According to a study by Feng, Ghafoor, Seo, Yang, and Park (2013), the UVC treatment in helix Teflon coil reactor reduced total aerobes, coliforms and yeast/mold in water melon juice by 2.6, 1.47 and 0.99 logs at the UVC dosage of 37.5 J/ml. Moreover, Müller, Noack, Greiner, Stahl, and Posten (2014) reported that the UVC treatment of apple and grape juices stopped browning and spoilage reactions and improved the shelf life and stability of products. In addition to germicidal effect, UVC treatment may also reduce the polyphenol oxidase (PPO) and peroxidase (POD) activity of coconut water (Augusto, Ibarz, Garvín, & Ibarz, 2015). Thus the literature shows a great potential for pasteurizing TCW by UVC technology, which has lower cost and less complicated design compared to

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filtration and other Nonthermal technologies. Recently, Gabriel, Aguila, and Tupe (2015) showed that the flow characteristics of coconut beverage had significant effects on the UVC inactivation of *Salmonella enterica*. In this study we used a continuous flow coiled tube UV reactor providing increased turbulence and mixing due to Dean effect (Choudhary et al., 2011). Accordingly, the goal of this research was to investigate the effect of UVC light as a pasteurization technology for tender coconut water in a continuous flow coiled UV reactor. Specific objectives were to study the effects of Reynolds number and the diameter of the coiled tube UVC reactor on log reduction of *E. coli* W1485 and *L. monocytogenes,* and evaluate the physico-chemical and sensory quality of treated TCW in comparison to fresh TCW.

2. Materials and methods

2.1. Coconut water extraction and quality evaluation

Fresh green coconuts (Global Best Produce- Thailand) were bought locally (International Grocery, Carbondale, IL) just before an experimental trial to avoid probable growth of any microbial contaminants. The endosperm of the fruit was exposed by removing parts of mesocarp and endocarp and sterilizing surface with 70% ethanol. The tender coconut water was then poured into a sterile glass bottle through a sterile muslin cloth filter yielding approximately 900 ml per fruit.

Natural presence of bacteria, if any, on TCW was tested by plating 100 μl of sample on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) plates incubated overnight at 37 °C followed by counting total aerobic CFUs as per Marshall (2004).

Observation of changes in physico-chemical properties (pH, soluble solids and density) of UV treated; heat treated and untreated TCW were done at time 0, and after 1, 2, 3, and 4 weeks of storage at 4 °C. The density of TCW was measured using a hydrometer (EW-082-9884, Cole Parmer, Vernon hills, IL) immediately after its extraction and filtration. The viscosity of TCW was measured with a rotational viscometer (Brookfield Engineering, Middleboro, MA, USA, Model: DV-I Prime). Absorbance of the TCW at 254 nm wavelength was measured with a UV–Vis spectrophotometer (Fisher Scientific, Hanover, Il, USA). A well-calibrated pH meter (Corning, NY, USA) was used for pH measurements. The TSS (%) of TCW before and after treatments were measured by a handheld refractometer (FS1394626, Fisher Scientific, Hanover, Il, USA). All the above measurements were recorded at room temperature of 25 °C.

2.2. Inoculation and enumeration

Master cultures of *Escherichia* coli (W1485) and *Listeria monocytogenes* were obtained from the Department of Microbiology at Southern Illinois University Carbondale. *E. coli* and *L. monocytogenes* were grown on Tryptic Soy Agar/Broth and Brain Heart Infusion Agar/Broth media respectively as described in Difco & BBL manual (Zimbro, Power, Miller, Wilson, & Johnson, , 2009), sub-cultured and incubated in Tryptic Soy Broth and Brain Heart Infusion Broth respectively for 24 h at 37 °C. The cultures were centrifuged at 10 000×*g* for 15 min at 4 °C and the resulting pellets were washed twice (with PBS buffer for *E. coli* and 0.1% peptone water for *L. monocytogenes*) and mixed with 450 ml TCW resulting in 10^8 CFU/ml.

Number of colony forming units before and after UV treatment were measured and the log reduction was calculated using the equation (1).

$$\text{Log Reduction} = log_{10}\left(\frac{A}{B}\right) = log_{10}(A) - log_{10}(B) \quad (1)$$

where, A is the number of CFU before treatment; B is the number of CFU after treatment.

