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Pulsed UV laser light on *Escherichia coli* and *Saccharomyces cerevisiae* suspended in non-alcoholic beer

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ABSTRACT

Background: The aim of this study was to investigate the effect of pulsed ultra-violet (UV) irradiation on inactivation of beer spoilage microorganisms. UV irradiation is nowadays cost effective enough to compete with traditional biological, physical, and chemical treatment technologies and has become an alternative to such methods.

Material and Methods: Photoinactivation effects of pulsed UV laser with the wavelengths of 355 and 266 nm, which inactivate typical prokaryotic (*Escherichia coli*) and eukaryotic (*Saccharomyces cerevisiae*) microorganisms, were examined with different doses and exposure times.

Results: A dose of 100 J/cm^2 of the 355 nm pulsed UV laser was able to reduce about 1 to 2 log (88.75%) of *E.coli* with the population of 1.6×10^8 colony-forming units (CFU/ml), and 97% of 3.2×10^7, 3×10^6, 5.5×10^5, and 9×10^4 CFU/ml. In the case of 266 nm, more than 99% reduction in *E. coli* serial dilutions was inactivated, using 10 J/cm^2 with exception of 7×10^4 CFU/ml which was not detected any bacterial growth using 5 J/cm2. In addition, 50, 40, and 20 J/cm^2 energy were used successfully to inactivate *S. cerevisiae* at the populations of 5.4×10^6, 7×10^5, 5×10^4 and 4×10^3 CFU/ml, respectively.

As a result, pulsed UV Laser with 266 nm was strong enough to inactivate a high titer of bacterial and yeast indicator standards suspended in non-alcoholic beer in comparison with 355nm doses.

Conclusion: Results indicate that pulsed UV technology, in principle, is an attractive alternative to conventional methods for the inactivation of indicator microorganisms and has potential in irradiation of unpasteurized beer.

Keywords: Pulsed UV laser light, Inactivation, *E. coli*, *S. cerevisiae*, and non-alcoholic Beer

NTRODUCTION

The effects of contamination range from comparatively minor changes in beer flavor and fermentation performance to gross flavor defects and super-attenuation of worts (1). Beer is a relatively hostile environment to many microorganisms. The antiseptic properties of hop compounds are increased by ethanol. Low redox and acid pH provide additional protection against many potential spoilage microorganisms. Ethanol is a powerful inhibitor of microbial growth, but low and zero alcohol beers have a much increased susceptibility to spoilage compared to their alcoholic counterparts. Several bacterial and some yeast species are capable of growth in beer. This can cause the formation of hazes, surface pellicles and many undesirable changes in beer flavor and aroma (1).

Sterility is traditionally achieved by pasteurizing the beer in the bottle after filling, (tunnel pasteurization), or before filling (flash pasteurization or sterile filtration) (2). A new sterilization technique based on the use of pulsed UV irradiation is suggested to have great potential in the development of a new method of sterilization (3). In addition, FDA has given premarket approval to use UV radiation for

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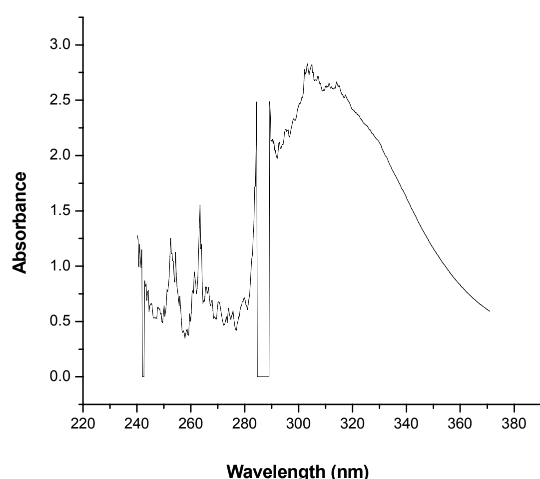

Fig. 1. Non-alcoholic beer absorption in the UV region, Distilled water was adjusted as a blank sample in order to obtain beer absorption.

