Ann Ig 2023 Jul-Aug; 35(4): 403-412 doi: 10.7416/ai.2022.2550. Epub 2022 Dec 8.

Disinfecting Slush Machines by an Innovative Near Ultraviolet Light Emitting Diode (UV LED) Technological System

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Key words: nUV system, UV technology, Food control, Food contamination, Foodborne disease
Parole chiave: Tecnologia nUV, controllo degli alimenti, contaminazione alimentare, malattie trasmesse dagli alimenti

Abstract

Background. *Microbial contamination of food and beverages is a topic of great interest. The most innovative technologies take advantage from UV light. This study aimed to evaluate a possible configuration of a nUV LED device at a wavelength of 405 nm installed on slush machines in order to reduce the microbial contamination.*
Study Design and Methods. *The study was conducted in the Department of Molecular and Developmental Medicine, University of Siena, Italy. A nUV LED device with 408 nm wavelength was installed and used on the slush machines. The inner walls of the machine tanks were fouled with contaminated slush, to evaluate the effectiveness of nUV radiation in reducing microbial contamination over time.*
Results. *Experiment results on the slush machine showed a statistically significant logarithmic microbial reduction, in relation with the distance from the nUV LED light source. It has also been shown that the reduction of microbes is possible with a proper management of some parameters: the exposure time, the power and wavelength of the light source, the distance and the obstacles between the light source and the target to be irradiated.*
Conclusion. *To reduce the incidence of foodborne diseases it is necessary to take all necessary precautionary measures, and the use of nUV technology has proved to be a crucial element in achieving this goal.*

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Annali di Igiene : Medicina Preventiva e di Comunità (Ann Ig)
ISSN 1120-9135 https://www.annali-igiene.it

Introduction

Concern about food contamination is a topic of wide and growing interest. Foodborne illnesses are a public health concern around the world (1). Symptoms resulting from bacterial food contamination include nausea, vomiting, and abdominal cramping with or without diarrhea. If significant fluid is lost, it can lead to dehydration and hypotension (2), a dangerous situation especially for the elderly. It is necessary to ensure adequate cleanliness of food preparation equipment to prevent "weak points" during food preparation that lead to the occurrence of food contamination. Even in slush machines, if proper hygienic conditions do not exist, a favorable environment can be created for bacterial proliferation that can be harmful to health. Contact between food and external machine surfaces can result in the transmission of bacterial species or spores that can cause illness in the consumer (3). Slush is a food similar to ice cream, and there are numerous studies in the literature showing foodborne disease from ice cream (4, 5). Slush, owing to intrinsic chemical and physical characteristics (pH, activity water, nutrient content), represents a substrate that allows microbial growth even if stored at low temperatures. Additional potential hazards are represented by secondary contaminations due to contact with inadequately sanitized containers, tools or machines, or to microorganisms transmitted by the operators (6). Blue-violet visible light at a wavelength of 405 nm (nUV, Near UV) has germicidal properties as it is absorbed by bacterial porphyrins, producing oxygen radicals and therefore bacterial cell death (7). Studies show that nUV at 405 nm inactivate numerous bacterial pathogens commonly associated with hospital-acquired infections, belonging to both gram-positive and gram-negative species (8).

Light-Emitting Diodes (LEDs) are an alternative source of ultraviolet light suitable for applications in the food industry (9, 10). Another advantage is the average lifetime of LEDs, which is longer than UV lamps currently used in industry and the heat emissions of LEDs are lower than those of UV lamps. Considering that the cost of LEDs has recently decreased due to technological advances, energy efficient and safe, LEDs are suitable for food applications (11).

The effectiveness of UV technology in reducing bacterial contamination on food is widely reported in the literature (12-15) and its use has been approved by the Food and Drug Administration as an effective method of inactivating the replication of pathogens and pernicious microorganisms in food, water and beverages (16). UV disinfection is a physical-free process, generates no by-products and barely affects the nutritional values of food, and does not present any risk of overdose (17). Furthermore, there are already studies in the literature that show its ability to reduce the microbial load in foods similar to slush such as juices (18, 19). A new process that involves UV technology is the photodynamic inactivation (PDI), a non-thermal photophysical and photochemical reaction that requires visible light, particularly in the 400–430 nm wavelength range and photosensitizers such as porphyrin molecules in the presence of oxygen (20, 21).