2.3. UVC treatment

Dean flow UVC reactors described by Choudhary et al. (2011) was used in this study. The reactors were made of UVC transparent PFA tubes spirally wound around a 60 cm long quartz glass sleeve enclosing a 60 cm long UVC lamp emitting at 254 nm (8.7 W, SBL 325 model, American Ultraviolet Company, Lebanon, IN). One reactor was constructed using a 1.6 mm ID (3/16″ OD) PFA tube, whereas the second reactor was made of 3.2 mm ID (4/16” OD) PFA tubing. Both of these reactors had two sections of PFA tubes. The Section A in the 3.2 mm ID reactor had 120 cm long tubing, connected by a three way valve to the section B of 240 cm. Similarly, the section A in the 1.6 mm reactor had 240 cm long tubing, connected by a three way valve to the section B of 480 cm. This design was adopted by Choudhary et al. (2011) to keep a constant residence time of liquid food under pasteurization when the flow rate of the liquid was increased to obtain next level of Reynolds number. Fig. 1 shows a schematic diagram of the UV reactor used in this study.

Before and after each experimental trial, the UV reactor was cleaned and sanitized by circulating 500 mL of hot water (70 °C) followed by 100 ml hypochlorite (200 ppm) for 10 min. The reactor was finally rinsed with sterile deionized water at room temperature for 4 min. The UV lamp or ballast was turned on 3 min before pumping the samples through the reactors. For each experimental trial, 450 mL of inoculated TCW samples were pumped through the UV reactors. The treated samples were stored in sterile glass bottles and immediately spread plated (100 μl) and incubated at 37 °C for the enumeration.

2.4. UV dose measurement

A UVX radiometer (UVP LLC, Upland, CA) was used to measure UV irradiance across the reactor. UV dosage (J L^{-1}) was calculated as per the following procedure. UV absorbed by the TCW being treated in UV reactors were estimated by taking difference in irradiance (mW/cm^2) of the UVC measured across the empty and TCW filled

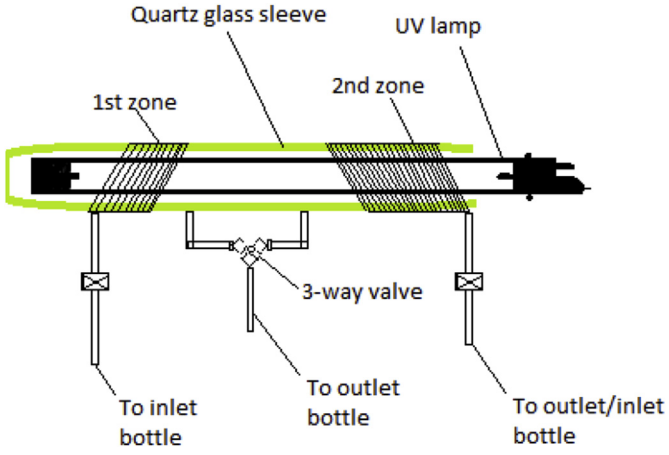

Fig 1. Schematic diagram of UVC reactor design used for treating tender coconut water.

PFA tubes. The UVC absorbed per unit area for the 1.6 mm reactor was 2.38 mW/cm^2, whereas for the 3.2 mm reactor it was 3.79 mW/cm^2. The UVC absorbed per unit area of reactor coil could be converted to per mL of TCW by estimating volume of TCW per unit cylindrical area of the Dean-flow UVC reactor. Since the volume of TCW in 1.6 mm PFA tubing per unit area of the reactor was 0.0415 mL/cm^2, the UVC power per mL for the 1.6 mm reactor would be 2.38/0.0415 = 57.35 mW/mL. Similarly, for the 3.2 mm reactor (with volume per unit area of 0.1247 mL/cm^2), the UVC power per mL would be 3.79/0.1247 = 30.39 mW/mL. The residence time of TCW in each reactor was 14 s, and hence the UV dose was 802 mJ/mL in 1.6 mm reactor and 425.46 mJ/mL in the 3.2 mm reactor.

2.5. Experimental design and statistical analysis

Previous research (Bandla, Choudhary, Watson, & Haddock, 2012) demonstrated the effect of liquid depth and flow characteristics on microbial inactivation. Therefore the tube diameter (D) and Reynold number (Re) were chosen as the independent variables.

