Role of Blue Light and Photosensitizers in The Reduction of Foodborne Bacteria

Sajesh T [1]

[1] Research Scholar, Department of Food Technology, Mansarovar Global University, Sehore, Madhya Pradesh.

Dr. Nirmal Sharma [2]

[2] Supervisor, Department of Food Technology, Mansarovar Global University, Sehore, Madhya Pradesh.

ABSTRACT

This research seeks to determine if Escherichia coli O157, Staphylococcus aureus, and Salmonella enterica may be inactivated by combining blue light (405 nm) with the natural photosensitizers riboflavin and chlorophyll. The bacteria and viruses were subjected to blue light and photosensitizers at concentrations of 5 µM for different amounts of time (5, 10, 15, and 20 minutes). The results showed that the combined treatment greatly decreased bacterial populations. At 20 minutes, the maximal log reductions for E. coli, S. aureus, and S. enterica were 5.6, 5.8, and 5.4 log CFU/ml, respectively. When compared to chlorophyll, riboflavin was the most effective photosensitizer. According to the results, a non-thermal method that shows promise for improving food safety and lowering the incidence of foodborne diseases is photodynamic inactivation utilizing blue light and natural photosensitizers.

Keywords: Photodynamic, Riboflavin, Chlorophyll, Foodborne, Bacterial.

I. INTRODUCTION

Researchers and food safety specialists are always looking for new ways to reduce the dangers of microbiological contamination in food items since the incidence of food poisoning is becoming an urgent issue in public health across the globe. Every year, millions of illnesses and hundreds of fatalities are caused by foodborne pathogens, which include germs like Escherichia coli, Salmonella, and Listeria monocytogenes. This highlights the need of finding effective ways to intervene. The use of heat treatment, chemical preservatives, and refrigeration—the three mainstays of traditional food preservation methods—has been crucial in lowering the microbial burden. The possible altering of food quality, nutritional deterioration, and the development of resistant bacterial strains are some of the disadvantages that come with these procedures.

Blue light, which is defined as light with a wavelength between 400 and 495 nanometers, has recently gained a lot of interest due to its unique antibacterial capabilities. Many different kinds of microbes, such as bacteria, viruses, and fungus, are susceptible to inactivation when exposed to this particular spectrum of visible light. One mechanism by which blue light may kill microbes is photodynamic inactivation (PDI), which works best when photosensitizing chemicals are present. Reactive oxygen species (ROS) like hydroxyl and singlet oxygen are formed when these agents absorb blue light and then transfer that energy to neighboring oxygen molecules. The interaction between these ROS and lipids, proteins, and nucleic acids, among other cellular components, may cause damage and, eventually, cell death because to their high reactivity.

Natural photosensitizers like chlorophyll, curcumin, or riboflavin are one kind of photosensitizer; another is a synthetic chemical developed for increased effectiveness. Because various chemicals have distinct absorption properties and reactivity profiles, choose the right photosensitizer is crucial. Factors such as photosensitizer concentration, light intensity, exposure period, and microbe type affect how well blue light and photosensitizer work together. Studies have shown that by combining blue light with the right photosensitizers, the viability of bacteria that may cause food poisoning can be drastically reduced in a variety of food matrices, such as meat, vegetables, fruits, and dairy products.

Blue light technology has several benefits as compared to older approaches to food safety. The primary benefit is that it eliminates microbes without using heat, which keeps food's flavor, texture, and nutritional content intact. For the growing number of people who want their food unprocessed and unladen with artificial flavors, colors, and preservatives, this is of paramount importance. Another great thing about blue light therapy is how adaptable it is. It can be used at many points in the food processing and storage chains to make sure that food stays safe. For example, it may be used to disinfect food items' surfaces, treat packaging materials, and even lower microbial contamination in food processing settings.

Blue light and photosensitizers have recently come to the attention of researchers as a possible practical way to improve food safety. Due to its susceptibility to contamination during harvesting, processing, and distribution, fresh fruit may be successfully reduced in microbial load by combining blue light with photosensitizers, according to research. Research on strawberries and lettuce, for instance, has shown that typical foodborne pathogen populations may be drastically reduced by exposing these foods to blue light while also exposing them to the right photosensitizer. Similarly, studies on the effects of blue light on meat have shown encouraging outcomes in terms of reducing the prevalence of harmful germs without compromising the meat's quality or freshness.

Nevertheless, in order to fully harness the power of blue light technology for food safety, we must first overcome its limits and overcome the obstacles that stand in the way. An important factor to think about is that complex food matrices might have light distributions that aren't perfectly even, which can cause certain regions to have less effective microbial inactivation than others. Ongoing study is needed to determine the long-term effectiveness of photodynamic therapy as there is still worry about the possibility of bacterial resistance. Additionally, regulatory norms and consumer acceptability must be carefully considered when using blue light and photosensitizers into current food safety processes.

