Impact of UV-C processing of raw cow milk treated in a continuous flow coiled tube ultraviolet reactor

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Abstract: Raw cow milk (RCM) was treated in a continuous flow coiled tube ultraviolet reactor at the Reynolds number (R_e) of 713 for a residence time of 17 s with cumulative UV-C dose of 16.822 mJ cm^{-2}. SPC in RCM was reduced by 2.3logs at these conditions. Sensory analysis of milk samples was conducted using triangle test. UV-C treated RCM and untreated RCM was compared with the fresh RCM (control) using triangle test method of olfactory sensory analysis right after the UV treatment and during storage periods (1, 3, 7 d) at 4℃. There were no significant differences among the odor of UV-light-treated, untreated, and control, right after the UV treatment. Perceivable change in the odor of UV-treated and untreated RCM compared to the fresh RCM was observed on the 3rd and 7th days after treatment. There was no significant difference between the malondialdehyde and other reactive substances (MORS) content in untreated and fresh milk right after the treatment and during storage. Lipid oxidation products content of UV-treated RCM was significantly higher than that of the fresh or untreated RCM.

Keywords: raw cow milk, ultraviolet processing, UV-C treatment, sensory test, lipid oxidation substances

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1 Introduction

Raw cow milk is heat pasteurized to reduce pathogenic bacteria and control lipase activity of milk. Although heat pasteurization is an effective process, it may cause cooked flavor and may require a relatively high investment for small scale operation. There is a need for an alternative process for producers working with smaller volumes of milk that would be less costly than traditional pasteurization methods (Matak et al., 2007).

Nonthermal processes are gaining importance as alternative technologies to traditional thermal processing of foods because of their ability to minimize the loss of flavors and nutrients, and improve energy efficiency. Ultraviolet light (UV-C) treatment is one of the nonthermal technologies to kill microorganisms (Sastry, Datta and Worobo, 2000). Photochemical changes in proteins and nucleic acids are responsible for inactivation of microorganisms when UV light is absorbed by the food during UV treatment process (Jay, 1996). The photochemical reactions may lead to mutations of nucleic acid sequences leading to disrupt DNA transcription and replication (Miller et al., 1999).

Ultraviolet (UV-C) light treatment has been established as a nonthermal germicidal process for water purification systems (Parrotta and Bekdash, 1998) and apple cider (Wright et al., 2000; Hanes et al., 2002; Basaran et al., 2004). In goat's milk, UV-C treatment inactivated *Listeria monocytogenes* by more than 5 $\log_{10}$ CFU mL^{-1} (Matak et al., 2005). It was observed that

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photochemical reactions in milk caused malodors due to UV and visible light with wavelengths between 280 and 700 nm (Bradley, 1980; Min and Boff, 2002). However, UV irradiation studies for enriching the vitamin D component at the germicidal wavelength of 254 nm (UV-C) did not report any negative sensory defects (Capstick, Hall and Neave, 1946; Burton, 1951; Caseiro et al., 1975).

Key factors influencing efficiency of UV-C treatment include reactor design, fluid dynamic parameters and UV-C absorbance of liquid food (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., 2004; Franz et al., 2009). Reynolds number ($R_{e)}$ is a measure of the ratio of inertial forces to viscous forces for fluid flow in a coiled tube. It is expressed as:

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

where, R_e 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 $R_e < 2100$, whereas $R_e > 4000$ indicates turbulent flow. Flow with R_e between these numbers is considered transient flow (Geankoplis, 1993).

Coiled tube reactors may provide secondary vortices and cause superior mixing of fluid flowing through the reactor depending on the design of the coiled tube reactors. The Dean number D_e is a similarity parameter governing the fluid motion in coiled tube flow configuration.

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

where, D is the tube diameter; D_c is the coil diameter, and R_e is the tube Reynolds number (Dean, 1927). When the flow of a fluid in a coiled tube reactor is accompanied by secondary flow vortices, it is called Dean flow. This occurs when the ratio (D/D_e) in Equation (2) is within $0.03 < D_e < 0.1$ (Dean, 1927). Franz et al. (2009) suggested Dean flow condition causes superior mixing and uniform processing conditions, leading to better exposure of liquid food to UV-C in a UV reactor.