The residence time (14 s) was a fixed variable. The response variable was colony forming unit (CFU) on the basis of standard plate count (SPC). Re is an indicator of turbulence inside the UV reactor for any liquid food. Following equation (2) defines Reynold number:

$$\mathrm{Re} = (\rho/\mu) \times \mathrm{VD} \quad (2)$$

where, Re is Reynolds number, ρ is density of fluid, μ is dynamic viscosity of fluid, D is diameter of wrapped tube, and V is velocity of flow. The Re levels, calculated using equation (2), are shown in Table 1.

A completely randomized factorial design was used with two levels of D and three levels of Re, with 3 replications each resulting in total of 18 experimental trials. One-way ANOVA was used to compare the effect of Re and D on microbial reduction. The analysis of simple effect was reported if interaction effect was significant. Statistical analyses was conducted using JMP®, Version 12.2 (SAS Institute Inc., Cary, NC).

3. Results and discussions

3.1. Sample quality and sterility test

Fresh tender coconut water after extraction from shell was tested for sterility, pH, Brix, density, viscosity and standard plate count (SPC). The SPC test showed no growth of organism when incubated and observed after 24 h. The results confirmed the procedure used for extraction did not contaminate the TCW. The pH of the fresh TCW was 5.09, which falls under the category of a non-acidic food (pH higher than 4.6). The total soluble solids (TSS) of fresh TCW was in the range of 6.7–6.9%. The density and Viscosity was measured to be 1015.6 kg/m^3 and 1.39 × 10^{-3} Pa s respectively. The absorption coefficient of UVC at 254 nm for the TCW was measured to be 1.90 cm^{-1}. Koutchma, Parisi, and Patazca (2007) determined that absorption coefficient of pineapple, guava and apple juices were in the range of 11–78 cm^{-1}. They reported desirable UV absorption coefficient should be less than 15 cm^{-1} for 5 log reduction of *E. coli* K12 in fruit juices.

Table 1
Reynolds number (Re) values used in this study.

Tube Diameter	Re	Flow rate (ml/min)	Speed (cm/s)	Calculated Re[a]
3.2 mm	1	40	8.57	198.8
	2	80	17.14	397.7
	3	120	25.71	596.4
1.6 mm	1	20	17.14	198.8
	2	40	34.29	397.7
	3	60	51.42	596.4

[a] Measured density and viscosity of TCW for Re calculation were 1015.6 kg/m^3 and 1.39 × 10^{-3} Pa s respectively.

3.2. Bactericidal effect of UV treatment

The mean log reduction of *E. coli* W1485 and *L. monocytogenes* after treatment by Dean-flow UVC reactors are shown in Figs. 2 and 3. Number of cells of *E. coli* and *L. monocytogenes* present in freshly harvested and inoculated TCW were 8.78 and 8.23 logs respectively before treatment in the UVC reactors.

The maximum and minimum log reduction observed in this study were 5.76 and 2.38 log CFU/mL. The highest log reduction was observed in *E. coli* with 1.6 mm reactor at Re 596.4 whereas the lowest log reduction was observed in *L. monocytogenes* with 3.2 mm reactor at Re 198.8 respectively. The higher log reduction in the 1.6 mm ID reactor could be attributed to thinner volume of TCW exposed to the UVC light in the 1.6 mm ID tubes than 3.2 mm ID tubes, resulting in higher practical dose obtained by the 1.6 mm reactor.

For *E. coli* inactivation, the ANOVA results denoted a significant interaction effect for D and Re ($p < 0.05$). Further analysis of simple effect at each Re level revealed that at Re 198.8, the 1.6 mm reactor caused significantly higher reduction of *E. coli* than the 3.2 mm reactor. On the contrary, at Re 397.7 and 596.4, the mean log reduction of *E. coli* in TCW for 1.6 mm reactor was not significantly different than 3.2 mm reactor. This could be because the increase in the secondary vortex and turbulence at higher Re levels causing better mixing of TCW in the reactors. This shows that after Re 397.7 the inactivation of *E. coli* becomes greater than 5 logs as desired by the FDA.

For *L. monocytogenes*, main effects of Re and D were significant ($p < 0.05$). As shown in Fig. 3, the highest log reduction of *L. monocytogenes* was found to be 4.7 logs in 1.6 mm ID reactor. The inactivation of *L. monocytogenes* was significantly higher in 1.6 mm reactor than the 3.2 mm reactor for all three levels of Reynold's number.

