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INATIVAÇÃO FOTODINÂMICA MEDIADA POR CURCUMINA SOLÚVEL EM ÁGUA CONTRA PATÓGENOS DE ORIGEM ALIMENTAR

Luana Carolina Martins Rosa

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Dissertação apresentada ao programa de Pós-graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de mestre em Ciência de Alimentos

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LUANA CAROLINA MARTINS ROSA

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Dissertação apresentada à Universidade Estadual de Maringá, como parte das exigências do Programa de Pós-graduação em Ciência de Alimentos, para obtenção do grau de Mestre em Ciência de Alimentos.

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Maringá – 2022

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BIOGRAFIA

Luana Carolina Martins Rosa, brasileira, nascida em 26/04/1996 na cidade de Maringá, no noroeste do estado do Paraná. Possui graduação em Biomedicina pela Universidade Estadual de Maringá (2018). Tem experiência nas áreas de Microbiologia e Controle de Qualidade, atuando principalmente no tema de microbiologia de alimentos.

Dedico

A todos que me apoiaram e me incentivaram para que eu concluísse mais uma etapa da minha vida.

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APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de dois artigos científicos.

1 Luana Carolina Martins Rosa, João Vitor de Oliveira Silva, Maíra Dante Formagio, Andreia Farias Pereira Batista, Camila Fabiano de Freitas, Benicio Alves de Abreu Filho, Miguel Machinski Junior, Fernanda Vitória Leimann, Jane Martha Graton Mikcha.
Artigo: **Antimicrobial photodynamic therapy by water-soluble curcumin against foodborne pathogens.**
Submetido ao periódico científico Food Science and Technology International.

2 Luana Carolina Martins Rosa, Letícia Graziela Zavaddzki, Camila Fabiano de Freitas, Adriele Rodrigues dos Santos, Luis Carlos Malacarne, Paula Aline Zanetti Campanerut-Sá, Farnanda Vitoria Leimann, Evandro Bona, Jane Martha Graton Mikcha.
Artigo: **Optimization of the antimicrobial photodynamic therapy mediated by water-soluble curcumin using the response surface methodology.**
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GENERAL ABSTRACT

INTRODUCTION. Safe and nutritious foods are essential to ensure a good quality of life, however, the presence of pathogenic microorganisms in food represents a serious public health problem. In addition, to having health consequences, it affects social, environmental, and economic development. Food can be contaminated at any stage of production and the methodologies currently used are not always effective for microbial control; often limited to the type of food as they result in changes in sensory and nutritional characteristics. A promising methodology for microbial control is the antimicrobial photodynamic therapy – aPDT. This technique consists of the use of a photosensitive compound that, in the presence of light, produces reactive oxygen species that result in damage that causes the death of microorganisms. Curcumin is a natural yellow colorant widely used by the food industry that, when applied in photodynamic therapy, has shown to be effective and promising against microorganisms. However, the use of curcumin is restricted as it is extremely hydrophobic. To overcome this problem, water-soluble formulations are a viable alternative. In addition, studies indicate better action of curcumin at low pH, potentiating antimicrobial activity.

AIMS. The present study aimed to evaluate the *in vitro* susceptibility of *Staphylococcus aureus* and *Escherichia coli* to water-soluble curcumin (WSC) and the compound exposed to visible blue light at acidic pH with organic acids. Furthermore, this work purposed to apply an experimental design and surface response methodology to evaluate the interaction of WSC concentration at pH 5.0 and illumination time against *Staphylococcus aureus*, *Salmonella* Typhimurium, and *Escherichia coli*.

MATERIAL AND METHODS. The WSC was obtained in partnership with the Federal University of Technology – Paraná (Campo Mourão *campus*). The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined using the broth microdilution method in 96-well microplates. Based on the results obtained, conditions for aPDT were established at acidic pH with concentrations of curcumin ranging from 3.90 to 125 µg/mL. The samples were submitted to treatments with different illumination times of 2.5, 5, and 10 min with a blue LED light system built to illuminate a 96-well plate, with an irradiance of 16 mW/cm² and a wavelength of 450 nm. The results found were expressed in log CFU/mL to determine the reduction in cell viability. In addition, an experimental model was proposed to determine the optimal conditions for the application of photodynamic therapy, using a Rotable Central Composite Design. The design proposed eight experiments with four replications of the center point to evaluate the combined effects of lighting time and curcumin concentration.

RESULTS AND DISCUSSION. For *S. aureus*, the combination of WSC with lactic acid and 2.5 min of illumination reduced the MIC from 500 µg/mL to 15.62 µg/mL, and WSC with citric acid and 5 min of exposure to light reduced the MIC from 125 µg/mL to 7.81 µg/mL. While for *E. coli*, curcumin alone did not inhibit bacterial growth, however, when exposed to 5 min of illumination with a concentration of 62.5 µg/mL curcumin with lactic acid and curcumin at 7.81 µg/mL with citric acid, no viable cells were recovered. The models sowed predictive capacity (R^2_{adj}) of 64% for *S. aureus*, 91% for *E. coli*, and 81% for *S.* Typhimurium. The models demonstrated that there was no significant interaction between exposure time and photosensitizer concentration for *S. aureus* and *E. coli*. The greatest reductions observed for *S. aureus* were approximately 5.16 log CFU/mL at 4.41 µg/mL and 6.77 min of illumination time, while higher concentrations and illumination times were able to inhibit *E. coli* and *S.* Typhimurium growth.

CONCLUSIONS. The results demonstrated that WSC when combined with blue LED light was more effective against foodborne pathogens than to non-irradiated compounds. Low illumination times and photosensitizer concentrations were sufficient to eradicate *Staphylococcus aureus* and *Escherichia coli*. The application of experimental design was effective for the optimization of photodynamic therapy, providing information on photodynamic activity against microorganisms

in different combinations of photosensitizer concentration and lighting time, reducing the number of experiments and costs.

Keywords: Curcumin, Foodborne pathogens, Photodynamic therapy.

RESUMO GERAL

INTRODUÇÃO. Alimentos seguros e nutritivos são fundamentais para garantir uma boa qualidade de vida, no entanto, a presença de microrganismos patogênicos em alimentos representa um grave problema de saúde pública. Além de gerar consequências na saúde, afeta o desenvolvimento social, ambiental e econômico. Os alimentos podem ser contaminados em qualquer etapa da produção e as metodologias utilizadas atualmente nem sempre são efetivas para o controle microbiano; muitas vezes limitadas ao tipo de alimento por resultarem em alterações nas características sensoriais e nutricionais. Uma metodologia promissora para o controle microbiano é a terapia fotodinâmica antimicrobiana – conhecida pela sigla do inglês aPDT. Essa técnica consiste na utilização de um composto fotossensível que, na presença da luz, produz espécies reativas de oxigênio que resultam em danos que ocasionam a morte dos microrganismos. A curcumina é um corante de cor natural amarela muito utilizado pela indústria de alimentos que quando aplicada na terapia fotodinâmica demonstra ser efetiva e promissora contra os microrganismos. No entanto, o uso da curcumina é restrito por ela ser extremamente hidrofóbica. Para superar esse problema, formulações solúveis em água são uma alternativa viável. Além disso, estudos indicam melhor ação da curcumina em pH baixo, potencializando a atividade antimicrobiana.