Although HACCP (Hazard Analysis Critical Control Point) regulation helps to reduce the probability of contamination by pathogenic micro-organisms in food and/or the production of toxins by them, the number of episodes of food-borne diseases continues to increase in most industrialised countries (22). Depending on this, any procedure that could help to improve the situation must be analysed.

The slush is a beverage consisting of crushed ice and coffee or fruit syrups, widely consumed especially during the summer season. This study aims to evaluate the functionality of a nUV LED system installed on slush machines in reducing the risk of microbial contamination of the drink.

Material and Methods

Setting

This cross sectional study, with an experimental pre-post design, was conducted between November 2019 and July 2020 at the Department of Molecular and Developmental Medicine, University of Siena, Italy.

1. The nUV system

A printed circuit board (PCB) equipped with 4 nUV LEDs having a wavelength peak at 408 nm, (Liteon LTPL-C034UVG405-PA 405 nm) was engineered and installed on the back of the tanks of 2 slush machines, provided by the company "SPA Drink System" (Figures 1-2). The slush machine consists of a large transparent tank (20x58x87 cm) into which the ingredients are placed and mixed by a spiral paddle to obtain the final product. The tanks are insulated to minimise the influence of external temperature and closed by a lid at the top. The instrument is equipped with a water cooling system and a mechanical keyboard to manually set the defrosting and cooling mode. A plastic tap at the front of the tool allows the slush to be collected at the end of its processing.

Each slush machine was equipped with two PCBs to irradiate mainly the side and front faces of the slush tank (Figure 3). For this purpose, the PCBs were slightly inclined forward and angled towards the side walls, allowing the light to be distributed from the top to the bottom of the entire canister. In order to measure in a heterogeneous way the biocidal capacity of the light, three spots of the canister were taken as reference: two on

Figure 1 - SP1 Slush machine front view. In red, the inner front sections of the tank spread with contaminated slush.

Figure 2 - SP1 Slush machine lateral view. In red, the inner lateral sections of the tank spread with contaminated slush. The inner section of the tank was contaminated using a special stamp to facilitate the adhesion of the contaminated slush to the walls.

Figure 3 - Slush machine disinfection system. The nUV light sources (yellow LEDs) were mounted on an angled PCB holder to uniformly irradiate the inner and front sections of the tank. Light uniformity was also optimised by positioning the LEDs on the PCB.

each of the side faces, at 10 cm and 20 cm from the light source (Figure 2), and one at the frontal level, at 35 cm from the light source (Figure 1); on these points, all just above the cylinder that moves the slush at about 2/3 from the highest part of the canister, the irradiances were measured. The measurement points were taken that way in order to simulate the conditions in which the product in the canister is running low and a refill is needed. In addition, to verify the proper functioning of the slush machines and the mounted nUV light sources, for five days (before starting the microbiological experiments) four different operators tasted and checked a slush batch constantly exposed to nUV radiation.

2. Microbiological experiments

The inner walls of the machine tanks were fouled with contaminated slush, to evaluate the effectiveness of nUV radiation in reducing microbial contamination over time. A total of two slush machines were used during the study: in the first, the tank walls were contaminated and the nUV PCB kept on for the duration of the experiment, while in the second machine, used as a positive control, the walls were contaminated and the PCB kept off all the time. Five different bacterial strains were selected for the following experiments: *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212) and *Clostridium perfringens* (ATCC 13124). Several conditions were established for testing: i) three distances (10, 20 and 35 cm) from nUV light sources, ii) one bacterial inoculum concentration for each microbe, and iii) three exposure times.

Each strain was plated on a solid medium and incubated in a thermostat at 36.5 °C overnight. Subsequently, some colonies were diluted in phosphate-buffered saline (PBS) and measured on the turbidimeter to achieve a 1.5×10^9 CFU/mL concentration. From the original inoculum 100 μL were transferred to 100 mL of slush (approximately 10^6 CFU/mL). The slush solution was prepared aseptically in a biological hood according to the recipe on the package (1 kg powder + 1 L trifiltrated water).

The contaminated slush was sprinkled at selected spots (10 cm, 20 cm, and 35 cm from the light source) on the inner surface of the machine tank (approximately 1 mL of slush spread on each spot), using a special stamp covered with sterile gauze, and allowed to dry for approximately 15 min. Subsequently, the nUV reactor was turned on in either machine, and at 2, 4, and 6 hr, sampling was performed at the different spots on the side and middle walls of the exposed and unexposed tanks (positive control), using contact plates with Plate Count Agar (PCA, Oxoid Ltd, Basingstoke, United Kingdom) as growth medium.

