Int.J.Curr.Microbiol.App.Sci (2024) 13(10): 50-58

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 13 Number 10 (2024)
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1310.007

Hurdle Technology Prototype to Reduce Microbial Load in Clarified Citrus Juice

Abhinav Sasi, N. K. Ahammed Zainy, Alan John, Ansa Shaji, V. V. Anujeslet, Jithin Mohan, Arya K. Salu*, Sasmila Bai and R. Ajay

VKIDFT, KVASU, Mannuthy – 680651, Kerala, India

**Corresponding author*

A B S T R A C T

Keywords

Hurdle technology, Clarified citrus juice, UV-Ozone-LED treatment

Article Info

Received:
10 August 2024
Accepted:
22 September 2024
Available Online:
10 October 2024

Hurdle technology is a food preservation approach that involves combining multiple preservation factors or hurdles to prevent the growth of microorganisms, enzymatic reactions, and other deteriorative processes in food. By employing several hurdles simultaneously, each set at sub-lethal levels, the overall effectiveness of preservation is enhanced. To enhance the microbial safety of clarified citrus juices by employing a combined treatment involving ultraviolet (UV) irradiation, ozone application, and light-emitting diode (LED) technology. Clarified citrus juices, known for their susceptibility to microbial contamination, pose challenges in maintaining microbial safety while preserving their fresh attributes. In this research, a multi-hurdle strategy was implemented, harnessing the synergistic effects of UV, ozone, and LED treatments. The UV component of the process aims to disrupt the DNA structure of microorganisms, hindering their ability to replicate. Simultaneously, ozone, a powerful oxidizing agent, contributes to the elimination of pathogenic microorganisms and spoilage agents. The incorporation of LED technology enhances the microbial reduction process by providing targeted light wavelengths that further contribute to microbial inactivation.

Introduction

Due to the many occurrences of unfortunate events because of the consumption of unhygienic or unsafe foods, more advanced technologies of food processing are evolving. The utilization of hurdle technology has gained its importance in food processing due to recent circumstances. Hurdle technology, according to Gordon and Williams (2017) is a process of application of a combination of technologies and approaches using which pathogens are eliminated or controlled and thereby ensuring the safety of foods for consumption, improving its quality, and extending its shelf life. It is also known as 'combined method technology'. A series of techniques known as hurdle technology is utilized to render microbes inert during the preservation of food. Hurdles are aspects of food preservation, such as temperature, pH, redox potential, water activity, preservatives, and competing microorganisms that must be coordinated to produce a specific level of food quality and stability. To meet consumer demands for quality, nutritional value, and product safety, traditional heat treatment (pasteurization) of fruit juices destabilizes the stability of the thermolabile BAC molecules. As a result, the use of non-thermal technologies using the "hurdle concept" may be a promising solution. Together with the impacts of hurdle technology on homeostasis, stress reactions, metabolic exhaustion of bacteria, and multitarget preservation—all of which are crucial for food safety—these reactions are being studied as techniques of food protection.

Ozone is a powerful broad spectrum antimicrobial agent that is effective against bacteria, fungus, viruses, protozoa, and bacterial and fungal spores (Khadre *et al.,* 2001). According to Graham (1997) ozone was affirmed as Generally Recognized as Safe (GRAS) for use in food processing, when used in accordance with good manufacturing practices. When ozone passes through water it becomes aqueous ozone which has high vibrational force which have high effectiveness against microbes. The ozone microbicide action can be related to the damage of several cellular constituents including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelops, enzymes and nucleic acid in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids (Khadre *et al.,* 2001). The acidity and PH did not change due to the different treatments in apple juice samples. 5 mins ozone application slightly increased apple juices soluble solids content to 13.01% ($P<0.5$). During the colour analysis of the apple juice samples there was no significant difference between control and ozonated samples (Ari *et al.,* 2020).

UV-C ranging from 200-280 nm is very well known for its antimicrobial effect (Cappozzo *et al.,* 2015). The germicidal property of UV-C radiation is because pathogens absorb the ultraviolet light and thymine dimers are formed, blocking transcription and replication, which compromises cellular functions and leads to cell death (Sanganamoni *et al.,* 2017). UV-C light inactivates microorganisms by damaging their nucleic acid that absorbs UV light from 200-310 nm.