OBJETIVOS. O presente estudo teve como objetivo avaliar a suscetibilidade *in vitro* de *Staphylococcus aureus* e *Escherichia* coli à uma curcumina solúvel em água (WSC, na sigla em inglês) e ao composto exposto a luz azul visível em pH ácido com ácidos orgânicos. Além disso, este trabalho teve como objetivo aplicar um planejamento experimental e a metodologia de resposta de superfície para avaliar a interação da concentração de WSC em pH ácido (pH 5.0) e tempo de iluminação contra *Staphyloccus aureus, Salmonella* Typhimurium e *Escherichia coli.*

MATERIAL E METODOS. A WSC foi obtida em parceira com a Universidade Tecnológica de Maringá campus Campo Mourão. A concentração inibitória mínima (CIM) e a concentração bactericida mínima (CBM) foram determinadas usando o método de microdiluição em caldo em microplacas de 96 poços. Com base nos resultados, foram estabelecidas as condições para a aPDT foram estabelecidas em pH ácido com as concentrações de curcumina variando de 3,90 a 125 µg/mL. As amostras foram submetidas a tratamentos com tempos de iluminação de 2,5, 5 e 10 min com um sistema de luz LED azul construído para iluminar uma placa de 96 poços, com irradiância de 16 mW/cm² e comprimento de onda de 450 nm. Os resultados encontrados foram expressos em log de UFC/mL para determinar a redução da viabilidade celular. Além disso, foi proposto um modelo experimental para determinar as condições ótimas para a aplicação da aPDT utilizando um Planejamento Composto Central Rotacional. O modelo propôs oito experimentos com quatro repetições do ponto central para avaliar os efeitos combinados do tempo de iluminação e concentração da curcumina.

RESULTADOS E DISCUSSÃO. Para *S. aureus*, a combinação de WSC com ácido lático e 2.5 min de iluminação, reduziu a CIM de 500 µg/mL para 15,62 µg/mL; e WSC com ácido cítrico e 5 min de exposição a luz reduziu a CIM de 125 µg/mL para 7,81 µg/mL. Enquanto para *E. coli*, a curcumina sozinha não inibiu o crescimento bacteriano, no entanto, quando exposta a 5 min de irradiação com concentrações de 62,5 µg/mL de curcumina com ácido lático e 7,81 µg/mL com ácido cítrico, nenhuma célula viável foi recuperada. O modelo apresentou capacidade preditiva (R^2_{adj}) de 64% para *S. aureus*, 91% para *E. coli* e 81% para *S.* Typhimurium. Os modelos demonstraram que não houve interação significativa entre o tempo de exposição e a concentração do fotossensibilizador para *S. aureus* e *E. coli*. As maiores reduções observadas para *S. aureus* foram de aproximadamente 5,16 log de UFC/mL com WSC a 4,41 µg/mL e 6,77 min de iluminação, enquanto maiores concentrações e tempos de iluminação foram capazes de inibir totalmente o crescimento de *E. coli* e *S.* Typhimurium.

CONCLUSÕES. Os resultados demonstraram que a WSC quando combinada com uma luz LED azul foi mais eficaz contra patógenos de origem alimentar do que contra o composto não irradiado. Baixos tempos de iluminações e concentrações do fotossensibilizador foram suficientes para erradicar o crescimento de *Staphylococcus aureus* e *Escherichia coli*. A aplicação de um planejamento experimental foi eficaz para a otimização da terapia fotodinâmica, fornecendo informações sobre a atividade fotodinâmica contra os microrganismos em diferentes combinações de concentração do fotossensibilizador e tempo de iluminação, reduzindo o número de experimentos e os custos.

Palavras chaves: Curcumina, Patógenos de Origem Alimentar, Terapia fotodinâmica.

ARTICLE I

Antimicrobial photodynamic therapy by water-soluble curcumin against foodborne pathogens

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ABSTRACT

Foodborne diseases and microbiological control represent the major challenge for the food industry. New technologies that employ natural agents have driven increasing interest. Therefore, this study aimed to evaluate the *in vitro* susceptibility of *Staphylococcus aureus* and *Escherichia coli* to water-soluble curcumin

(WSC) associated with acidic pH and blue LED light. The minimum inhibitory concentration (MIC) and bacterial photoinactivation were conducted using different photosensitizer concentrations. For *S. aureus*, the combination of WSC with lactic acid and 2.5 min of illumination time reduced the MIC from 500 µg/mL to 15.62 µg/mL, and WSC with citric acid reduced the MIC from 125 µg/mL to 7.81 µg/mL after 5 min of exposure light. WSC without illumination did not inhibit *E. coli* growth (MIC > 1000 µg/mL), however, when applied in photodynamic therapy (5 min illumination blue LED) WSC at 62.5 µg/mL with lactic acid and WSC 7.81 µg/mL with citric acid eradicated *E. coli* cells. The results obtained suggest that water-soluble curcumin associated with organic acids and combined with a blue LED light was effective against foodborne pathogens.

Keywords: Photodynamic therapy; Curcumin; Foodborne pathogens

1. Introduction

Safe and nutritious foods are essential to ensure the quality of life, however, a serious health problem faced are the foodborne diseases, caused by the presence of pathogenic microorganisms or toxic substances in water and food (Fung et al., 2018). Foodborne diseases are capable of causing gastrointestinal symptoms, such as vomiting, diarrhea, and other more serious illnesses, which can lead to even death (World Health Organization, 2020). According to the World Health Organization (2015), it is assumed that 600 million people fall after eating contaminated food, and of these, 420,000 die each year.

In addition to having health consequences, food contamination affects social, environmental, and economic development; therefore, the food sectors seek to adopt the culture of food safety and implement microbial control alternative methods to ensure the quality of the product offered (Fung et al., 2018; Scharff, 2015; World Health Organization, 2015; Powell et al., 2011). In fact, the methodologies currently used neither are always effective for microbial control and are often limited to the type of food, resulting in changes in the sensory characteristics and in the emergence of microorganisms resistant to antibiotics or sanitizing agents, generating a health and food safety risk (Faille et al., 2018; Gutiérrez-del-Río et al., 2018; Yang et al., 2017). Thus, there is a need to explore alternative methods that guarantee a better antimicrobial action.