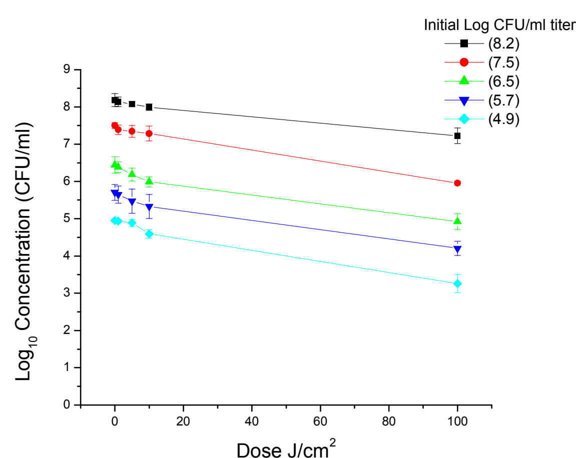

Fig. 2. Log_{10} CFU/ml survival curves of *E. coli* at 355-nm wavelength of pulsed UV laser.

the treatment of water and food including juices, and to achieve a 5-log reduction in numbers of the most resistant microorganisms, under specific conditions of use (4-6).

In this study, the photoinactivation property of pulsed UV laser radiation at wavelengths of 355 and 266 nm, used as a physical means to inactivate two typical microorganisms, prokaryotic (*Escherichia coli*) and eukaryotic (*Saccharomyces cerevisiae*), with respect to dose and exposure times, was examined. Furthermore, the effectiveness of UV laser treatments with respect to their doses and exposure times was determined.

MATERIAL AND METHODS

Laser operation. A Q-switched Nd:YAG laser (NL 301G, EKSPLA) was used for experimental realization. The diameter of the beam was 6 mm, and pulse duration was 5 ns, with a repetition rate of 10 Hz, which comes with third and fourth harmonics; 355 nm, 266 nm. The output energy of the laser at the third and fourth harmonics, adjusted to 80% of its maximum energy, were 60 & 10 mJ/pulse, respectively.

Preparation of Test Microorganisms Suspension. *Escherichia coli* (K12) was grown under optimal conditions in Nutrient broth (Merck), at 37°C, in a shaker to ensure sufficient cell density. An 18 hour culture was used for experimental purposes to mimic environmental conditions. The cell suspension was centrifuged and the supernatant removed. The pellet was resuspended in non-alcoholic beer (Behnoosh Inc. Iran) to obtain an *E. coli* concentration of approximately 1.5×10^8 Colony- Forming Unit (CFU/ml) as determined by 0.5 McFarland standard and spectrophotometric assays. Serial dilutions of 10^8, 10^7, 10^6, 10^5, and 10^4 CFU/ml of tested microorganisms were prepared. The samples were mixed thoroughly, and 150 µl of each dilution was poured into sterilized quartz tubes for radiation experiments. After exposure, 100 µl of irradiated and nonirradiated (control) cells were cultured on MacConkey Agar (Merck). CFU number was determined by pour plate method after incubation at 37°C for 72 hours.

Saccharomyces cerevisiae, wild-type (commercial bakery yeast) strain, was cultivated in yeast extract-peptone-dextrose broth at 37°C for 14 to 18 hours. The cell suspension was centrifuged and pellet resuspended in non-alcoholic beer to obtain a concentration of approximately 1.5×10^6 CFU/ml as determined by 0.5 McFarland standard and spectrophotometric assays. Serial dilutions of 10^6, 10^5, 10^4, and 10^3 CFU/ml were used for the irradiation experiment. Treated and untreated samples were then plated on peptone dextrose agar (Merck). CFU number was determined by pour plate method after incubation for 72 hours at 37°C.

Sample irradiation. Quartz tubes with an inner diameter of 5 mm were used for irradiation of microorganisms. Absorption of non-alcoholic beer was obtained by spectrophotometer (Ocean Optics HR4000, Florida, USA) in the UV region, which was 0.753 at the 266 nm and 0.962 at the 355 nm wavelength (Fig. 1). Continuous stirring kept the

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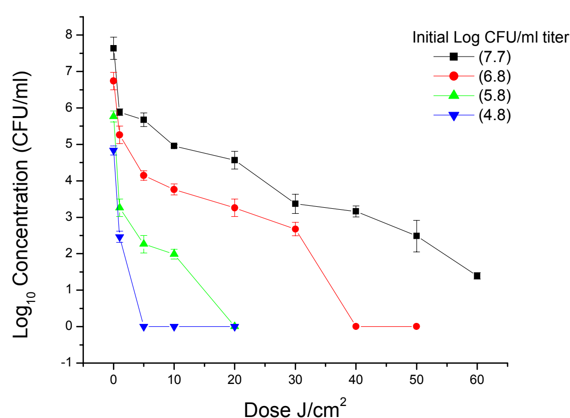

Fig. 3. Log_{10} CFU/ml survival curves of *E. coli* at 266-nm wavelength of pulsed UV laser.

