Inactivation of pathogenic and heat resistant microorganisms in milk by a non-thermal UV treatment system.

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ABSTRACT

UV processing is a disinfection method used for various foods and beverages and related products, but this process is the least studied. Its capability to inactivate microorganisms is arises principally from exposure of UV-C with the germicidal range between 250 and 280 nm. This UV causes dimers to form between pyrimidine bases in the DNA molecule resulting in the replication and transcription process being blocked, leading to cell inactivation of bacteria, fungi, moulds and viruses. In this study, the efficiency of UV-C technology as a novel process in the inactivation of food-borne pathogens in milk has been determined. Milk was inoculated with cultures of Salmonella spp., Bacillus spp., B. sporothermodurans, B. subtilis and E. coli K12 and were treated with at UV doses between 0 and 13,400 J L^{-1} at a flow rate of 4,000 L h^{-1}. Samples were drawn after each UV dose delivered and tested microbiologically. Bacillus is considered as among some of the most heat-resistant organisms that can potentially contaminate milk. In the inoculation trials, the initial total viable count in the milk was 10^6 cfu mL^{-1}. The UV system was able to achieve a mean 3-4 log_{10} reduction for the Bacillus and up to a mean 5 log_{10} reduction for E. coli K12 and Salmonella spp. The UV-C system was able to achieve microbial reduction in milk similar to that achieved with pasteurization. These results suggest that UV-C treatment at 254 nm decreased microbial levels to an acceptable level. This UV technology could be used as an alternative, or adjunct to, conventional pasteurization technology in conjunction with good manufacturing and agricultural practices.

Keywords: UV-C; inactivation; Bacillus

INTRODUCTION

Ultraviolet (UV) light, specifically UV-C, has been successfully used as a process for the disinfection of air, water and surfaces (Guerrero-Beltran and Barbosa-Canovas, 2004; Keyser et al., 2008). In recent years, the use of UV-C to sterilize liquids has been studied (Choi and Nielsen, 2005). The UV disinfection system (SurePure Photopurification System) used in this study emits ultraviolet light (UV-C) in the germicidal wavelengths between 240 and 280nm that is of sufficient energy and time to inactivate contaminating microorganisms, such as bacteria, yeasts, moulds and viruses in treated liquids. In this range, UV-C is able to cause DNA lesions by the formation of dimers between pyrimidine units. These dimers inhibit replication and transcription processes, which lead to no cell growth and ultimately cell death of the organism (Koutchma et al., 2009). UV-C has proven to be effective in inactivating contaminating organisms without any secondary effects on the nutritional, sensory and marketing attributes of the product (Bintis et al., 2000).

The color and turbidity of the liquid being treated, however, plays a role as UV-C can only penetrate roughly 1 mm into juice and even less so into milk. The efficacy of the UV-C is therefore dependent on the properties of the liquids being treated (i.e., color, viscosity, absorbance and presence of suspended solids) (Fredericks et al., 2011).

Spore-forming bacteria, such as *Bacillus*, are a concern in the food industry, since they can survive various methods of processing, such as heat, chemicals, desiccation and radiation and can cause food poisoning and spoilage (Blatchley et al., 2005). Certain species of *Bacillus*, such as *B. sporothermodurans*, are of particular concern, since they are heat resistant and can survive the process of pasteurization (Schelderman et al., 2006). Dairy industries are therefore seeking alternative methods to render their products microbiologically safe for consumption. The aim of this study was to determine the efficiency of the UV-C technology as a novel process in the activation of food-borne pathogens in milk.

METHODOLOGY

Batches of fresh milk were received and tested within two hours of receipt. Cultures of the *Bacillus* species, *B. subtilus* and *B. sporothermodurans*, were grown up in Tryptic Soy Broth (TSB) and cultures of *E. coli* K12 and Salmonella species in Buffered Peptone Water (BPW) to a concentration of 10^6 cfu mL^{-1} for each culture. Individual twenty-liter batches of milk were inoculated with each culture and circulated through the UV system to ensure an even distribution of the inoculums though the milk.

UV light was used initially to disinfect surfaces, therefore the irradiance is generally expressed as watts per square meter (W m^{-2}), whilst the radiant exposure (UV dose) is expressed as watts-second per square meter (W s m^{-2}) or joules per square meter (J m^{-2}) and characterizes the energy delivered per surface area of the treatment device. UV dose *(D)* is, therefore, determined as time *(t)* multiplied by irradiance *(E)*. As the UV-C energy penetrates into the medium, therefore working with volume rather than area, Keyser et al. (2008) proposed an alternative method to characterize UV as dose per volume of liquid. For liquids, the UV dose was expressed as J L^{-1}.