For all these reasons, the number of studies seeking to find natural alternatives for food preservations is increasing (Bourab Chibane et al., 2019; Gutiérrez-del-Río et al., 2018). Among them, curcumin, a yellow compound derived from the *Curcuma longa* L. rhizome, is widely used by the food industry as a food additive with coloring, flavoring, and preservative properties. Also, it is applied in the pharmaceutical industry for its anti-inflammatory and antioxidant properties (Delgado et al., 2021; Hewlings and Kalman, 2017). Curcumin and its derivatives have shown antimicrobial activity against both Gram-positive and Gram-negative bacteria, and when

combined with visible light at a specific wavelength it is photoexcited and its antimicrobial action is enhanced by the production of reactive oxygens species (ROS) and resulting in cells damage and microorganism's death (Polat an Kang, 2021; Adamczak et al., 2020;Praditya et al., 2019; de Oliveira et al., 2018).

The use of curcumin is restricted because it is extremely hydrophobic and unstable (Silva et al., 2018). As result, new alternatives, such as formulations encapsulated in nanoparticles and water-soluble formulations, are sought to facilitate its solubilization, improve its stability, obtain better uptake release and antimicrobial activity compared to free curcumin (Dias et al., 2021; Gao and Matthews, 2020; Mirzahosseinipour et al., 2020; dos Santos et al., 2019; Mangolim et al., 2014). Studies also indicate the highest action of curcumin at low pH. improving the antimicrobial activity (Wang et al., 2021; de Oliveira et al., 2018). Similarly, our research group has demonstrated the greatest photoinhibition effect by curcumin in nanoparticles in acid environments (Dias et al., 2021).

So, in the present study, we evaluated the association of WSC with citric and lactic acids, as they are natural food additives considered generally recognized as safe (GRAS) by the U.S Food and Drug Administration (Food and Drug Administration, 2021). It was evaluated the *in vitro* susceptibility of *Staphylococcus aureus* and *Escherichia coli* to non-irradiated and photoactivated water-soluble curcumin as photosensitizer (PS) combined with organic acids.

2. Methodology

2.1 Compound and Light source

Water-soluble curcumin (WSC, Natural Powder Curcumin Water Soluble) was kindly provided from IFC Solutions (Liden, NJ, USA) and obtained in partnership with Federal University of Technology - Paraná (Campo Mourão *campus*).

The blue LED light system used in the assays was constructed to illuminate 96-well plates and was composed of 20 LEDs (3W) with an irradiance of 16mW/cm², and a wavelength of 450 nm. The absolute irradiance of LEDs was evaluated in a Spectroradiometer USB2000 + RAD (Ocean Optics, Winter Park, FL, USA) and the spectral emission was obtained using a spectrofluorometer (Varian Gary Eclipse, San Diego, United States). The maximum light dose (fluency) was 15.0 J/cm², calculated by multiplying irradiance by illumination time.

2.2 Microorganisms and culture conditions

The bacterial strains used were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 provided by the Laboratory of Food Microbiology, State University of Maringá, Paraná, Brazil. The strains were stored at -20ºC in Brain Heart Infusion (BHI, Difco, Le Pont-de-Claix, France) supplemented with 20% of glycerol (v/v).

2.3 Determination of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration and minimum bactericidal concentration of WSC were determined according to the Clinical and Laboratory Standards Institute (CLSI, 2020), using the broth microdilution method in 96-well microplates. WSC was serially diluted in 100 µL of Mueller Hinton Broth (MHB, Difco, Le Pont-de-Claix, France) with final pH adjusted to 7.0 or to 5.0 (with 1 M lactic or citric acids) at concentrations of 1.95 – 1000 µg/mL. Standardized bacterial suspensions were made in 0.85% saline using the 0.5 McFarland scale and diluted to 1:20, and 10 µL was inoculated in each well of the microplate. The microplates were incubated at 35 ºC for 24 h, and the MIC was visually determined as the lowest concentration of WSC at which bacterial growth was not observed. The positive control consisting of MHB and bacterium inoculum, and controls with lactic acid and citric acid in MHB and bacterium inoculum were included. After MIC determination, 20 µL were removed from wells in which bacterial growth was not reported and inoculated into Trypticase Soy Agar (TSA, Difco, Le Pont-de-Claix, France), incubated for 24 h at 35 ºC. MBC was determined as the lowest concentration, where no bacterial growth was observed on TSA medium. The experiment was performed in triplicate with three repetitions.

2.4 Antimicrobial photodynamic therapy

The conditions for antimicrobial photodynamic therapy (aPDT) were established based on the results obtained in MIC assays and previous studies from our research group. Before assays, each bacterial strain was cultivated in BHI overnight at 35 ºC. Then, centrifugated at 4500g for 5 min, washed three times and resuspended in 0.85% saline solution. The bacterial inoculum was standardized at 10^7 CFU/mL and the aPDT was performed according to Dias et al. (2021), with some modifications. For that, 7.9 µL of the inoculum was mixed with 150 µL of WSC solution (WSC in acidic saline pH 5.0, adjusted with lactic or citric acid) in 96-well microplates, the concentrations ranging from 3.90 to 125 µg/mL and kept in the dark for 10 min. The samples were subjected to treatments with different illumination times (2.5, 5, and 10 min).

After treatment, samples were serially diluted in 0.85% saline solution, plated on TSA, and incubated at 35 ºC for 24 h. The results were expressed as log CFU/mL. Each assay was performed in duplicate with at least three repetitions.

Seven control groups were used: bacterium inoculum without curcumin or irradiation – $WSC_{(-)}L_{(-)}$ (positive control), bacterium inoculum and WSC without irradiation – $WSC_{(+)}L_{(-)}$ (dark control), bacterium inoculum exposed to LED – $WSC_{(-)}L_{(+)}$ (light control), bacterium inoculum in acidic saline with lactic acid without curcumin or irradiation – $LA_{(+)}WSC_{(-)}L_{(-)}$ (lactic acid control), bacterium inoculum in acidic saline with lactic acid without curcumin exposed to LED – $LA_{(+)}WSC_{(-)}L_{(+)}$ (lactic acid light control), bacterium inoculum in acidic saline with citric acid without curcumin or irradiation – $CA_{(+)}WSC_{(-)}L_{(-)}$ (citric acid control) and bacterium inoculum in acidic saline with citric acid without curcumin exposed to LED – $CA_{(+)}WSC_{(-)}L_{(+)}$ (citric acid light control).

2.5 Statistical analysis

The results were expressed as mean and standard deviation and the data were subjected to analysis of variance (ANOVA) with a 5% level of significance and compared using Tukey's test. Statistical analyses were performed using GrandPad Prism 7.04 Software.

3. Results and Discussion

3.1 Determination of minimum inhibitory concentration and minimum bactericidal concentration

The MIC and the MBC of WSC are shown in Table 1. For *S. aureus*, the most effective activity occurred using WSC in acidic pH with citric acid (MIC 125 µg/mL). For *E. coli*, there was no growth inhibition at the highest concentration evaluated regardless of pH.