The primary mechanism of inactivation by UV is the creation of pyrimidine dimers which prevent microorganisms from replicating, thereby rendering them inactive and unable to cause infection (Koutchma, 2009). The use of UV light is well established for air and water treatment and surface decontamination. UV technologies were used for non-thermal processing of liquid foods, used to sterilize water and acceptable irradiation treatment for the processing of juice. It is also used for air treatment (Nicolau *et al.,* 2022). It was reported that vitamin D is photo-chemically altered by UV light. The highest destruction of riboflavin and beta-carotene (~50%) may be observed; however, in terms of vitamins C, B6, and A, only 16.6% to 11% of those vitamins destroyed after exposure to UV light (Koutchma, 2009). The reduction of 1.34 log, 4.29 log and 5.10 log after 30 minutes of UV treatment was reported for *S. cerevisiae, E. coli and L. innocua*. A 3 log reduction of *S.cerevisiae* and polyphenol oxidase was reported (Koutchma, 2009). 3.7- 3.8 and 6.4-5.6 log reductions *E. coli* in turbid fruit juice were observed.

Another form of non-thermal food processing method is pulsed LED treatment. Pulsed operating modes of light can remove hazardous germs in food and water and thus making LEDs very effective. Blue LEDs have effect on several metabolic pathways and accumulation of phenolic compounds, anthocyanin, etc (Taulavuori *et al.,* 2017). It is a novel technology that may be employed in a wide range of food processing applications, including the disinfection of solid and liquid food items (Poonia *et al.,* 2022). LEDs emitting visible light can excite light sensitive compounds, e.g., porphyrins, present in the bacterial cell wall. These excited compounds collide with, and transfer energy to, oxygen molecules, producing reactive oxygen species (ROS) such as hydroxyl radicals, hydrogen peroxide, and singlet oxygen. ROS further react with cellular components causing cell death (Ghate *et al.,* 2013).

LEDs have been applied to air disinfection, water treatment, surface decontamination and curing (Koutchma & Orlowska, 2012). Quality changes have been evaluated by Kim *et al.,* (2017) on the surface of fresh-cut 13 mango treated with a 405 nm LED to test its anti-bacterial effects against E. coli, *Listeria monocytogenes*, and Salmonella. There was no significant difference observed between treated and untreated mango in terms of color, antioxidant capacity, and ascorbic acid content regardless of the storage temperature.

By understanding the advantages of non-thermal hurdle technologies like UV-C, Ozone and LED treatments over the thermal processes, for the current study hurdles used are Ozone, UV-C and LED to reduce the microbial load in clarified citrus juice. The sample used to test the equipment efficiency by checking microbial count is clarified citrus juice.

Materials and Methods

Sample Required

Clarified citrus juice is taken as sample for the treatment. Cleaned citrus fruits are pulped in a juicer. The pulp obtained is then clarified using a muslin cloth which then undergoes treatment.

Proposed prototype design for hurdle technology using Ozone, UV and LED is shown in figure 1.

Prototype Designing

Electronics

- Ozone generator
- Pulsed Blue LED strip
- Arduino Uno clone (ADIY)
- 5V HL Relay
- Ultrasonic module SR04
- Solenoid valves (12V)
- UV Tube (11V, 12A)
- UV Choke

Non-Electronics

- SS Ozone chamber
- SS UV Chamber
- Spiral quartz glass

Outer Chamber

57cm (L) x 30cm (W) plywood box is used for the enclosure which is covered by 22 forex sheets. Forex sheets are suitable for long-term interior and exterior applications, and it has optimum mechanical properties and surface quality. It is also food contact approved. The plywood sheet is of 1cm thickness.

Figure 2 depicts the outer chamber. 2 holes are present in the outside enclosure box; one at the top to place sample funnel and one at the side to pass connection wire. The box is divided into top and bottom part. The top part holds the ozone chamber and UV-LED chamber while the bottom part of the box again has 2 parts.

One is to place an ozone generator and the one behind it is for circuit connections. The chamber is closed with a suitable plywood door.

Ozone Chamber

Food grade stainless steel bottle of capacity 500 ml is used as ozone chamber which is placed at the top of the equipment with the support of a bottle holder clamped to the upper left corner wall. There are 4 openings on the chamber which have different purposes. The opening at the centre of the top of the bottle is for inlet of the sample and the one at the centre of the bottom is to fix solenoid valve 1 through a food grade PVC flexible transparent pipe fixed to it using M-seal. Another opening at the side is to pass insulated silicon rubber tube from ozone generator for ozone treatment of the sample and the one at the other side is for exhaustion. Figure 3 shows the ozone chamber at the top and ozone generator at the bottom.

