A New Colored Beverage Disinfection System Using UV-A Light-Emitting Diodes

XIN LIAN1, 3, KAYO TETSUTANI2, MAI KATAYAMA1, 3, MASAYUKI NAKANO2, KAZUAKI MAWATARI1, NAGAKATSU HARADA2, AKIKO HAMAMOTO3, MASAYUKI YAMATO1, MASATAKE AKUTAGAWA1, YOHSUKE KINOUCHI1, YUTAKA NAKAYA2, AND AKIRA TAKAHASHI3*

1Japan Science and Technology Agency Innovation Satellite Tokushima
2-1 Minami Jyosanjima-cho, Tokushima City, Tokushima 770-8506, Japan
2Department of Nutrition and Metabolism, Institute of Health Biosciences,
The University of Tokushima Graduate School, 3-18-15 Kuromoto-cho,
Tokushima City, Tokushima 770-8503, Japan
3Department of Preventive Environment and Nutrition, Institute of Health Biosciences,
The University of Tokushima Graduate School, 3-18-15 Kuromoto-cho,
Tokushima City, Tokushima 770-8503, Japan
4Department of Electrical and Electronic Engineering, Institute of Socio- Techno Sciences,
University of Tokushima Graduate School, 3-18-15 Kuromoto-cho,
Tokushima City, Tokushima 770-8503, Japan

Received 10 April, 2009/Accepted 23 October, 2009

In this study we evaluated the ability of the UV-A-LED to eliminate bacteria in a colored beverage. Ten edible pigments were used to make a colored solution at concentrations of 1.0%, 0.1%, 0.01% and 0.001%. We used a colony-forming assay to monitor the bactericidal action against the bacteria. The bactericidal effect of UV-A-LED against *Escherichia coli* DH5α decreased with the increasing concentration of almost all of the edible pigments.

Although less effective in colored solutions and commercially available orange juice than in the positive control PBS, it holds potential for further development and use to ensure food and water safety.

Key words: Colored beverage / UVA-LED / Disinfection system.

The exclusion of pathogenic bacteria from drinking water is a concern shared by water utilities worldwide. While every effort should be made to prevent the contamination of drinking water, much improvement is still needed in some parts of the world. Food manufacturing and processing industries employ a variety of decontaminating agents, including chlorine, chlorine dioxide (ClO₂), ionizing irradiation, and others. Each method has its advantages and disadvantages.

UV disinfection technology has drawn increasing interest in the water industry since finding that UV radiation can inactivate oocysts of *Cryptosporidium* and *Giardia*, which are pathogenic microorganisms of major concern in processing safe drinking water (Craik et al., 2001; Drescher et al., 2001). Unlike chemical disinfectants, low pressure lamps do not affect the biological stability of water. UV is considered to be broadly effective against all pathogens that can be transmitted through drinking water, including bacteria, protozoa and viruses (Piriou et al., 2004).

UV-A has been reported to have a bactericidal effect (Berney et al., 2006). Recently, we developed a UV-A irradiation system and found that UV-A by itself can disinfect water efficiently. *Vibrio parahaemoly-
Enteropathogenic Escherichia coli, Staphylococcus aureus and E. coli DH5α were reduced by more than 5-log₁₀ within 75 min at 315 J cm⁻² of UV-A. Salmonella was reduced by more than 4-log₁₀ within 160 min at 672 J cm⁻² of UV-A (Hamamoto et al., 2007; Mori et al., 2007). This UV-A disinfection system uses a wavelength band distinct from that of UVC, and does not require mercury as the illuminant, which greatly reduces the waste problem. A high-quality UV-A disinfection system would present a sustainable, environmentally safe tool for the food industry and for the protection of public water supplies.

To date, we have confirmed the bactericidal effect of the UV-A-LED only in phosphate buffer (−), a transparent, colorless liquid (Hamamoto et al., 2007; Mori et al., 2007; Yagi et al., 2007). The typical commercially sold beverage, however, is a complex mixture of soluble and particulate components, including edible pigments and fibers, which may attenuate the killing effect through the absorption, reflection, or scattering of the light. The edible pigments, in particular, are likely to influence the efficacy of the UV-A-LED disinfection system.

We designed this study to define the effects of edible pigments on the bactericidal action of the UV-A-LED sterilization method. We also tested the effectiveness of the UV-A-LED system in commercially sold orange juice.

E. coli DH5α was purchased from Takara Bio Inc. (Osuka, Japan). Bacteria were cultured in Luria-Bertani (LB) broth (1% tryptone, 1% NaCl, 0.5% yeast extract) at 37°C for 18h. Cells were centrifuged (5000g, 10min, 4°C), washed three times with sterilized phosphate-buffered saline (PBS, pH 7.4) and suspended in PBS at an initial concentration of 1 x 10⁶ CFU/ml. Artificial food colorant was added to make the colored solution at concentrations of 0.001%, 0.01%, 0.1% and 1.0%. None of the colorants affected the growth rate of the E. coli (data not shown). An aliquot (150 µl) of the bacterial suspension was placed into each well of a sterilized 96-well plate (Falcon, Franklin Lakes, NJ, USA) and exposed to UV light.