For the 1.6 mm reactor, the log reduction of *L. monocytogens* significantly increased when Reynolds number increased from 198.8 to 397.7 but further increasing Re to 596.4 did not cause significant increase in the log reduction. This indicates that merely by increasing the Re beyond 397.7, log reduction of *L. monocytogenes* could not be improved. However, it is suggested that further improvement in log reduction of *L. monocytogenes* in TCW could be obtained by increasing UV dosage either by increasing the intensity of UV lamp or by prolonging the residence time.

Overall, in case of both the organisms, there was a significant difference in reduction between the highest and lowest level of Re. Moreover, there was no significant difference between the inactivation of microbial cells for the two tube sizes at the highest level of Re. This could be because the mixing condition in the 3.2 mm reactor might have allowed higher reduction of organisms. Thus for future design of UVC reactors for TCW, higher diameter UV reactor will be more efficient because of its higher capacity (flow rate).

According to Choudhary et al. (2011), Dean flow UVC treatment on raw cow milk (RCM), skimmed cow milk (SCM) and raw soymilk (RSM), 1.6 mm UV reactor was reported to be more effective on reduction of both *E. coli* and *B. cereus* in 11.3 s. They used higher level of Re (1372) compared to this study (Re = 596.4) to obtain similar reduction of *E. coli* (5.6 log) in SCM. Using a Dean vortex UV

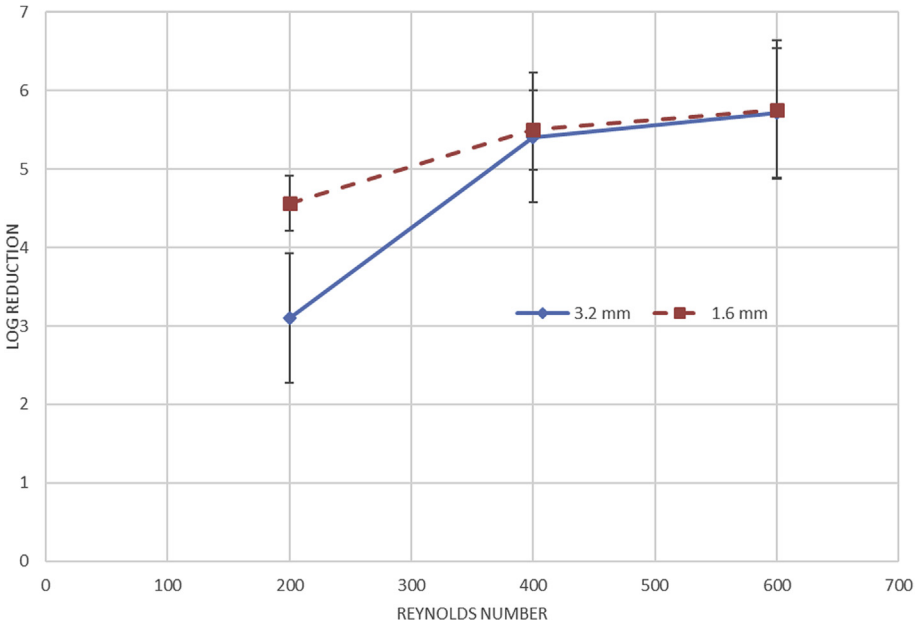

Fig. 2. Mean value of log reduction and standard error of *E. coli* W 1485 in tender coconut water in response to UVC treatment in 3.2 mm ID and 1.6 mm ID reactors. The population of *E. coli* prior to UV treatment were 8.78 log CFU per ml.