Ozone Generator

KENT vegetable cleaner (counter-top) is used as ozone generator. It is placed at the bottom of the outer chamber. The generator is a single phase 220-240V AC voltage with an ozone concentration of 200mg/hr. Figure 4 shows the main parts if the ozone generator. The main parts of ozone generator are:

- Main body
- Cable hanger
- Silicon rubber tube
- Plug
- Power switch
- Timing switch

The time settings of this ozone generator are 15 and 30 minutes.

UV-LED Chamber

Figure 5 & 6 shows the UV-LED chamber and LED strips inside the chamber respectively. Stainless steel UV chamber (19.5cm length and 5cm diameter) used in water filters are used here. It is placed on the top of an ozone generator on plywood in a slanting position clamped to it. Both sides of the chamber are closed using metal tins sealed to it using insulator. Both tin cans have openings at their sides; top one works as ozone treated sample inlet and the bottom one works as outlet of UV-LED treated sample. The inner walls of UV-LED chamber contain 8 strips of 12V pulsed blue LEDs stick to it. The +ve blue wire and -ve black wire of LED are soldered together in series. The chamber contains quartz glass spiral tube with a 11V 12A UV-C tube to UV treat sample. Figure 7 shows the image of quartz glass spiral tube. The specifications of quartz glass spiral tube are:

• Tube dimensions: ID 8mm X OD 10mm. • Spiral dimensions: ID 20mm X OD 40mm X Length 175mm
• At both ends open, clear tube.
• Inlet end- 20 mm
• Outlet end-10 mm
•Inlet and outlet should be perpendicular to the spiral tube.

Solenoid Valves

There are two 12V solenoid valves (V1 and V2), and these are used to control the flow of ozone treated sample from ozone chamber to UV-LED chamber. It is fixed to the left wall below ozone chamber, tighter using nylon cable tie. V1 controls the outlet of UV-LED treated sample from UV-LED chamber.

Circuit System

The power source of Ozone, UV and 12V are connected together at the bottom of the outer chamber. Relay, transistor and diode are the major components for AC &high power DC circuits.

Dot board 1

• For Ozone: Phase line enters from ozone generator to relay1 and comes out to the power source, neutral line directly towards power source.
• For UV: Phase line passes through relay 2.
• For LED: Positive line passes through relay 3.

Dot board 2

• For solenoid valve 1: Positive line passes through relay 4.
• For solenoid valve 2: Positive line passes through relay 5.

Control System

Arduino nano is a micro controller that controls a practical system using software. It is an Arduino classic breadboard friendly designed board with small dimensions. It comes with pin headers that allow for an easy attachment onto a bread board features a mini USB 26 connector. Figure 8 shows Arduino nano controller. The Arduino IDE is open-source software, used to write and upload code to Arduino boards. It supports the programming languages C and C++.

2 switches are placed on the outer right wall of equipment chamber in case of emergency. 1st switch is to control V1 and the 2nd one to control V2. The power supply for the switches is from 12V batteries placed inside the equipment chamber.

Proposed Prototype Analysis

First AC devices and Arduino nano are powered and the specific switches to run the programs are selected. Ozone generator is powered on and along with the working of the ozone, ensures the pretreatment of UV chamber.

Pour 13 ml sample into the ozone chamber as it is the capacity of quartz glass used. V1 and V2 will be opened after ozone treatment for a period of 8 seconds. After that sample will be occupied at the UV-LED chamber. Then UV-LED treatments take place for specific time. V1 & V2 will open simultaneously to remove air in the pipe for a period of 8 seconds and the juice can be collected out from V2. Simultaneously next batch is poured into the ozone chamber and the process continuous.

Results and Discussion

Sample was poured into ozone chamber through sample funnel where it was treated using ozone followed by UV-LED treatment in the UV-LED chamber. Citrus juice was hurdle treated using ozone, UV and LED in 3 different combinations. 1st sample was treated using a combination of ozone, UV and LED, each treatment for 2 min ($O_2U_2L_2$). 2nd sample was treated using a combination of ozone for 2 min, and UV and LED for 3 min each ($O_2U_3L_3$). 3rd sample used the combination of ozone, UV and LED each treatment for 3 min ($O_3U_3L_3$). Time combinations of treatments were determined after various trials of experimental analysis and their results.

Microbial analysis

Table 1 show the total mesophilic bacterial count. 5 times reduction in the microbial count is achieved after $O_3U_3L_3$ treatment of sample and hence can be considered more effective.