UV dose per area

The length of the quartz sleeve used was 0.860 m, with an outer surface area (A_S) of 661.93 cm^2. The area between the quartz sleeve and corrugated spiral tubing is termed the annulus and the volume thereof was determined to be 0.675 L or 0.00068 m^3. The effective area (A_S) of UV-C is at a distance of 5 mm for the UV lamp is 5 mm away from the outer surface of the sleeve. According to the manufacturers, the total UV-C output to the constant surface of the quartz sleeve (A_S = 661.93 cm^2) from the UV lamp is 30 W UV-C. Ignoring the volume of the annulus and disregarding the type of product in the annulus, the following calculations is based on the effective A_S of the quartz sleeve alone, not taking into account the volume of the annulus and the type of liquid treated.

Since the internal volume *(V)* of the reactor is 0.675 L and the flow rate *(Q)* is 4,000 L h^{-1}, the hydraulic retention time *(t)* is 0.608 s.

Therefore the UV dose *(D)* for the surface area of one reactor with continuous flow is given by:

UVDose = Irradiance *(E)* × Time *(t)*

= 38.50 mW cm^{-2} × 0.608 s = 23.408 mJ cm^{-2}

UV dose per volume

At a flow rate (Fr) of 4000 L h^{-1} the product retention time (T) is 0.608 s per reactor), therefore the UV dose per L of liquid treated for one reactor with continuous flow is calculated as follows:

UV Dose = Total UV-C output per unit (W) / Flow rate (L s^{-1})

= 25.50 W / 1.11 L s^{-1} = 22.97 J L^{-1}

Note that the UV dose on a volume basis is numerically almost the same as the UV dose on an area basis.

Inoculated milk was then processed through the pilot scale UV disinfection system (**see Figure 1**). The commercial adaptation of this system is the SP40 which consists of 40 turbulators (**see Figure 2**).

The SP5 unit consists of a five turbulators or UV lamps, which are connected in series (**see Figure 3**). Each turbulator consists of a single germicidal low-mercury UV lamp (30 W output at 254 nm) housed in a quartz sleeve and encased in a stainless steel chamber.

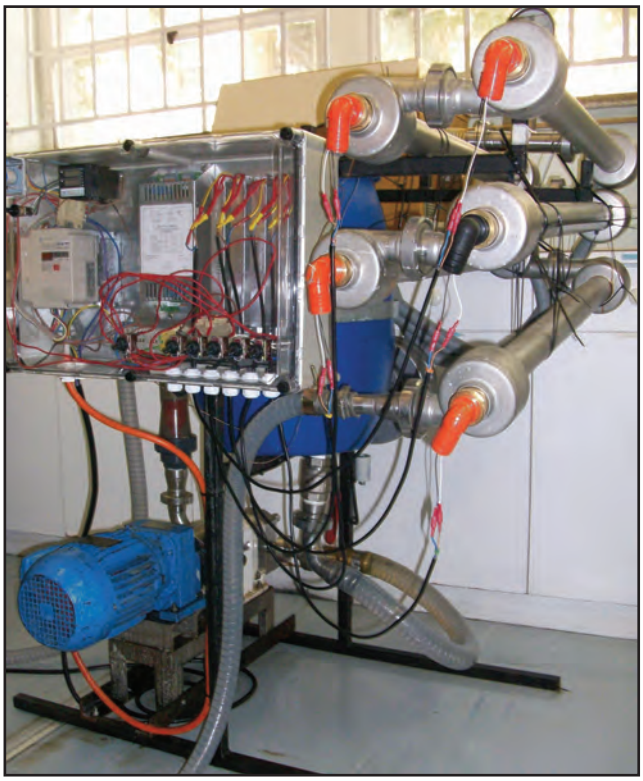

Figure 1: UV disinfection system (Sure Pure Photopurification System [SP5] lab scale model)