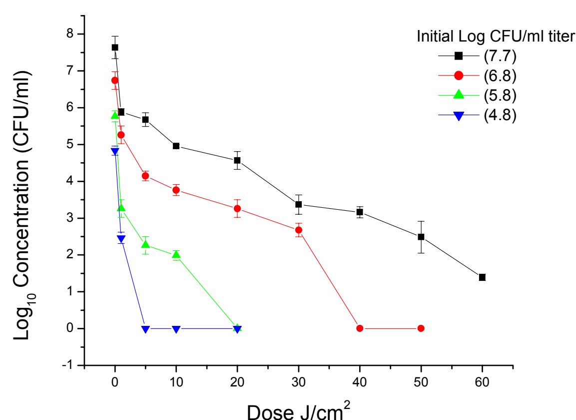

Fig. 4. Log_{10} CFU/ml survival curves of *S. cerevisiae* at 266-nm wavelength of pulsed UV laser.

cells suspended and obtained good homogeneity, and the speed of the magnet was well adjusted to avoid turbidity.

A laser beam, 6-mm diameter, was adjusted to equal the height of the cell suspension in the quartz tubes to cover the entire solution. Samples were exposed UV radiation, 0 to 100 J/cm^2, at room temperature (20 - 25°C), and the repetition rate was 10 Hz.

The dose for each microorganism was determined by maintaining exposure intensity while varying exposure time. For positive controls, identical samples were incubated for the duration of the exposure time at room temperature. Concentration of test microorganisms were expressed as CFU/ml in log-10 manner and viable organisms were counted before and after irradiation. Exposed and non-exposed samples were maintained at 4°C until enumeration. The time intervals between completion of irradiation and incubation of microorganisms were < 20 minutes. Each experiment was repeated three times, and the average values are presented in the results section.

Statistical Analysis. Each experiment was performed three times, and the average and SD of the mean are presented for each sample. Statistical analysis (general linear model analysis of variance [ANOVA]) was performed using SPSS software (standard version 9.0; SPSS, Chicago, IL) for each experimental condition.

RESULTS

The log reductions (survival curve) for each microorganism were plotted as a function of pulsed UV dose for each wavelength and are presented in Fig. 2 to 4.

***E. coli* Inactivation Curves.** As shown in Fig. 2, a dose of 100 J/cm^2 of the 355 nm pulsed UV laser was able to reduce an *E. coli* population of 1.6×10^8 CFU/ml by about 1 log (88.75%), and this decrease was increased to about 2 log (97%) for populations of 3.2×10^7, 3×10^6, 5.5×10^5, and 9×10^4 CFU/ml. However, using similar doses, higher inactivation rates in different populations could not be achieved. Independent of cell density and when using 10 J/cm^2 energy, more than 50% reduction in cell numbers was observed in bacterial populations of 3×10^6, 5.5×10^5, and 9×10^4 CFU/ml. Nonetheless, the log reductions at higher cell densities were not the same as above results and remained at 37.5%.

As shown in Fig. 3, when using 266-nm wavelength, and a dose of 10 J/cm^2, more than 3 log inactivation was detected in *E. coli* 5×10^7, 6×10^6, and 6×10^5 cell numbers, however, in cell density of 7×10^4 CFU/ml no bacterial growth was observed using 5 J/cm2 energy dose. A 5 log reduction (99.999%) of *E. coli* with population of 5×10^7 was achieved with the dose of 50 J/cm2. Other *E. coli* populations, 6×10^6 and 6×10^5 CFU/ml were completely inactivated using 40 and 20 J/cm^2, as no bacterial growth was observed in pour plate method after 72 hours.

***S. cerevisiae* Inactivation Curves.** Due to the fact that irradiation of *E. coli* at 355 nm was not able to inactivate the 5-log of bacterial population, in reference to new rules which are needed (6-8), irradiation of *S. cerevisiae* at 355 nm wavelength was

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not performed further.

As shown in Fig. 4, a 30 J/cm^2 energy dose at 266 nm resulted in 4 log of inactivation of *S .cerevisiae* at cell densities of 5.4×10^6 (99.999%), 7×10^5 (99.99%), and 5×10^4 (99.97%) CFU/ml. Increasing the energy dose to 20, 40, and 50 J/cm^2 resulted in full inactivation of *S. cerevisiae* population at cell density of 5.4×10^6, 7×10^5, and 5×10^4 CFU/ml yeast, respectively.