To the best of our knowledge, the antimicrobial activity of curcumin has been reported only at neutral pH. Bhawana et al. (2011) investigated the antimicrobial effect of curcumin dissolved in DMSO compared to nanocurcumin soluble in the water against *S. aureus* and *E. coli*. Curcumin in DMSO demonstrated a MIC of 150 µg/mL for *S. aureus* and 300 µg/mL for *E. coli*, while nanocurcumin soluble in water, the MIC was 100 µg/mL for *S. aureus* and 250 µg/mL for *E. coli*. On other hand, Adamczak et al. (2020) observed an inhibitory concentration of 250 and 2000 µg/mL for *S. aureus* ATCC 29213 and *E. coli* ATCC 25922, respectively. Alippilakkote and Sreejith (2018) tested a curcumin loaded poly (lactic acid) nanocapsule and demonstrated a

MIC of 468 µg/mL for *S. aureus* and 937 µg/mL for *E. coli*. When investigating the antimicrobial activity of curcumin by zone of inhibitions measurements, Belma et al. (2021) reported that the strongest activity of curcumin solutions was observed at a concentration of 500 µg/mL, resulting in a zone of inhibition of 14.7 mm against *S. aureus* and 13.7 mm against *E. coli*.

Table 1 – Minimum inhibitory concentration and minimum bactericidal concentration for water-soluble curcumin against *Staphylococcus aureus* and *Escherichia coli.*

Species	**WSC (neutral pH)**		**WSC and lactic acid**		**WSC and citric acid**	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
Staphylococcus aureus	1000	> 1000	500	500	125	250
Escherichia coli	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000

According to Holetz et al. (2002), the antimicrobial activity of natural compounds was classified into following groups regarding to the MIC values: i) good antimicrobial activity: MIC < 100 µg/mL; ii) moderate activity: MIC: 100 – 500 µg/mL; iii) weak activity: MIC: 500 - 1000 µg/mL and iv) inactive: MIC >1000 µg/mL. Despite the variation in biological activity found in the studies above, according to Holetz et al. (2002), all presented a moderate antimicrobial activity of curcumin against *S. aureus*, while for *E. coli* it demonstrated a weak active or inactive activity. These findings agree with the literature available, that the antimicrobial activity of curcumin is more effective on Gram-positive than Gram-negative bacteria, which could be explained by the difference in the structure of bacterial cell walls (Belma et al., 2021; Bhawana et al., 2011).

Another point that should be considered is the result obtained against *S. aureus* associating WSC and lactic and citric acids. Hilmi et al. (2019) when evaluating citric acid as a potential antimicrobial agent, found that *S. aureus* was more sensitive to citric acid treatment than *E. coli*. Although the mechanism of action is not fully elucidated, it is believed that undissociated forms of organics acids can easily penetrate the bacterial cell. And once internalized into the cytoplasm, they dissociate into protons and anions, decreasing the intracellular pH and causing changes in cytoplasm and cellular metabolism (Burns et al., 2021; Kim et al., 2020; Lund et al., 2014). However, Gram-negative may have reduced susceptibility to organics acids due to the presence of an outer membrane that prevents the passage of low lipid-soluble organics acids (Cherrington et al., 1991). According to Burel et al. (2020), *E. coli* showed greater sensitivity to citric acid when exposed at neutral and high pH values, where acids are in their tribasic form and able to chelate a large number of divalent cations presents in the membrane, causing a rupture of the cell membrane.

3.2 Antimicrobial photodynamic therapy

From the results obtained in MIC and MBC assays and considering curcumin as a potential PS, we sought to evaluate the potentiation of antimicrobial activity, associating the compound with visible blue light.

Figure 1. shows the visible absorption spectrum of WSC and the light-emitting diode potency (P_{LED} emitted). The absorption maxima were identified and were consistent with the literature (Priyadarsini, 2009), that curcumin in polar solvents the absorption maximum is at ~420 nm, and in hydrogen bond donor and acceptor solvents is around 430 – 434 nm. The power density was 16 mW/cm², as a result, light doses of 2.5, 5, and 10 J/cm² corresponded to 2.5, 5, and 10 minutes of light exposure.