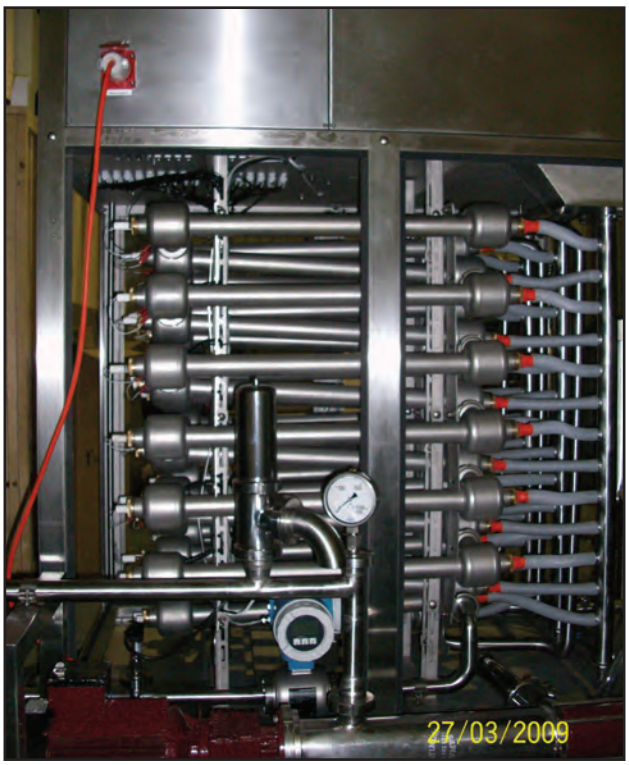

Figure 2: Sure Pure Photopurification System [SP40] commercial application

Figure 3: Single SP turbulator

This results in a turbulent flow of the liquid product between the liquid and the stainless steel chamber resulting in the maximum exposure of the liquid to the UV (**Figure 4**). Inoculated milk was processed through the SP5 system at a flow rate of 4000 L h^{-1}. Various UV doses were applied for each organism: *B.* spp. 0–2,500 J L^{-1}; *B. subtilis* 0–3500 J L^{-1}; *B. sporothermodurans* 0–13,800 J L–*1*; *E. coli* K12 0–2,100 J L^{-1} and *Salmonella* spp. 0–3,500 J L^{-1}.

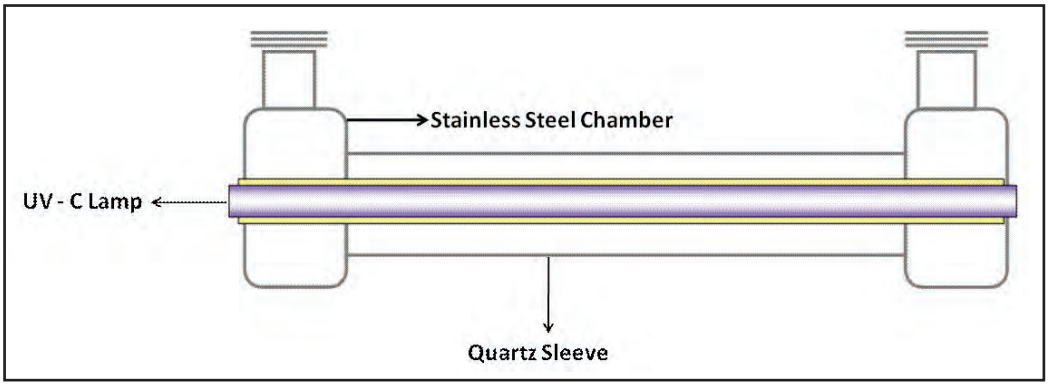

Figure 4: Schemmatic representation of the UV-C turbulator.

Samples (50 mL) were drawn after the application of each UV dose and serially diluted in ¼ strength Ringers solution. For *Bacillus*, tubes were placed in a water bath at 80 ˚C for 10 min. Samples were then pour plated onto Tryptic Soy Agar (TSA) and incubated at 37 ˚C for 24–48 hours. Results were reported as colony-forming units per milliliter (cfu mL^{-1}).

RESULTS AND DISCUSSION

The SurePure Photopurification Technology was able to effectively reduce the number of the artificially inoculated bacteria in milk. The initial concentration of the bacterial inoculate was 10^6 cfu mL^{-1}. After exposure to the UV there was a 3–4 log_{10} reduction for *Bacillus* species, *B. sporothermodurans* (**see Figure 5**) and *B. subtilis*. For the heat resistant *Bacillus sporothermodurans*, a UV dose of 13,770 J L^{-1} was required to achieve a 4-log_{10} reduction in milk, while for the Bacillus species only 2,448 J L^{-1} was required to achieve a 2-log_{10} reduction. This is indicative of the fact that heat-resistant microorganisms bear a greater resistance to UV-C than other organisms; hence a higher UV dose is required to inactivate these organisms.