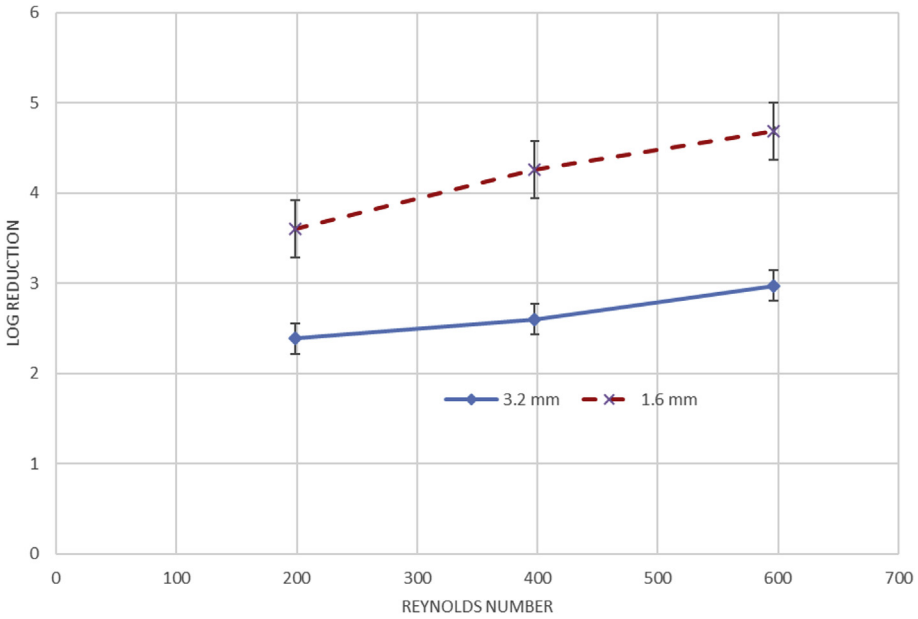

Fig. 3. Mean value of log reduction and standard error of L. monocytogenes in tender coconut water in response to UVC treatment in 3.2 mm ID and 1.6 mm ID UV reactors. The population of *L. monocytogenes* prior to UV treatment were 8.23 log CFU per ml.

reactor, Franz, Specht, Cho, Graef, and Stahl (2009) reported more than 6 $\log_{10}$ CFU/ml reduction of *E. coli* in cloudy apple juice, which was higher than milk because of better transmission of UVC through apple juice.

Inactivation of *L. monocytogenes* was lesser than *E. coli* W1485 at leach level of Re and D. The main reason that could be attributed to this phenomenon is the peptidoglycan layer, present on cell wall of the Gram positive *L. monocytogenes,* blocking UVC absorption (Bank, John, Schmehl, & Dratch, 1990). Moreover, the presence of sigma b factor in Gram positive bacteria like *Bacillus* species and *L. monocytogenes* regulates various stress responses leading to better survival of *L. monocytogenes* (van Schaik & Abee, 2005).

Feng et al. (2013) treated watermelon juice in a Teflon coil UVC reactor and reported total coliform bacteria, aerobes and yeast molds were reduced by 2.6, 1.47 and 0.99 $\log_{10}$ CFU/mL respectively by the UVC dosage of 37.5 J/ml. This shows that the *E. coli* were not inactivated like in our research but comes out to be similar reduction to that of *L. monocytogenes*, even though we had much lower UVC dosage (maximum of 802 mJ/L). The effect of Teflon coil diameter (which helps to predict the flow rate in the Teflon coil) was not studied by Feng et al. (2013), hence the flow condition in their study might may not be enough to attain good mixing. It can be further attributed to less transparent quality of watermelon juice and its physico-chemical nature.

3.3. Quality evaluation

In order to ensure the retention of natural quality of UV treated TCW samples, pH and total soluble solids were measured on UV treated and untreated samples. A slight change in pH (5.1–5.4) compared to the fresh TCW in 28 days indicated that the UV treatment effectively reduced acid producing bacteria and enzymes in samples. Minute settling of solids might be the reason for pH increase. Augusto et al. (2015) recently studied effect of coconut water on PPO and POD enzymes and indicated that UV is an effective method of deactivating enzymes in coconut water. Moreover, the TSS value of UV treated samples did slightly decrease (6.2%–5.9%) with storage time of 28 days indicating that the solids of coconut water might have settled as the samples were drawn from top layers for solids test.

Our results indicate that UVC treatment did not significantly change the physico-chemical properties of tender coconut water indicating retention of aroma and flavor. Therefore, the natural properties of coconut water were not disturbed by UVC pasteurization. Although we did not evaluate the change of enzymatic activities, the retention of physicochemical properties indirectly indicated that food spoilage enzymes might have been inactivated by UVC treatment.

4. Conclusion

The maximum reduction of *E. coli* W1485 was above 5 logs in tender coconut water in both the reactors for Re levels 397.7 and 596.4. However, the reduction of *L. monocytogenes* was slightly less than 5 logs (4.7 logs CFU/ml) in 1.6 mm ID UVC reactor. Higher reduction of *L. monocytogenes* may be achieved by increasing UV dose by either increasing intensity of UV or increasing the residence time. In conclusion, UVC treatment in Dean Flow reactor was capable of pasteurizing and retaining physio-chemical properties of tender coconut water if exposed with adequate UVC dosage and flow rate. UV treatment of coconut water by a Dean Flow reactor is an effective method of pasteurization that can inactivate food pathogens as well as spoilage organisms and enzymes.

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