Innovative and effective ways of microbial reduction are more sought after than ever before due to the growing concern of consumers about food safety and quality. An eco-friendly and long-term replacement for traditional techniques, the use of blue light and photosensitizers to decrease food-borne germs is a huge step forward in food safety technology. This cutting-edge method uses light and chemistry to effectively manage microbes, which is both a pressing issue and in line with consumer tastes for less processed, healthier food.

II. REVIEW OF LITERATURE

Lena, Alessia et al., (2023) The use of blue light to disinfect items and surfaces that come into touch with food is a relatively new technique. The process relies on the light-activated photosensitizers that determine the ROS release. ROS are responsible for cell death in bacteria by damaging their cells. Planktonic and biofilm forms of several microorganisms, including viruses, yeasts, molds, and bacteria, are treatable. Light characteristics (irradiance, dosage, wavelength), microbiological parameters (pH, temperature, starting inoculum, grade of biofilm maturity), and surface parameters (material, roughness, and optical qualities) all have an impact on blue light technology. Plus, it's versatile enough to work with or without additional technologies. One of the many benefits of using blue light is that it reduces chemical emissions and makes food handling safer for workers. Also, it's very improbable that germs would evolve a resistance to the blue light treatment.

Zhu, Shengyu et al., (2021) Infectious disorders caused by foodborne bacteria are on the rise, which is a major reason why food safety concerns have recently received more attention. At the same time, it's concerning that more and more bacteria are developing resistance to antibiotics. Consequently, novel, cost-effective methods to inactivate pathogenic microbes and avoid cross-contamination must be developed without delay. Photodynamic inactivation (PDI) is a new and exciting way to kill food-borne viruses. It's safer than standard preservatives, has lower microbial resistance, and satisfies the need for environmentally friendly treatments among modern customers. Reports of this technology being used to ensure food safety have been on the rise in recent years. Recent advances in photodynamic inactivation of food-borne microbes are summarized in this article. This review looks at the processes, variables that affect photodynamic inhibition (PDI), and the use of various photosensitizers (PSs) on various dietary substrates.

Phasupan, Pimonpan et al., (2021) With more and more people looking at photodynamic therapy (PDT) as a means of food preservation, there has to be a comprehensive way to assess and compare various PS-light treatments. This study evaluates the antimicrobial photodynamic efficacy of two PSs, chlorophyllin sodium magnesium salt (Chl-Mg) and chlorophyllin sodium copper salt (Chl-Cu), under blue and white light. The pathogens tested were Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus, which are common in food poisoning. The findings demonstrated that, rather than light source properties, absorbed photons mostly determined a PS's phototoxicity. In terms of antibacterial activity, photosensitized Chl-Mg outperformed Chlsingle bondCu. The therapies that were given had a greater impact on S. aureus bacteria than on E. coli bacteria. The Weibull model can explain the kinetics of bacterial inactivation in relation to the quantity of absorbed photons, with an R2 value ranging from 0.947 to 0.962 and kinetics constants D ranging from 0.202 × 1017 photons/cm2 to 2.409 × 1018 photons/cm2. Potentially useful areas for the kinetics models include PDT process optimization, evaluation, and design.

Hadi, Joshua et al., (2020) The main way that blue light may kill microbes is by triggering the body's own photosensitizers, which in turn create reactive oxygen species that can damage bacterial cell walls. Blue light may cause photodamage to the eyes, but it has no effect on the skin, according to recent studies. Experimental evidence suggests that antimicrobial blue light has no impact on food sensory and nutritional qualities; nevertheless, this has to be confirmed in future studies with human panels. Antimicrobial blue light's effectiveness may be influenced by food qualities as well. Bactericidal activity can be reduced or increased depending on whether there are absorptive materials (like proteins on meats) or photosensitizers (like riboflavin in milk). Combinations of blue light with other therapies, including organic acids, essential oils, or polyphenols, are also possible. Although there hasn't been any case of bacteria developing a full immunity to blue light, there is some indication that this may happen over time, particularly via gene alterations, but it would be far slower than antibiotic resistance. Investigating the oxygen-independent mechanism of blue light, such as its ability to inactivate spoilage bacteria in vacuum-packed meats, and determining the number and kind of intracellular photosensitizers across different bacterial species may be goals of future research.