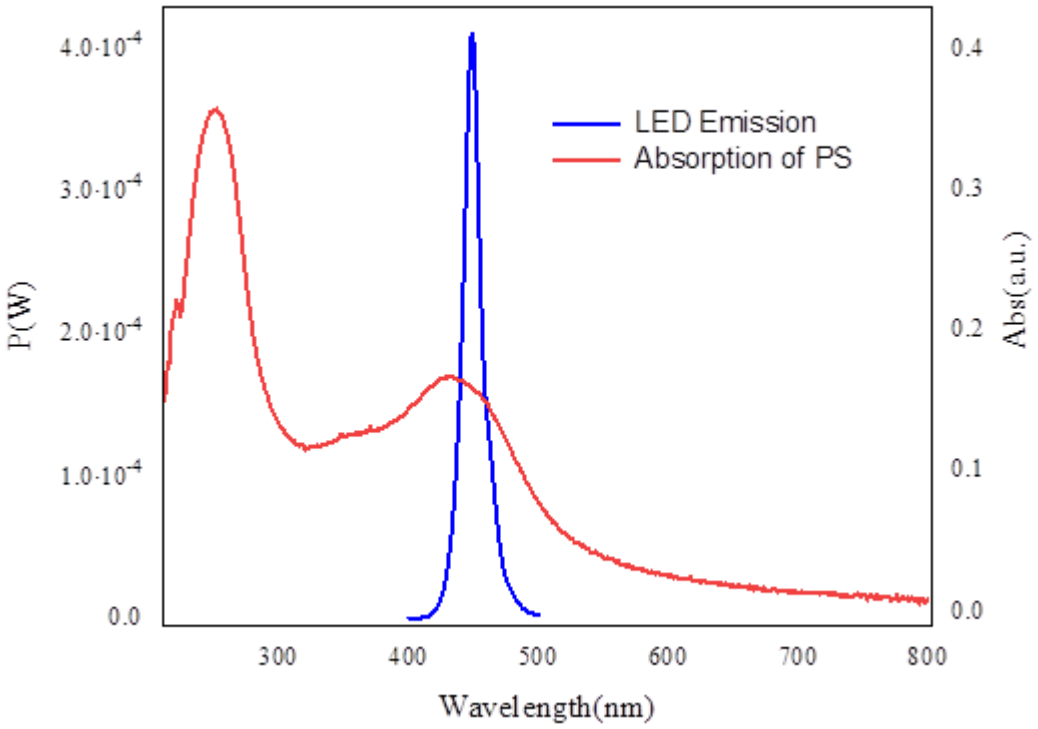


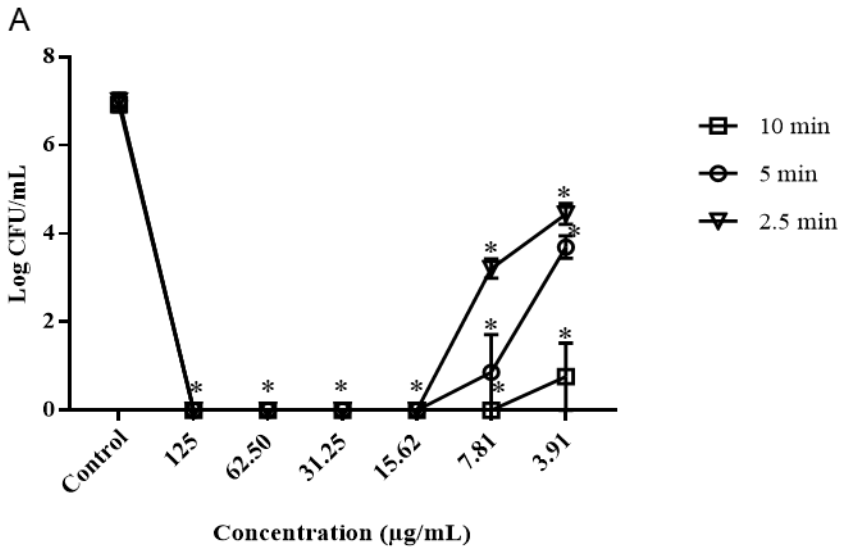
Figure 1. Light-emitting diode emitted potency (P_{LED} emitted) and PS electronic absorption spectra.

Although the efficacy of curcumin as a PS to control foodborne pathogens (Gao and Matthews, 2020; dos Santos et al., 2019; Lin et al., 2019), few studies have evaluated the effect of curcumin at different pH (Dias et al., 2021; de Oliveira et al., 2018). Furthermore, previous studies in our laboratory demonstrated a better action of curcumin as a PS at acidic pH (Dias et al., 2021). So, in this study, we sought to investigate the potential action of curcumin associated

with organic acids (lactic and citric acids) in different photosensitizer concentrations (ranging from 3.90 to 125 µg/mL) and illumination times (2.5 – 10 min) (Figure 2).

aPDT mediated by WSC was efficient in inactivating *S. aureus* and *E. coli*. The evaluated controls had counts of approximately 7 log CFU/mL, and no reduction in bacterial viability was observed when compared to the positive control, indicating that the acids, light, and WSC alone do not present antimicrobial activity.

Total inactivation of *S. aureus* was founded after 10, 5, and 2.5 min of LED light exposure when concentrations of 125 – 15.62 µg/mL WSC with lactic acid were used (Figure 2A). Cell recovery occurred after 5 and 2.5 min of illumination at a WSC concentration of 7.81 µg/mL, with the growth of approximately 1.0 and 3.5 log CFU/mL, respectively. The treatment with citric acid and WSC at a lower concentration (3.90 µg/mL) after 10 min of exposure to light was able to lead to complete inactivation of microorganisms. Bacterial eradication was also possible to observe with WSC at 7.81 µg/mL at an illumination time of 5 min (Figure 2B).



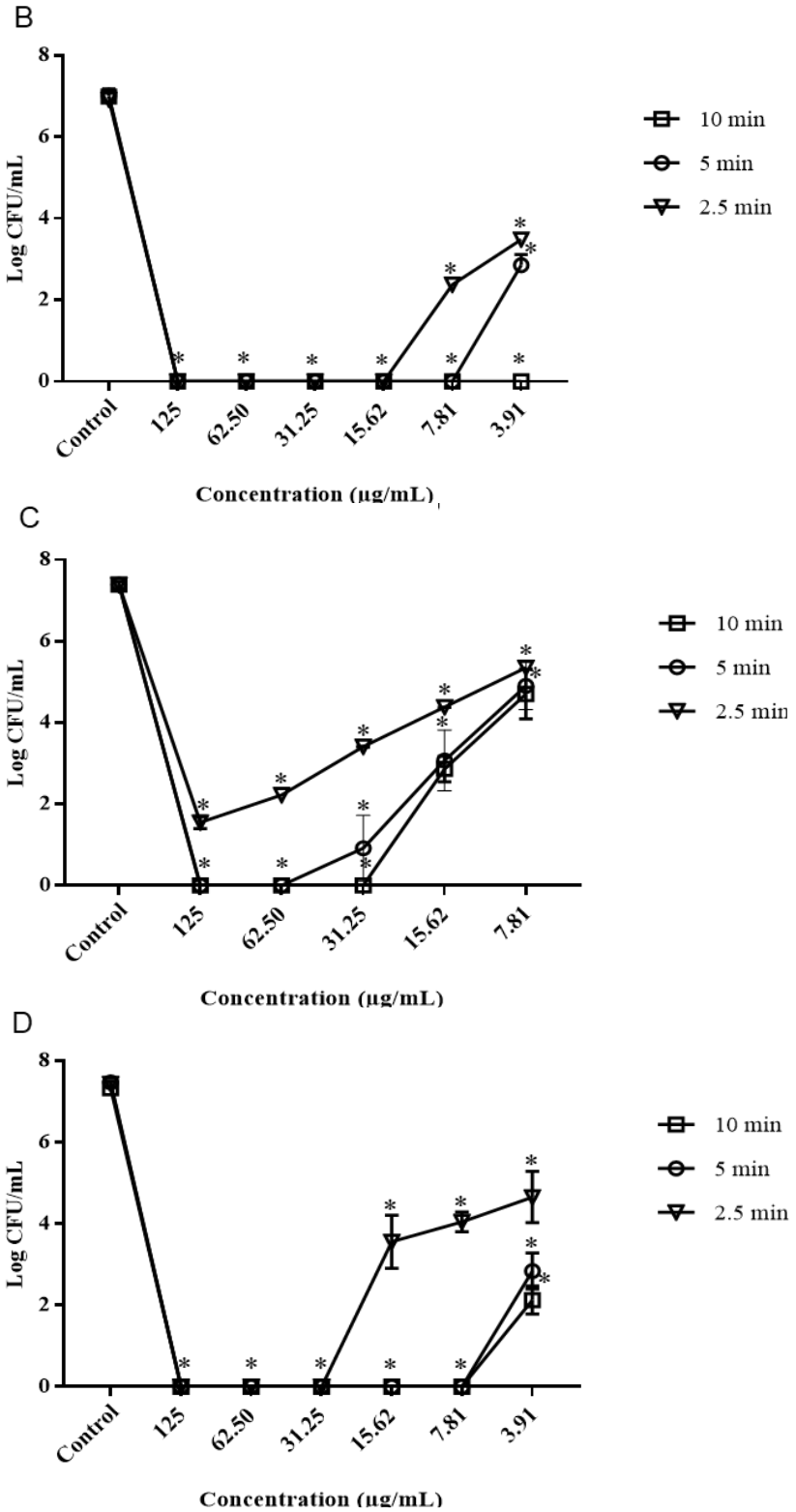


Figure 2. Effect of different illumination times and WSC concentrations on the photodynamic inactivation of *Staphylococcus aureus:* (A) WSC with lactic acid and (B) WSC with citric acid (B); *Escherichia coli:* (C): WSC with lactic acid and (D) WSC with citric acid. The control group represents cells in saline solution. Data are presented means values and error bars indicate standard deviations (* $p < 0.05$).