At the end of exposure, all nUV light-treated samples and their respective positive controls were incubated at 36.5 °C (anaerobic microorganisms were placed in a sealed jar with a CO_2 generator) and counted with a manual colony counter after 48 hours. The experiment was repeated in triplicate for each bacterial strain. The percentages of reduction of each microorganism were calculated from the logarithmic reduction of CFU of the exposed samples compared with that of the positive controls.

Statistical analyses were carried out using Stata Ver 14 software. Final results were expressed as percentage and logarithmic reduction mean with 95% confidence interval.

Results

The irradiance measured in the 3 sampling spots at 10, 20 and 35 cm was respectively 1.8, 1.1 and 3.3 mW/cm^2.

Experiment results on the slush machine showed a statistically significant logarithmic microbial reduction for all five strains, in relation to the distance from the nUV LED light source (Tables 1-5). The highest level of reduction was achieved with *P. aeruginosa* at 6 hr of exposure, at all distances tested. The light source exposure produced a bacterial inactivation effect of about 4 to 6 $\log_{10}$ at 6 hr, even at 35 cm distances, for all five strains (with the only exception of *E. coli*). The most significant level of abatement at 4 hr was achieved by *P. aeruginosa* and *E. coli*, reaching a mean logarithmic reduction of 6.48 $\log_{10}$ (99.9999% reduction) each, even at the most distant spot from the light source (35 cm). At 2 hr, the reduction effect is evident for only 2 of the 5 bacteria: for *P. aeruginosa*, the maximum abatement was achieved after 2 hr at 20 cm from the nUV source (mean reduction of 6.48 $\log_{10}$); for *C. perfringens*, the maximum level of decontamination was observed at a distance of 10 cm, with a mean reduction of 4.09 $\log_{10}$ (99.99% reduction). For the remaining microbes, no significant reduction in microbial concentration could be observed after 2 hr of exposure.

Table 1 - *Staphylococcus aureus* ATCC 43300 CFU/mL logarithmic reduction after nUV exposure

Distance	2 hours Log_{10} reduction		4 hours Log_{10} reduction		6 hours Log_{10} reduction	
	mean	95% IC	mean	95% IC	mean	95% IC
10 cm	0.00	0.00 – 0.00	4.70	4.04 – 5.35	5.42	3.35 – 7.49
20 cm	0.00	0.00 – 0.00	4.30	4.04 – 4.56	5.32	3.06 – 7.59
35 cm	0.00	0.00 – 0.00	5.05	3.77 – 6.32	5.44	3.40 – 7.48

Table 2 - *Escherichia coli* ATCC 8739 CFU/mL logarithmic reduction after nUV exposure

Distance	2 hours Log_{10} reduction		4 hours Log_{10} reduction		6 hours Log_{10} reduction	
	mean	95% IC	mean	95% IC	mean	95% IC
10 cm	0.00	0.00 – 0.00	6.48	6.48 – 6.48	5.18	2.56 – 7.72
20 cm	0.00	0.00 – 0.00	1.52	0.00 – 4.51	2.14	0.00 – 6.32
35 cm	0.00	0.00 – 0.00	0.00	0.00 – 0.00	0.00	0.00 – 0.00

Table 3 - *Pseudomonas aeruginosa* ATCC 27853 CFU/mL logarithmic reduction after nUV exposure

Distance	2 hours Log_{10} reduction		4 hours Log_{10} reduction		6 hours Log_{10} reduction	
	mean	95% IC	mean	95% IC	mean	95% IC
10 cm	0.00	0.00 – 0.00	6.48	6.48 – 6.48	6.48	6.48 – 6.48
20 cm	6.48	6.48 – 6.48	6.48	6.48 – 6.48	6.48	6.48 – 6.48
35 cm	5.74	4.29 – 7.19	6.48	6.48 – 6.48	6.48	6.48 – 6.48

Table 4 - *Enterococcus faecalis* ATCC 29212 CFU/mL logarithmic reduction after nUV exposure

Distance	2 hours Log_{10} reduction		4 hours Log_{10} reduction		6 hours Log_{10} reduction	
	mean	95% IC	mean	95% IC	mean	95% IC
10 cm	0.00	0.00 – 0.00	3.81	3.48 – 4.13	3.70	3.53 – 3.87
20 cm	0.00	0.00 – 0.00	1.51	0.00 – 4.46	3.64	3.36 – 3.91
35 cm	0.00	0.00 – 0.00	3.42	3.41 – 4.43	3.97	3.71 – 4.23