Bhavya, Ml & Hebbar, Umesh. (2018) The effectiveness of blue (462 ± 3 nm) light from light-emitting diode (LED) lighting in killing food-borne pathogens such as Escherichia coli and Staphylococcus aureus when combined with an external photosensitizer (curcumin) was examined in a laboratory setting. They looked at the sublethal damage of cells and the influence of temperature, photosensitizer concentration, and incubation time on microbial inactivation. Additionally, the inactivation mechanism by blue light and photosensitizer was investigated. When exposed to photosensitizer (20 µM) at 13 J/cm² of blue light, E. coli and S. aureus achieved a maximum decrease of 5.94 ± 0.22 and 5.91 ± 0.20 log CFU/ml, respectively. The inactivation of these pathogens was unaffected by the presence of photosensitizer at both 9 °C and 27 °C. The inactivation of these food-borne pathogens was not significantly affected by incubation with the photosensitizer. Cells exposed to photosensitizer and blue light at the same time also showed sublethal harm (>90% injury). The investigation using confocal laser scanning microscopy showed that the photodynamic activity of curcumin disrupted the integrity of the bacteria's membranes. In addition, the combination of photosensitizer and blue light caused both cells to release reactive oxygen species into the surrounding cell membrane. When comparing the photosensitizer and blue light-treated and untreated groups of Escherichia coli and Staphylococcus aureus cells, scanning electron microscopy revealed morphological alterations in the cell wall. According to the research, one possible method for ensuring food safety is the photodynamic inactivation of food-borne viruses by the use of LED-based photosensitization.

III. MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

We used Salmonella enterica, Staphylococcus aureus, and Escherichia coli O157 as our model organisms. Prior to the trials, each strain was cultured for 24 hours at 37°C in nutritional broth.

Photosensitizers and Blue Light

- **Photosensitizers**: Because of their efficacy in photodynamic inactivation and their natural origins, riboflavin (Vitamin B2) and chlorophyll were chosen.
- **Blue Light Source**: All of the studies made use of a 405 nm LED with an intensity level of 40 mW/cm².

Experimental Design

Bacterial suspensions with a concentration of 10^8 CFU/ml were exposed to blue light for 5, 10, 15, and 20 minutes after being treated with 5 µM of photosensitizer. Two groups served as controls: one treated with photosensitizer alone (i.e., no blue light) and another treated with blue light alone (i.e., no photosensitizer).

Inactivation Assay

To test bacterial survival, samples were plated on nutrient agar and counted for colony-forming units (CFUs) after 24-hour incubation.

Statistical Analysis

To determine which treatments were most effective in reducing germs, data were evaluated using analysis of variance (ANOVA). A significant result was defined as a p-value less than 0.05.

IV. RESULTS AND DISCUSSION

Table 1: Inactivation of Pathogens with Blue Light and Photosensitizers

Time (min)	E. coli Reduction (log CFU/ml)	S. aureus Reduction (log CFU/ml)	S. enterica Reduction (log CFU/ml)
Control	0.2 ± 0.05	0.1 ± 0.03	0.3 ± 0.04
5 min	1.5 ± 0.1	1.8 ± 0.2	1.6 ± 0.1
10 min	2.8 ± 0.2	3.0 ± 0.3	2.9 ± 0.2
15 min	3.9 ± 0.3	4.5 ± 0.2	4.1 ± 0.3
20 min	5.6 ± 0.4	5.8 ± 0.4	5.4 ± 0.5

Table 1 displays the results of exposure periods for E. coli O157, Staphylococcus aureus, and Salmonella enterica, as well as the efficacy of blue light and photosensitizers in eliminating these bacteria. The control group did not see any decrease in germs, but after 5 minutes of treatment, there was considerable inactivation, with S. aureus exhibiting the largest reduction of 1.8 log CFU/ml. Bacterial reduction improved for all pathogens as exposure duration increased. At 20 minutes, the most significant inactivation was seen for S. aureus, with a decrease of 5.8 log CFU/ml. E. coli and S. enterica showed reductions of 5.6 and 5.4 log CFU/ml, respectively.

Table 2: Effectiveness of Different Photosensitizers

Photosensitizer	E. coli Reduction (log CFU/ml)	S. aureus Reduction (log CFU/ml)	S. enterica Reduction (log CFU/ml)
Riboflavin	5.6 ± 0.4	5.8 ± 0.4	5.4 ± 0.5
Chlorophyll	4.9 ± 0.5	4.5 ± 0.3	4.7 ± 0.4

In Table 2, we can see how chlorophyll and riboflavin, two photosensitizers, fared in lowering the concentrations of Salmonella enterica, Staphylococcus aureus, and E. coli O157. Greater bacterial reductions were seen across all three pathogens when riboflavin was used, with S. aureus exhibiting the largest decrease of 5.8 log CFU/ml. While chlorophyll reduced E. coli, Staphylococcus aureus, and Salmonella enterica to 4.9, 4.5, and 4.7 log CFU/ml, respectively, it was marginally more effective. In comparison to chlorophyll, riboflavin showed better photodynamic inactivation overall.