Among the studies that evaluated aPDT mediated by WSC at neutral pH as an alternative to microbial control, dos Santos et al. (2019) found a greater reduction in *S. aureus* with 750 µg/mL and 5 min of LED illumination (450 nm; 32.1 mW/cm²; 10 J/cm²). In contrast, Mirzahosseinipour et al. (2020) did not observe a significant reduction in *S. aureus* population with nanocurcumin-silica (50 µg/mL) illuminated for 10 min with a LED source. Regarding the influence of pH, few studies demonstrated the action of aPDT in acidic pH. Dias et al. (2021), worked with curcumin in Pluronic® P123 nanoparticle against *S. aureus* and compared the influence of pH in its antimicrobial activity. At pH 7.2, bacterial counts were reduced by 1.5 – 2.0 log CFU/mL with 15 and 30 min of LED exposure and curcumin nanoparticle at 31.25, 15.62, and 7.80 µmol/L. At pH 5.0 with hydrochloric acid, a greater antimicrobial reduction was demonstrated. At the same conditions of illumination time and curcumin concentrations, a total inactivation of *S. aureus* was observed.

Gram-negative bacteria demonstrated greater resistance to treatment. So higher concentrations of WSC (125 and 62.5 µg/mL) and exposure time to illumination (10 and 5 min) were necessary to completely inactivate the *E. coli* when WSC was associated with lactic acid (Figure 2C). Under the same treatment conditions, WSC with citric acid at 31.25 and 15.62 µg/mL, and 10 and 5 min of light exposure resulted in the complete elimination of *E. coli* (Figure 2D). De Oliveira et al. (2018) compared the effect of pH on curcumin-mediated photoinactivation against *E. coli* O157:H7, and found that, at pH 3.0, the combination of UV-A light and curcumin (5 mg/L) showed a reduction of more than 5 log CFU/mL after 2 min of illumination while at pH 6.0 no reduction was observed.

It is important to highlight those results demonstrated that the use of WSC as PS was significantly more effective for inhibiting microbial growth, requiring lower concentrations when compared to MIC. For *S. aureus* the combination of WSC with lactic acid and 2.5 min of illumination time reduced the MIC from 500 µg/mL to 15.62 µg/mL, and WSC with citric acid and 5 min of exposure to light reduced the MIC from 125 µg/mL to 7.81 µg/mL. While for *E. coli*, curcumin that previously did not inhibit bacterial growth, when exposed to 5 and 2.5 min of illumination time, inhibited growth with concentrations of 62.5 µg/mL of WSC with lactic acid and 31.25 µg/mL with citric acid, respectively.

Photosensitive compounds, in the presence of oxygen, are excited when exposed to light, producing energy that results in the production of reactive species of oxygen, such as singlet oxygen and/or hydroxyl radicals, superoxide, and hydrogen peroxide, causing several oxidative reactions that are cytotoxic in the cell and lead to an apoptotic response (do Prado-Silva et al., 2022; Wang et al., 2021; Cieplik et al., 2018).

Association of WSC with organic acids showed good results in aPDT, citric acid promoted a greater reduction in bacterial counts compared with lactic acid. The results were consistent with Wang et al. (2021) that pH lower than 5.2 with phosphoric acid-citric acid buffer could lead to an enhanced inhibitory effect of curcumin, reducing cell viability. According to de Oliveira et al. (2018), citric acid can increase the susceptibility of cells to PS and/or improve curcumin photoactivity. Furthermore, the acidic pH contributes to increasing the solubility and stability of curcumin and improve cell permeability, making the cell more susceptible to aPDT (Lund et al., 2014).

The mechanism of action that improved aPDT in acidic pH has not been elucidated in the present study, but according to Lund et al. (2014), although organic acids are considered weak, causing stress in bacteria, as they are less dissociated, they freely cross the membrane causing a collapse of the pH gradient in bacterial cells. Thus, lactic and citric acids can disrupt the integrity of the cell wall, promoting the entry of PS into the cell (Zhu et al., 2021). Lactic acid can act by releasing lipopolysaccharides from the cell wall, compromising the integrity, and exposing membrane lipids, while citric acid, being a chelating agent, removes Ca^{2+} and Mg^{2+} ions from the cell wall, increasing membrane permeability and releasing phospholipids and lipoproteins. Since lipids and proteins are the main targets of ROS during aPDT, citric acid may have potentiated the aPDT by exposing these macromolecules (Ghate et al., 2015).

4. Conclusion

The results demonstrated that WSC combined with a blue LED light is effective against *S. aureus* and *E. coli*, requiring lower concentrations compared to MIC. Low illumination time and WSC concentration associated with organic acids were enough for eradication of bacterial growth, being *S. aureus* more susceptible to aPDT. WSC mediated aPDT was most effective when combined with citric acid. Therefore, when WSC was applied in aPDT, it enables the development of a promising alternative to be applied in foodborne pathogens control.

Declaration of Conflicting Interests

The authors declare that there are no conflicts of interest.

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References

Adamczak A, Ożarowski M and Karpiński TM (2020) Curcumin, a Natural Antimicrobial Agent with Strain-Specific Activity. *Pharmaceuticals* 13(7): 153. DOI: 10.3390/ph13070153.

Alippilakkotte S and Sreejith L (2018) Pectin mediated synthesis of curcumin loaded poly (lactic acid) nanocapsules for cancer treatment. *Journal of Drug Delivery Science and Technology* 48. Elsevier: 66–74. DOI: 10.1016/j.jddst.2018.09.001.

Belma P, Kenan Č, Dina L, et al. (2021) Curcumin: Natural Antimicrobial and Anti Inflammatory Agent. *Journal of Pharmaceutical Research International* 32(43): 1–8. DOI: 10.9734/jpri/2020/v32i4331060.

Bhawana, Basniwal RK, Buttar HS, et al. (2011) Curcumin Nanoparticles: Preparation, Characterization, and Antimicrobial Study. *Journal of Agricultural and Food Chemistry* 59(5): 2056–2061. DOI: 10.1021/jf104402t.

Bouarab Chibane L, Degraeve P, Ferhout H, et al. (2019) Plant antimicrobial polyphenols as potential natural food preservatives. *Journal of the Science of Food and Agriculture* 99(4): 1457–1474. DOI: 10.1002/jsfa.9357.