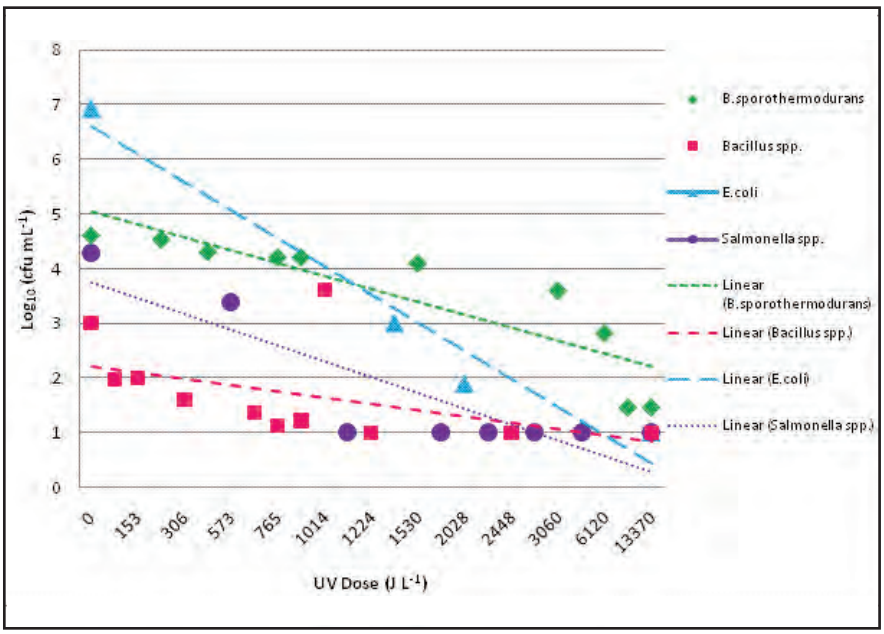

Figure 5: Log_{10} reduction of various microorganisms in milk treated with UV

Raw milk is a highly nutritious food ideally suited for the growth of disease causing bacteria such as *Salmonella*. A 5-log_{10} reduction for *E. coli* K12 (**see Figure 5**) was achieved after a UV dose of 2,000 J L^{-1} and for *Salmonella* spp. after a UV dose of 3,400 J L–1 (**see Figure 5**).

For *Bacillus* spp., a 2-log_{10} reduction was achieved at UV dose of 2,448 J L^{-1}, but a similar result was obtained for *E. coli* and *Salmonella* spp. at UV doses of 1,014 and 1,147 J L^{-1}, respectively.

From these results, it is apparent that both *E. coli* K12 and *Salmonella* spp. possess a lower resistance to UV-C resulting in a higher log_{10} reduction when compared to *Bacillus*, which exhibits a higher resistance. *Bacillus* species, *B. sporothermodurans* and *B. subtilis* are considered to be some of the most heat-resistant bacteria in milk, which is a problem for the industry as they are able to survive the pasteurization process. As milk is an opaque medium, the penetration of the UV-C into the liquid is minimal. An effective reactor design, such as that of the SurePure Photopurification System, circumvents this problem, since it produces a turbulent flow of the liquid which ultimately results in the liquid being exposed to the maximum amount of UV. Very few non-thermal studies have been carried out to establish how heat-resistant organisms might be reduced in food products As a result, the new phootopurification technology, such as that from SurePure, might be one such effective method and could gain acceptance in the dairy industry as an adjunct or alternative to the pasteurization process.

These results suggest that UV-C treatment at 254 nm decreases microbial loads to very low numbers. This non-thermal cold pasteurization method is fast gaining acceptance in eliminating food spoilage and pathogenic microorganisms including bacteria, yeast and moulds. It is also easily incorporated into any production line. UV-C has the advantage that it does not produce chemical residues, by-products or radiation; it is a cold process requiring very low maintenance at low cost, and it does not

require energy to heat the substrate. It has the potential to reduce heat-resistant microorganisms successfully in milk. This UV technology could be used as an alternative pasteurization technology in conjunction with Good Agricultural Practices.

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