High-quality fresh milk has a delicate flavor. Minor variations in chemical composition can render it unacceptable by consumers (Walstra and Jenness, 1984). Triangle test is a method of sensory analysis of dairy products used to find the odd sample in a set of three samples (two samples smell the same and one sample smells different) (Meilgaard, Civille and Carr, 1999). The British Standard for Triangle Test (BSI, 2004) states that it is “a procedure for determining whether a perceptible sensory difference or similarity exists between samples of two products.” The standard further claims that “It is statistically sound and applicable even when the nature of the differences is unknown.” The method requires the products to be fairly homogeneous. It is therefore expected to be an effective method to test whether UV-C processing of raw cow milk causes any change in flavor. This method was reported by Matak et al. (2005) to test sensory changes of UV treated goat milk.

The objective of the study was to determine the effect of continuous UV-C treatment of raw cow milk on inactivation of natural flora of milk, and to determine if chemical and sensory properties of RCM were affected by UV-C treatment of raw cow milk in a coiled tube UV reactor.

2 Materials and methods

2.1 Milk collection

RCM (20 liters) was collected in a sterilized stainless steel can from the dairy farm of Southern Illinois University, Carbondale. Composition of milk (fat, protein and total solids) was analyzed using an infrared milk analyzer (Multispec; Foss Food Technology Corp., Eden Prairie, MN) by the lab technicians of Prairie Farms (Carbondale, IL, 62901). The milk samples were refrigerated until processed, which occurred within 2-3 hours.

2.2 UV irradiation

Choudhary et al. (2011) designed and described a coiled tube UV reactor. The same reactor was used for this study (Figure 1). The UV-C source 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 UV lamp was enclosed within a quartz glass sleeve (American

Ultraviolet Company, Lebanon, IN, USA) with a 22 mm OD and an air gap of 2.4 mm between UV lamp and sleeve. Perfluoroalkoxy polymer resin (PFA) tubing with 1.6 mm inside diameter (ID) and 3.2 mm OD was selected to wrap around the glass sleeve based on Geveke (2008) that PFA tubing is highly transparent to UV light and has more chemical and heat resistance than polytetrafluoroethylene and fluorinated ethylene propylene. The UV reactor was covered with aluminum foil to prevent exposure of personnel to UV light.

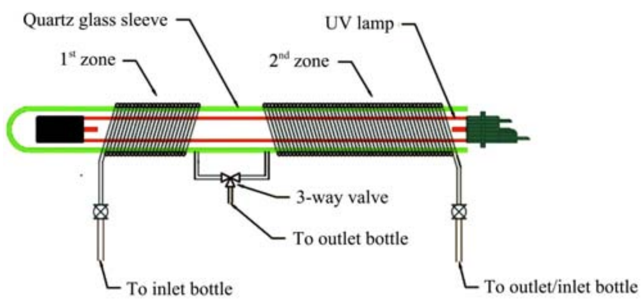

Figure 1 Diagram of the coiled tube UV reactor used for this study (drawing not to scale)

The inside diameter and thickness of the PFA tube was 1.6 mm and 0.8 mm respectively. 1st zone and 2nd zone of coil were used to vary residence time of milk while keeping the Reynolds number constant. The 3-way valve facilitates flow of milk through either 1st zone or 2nd zone or both the zones.

The Reynolds number (R_e) was calculated using Equation (1). The density (ρ) and viscosity (μ) of RCM estimated according to the relationship developed by Bakshi and Smith (1984) were 1,021.46 kg m^{-3} and 1.941×10^{-3} N s m^{-2} respectively.

The UV reactor produced dean flow condition with the calculated D/D_c (equation (2)) value of 0.06. The UV-C dose was calculated using the following equation of multiplying the residence time with the irradiance intensity at the milk surface per Quintero-Ramos et al. (2004).

$$\text{UV-C dose (mJ cm}^{-2}) = \text{Irradiance intensity (mW cm}^{-2}) \times \text{Exposure time (s)} \quad (3)$$

where, *irradiance intensity* is the incident intensity of UV-C light on the surface being treated. Irradiance intensity may be either directly measured using a UV-C sensor or estimated if the intensity of the source and optical properties of transmission medium are known. 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).

Choudhary et al. (2011) reported a 4.1 $\log_{10}$ CFU ml^{-1} reduction of *E. coli* W 1485 in raw cow milk by UV-C treatment in the same coiled tube UV reactor at a flow rate of 100 lpm, corresponding to a R_e of 713 at a UV dose of 11.187 mJ cm^{-2}. In this study, the residence time of milk through the UV reactor was increased to 17 s to provide increased UV dose (16.822 mJ cm^{-2}), as may be required to achieve greater more than 5-log reductions of *E. coli* in RCM.