Burel C, Kala A and Purevdorj-Gage L (2021) Impact of pH on citric acid antimicrobial activity against Gram-negative bacteria. *Letters in Applied Microbiology* 72(3): 332–340. DOI: 10.1111/lam.13420.

Burns J, McCoy CP and Irwin NJ (2021) Synergistic activity of weak organic acids against uropathogens. *Journal of Hospital Infection* 111. Elsevier Ltd: 78–88. DOI: 10.1016/j.jhin.2021.01.024.

Cherrington CA, Hinton M, Mead GC, et al. (1991) Organic Acids: Chemistry, Antibacterial Activity and Practical Applications. In: *Advanced in Microbial Physiology*, pp. 87–108. DOI: 10.1016/S0065-2911(08)60006-5.

Cieplik F, Deng D, Crielaard W, et al. (2018) Antimicrobial photodynamic therapy – what we know and what we don't. *Critical Reviews in Microbiology* 44(5). Informa Healthcare USA, Inc: 571–589. DOI: 10.1080/1040841X.2018.1467876.

CLSI (2020) *Performance Standards for Antimicrobial Susceptibility Testing, 30th Ed. CLSI Supplement M100*. Clinical and Laboratory Standards Institute, Wayne, PA.

de Oliveira EF, Tosati J V, Tikekar R V, et al. (2018) Antimicrobial activity of curcumin in

combination with light against Escherichia coli O157:H7 and Listeria innocua : Applications for fresh produce sanitation. *Postharvest Biology and Technology* 137. Elsevier: 86–94. DOI: 10.1016/j.postharvbio.2017.11.014.

Delgado Y, Cassé C, Ferrer-Acosta Y, et al. (2021) Biomedical Effects of the Phytonutrients Turmeric, Garlic, Cinnamon, Graviola, and Oregano: A Comprehensive Review. *Applied Sciences* 11(18): 8477. DOI: 10.3390/app11188477.

Dias VHC, Malacrida AM, dos Santos AR, et al. (2021) pH interferes in photoinhibitory activity of curcumin nanoencapsulated with pluronic® P123 against Staphylococcus aureus. *Photodiagnosis and Photodynamic Therapy* 33. Elsevier B.V.: 102085. DOI: 10.1016/j.pdpdt.2020.102085.

do Prado-Silva L, Brancini GTP, Braga GÚL, et al. (2022) Antimicrobial photodynamic treatment (aPDT) as an innovative technology to control spoilage and pathogenic microorganisms in agri-food products: An updated review. *Food Control* 132. Elsevier Ltd: 108527. DOI: 10.1016/j.foodcont.2021.108527.

dos Santos RF, Campos BS, Rego Filho F de AMG, et al. (2019) Photodynamic inactivation of S. aureus with a water-soluble curcumin salt and an application to cheese decontamination. *Photochemical & Photobiological Sciences* 18(11). Royal Society of Chemistry: 2707–2716. DOI: 10.1039/C9PP00196D.

Faille C, Cunault C, Dubois T, et al. (2018) Hygienic design of food processing lines to mitigate the risk of bacterial food contamination with respect to environmental concerns. *Innovative Food Science & Emerging Technologies* 46. Elsevier: 65–73. DOI: 10.1016/j.ifset.2017.10.002.

Food and Drug Administration (2021) Code of Federal Regulations, Title 21. Available at: p://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm.

Fung F, Wang H and Menon S (2018) Food safety in the 21st century. *Biomedical Journal* 41(2). Elsevier Ltd: 88–95. DOI: 10.1016/j.bj.2018.03.003.

Gao J and Matthews KR (2020) Effects of the photosensitizer curcumin in inactivating foodborne pathogens on chicken skin. *Food Control* 109. Elsevier: 106959. DOI: 10.1016/j.foodcont.2019.106959.

Ghate V, Kumar A, Zhou W, et al. (2015) Effect of organic acids on the photodynamic inactivation of selected foodborne pathogens using 461 nm LEDs. *Food Control* 57.

Elsevier Ltd: 333–340. DOI: 10.1016/j.foodcont.2015.04.029.

Gutiérrez-del-Río I, Fernández J and Lombó F (2018) Plant nutraceuticals as antimicrobial agents in food preservation: terpenoids, polyphenols and thiols. *International Journal of Antimicrobial Agents* 52(3): 309–315. DOI: 10.1016/j.ijantimicag.2018.04.024.

Hewlings S and Kalman D (2017) Curcumin: A Review of Its Effects on Human Health. *Foods* 6(10): 92. DOI: 10.3390/foods6100092.

Hilmi B, Bustami Y, Trongsatitkul T, et al. (2019) The Effect of Natural Antimicrobial Agents on Staphylococcus aureus and Escherichia coli Growth. *Journal of Physical Science* 30(Supp.2): 55–63. DOI: 10.21315/jps2019.30.s2.5.

Holetz FB, Pessini GL, Sanches NR, et al. (2002) Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. *Memórias do Instituto Oswaldo Cruz* 97(7): 1027–1031. DOI: 10.1590/S0074-02762002000700017.

Kim HW, Lee NY, Park SM, et al. (2020) A fast and effective alternative to a high-ethanol disinfectant: Low concentrations of fermented ethanol, caprylic acid, and citric acid synergistically eradicate biofilm-embedded methicillin-resistant Staphylococcus aureus. *International Journal of Hygiene and Environmental Health* 229. Elsevier GmbH: 113586. DOI: 10.1016/j.ijheh.2020.113586.

Lin Y, Hu J, Li S, et al. (2019) Curcumin-Based Photodynamic Sterilization for Preservation of Fresh-Cut Hami Melon. *Molecules* 24(13): 2374. DOI: 10.3390/molecules24132374.

Lund P, Tramonti A and De Biase D (2014) Coping with low pH: molecular strategies in neutralophilic bacteria. *FEMS Microbiology Reviews* 38(6): 1091–1125. DOI: 10.1111/1574-6976.12076.

Mangolim CS, Moriwaki C, Nogueira AC, et al. (2014) Curcumin–β-cyclodextrin inclusion complex: Stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application. *Food Chemistry* 153. Elsevier Ltd: 361–370. DOI: 10.1016/j.foodchem.2013.12.067.

Mirzahosseinipour M, Khorsandi K, Hosseinzadeh R, et al. (2020) Antimicrobial photodynamic and wound healing activity of curcumin encapsulated in silica nanoparticles. *Photodiagnosis and Photodynamic Therapy* 29. Elsevier: 101639. DOI: 10.1016/j.pdpdt.2019.101639.

Polat E and Kang K (2021) Natural Photosensitizers in Antimicrobial Photodynamic Therapy. *Biomedicines* 9(6): 584. DOI: 10.3390/biomedicines9060584.