UV-A-LED irradiation was conducted with a device that we developed (Hamamoto et al., 2007). The peak wavelength of the diode was 365nm. The maximum current of one diode was 0.5A, and the intensity of UV-A light at that time was 70mWcm⁻², which was measured by an accumulated UV meter (UIT-250; Ushio Corp. Tokyo, Japan).

UV-A-LED irradiation was performed in a dark room at 25°C for 30 minutes, and control samples were kept in a completely dark environment in the same room for the same periods of time. Bacterial solutions were not stirred during the UV exposure.

Food color, like flavoring and seasoning ingredients, is added to improve the appeal and quality of foods. Food coloring agents include both natural and synthetic substances, although to satisfy consumer concerns for safety, natural pigments have recently replaced many synthetic forms. For this study, we used artificial food coloring agents (Provided by San-Ei Gen F.F.I., Inc.), which are widely accepted in the food industry, to make the colored solutions:

1. Carotenoids. β-carotene: carotene base No.35468, Paprika color: Paprika base No.34007 and Sun clear paprika No.34592, Marigold: Marigold base No. 33380;

2. Flavonoids Carthamus yellow. Sun yellow No.25FU, Red cabbage color: sun red® RCFU, Purple red pigment: sun redR No.2L, Grape juice color: sun red® GRF;


To investigate the bactericidal effect of UV-A-LED on typical beverages, two kinds of commonly available orange juice were used. Escherichia coli DH5α was suspended in orange juice at an initial concentration of 1 x 10⁶ CFU/ml. UV-A-LED irradiation was performed as described earlier.

To fully evaluate the bactericidal effect of UV-A-LED, we determined the effects of the artificial coloring agents on both cell proliferation and cell death. The disinfection level was determined by a colony-forming assay. After UV irradiation, bacterial suspensions were diluted appropriately, plated on LB agar plates and incubated at 37°C for 18h. After incubation, the numbers of colonies were counted, and log survival ratios or disinfection percentages were calculated using the following equation:

Log survival ratio = log(Nf/Ni)

Disinfection percentage (%) = 100 – (Nf/Ni) × 100

Nf is the colony count of the UV irradiated sample, and Ni is the colony count of the sample before UV irradiation.

We estimated the capacity of UV-A-LED light to kill E. coli DH5α in LB broth prepared with the different artificial food colors at different concentrations. The bacterial suspension was adjusted to a concentration of 10⁶ CFU per milliliter. At 126Jcm⁻² of UV-A irradiation, the disinfection efficiency reached 1.5-2.0 log₁₀ in β-carotene: carotene base No.35468, at 0.001% and 0.01%, and 1.0-1.5 log₁₀ in β-carotene: carotene base No.35468 at 0.1% (Fig. 1 A). However, these values are significantly lower than those achieved with PBS (−), a transparent colorless liquid. Similar results were achieved in LB broth made with each of
the food colors (Fig 1 B-F).

It was noted that some of the food colors inhibited the bactericidal effect to a significantly greater extent than others did. In these preparations, the irradiation produced less than a 0.5 log$_{10}$ reduction (Paprika base No.34007, Sun yellow No.2SFU, Sun red® No.2L, data not shown).

The coloring concentration used most frequently in

A. β-carotene: carotene base No.35468

B. Marigold: Marigold base No. 33380

C. Red cabbage color: sun red® RCFU

D. Grape juice color: sun red® GRF

E. Green colorant: Melon color-L

F. Purple colorant: Grape color RCG

**FIG. 1.** Inactivation of bacteria by UVA-LED irradiation in colored LB broth. The inactivation of *E. coli* DH5α by UVA-LED light was estimated in colored LB broth made with coloring agents at different concentrations. The bacterial suspension was adjusted to a concentration of 10$^3$ CFU per milliliter. Inactivation efficiency was measured at 126 J cm$^{-2}$ of UVA irradiation. A. Results for β-carotene: carotene base No.35468: 1.5-2.0 log$_{10}$ reductions at 0.001% and 0.01%, and 1.0-1.5 log$_{10}$ reductions at 0.1%; B. Results for Marigold: Marigold base No. 33380: 1.0-1.5 log$_{10}$ reductions at 0.001%, 0.01%, and 0.1%; C. Results for Red cabbage color: sun red® RCFU: 1.0-1.5 log$_{10}$ reductions at 0.01% and 0.1%, and 0.5-1.0 log$_{10}$ reductions at 1.0%; D. Results for Grape juice color: sun red® GRF: 1.0-1.5 log$_{10}$ reductions at 0.01% and 0.1%, and 0.5-1.0 log$_{10}$ reductions at 1.0%; E. Results for Green colorant: Melon color-L : 1.0-1.5 log$_{10}$ reductions at 0.01%, and 0.5-1.0 log$_{10}$ reductions at 0.1% and 1.0%; F. Results for Purple colorant: Grape color RCG: 0.5-1.0 log$_{10}$ reductions at 0.01%, 0.1% and 1.0%. Data were mean±SD. The results represented are from 5 independent experiments.
the food industry is about 0.1%. We therefore compared the bactericidal effect of UV-A-LED in the media prepared with each of the artificial colors at 0.1% (Fig 2). The level of the bactericidal effect varied, depending on the color used. At a concentration of 0.1%, the chemical structures of the flavonoid and carotenoid coloring agents are not thought to influence the bactericidal effect of UV-A. However, we performed an analysis of correlation and found a positive correlation between the log survival ratio and the absorbance of hydrophilic food colors at 0.1%, $r = 0.694$. No such correlation emerged from the analysis of other food colors (data not shown).