For comparison purposes, a separate batch of milk was passed through the reactor with UV lamp off. Agitation caused by the act of pumping the milk through the UV reactor might have an effect on milk properties due to shear and lipase activity that can be observed from milk pumped through the reactor without UV light on. An aliquot (100 mL) was sampled for chemical analyses after UV treatment for subsequent chemical analyses such as changes in pH, and lipid oxidation using TBARS test right after the treatment and during storage period. Treated samples were collected in 1000-mL HDPE containers (Nalge Nunc Int., Rochester, NY), fitted with lids and stored in the dark cold room maintained at 4℃ for conducting sensory analysis during storage period up to seven days. The untreated (control) sample was stored together with the treated samples in each case except the sensory evaluation, when always fresh RCM samples were used.

2.3 Sensory testing

As our motivation to develop a nonthermal UV processing was to obtain safe milk with fresh taste of raw milk, fresh raw milk was used as our bench mark for sensory evaluation. Therefore, triangle test of sensory analysis was conducted for comparison of odor of UV treated, untreated RCM, and fresh RCM (control). The fresh RCM was collected each day the sensory analysis was performed (not stored along with treated RCM). The untreated milk (pumped through the reactor without UV bulb turned on) was stored for seven days to study the effect of agitation caused by coiled-tube arrangement. UV-C treated milk was obtained by pumping RCM through the reactor with UV bulb turned on, and stored for seven days. Milk samples (>15 mL) were poured into 20-mL semiopaque plastic cups immediately after processing, fitted with plastic lids, assigned a random 3-digit code, and stored at 4℃ until sensory test was concluded.

A sensory panel of 30 members was constituted after the approval of the human subjects committee, Southern Illinois University, Carbondale. The panelists were chosen from a pool of volunteers across the SIUC campus, primarily consisting of college students who were not trained in sensory analysis. Sensory analysis of milk was performed right after the treatment and during storage at one, three and seven days after treatment. Because milk samples were not heat pasteurized, and UV-C processing is not yet an approved process for milk, samples were assessed for differences in odor, but not taste. Triangle test of sensory analysis was conducted with the following three comparisons: (1) fresh RCM (control) vs. UV treated; (2) UV treated vs. untreated (lamp off); (3) control vs. untreated (lamp off). Each of the 30 panelists was presented with all three sets of comparisons. Each of the above mentioned comparisons had two sets of three samples (one different sample and two same samples). One example comparison is shown in Figure 2. Sample presentations for each of the comparisons were as follows:

Comparison (1): UV treated versus fresh RCM (Set 1 - UV treated, fresh RCM, fresh RCM; Set 2- UV treated, UV treated, fresh RCM).

Comparison (2): Untreated versus fresh milk (Set 1- Fresh RCM, fresh RCM, untreated; Set 2- untreated, untreated, fresh RCM).

Comparison (3): Untreated versus UV treated (Set 1- UV treated, untreated, untreated; Set 2- UV treated, UV treated, untreated).

Panelists were instructed to identify the sample that smelled odd and different in each group of three. There was additional space for comments with instructions to describe any odors associated with the unique sample.

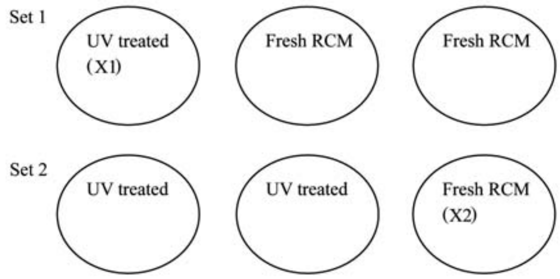

Figure 2 Showing two sets of milk samples presented to a sensory panelist for a comparison

A correct response was recorded only when the panelist chose both the samples marked as X1 and X2.

2.4 Standard plate count (SPC)

SPC was enumerated in fresh RCM. Dilution blanks were made up of phosphate-buffered saline (PBS). A series of dilutions were spread plated (0.1 mL) in duplicate with tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) for fresh RCM, UV-C treated, and untreated RCM. Plates were incubated at 33℃ for 24 - 48 hours (Marshall, 2004) and colonies were counted manually to report the number of colony forming units per ml ($CFUmL^{-1}$) of sample.

2.5 Malondialdehyde and other reactive substances

The thiobarbituric acid reactive substances (TBARS) test kit (Zeptometrix corporation, Oxitek, TBARS assay kit, Buffalo, New York, USA) was used to measure malondialdehyde and other reactive substances (carbonyl oxidation products of lipid oxidation reactions in milk). The test determined the level of production of malondialdehyde, which was a secondary product of oxidation in milk. The lipid oxidation products were presented as the malondialdehyde content obtained from the calibration curve of standard malondialdehyde content vs optical absorbance at 532 nm. Samples were analyzed by taking absorbance values at 532 nm in two replicates. This test was nonspecific because color reactions due to other products such as secondary oxidation products (heptanal and pentanal) might occur in milk (Frankel, 1991). The results of this test were reported as nmol ml^{-1} of Malondialdehyde content in the milk samples.