V. CONCLUSION

The research shows that some food-borne infections, such E. coli O157, Staphylococcus aureus, and Salmonella enterica, may be effectively inactivated by combining blue light with photosensitizers, particularly riboflavin and chlorophyll. The findings show that the bacterial populations are significantly reduced when exposed to blue light for longer periods of time, suggesting that it might be a practical way to improve food safety. The most effective photosensitizer, according to the results, was riboflavin, which killed the most germs of all the studied pathogens.

REFERENCES

1. A. Lena, M. Marino, M. Manzano, C. Comuzzi, and M. Maifreni, "An overview of the application of blue light-emitting diodes as a non-thermic green technology for microbial inactivation in the food sector," *Food Eng. Rev.*, vol. 16, no. 1, pp. 1-26, 2023.
2. S. Huang, S. Lin, H. Qin, H. Jiang, and M. Liu, "The parameters affecting antimicrobial efficiency of antimicrobial blue light therapy: A review and prospect," *Biomedicines*, vol. 11, no. 4, pp. 11-97, 2023.
3. S. Zhu, Y. Song, J. Pei, F. Xue, C. Xiaowen, X. Xiong, and C. Li, "The application of photodynamic inactivation to microorganisms in food," *Food Chem.*, vol. 12, no. 1, pp. 100-150, 2021, doi: 10.1016/j.fochx.2021.100150.
4. M. Lu, S. Wang, T. Wang, S. Hu, B. Bhayana, M. Ishii, Y. Kong, Y. Cai, T. Dai, W. Cui, and M. Wu, "Bacteria-specific phototoxic reactions triggered by blue light and phytochemical carvacrol," *Sci. Transl. Med.*, vol. 13, no. 1, pp. 35-71, 2021.
5. P. Phasupan, T. Le, and L. T. Nguyen, "Assessing the photodynamic efficacy of different photosensitizer-light treatments against foodborne bacteria based on the number of absorbed photons," *J. Photochem. Photobiol. B Biol.*, vol. 221, no. 3, pp. 112-249, 2021.

6. J. Hadi, S. Wu, and G. Brightwell, "Antimicrobial blue light versus pathogenic bacteria: Mechanism, application in the food industry, hurdle technologies and potential resistance," *Foods*, vol. 9, no. 3, pp. 1-20, 2020.
7. J.-E. Hyun and S. Lee, "Blue light-emitting diodes as eco-friendly non-thermal technology in food preservation," *Trends Food Sci. Technol.*, vol. 105, no. 7, pp. 284-295, 2020.
8. J. Gao and K. Matthews, "Effects of the photosensitizer curcumin in inactivating foodborne pathogens on chicken skin," *Food Control*, vol. 109, no. 5, pp. 106-959, 2019.
9. M. Bhavya and U. Hebbar, "Efficacy of blue LED in microbial inactivation: Effect of photosensitization and process parameters," *Int. J. Food Microbiol.*, vol. 290, no. 7, pp. 1-10, 2018.
10. C. Penha, E. Bonin, A. Silva, N. Hioka, É. Zanqueta, T. Nakamura, B. Filho, P. Campanerut-Sá, and J. Mikcha, "Photodynamic inactivation of foodborne and food spoilage bacteria by curcumin," *LWT - Food Sci. Technol.*, vol. 73, no. 3, pp. 1-10, 2016.
11. M. Hessling, B. Spellerberg, and K. Hönes, "Photoinactivation of bacteria by endogenous photosensitizers and exposure to visible light of different wavelengths – A review on existing data," *FEMS Microbiol. Lett.*, vol. 364, no. 3, pp. 1-30, 2016.
12. K. Aponiene and Z. Luksiene, "Effective combination of LED-based visible light, photosensitizer and photocatalyst to combat Gram (−) bacteria," *J. Photochem. Photobiol. B Biol.*, vol. 142, no. 5, pp. 257-263, 2014.
13. F. Cieplik, A. Späth, C. Leibl, A. Gollmer, J. Regensburger, L. Tabenski, K.-A. Hiller, T. Maisch, and G. Schmalz, "Blue light kills *Aggregatibacter actinomycetemcomitans* due to its endogenous photosensitizers," *Clin. Oral Invest.*, vol. 18, no. 6, pp. 1763-1769, 2013.
14. Z. Luksiene and L. Brovko, "Antibacterial photosensitization-based treatment for food safety," *Food Eng. Rev.*, vol. 5, no. 4, pp. 10-20, 2013.
15. I. Buchovec, E. Paskeviciute, and Z. Luksiene, "Photosensitization-based inactivation of food pathogen *Listeria monocytogenes* in vitro and on the surface of packaging material," *J. Photochem. Photobiol. B Biol.*, vol. 99, no. 1, pp. 9-14, 2010.