Outbreaks of illness associated with consumption of fruit juice have increased since the early 1990s. In response to epidemiologic investigations of these outbreaks, the U.S. Food and Drug Administration implemented process control measures for the production of fruit juice (Vojdani et al., 2008). Thus, the capacity of UV-A-LED light to kill *E. coli* DH5α in different orange juice samples was checked. The bacterial suspension was adjusted to a concentration of 10^5 CFU per milliliter. At 126 J cm⁻² of UV-A irradiation, the disinfection efficiency reached 0.35 log₁₀ in Juice A and 1.58 log₁₀ in Juice B (Fig. 3). The disinfection efficiency of UV-A-LED in these two kinds of commercially available orange juice was lower than in the positive control (PBS), a transparent colorless liquid, in which a log₁₀ reduction between 2.5 and 3.0, and differed between juice samples (Fig. 3).

It has been reported that exposing drinking water in polyethylene terephthalate (PET) bottles to sunlight (ca. 6h) kills enteric bacteria present in the water. The two primary causes for bacterial disinfection in

**FIG. 2.** Comparison of the disinfection efficiency of UVA-LED in LB broth containing different food colors at 0.1% concentration. The bactericidal effect varied with the artificial color. A. Carthamus yellow No.2SFU, B. Sun red® No.2L, C. Grape juice color; sun red® GRF, D. Red cabbage color: sun red® RFCU, E. β-carotene: carotene base No.35468, F. Marigold base No.33380, G. Paprika base No.34007, H. Sun clear paprika No.34592, I. Green colorant: Melon color-L, J. Purple colorant: Grape color RCG. Data were mean±SD. The results represented are from 5 independent experiments.

**FIG. 3.** Comparison of the disinfection efficiency of UVA-LED in different commercially available orange juice samples. The bactericidal effect varied with the type of orange juice. Data were mean±SD. The results represented are from 5 independent experiments.

This method are believed to be mild heat and UV-A light (Wegelin et al., 1994). Also, the sensitivity of *E. coli* to thermal, UV-A and solar disinfection methods is determined by the specific growth rate (Berney et al., 2006). In this study we found that a UV-A-LED sterilization system can kill bacteria in a colored solution made of artificial food colors, and in liquids including other compounds. This disinfection effect was significantly greater, however, in a transparent colorless liquid, which suggests that additional sterilization methods, or methods combined, should be used to ensure the safety of colored liquids.

The colored compounds may decrease the efficiency of the UV-A-LED in several ways. First, the absorbance band of the food color may overlap with the ultraviolet emission peak (365nm), and thereby decrease the effective dose on the bacteria. Although not all of the coloring compounds show a large attenuating effect, this factor may significantly influence the bactericidal action of UV-A-LED in a solution of a hydrophilic pigment.

Second, several researchers have shown that UV-A induces the production of the intracellular reactive oxygen species (ROS) (Sander et al., 2004, Cooper et al., 2009). As previously reported, the UV-A-LED-induced reactive oxygen species are central to the bactericidal effect (Hamamoto et al., 2007). Some carotenoids, including beta-carotene, quench highly reactive singlet oxygen under certain conditions and can block free radical-mediated reactions (Bendich and Olson, 1989). Studies also confirm the antioxidant activity of flavonoids from fruits and plants, for example, banana and Bidens tripartite (Wolniak et al., 2007, Vijayakumar et al., 2008). There is a possibility that the antioxidant activity of some food colors inhibited the oxidative effects of the UV-A-LED-induced
reactive oxygen species.

Finally, the pigments and other particles suspended in the liquid may reflect and scatter the radiation from the UV-A-LED. Commercially, these artificial colors are provided in lipophilic emulsified forms, i.e., colloidal dispersions in which particle size is sufficiently large to significantly decrease the disinfection efficiency.

In conclusion, we have shown that a UV-A-LED sterilization system can eliminate bacteria in colored solutions containing artificial food colors, and in liquids that include other compounds. The disinfection efficiency is lower in these preparations, however, than in a transparent, colorless liquid. Hence additional sterilization methods, or a combination of methods, should be used to ensure the safety of colored liquids.

REFERENCES