2.6 PH test

The pH of fresh RCM, UV-C treated RCM, and untreated RCM were measured right after the treatment and during storage period (1, 3 and 7 d) at 24-26℃ using a pH meter (Corning, NY, USA) after calibration in buffer solutions.

2.7 Statistical analysis

The data for each triangle test were analyzed by the number of correct responses out of 30 total responses (from 30 panelists). Responses were considered correct only when the panelist was able to identify the odd sample (smells different than the other two samples in the set) in both the sets of three samples. As per Meilgaard, Civille and Carr (1999), for a sensory evaluation consisting of 30 panelists, a treatment was considered significant when at least 15 panelists responded correctly during triangle test. The general linear models procedure of SAS 9.2 was used to analyze malondialdehyde content for each storage period. The Tukey's HSD method was used to separate means when significance (α = 0.05) was found among the treatments.

3 Results and discussion

3.1 Quality of milk

Average composition of RCM during experiments was total solids 13%, fat 3.9% and protein 3.2%. The pH of fresh, untreated, and UV treated cow milk at 24℃ was in the range of 6.6 - 6.7, which was within the range of normal pH (6.6 - 6.8). The pH of milk remained within the normal range during the storage period of seven days.

3.2 Standard plate count

Standard plate count (SPC) is an indicator of natural microbial quality of milk. The SPC of fresh RCM was 1.4×10^4 CFU mL^{-1}. This indicates that the milk used was of grade A quality (SPC<50,000 CFU mL^{-1}) (Marshall, 2004). For the UV-treated milk, SPC was reduced from 4.2 $\log_{10}$ CFU mL^{-1} of initial flora present in RCM to 1.9 $\log_{10}$ CFU mL^{-1}. Reinmann et al (2006) reported that 2.9 logs were obtained from seven logs of initial flora present in raw cow milk, which was treated at 25℃ at an UV dose 1.5 J mL^{-1} in the UV reactors (Pure UV version 1 and 2). They used higher UV dose than the UV dose used in this study, which may be the major reason for their reported higher level of inactivation of SPC. SPC present in RCM may need more UV dose than E. coli because RCM contains different species such as coliforms, mesophiles, and thermodurics.

3.3 Sensory changes

Table 1 shows the results of the olfactory sensory test. There was no difference in odor between the combinations of fresh RCM (control), UV-C treated RCM, and untreated RCM on the same day (Day 0) as UV-C treatment. The olfactory sensory analysis indicated that there was no significant difference between the odors of UV-C treated and untreated samples on the day the milk was treated, but on day 1 of storage, there was a significant difference only between the UV-C treated and untreated samples. The UV-C treated and untreated samples had significantly different odor than the fresh milk on the 3rd and 7th days after treatment. Changes occurred in both the treated and untreated milk during storage from the first day. This might be due to the action of active enzymes present in the milk, which are considered highly resistant to UV-C (Matak et al., 2007). This might also influence the milk flavor during the storage period. Matak et al. (2007) reported that the UV-C exposure caused changes in the milk that affected the milk odor right after the treatment in the sensory analysis using triangle test. But, panelists in our study did not detected off-flavors in either the UV-treated or untreated milk until the first 24 hours (Day 1) of storage. Off flavor after day 1 could be attributed to hydrolytic rancidity and change in volatile compounds in milk lipids during storage period. Panelists commented that the UV treated milk samples smelled sour (two panelists), oxidized (one panelist), rancid (one panelist), and plastic (one panelist), whereas the untreated milk smelled sour (two panelists), animal feed (two panelists), stinky (two panelists) and unusual smell (one panelist). All these comments about UV treated milk and untreated were on Days 3 and 7. Panelists also commented that the fresh milk smelled like animal feed (two panelists). The sensory panel members were not trained to report a particular odor type but their response was based on their own previous experience with smell types.