However, a 20 J/cm^2 energy dose of pulsed UV killed all cells at a density of 4×10^3 CFU/ml. General linear model ANOVA was employed to statistically analyze the results, which indicated that the tested microorganisms showed significant effects for different dose levels and within different populations; however, when microorganisms exposed to low -dose levels, i.e., 355 nm (≤ 10 J/cm^2), the inactivation rates were not significant. The inactivation rates at 266-nm wavelength were statistically significant both within and between different doses and microorganism populations ($P \leq 0.05$).

DISCUSSION

UV processing involves the treatment of foods with radiation from the UV region of the electromagnetic spectrum to inactivate microorganisms. UV treatments have been applied effectively to water supplies and food contact surfaces (9), and to apple juice (10-12). Pulsed UV has a higher penetration depth and may be more effective than continuous UV light (13).

Monochromatic pulsed UV has been shown to inactivate bacteria in milk (14, 15), however, its effectiveness against the target microorganism for traditional pasteurization methods has not been evaluated in such fluids e.g., milk, beer, and fruit juices, so, equivalence to thermal pasteurization has not been known properly (6).

In this study, the pulsed UV irradiation effects on *E. coli* and *S. cerevisiae* were examined. A 355 nm laser dose of 100 J/cm^2 was sufficient to inactivate *E. coli* population of 3.2 ×10^7 CFU/ml by more than 1 log. Moreover, in parallel experiments, a lower-energy dose (60J/cm^2) at 266 nm wavelength significantly inactivated a larger population of *E. coli* (5×10^{7}CFU/ml) by more than 6- log. In addition, a lower population of *E. coli* was completely inactivated when an even lower energy dose (40 J/cm^2) was employed at 266 nm. Also, other *E. coli* populations (6×10^5 and 7×10^4 CFU/ml) were inactivated completely by 10 and 5 J/cm^2, respectively. These results are in agreement with previous studies (3, 16-18) using pulsed light. In this case, inactivation of *E. coli* was achieved at around 270 nm, whereas wavelengths above 300 nm were inefficient for inactivating *E. coli*. Also, the most significant and effective inactivation of *S. cerevisiae* at 5.4 × 10^6 CFU/ml was observed at 266 nm and 50 J/cm^2 energy dose. Other populations of *S. cerevisiae* (7 × 10^5, 5 ×10^4, and 4 × 10^3 CFU/ml), were completely inactivated with energy doses of 40, 40, and 20 J/cm^2, respectively. In these studies, a 7-log concentration of *E. coli* is almost similar to a 5- log concentration of *S. cerevisiae*. The difference in population between these two microorganisms might have been caused by their sizes because *S. cerevisiae* is 100 times larger than *E. coli*. The maximum population of the test microorganisms in this study was designed to be greater than that of untreated food, beverages, and running water. Therefore, the irradiation protocols outlined in this study were designed for the worst case.

The laser employed in our experiment was operated in pulse mode. It has been shown that high-peak pulse power has irreversible effects on DNA and produces active species, which can damage the genome and other intracellular components, causing lethal and sublethal effects (3, 14, and 15, 19-22). In contrast, Ougoma et al. (19) reported that the time required for photoreactivation is 1 to 3 hours. However, in our study, irradiation time was less than 10 minutes. Therefore, it could be concluded that the photoinactivation process in our study could not be revoked.

The data which is obtained from irradiation of *E. coli* and yeast indicate that, irradiation effect on yeast was not as dramatic as the effect on *E. coli*. As mentioned by Vasilenko (2001) (23), this is good for the commercial application on the laser treatment. The laser treatment can be used on all kind of beers that are not normally pasteurized or sterilized because the laser irradiation will not greatly affect yeast viability but will kill bacteria. So, it is important to have some living yeast in bottle conditioned beer to continue natural fermentation (23).

In summary, pulsed UV disinfection has many advantages over alternative methods. Unlike chemical biocides, UV does not introduce toxins or residues and does not alter the chemical composition, taste, odor or pH of the fluid being disinfected. This feature is especially important in the brewing and beverage

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industries where the beer needs to be kept free from contamination by gram negative bacteria, which can cause off-flavors and acidity and alter the chemical properties of the product or, in the case of brewing specifically, affect the fermentation process.