Table.1 Total bacterial count for various time combinations using hurdle technology**.**

Time combinations of treatment	pH value
Control	4.34×10^6
$O_2U_2L_2$	3.70×10^6
$O_2U_3L_3$	1.10×10^6
$O_3U_3L_3$	8.16×10^6

Table.2 pH values of samples for various time combinations using hurdle technology

Time combinations of treatment	pH value
Control	6.63 ± 0.01
$O_2U_2L_2$	6.64 ± 0.02
$O_2U_3L_3$	6.63 ± 0.02
$O_3U_3L_3$	6.64 ± 0.01

Table.3 Color values (L, a& b) of samples for various time combinations using hurdle technology

Time combinations	L	a	b
Control	51.07 ± 0.18	3.50 ± 0.06	43.49 ± 0.77
$O_2U_2L_2$	50.32 ± 0.02	2.98 ± 0.04	41.25 ± 0.13
$O_2U_3L_3$	49.57 ± 0.06	2.07 ± 0.02	40.33 ± 0.07
$O_3U_3L_3$	49.65 ± 0.04	3.06 ± 0.03	439.65 ± 0.05

Figure.1 Proposed prototype design for hurdle technology using Ozone, UV and LED**.**

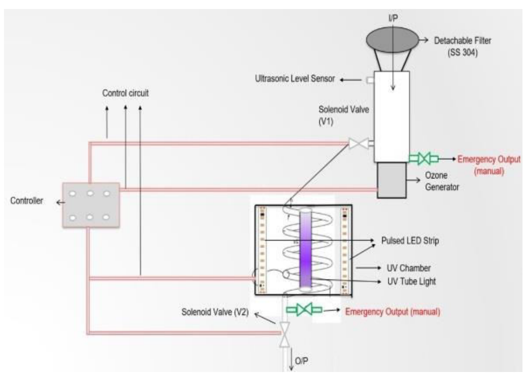

Figure.2 Outer Chamber for proposed design

Figure.3 Ozone generator along with ozone chamber

Figure.4 Parts of ozone generator.

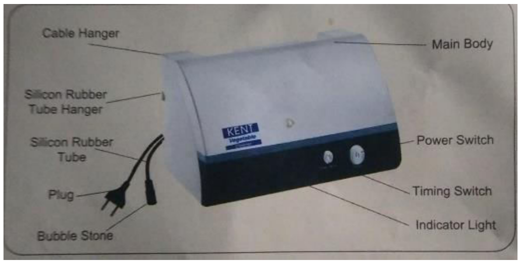

Figure.5 UV-LED Chamber**.**

Figure.6 LED strips inside the chamber

Figure.7 Quartz glass spiral tube.

Figure.8 Arduino nano controller

Chemical Analysis

Chemical analysis results of citrus juices samples are given in table 2. No relevant difference occurred in the pH value of citrus juice samples due to different treatments.

Color Analysis

Color analysis of citrus juice samples is shown in table 3. According to Arı *et al.,* (2020), the duration of ozone application was an important factor in determining the color values of sample. Pasteurization has created negative changes in color. It was reported that an increase occurred in the content of Maillard reaction products such as HMFs after heat treatments (Ertekin Filiz & Seydim, 2018). The color change in pasteurization process is thought to be related to the Maillard reaction.

The application of hurdle technology on the citrus juice was effective for both decreasing the total viable microorganisms to a 5 times reduction and maintaining the quality characteristics of the juice. Ozone, UV and LED treatments of 3 minutes each were sufficient and more effective to reduce the microbial count. Ozone treatment for more than 3 minutes caused a pungent order and therefore was avoided. There was no significant difference in the pH and colour of the treated sample. Thermal treatments like pasteurization can destroy the nutritional contents and reduce the quality of the sample. Consumers of today demand more nutritional, fresh, high quality food products. For this reason alternate technologies like ozone, UV-C, LED or a combination of them can be used to reduce the microbial count of clarified citrus juice.