Table 1 Number of correct responses from olfactory triangle test the same day as UV-C treatment and during storage days 1, 3, and 7 for UV-C treated RCM, untreated RCM, and fresh RCM (control)

Comparison	Day 0	Day 1	Day 3	Day 7
Control vs. UV-C treated	12	14	18[y]	20[y]
Control vs. untreated	9	12	20[y]	22[y]
UV-C treated vs. untreated	11	15[y]	21[y]	22[y]

Note: n = 30 observations. Critical correct response number = 15; α = 0.05; β = 0.05 and probability distribution (pd) = 50% (Meilgaard, Civille and Carr, 1999). Correct responses designated [y] are significant.

The SPC of UV-treated milk remained below 300 CFU mL^{-1} during the seven days storage period. The SPC of control (fresh raw milk collected on the same day as sensory test) was between 1.3 to 1.4×10^4 CFU mL^{-1}. The SPC of untreated milk was not measured during refrigerated storage at 4℃. However; there might have been some growth of psychrotrophs during the storage period of three days and later causing change in flavor of untreated milk due to enhanced enzymatic activities (Kumaresan, Annalvilli and Sivakumar, 2007). No coliforms were detected in the UV-treated milk during the storage period. There was no evidence relating sensory defects with microflora of RCM. Matak et al. (2007) reported significant sensory responses showing malodor of UV-C treated milk within 6 hours. In this study, the odd smell of the stored samples may be due to the enzymatic degradation because of insufficient inactivation of active enzymes as well as production of active enzymes by the bacteria surviving the UV treatment. A higher dose of UV or a combination treatment with other nonthermal processing technology may help in inactivating enzymes of milk system responsible for odor changes.

3.4 Chemical analyses

Table 2 shows malondialdehyde and other reactive substances (MORS) contents of fresh, UV-C treated, and untreated raw cow milk (RCM) for each storage period tested. For all four storage periods, there was a significant difference among treatments (F(2,6) = 55.52 to 100.32, p < 0.0001). In each case, UV-C treated milk had a higher MORS value than the fresh or untreated milk. There was no difference between fresh and untreated milk, indicating that the agitation of the UV reactor alone did not cause a difference in secondary products of oxidation, such as malondialdehyde. The increased production of oxidative products in UV-C treated milk could result from a photochemical reaction causing oxidation of unsaturated fatty acid residues in milk lipids and phospholipids (Koutchma et al., 2009).

Table 2 Mean values of malondialdehyde and other reactive substances (MORS) in nmol ml^{-1} of fresh RCM, UV-C treated, and untreated raw cow milk (RCM) during storage period

Storage period (days)	Fresh RCM*	Untreated RCM*	UV-C treated*
0	41.0[a]	45.2[a]	75.5[a]
1	48.2[b]	49.3[a]	73.1[a]
3	51.1[b]	53.3[ab]	74.8[a]
7	58.0[c]	63.2[b]	82.1[b]

Note: * Means followed by different letters within each treatment are significantly different. Fresh RCM was used as a control each day. Untreated milk was RCM pumped through the UV reactor without turning on the UV lamp. UV treated milk was exposed to UV-C for a residence time of 17 s (UV dose = 16.822 mJ cm^{-2}). All milk samples were refrigerated and taken out just before MORS analysis. For each storage period, UV-C treated is significantly higher than fresh or untreated RCM.

Within each treatment, there were differences in MORS values for the days of storage (Table 2). For fresh RCM there were significant differences (F(3,8) = 61.56, p < 0.0001), with Day 0 having a significantly lower MORS value than the other days and Day 7 having a significantly higher MORS value than the other days. For untreated RCM there were significant differences (F(3,8) = 10.38, p < 0.0039), with Day 7 having a significantly higher MORS value than Days 0 or 1. There were significant differences (F(3,8) = 8.44, p < 0.0074) among the storage period for UV-C treated RCM, with day 7 having a significantly higher MORS value than the other days. Although the MORS value was initially higher for UV-C treated RCM, the value did not change until Day 7.

Lee (2002) reported that the concentration of riboflavin in milk was directly related to the extent of off-flavors when milk was exposed to UV light. This could be the reason in this study also. There was no increase in MORS values in untreated samples, which implied that UV-C exposure was a significant factor for increasing production of oxidative products.

4 Conclusions

UV-C treatment of raw cow milk for 17 s in the coiled tube UV reactor (equivalent to an UV-C dose of 16.822 mJ cm^{-2}) was capable of reducing SPC by 2.3 log_{10} CFU mL^{-1}. The olfactory sensory analysis indicated that there was no significant difference between the odors of UV-C treated and untreated RCM right after UV-C treatment but after one day of storage the UV-C treated sample had significantly different smell than untreated sample. The higher values of MORS in UV-C treated RCM indicated that the oxidative degradation might have taken place in UV-C treated raw cow milk.

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