Table 5 - *Clostridium perfringens* ATCC 13124 CFU/mL logarithmic reduction after nUV exposure

Distance	2 hours Log_{10} reduction		4 hours $Log_{1}0$ reduction		6 hours Log_{10} reduction	
	mean	95% IC	mean	95% IC	mean	95% IC
10 cm	4.09	3.99 – 4.19	5.53	5.43 – 5.62	5.43	3.39 – 7.48
20 cm	3.51	3.48 – 3.54	4.31	4.21 – 4.41	4.75	2.90 – 6.61
35 cm	3.67	3.59 – 3.75	4.08	3.12 – 5.05	4.53	4.39 – 4.68

Discussion

Blue light with a wavelength of 405 nm has been shown in numerous studies to be effective in reducing bacterial load, proving to be a promising strategy with the potential for numerous applications (23-25). Our study shows one of the possible applications of this innovative technology in the food industry.

Other studies have already analysed with positive results the effectiveness of nUV at 405 nm in reducing the bacterial load of food (26-28), thus minimizing the risk of foodborne infections (3, 29). It has been shown that the intensity of UV treatment must be reduced to a minimum to prevent loss of quality as an undesirable effect (30). The use of nUVs reduces these risks, although the time required for an appreciable disinfection effect is longer. In our study we did not evaluate the change in nutritional and qualitative properties of the product; it was beyond the scope of the study. However, several experiences found that nUV does not diminish the organoleptic qualities of food (26, 31). In particular, a study by Towery et al. (31) analysed the effect of a narrow band of visible light of 405 nm on the colour, texture and organoleptic properties of cucumbers. The results showed that nUV light did not reduce the organoleptic qualities of the vegetables tested and appeared to be at least as acceptable organoleptically as other light decontamination methods. Concern about microbial food safety has increased dramatically as food contaminated with pathogenic bacteria can cause serious illness. For this reason, a tool that allows for a reduction in the overall battery charge of the slush would be useful. From these observations, we analysed the effectiveness of a nUV LED device in reducing the bacterial contamination of the drink in a slush machine.

The slush inside the tank is not uniformly subjected to the same temperature: in the centre of the machine the temperature is lower, while towards the walls the temperature is higher. The latter situation is more critical because this difference in temperature creates potential favourable conditions for the growth of bacterial colonies, especially in Summer, when the temperature outside is significantly higher. The inner wall of the slush, especially during the emptying of the tank, becomes a weak point in maintaining the quality of the product; in fact, the substances contained in the slush: sugars, proteins and lipids, depending on the type of product, will be deposited on the inner face of the tank, will remain exposed to the air inside the chamber, potentially subject to contamination, as well as to the heat coming from outside and the humidity coming from inside the tank. The possibility of condensation on the walls and contamination from the air is therefore not negligible, as well as contamination of the lower part of the tank with the product. Being able to direct the UV light source specifically on the walls allows to disinfect these most critical points and at the same time to have less impact on the product itself.

We have performed voluntary contamination to see if there could be bacterial proliferation or killing despite nUV LED device. Our microbiological experiments on the slush machine showed that the higher bacterial inactivation effect is reached for all five strains at 6 hr, but already at 4 hr there is a significant reduction in the bacterial load of more than 97%. Our study showed that the greatest reduction in bacterial contamination was at the point closest to the LED (10 cm), but also at the point farthest from the light (35 cm) there was a significant reduction in microbial load, sometimes even greater than that obtained at shorter distances. This is hypothesized to be due to the angle of the nUV light, which arrives on the walls in a more tangent way than that which impacts on the front of the tank, the most distant, where it is direct and quantitatively greater as also showed by the irradiance measurement that was 3,275 $\mu W/cm^2$. The distance is compensated for by the amount of light.

The present work has also some limitations.

The experiments were conducted on a coffee flavored slush. The composition of the product could be different from others and their role could have a weight on microbial growth, however it is believed that the real difference could be more on water or milk-based products than on single products. In our case having tested coffee we provided the condition that should favour the best growth conditions. Another limitation of the study involves the possible adverse effects of nUVA on the organoleptic properties of slush. Nevertheless, several studies report minimal changes in taste and smell in products treated with nUVA radiation. In addition, to better verify this hypothesis, in a preliminary phase, 4 subjects tasted the slush and check changes in colour and odour for about 5 days of continue exposition to the light source. In our case we did not appreciated significant variations. It is possible that differences could exist with other flavours and ingredients that we did not test.