Author Contributions

Abhinav Sasi: Investigation, formal analysis, writing—original draft. N. K. Ahammed Zainy: Validation, methodology, writing—reviewing. Alan John:—Formal analysis, writing—review and editing. Ansa Shaji: Investigation, writing—reviewing. V. V. Anujeslet: Resources, investigation writing—reviewing. Jithin Mohan: Validation, formal analysis, writing—reviewing. Arya K. Salu: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript. Sasmila Bai: Investigation, formal analysis, writing—original draft. R. Ajay: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Arı, B., Budak, N. H., Seydim, A. C., & Güzel-Seydim, Z. (2020). Effects of Ozonation on Apple Juice Quality. International Journal of Fruit Science, 20(sup3), S1570–S1578. https://doi.org/10.1080/15538362.2020.1822263

Cappozzo, J. C., Koutchma, T., & Barnes, G. (2015). Chemical characterization of milk after treatment with thermal (HTST and UHT) and nonthermal (turbulent flow ultraviolet) processing technologies. Journal of Dairy Science, 98(8), 5068–5079. https://doi.org/10.3168/jds.2014-9190

Ertekin Filiz, B., & Seydim, A. C. (2018). Kinetic changes of antioxidant parameters, ascorbic acid loss, and hydroxymethyl furfural formation during apple chips production. Journal of Food Biochemistry, 42(6), e12676. https://doi.org/10.1111/jfbc.12676

Ghate, V. S., Ng, K. S., Zhou, W., Yang, H., Khoo, G. H., Yoon, W.-B., & Yuk, H.-G. (2013). Antibacterial effect of light emitting diodes of visible wavelengths on selected foodborne pathogens at different illumination temperatures. International Journal of Food Microbiology, 166(3), 399–406. https://doi.org/10.1016/j.ijfoodmicro.2013.07.018

Gordon, A., & Williams, R. (2017). 5 - Case study: formula safe foods—sauces (A. B. T.- F.S. and Q. S. in D. C. Gordon (ed.); pp. 117–148). Academic Press.

Graham, D. M. (1997). Use of ozone for food processing. Food Technology (Chicago), 51(6), 72–75.

Khadre, M. A., Yousef, A. E., & Kim, J.-G. (2001). Microbiological Aspects of Ozone Applications in Food: A Review. Journal of Food Science, 66(9), 1242–1252. https://doi.org/10.1111/j.1365-2621.2001.tb15196.x

Kim, M.-J., Tang, C. H., Bang, W. S., & Yuk, H.-G. (2017). Antibacterial effect of 405±5 nm light emitting diode illumination against Escherichia coli O157: H7, *Listeria monocytogenes*, and Salmonella on the surface of fresh-cut mango and its influence on fruit quality. International Journal of Food Microbiology, 244, 82–89. https://doi.org/10.1016/j.ijfoodmicro.2016.12.023

Koutchma, T. (2009). Advances in ultraviolet light technology for non-thermal processing of liquid foods. Food and Bioprocess Technology, 2, 138–155. https://doi.org/10.1007/s11947-008-0178-3

Koutchma, T., & Orlowska, M. (2012). Ultraviolet light for processing fruits and fruit products. Advances in Fruit Processing Technologies, 2–27. https://doi.org/10.1201/b12088-2

Nicolau, T., Gomes Filho, N., Padrão, J., & Zille, A. (2022). A comprehensive analysis of the UVC LEDs' applications and decontamination capability. Materials, 15(8), 2854. https://doi.org/10.3390/ma15082854

Poonia, A., Pandey, S., & Vasundhara. (2022). Application of light emitting diodes (LEDs) for food preservation, post-harvest losses and production of bioactive compounds: a review. Food Production, Processing and Nutrition, 4(1), 8. https://doi.org/10.1186/s43014-022-00086-0

Sanganamoni, S., Purohit, S., & Rao, P. S. (2017). Effect of ultraviolet-c treatment on some physico-chemical properties of tender coconut water. International Journal of Current Microbiology and Applied Sciences, 6(5), 2893–2904. https://doi.org/10.20546/ijcmas.2017.605.329

Taulavuori, E., Taulavuori, K., Holopainen, J. K., Julkunen-Tiitto, R., Acar, C., & Dincer, I. (2017). Targeted use of LEDs in improvement of production efficiency through phytochemical enrichment. Journal of the Science of Food and Agriculture, 97(15), 5059– 5064. https://doi.org/10.1002/jsfa.8492

How to cite this article:

Abhinav Sasi, N. K. Ahammed Zainy, Alan John, Ansa Shaji, V. V. Anujeslet, Jithin Mohan, Arya K. Salu*, Sasmila Bai and Ajay, R. 2024. Hurdle Technology Prototype to Reduce Microbial Load in Clarified Citrus Juice. *Int.J.Curr.Microbiol.App.Sci.* 13(10): 50-58. **doi:** https://doi.org/10.20546/ijcmas.2024.1310.007