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REFRENCES

1. Briggs DE, Boulton CA, Brooks PA, Stevens R (2004) *Brewing Science and Practice*. Cambridge CB1 6AH, England: Woodhead Publishing Limited and CRC Press, LLC.
2. HYDE A. Plate Pasteurization for Keg and Smallpack. *The Brewer* 2000; 86: 248-250.
3. Takeshita K, Shibato J, Sameshima T, Fukunaga S, Isobe S, Arihara K, et al. Damage of yeast cells induced by pulsed light irradiation. *Int J Food Microbiol* 2003; 85: 151-158.
4. US FDA. Irradiation in the production, prcessing and handling of food. 21 CFR part 179. *Fed Regist* 2000; 65: 71056-71058.
5. US FDA. Hazard analysis and critical control point (HAACP): Procedures for the safe and sanitary processing and importing of juice. Final rule. *Fed Regist* 2001; 66: 6137-6202.
6. US FDA. Guidance for Industry, Juice HACCP Hazards and Controls Guidance. 21 CFR part 120. *Fed Regist* 2004; 69: 10051.
7. Koutchma T, Murakami E, Adhikari C. Summary: UV disinfection of juices. Food Safety Watch. *National Center for Food Safety and Technology* 2002; 13:4
8. Koutchma T, Keller S, Chirtel S, Parisi B. Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. *Innovative Food Science and Emerging Technologies* 2004, 5: 179-189
9. Sastry SK, Datta AK, Worobo RW. Ultraviolet light. *J Food Sci. Suppl* 2000; 65: 90-92.
10. Hanes DE, Worobo RW, Orlandi PA, Burr DH, Miliotis MD, Robl MG, et al. Inactivation of *Cryptosporidium parvum* oocysts in fresh apple cider by UV irradiation. *Appl Environ Microbiol* 2002; 68: 4168-4172
11. Quintero-Ramos A, Churey JJ, Hartman P, Barnard J, Worobo RW. Modeling of Escherichia coli inactivation by UV irradiation at different pH values in apple cider. *J Food Prot* 2004; 67: 1153-1156
12. Wright JR, Sumner SS, Hackney CR, Pierson MD, Zoecklein BW. Efficacy of ultraviolet light for reducing *Escherichia coli* O157:H7 in unpasteurized apple cider. *J. Food Prot* 2000; 63: 563-567.
13. Krishnamurthy K, Demirci A, Irudayaraj J. Inactivation of *Staphylococcus aureus* by pulsed UV-light sterilization. *J Food Prot* 2004; 67: 1027-1030.
14. Smith WL, Lagunas-Solar MC, Cullor JS. Use of pulsed ultraviolet laser light for the cold pasteurization of bovine milk. *J Food Prot* 2002; 65:1480-1482.
15. Krishnamurthy K, Demirci A, Irudayaraj JM. Inactivation of *Staphylococcus aureus* in Milk Using Flow-Through Pulsed UV-Light Treatment System. *J Food Prot* 2007; 72: 233-239.
16. Sosnin EA, Lavrent'eva LV, Yusupov MR, Masterova YV, Tarasenko VF (2002) Inactivation of escherichia coli using capacitive discharge excilamps. 2nd International Workshop on Biological Effects of Electromagnetic Field, Rhodes, Greece, pp 384-388.
17. Wang T, Macgregor SJ, Anderson JG, Woolsey GA. Pulsed ultraviolet inactivation spectrum of *Echerichia coli*. *Water Res* 2005; 39: 2921-2925.
18. Karimi Azar DaryaniM, Massudi R, Hosseini M. photoinactivation of *Escherichia coli* and *Saccharomyces cervisiae* suspended in Phosphate- Buffered Saline- A using 266- and 355-nm Pulsed Ultraviolet Light. *Curr Microbiol* 2008; 56: 423-428.
19. Oguma K, Katayama H, Ohgaki S. Photoreactivation of *Escherichia col* after low- or medium-pressure UV disinfection determined by an endonuclease sensitive site assay. *Appl Environ Microbiol* 2002; 68: 6029-6035.
20. Oppezzo OJ and Pizarro RJ. Sublethal effects of ultraviolet A radiation on *Enterobacter cloacae*. *J Photochem Photobiol B* 2001; 62: 158-165
21. Petin VG, Kim JK, Rassokhina AV, Zhurakovskaya GP. Mitotic recombination and inactivation in *Saccharomyces cerevisiae* induced by UV-radiation (254 nm) and hyperthermia depend on UV fluence rate. *Mutat Res* 2001; 478: 169-176.
22. Rice JK and Ewell M. Examination of peak power dependence in the UV inactivation of bacterial spores. *Appl Environ Microbiol* 2001; 67: 5830-5832.
23. Vasilenko V. The pasteurization effect of laser infrared irradiation on beer. *MBAA TQ* 2001; 38: 212-215.

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