A possible undesired effect, that has not been carefully verified, is the possible deterioration of the product due to a long and repeated exposure to nUV rays; nevertheless, no visually perceptible alterations have been observed during the experimentation time, equivalent to the common permanence of the product in the tank (a few days). While this may be a limitation, it is also true that the results show that 4 hours are sufficient to reduce most of the contamination; therefore, the disinfection system should be designed not to work continuously, but for programmable shorter periods.

A limitation not attributable to our study itself, but to the technology, is the possible photobiological risk. In fact, LED light sources, although centered on a certain wavelength, in our case 405 nm, still have relevant light tails above and below that wavelength. According to EN 62471, it is important to manage the energy level to ensure safe technology. This factor can be monitored and controlled using special UV sensors to reduce it below safe limits, or LEDs with a wavelength of 415 nm, which have a similar disinfection capacity. In this way, the tail to the left of the peak does not fall within the UV spectrum, or at least the percentage of UV in the beam is so low that it is not associated with photobiological risks.This aspect must therefore be managed with appropriate measures such as: i) the preparation of a risk manual to minimize possible consequences, which indicates the precautions to be taken to avoid a prolonged and close presence of people in the vicinity of the irradiation LED sources; ii) the use of LEDs that have minimum tails below 400 nm; iii) the prevalent use of the irradiating system programmed at times of the day when the human presence in its vicinity is unlikely; iv) a reduction of the irradiating power during times of the day when there is the presence of people. These are all easily implemented conditions that make the use of the technology effectively safe.

Conclusions

Despite various protocols, foodborne illnesses continue to occur in most industrialized countries. Visible blue-violet light at a wavelength of 405 nm is a safe and viable source for applications in the food industry when used properly. Our study with an nUV LED device inside a slush machine has shown that the reduction of microbes is possible with a proper management of some parameters: the exposure time, the power and wavelength of the light source, the distance and the obstacles between the light source and the target to be irradiated. The study showed that a well-engineered nUV LEDs system can be a viable method to significantly reduce bacterial contamination on slush.

Acknowledgements: SPA Drink System srl financed the University of Siena, with G.M. as the principal investigator for conducing the tests. G.M. did not receive any personal funds for the research. G.M. and G.C. are the co-founders of the company egoHEALTH. EgoHEALTH received funds for the engineering of the nUV module. SPA drink system srl had no role in the test design, data collection or analysis, decision to publish, or preparation and discussion of the test results in the manuscript.

Riassunto

Disinfezione delle macchine per granite mediante un innovativo sistema tecnologico con diodi ad emissione di luce vicina all'ultravioletto (nUV LED)

Background. La contaminazione microbica di cibi e bevande è un argomento di sanità pubblica molto rilevante. Le tecnologie più innovative nel campo sfruttano la luce UV. Questo studio mira a valutare l'efficacia di un dispositivo LED nUV alla lunghezza d'onda di 405 nm installato su una macchina per granita al fine di ridurre la contaminazione microbica della bevanda.

Disegno e metodi dello studio. Lo studio è stato condotto presso il Dipartimento di Medicina Molecolare e dello Sviluppo, Università di Siena, Italia. Il dispositivo nUV LED con lunghezza d'onda specifica di 408 nm è stato utilizzato e installato sulle macchine per granita. Le pareti interne dei serbatoi della macchina sono state sporcate con materiale contaminato da batteri, al fine di valutare l'efficacia delle radiazioni nUV nel ridurre la contaminazione microbica nel tempo.

Risultati. I risultati dell'esperimento sulla macchina per granite hanno mostrato una riduzione microbica logaritmica statisticamente significativa, in relazione alla distanza dalla sorgente luminosa nUV LED. Hanno inoltre dimostrato che la riduzione dei batteri è possibile con una corretta gestione di alcuni parametri: il tempo di esposizione, la potenza e la lunghezza d'onda della sorgente luminosa, la distanza e gli ostacoli tra la sorgente luminosa e il bersaglio da irradiare.

Conclusione. Per ridurre l'incidenza di malattie a trasmissione alimentare è necessario adottare tutte le misure di precauzione necessarie; l'utilizzo della tecnologia nUV si è dimostrato essere un elemento determinante al raggiungimento di tale obiettivo.

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