Powell DA, Jacob CJ and Chapman BJ (2011) Enhancing food safety culture to reduce rates of foodborne illness. *Food Control* 22(6). Elsevier Ltd: 817–822. DOI: 10.1016/j.foodcont.2010.12.009.

Praditya D, Kirchhoff L, Brüning J, et al. (2019) Anti-infective Properties of the Golden Spice Curcumin. *Frontiers in Microbiology* 10: 1–16. DOI: 10.3389/fmicb.2019.00912.

Priyadarsini KI (2009) Photophysics, photochemistry and photobiology of curcumin: Studies from organic solutions, bio-mimetics and living cells. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews* 10(2): 81–95. DOI: 10.1016/j.jphotochemrev.2009.05.001.

Scharff RL (2015) State Estimates for the Annual Cost of Foodborne Illness. *Journal of Food Protection* 78(6): 1064–1071. DOI: 10.4315/0362-028X.JFP-14-505.

Silva AC da, Santos PD de F, Silva JT do P, et al. (2018) Impact of curcumin nanoformulation on its antimicrobial activity. *Trends in Food Science & Technology* 72. Elsevier: 74–82. DOI: 10.1016/j.tifs.2017.12.004.

Wang Z, Jia Y, Li W, et al. (2021) Antimicrobial photodynamic inactivation with curcumin against Staphylococcus saprophyticus, in vitro and on fresh dough sheet. *LWT* 147. Elsevier Ltd: 111567. DOI: 10.1016/j.lwt.2021.111567.

World Health Organization (2015) *Who estimates of the global burden of foodborne diseases: foodborne diseases burden epidemiology reference group 2007-2015*. DOI: 10.1007/978-3-642-27769-6_3884-1.

World Health Organization (2020) Food Safety. Available at: https://www.who.int/NEWS-ROOM/FACT-SHEETS/DETAIL/FOOD-SAFETY.

Yang S-C, Lin C-H, Aljuffali IA, et al. (2017) Current pathogenic Escherichia coli foodborne outbreak cases and therapy development. *Archives of Microbiology* 199(6). Springer Berlin Heidelberg: 811–825. DOI: 10.1007/s00203-017-1393-y.

Zhu S, Song Y, Pei J, et al. (2021) The application of photodynamic inactivation to microorganisms in food. *Food Chemistry: X* 12. Elsevier Ltd: 100150. DOI: 10.1016/j.fochx.2021.100150.

ARTICLE II

Optimization of the antimicrobial photodynamic therapy mediated by water-soluble curcumin using the response surface methodology

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Highlights

- aPDT mediated by water-soluble curcumin and LED at acid pH was effective against foodborne pathogens.
- *Staphylococcus aureus* was the most sensitive bacterial tested to photodynamic therapy mediated by water-soluble curcumin.
- Experimental design optimized the aPDT process.

Abstract

Antimicrobial photodynamic therapy has been considered a promising technique for microbiological control and it can be applied in the prevention of foodborne diseases. This work aimed to evaluate the interaction of different water-soluble curcumin (WSC) concentrations and illumination times at acid pH in photodynamic therapy against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* Typhimurium using an experimental design and response surface methodology. The adjusted model predicted the combined influences of these factors, in accordance with predictions and experimental observations (R^2_{adj} = 0.6448 for *S. aureus*; R^2_{adj} = 0.9176 for *E. coli;* R^2_{adj} = 0.8196 for *S.* Typhimurium). The highest reductions of *S. aureus* were observed with curcumin at 4.41µg/mL and an illumination time of 6.77 min, while higher concentrations and illumination time were needed to total inhibit of *E. coli* (14.74 µg/mL and 9.51 min) and *Salmonella* Typhimurium (60 µg/mL and 15 min) growth. It was concluded that the response surface methodology was effective for optimization of aPDT and the treatment mediated by WSC could potentially be applied to control foodborne pathogens.

Keywords: Curcumin; Experimental design; Foodborne pathogens; Photodynamic inactivation

1. Introduction

Antimicrobial photodynamic therapy (aPDT) has been considered a promising technique for microbiological control and has been widely used in different areas, such as dentistry (1), medicine (2), environmental (3), and industry (4).

In the food industry, aPDT can be applied in the prevention of foodborne diseases, controlling pathogens such as *Staphylococcus aureus*, *Salmonella* spp., and *Escherichia coli* (5-7). According to the Centers for Disease Control and Prevention (CDC), they are among the main bacterial agents transmitted by food and responsible for outbreaks and hospitalization (8).

aPDT is considered advantageous for microorganism's control in the production chain, as it is a low-cost, non-thermal processing that allows, in most cases, to preserve food's sensory characteristics, especially texture, flavor, and color (9, 10). The process involves a photochemical reaction that consists of combining a photosensitive substance (PS) with visible light at a suitable wavelength that in the presence of molecular oxygen produces reactive oxygen species (ROS), including singlet oxygen and/or hydroxyl radicals, superoxide, and hydrogen peroxide, which cause the microorganism's death by promoting irreversible molecular damage to cells (11-14).

The efficiency of aPDT mainly depends on the photosensitizer to absorb light energy and generate ROS (15). Several compounds have been studied as PS, including natural-photoactivable compounds (12). Curcumin is a natural dye widely used by industry due to its low cost and safety (9, 16). It has been used as PS for assuring the microbiological quality of food – vegetables, fruits, meat, and dairy products - as well as on surfaces and packaging (4, 9, 10, 17, 18). Its use is still restricted because it is considered very hydrophobic, however, new alternatives sources have been developed to facilitate its solubilization and improve its stability, as water-soluble curcumin formulations (17,18).

In addition to new methodologies to overcome curcumin hydrophobicity, several studies have demonstrated the efficiency of aPDT associated with acids. Wang et al. (14), Dias et al. (19), and de Oliveira et al. (20) demonstrated that low pH can increase the aPDT efficacy mediated by curcumin, improving the antimicrobial activity.

Considering the potential of curcumin as PS, the application of an experimental design could determine the optimal conditions to inactivate microorganisms in aPDT (21, 22). The central composite design allows for the multivariate variation of independent parameters simultaneously leading to an objective, meaningful response, with less experimentation and costs. This method uses quantitative data from experiments designed to define the relationships between

the response and the variables (23, 24).

So, the present study aimed to apply an experimental design to evaluate the interaction of water-soluble curcumin concentration at acidic pH (pH 5.0) and illumination time using blue light-emitting diodes in aPDT against *Staphylococcus aureus* ATCC 25923, *Salmonella enterica* serovar Typhimurium ATCC 14028 and *Escherichia coli* ATCC 25922.

2. Materials and Methods

2.1 Photosensitizer

Water-Soluble Curcumin (WSC, Natural Powder Curcumin Water Soluble) was kindly provided by the company IFC Solutions (Liden, NJ, USA) and obtained in partnership with Federal University of Technology - Paraná (Campo Mourão *campus*). It was dissolved and diluted using sterile acidic saline 0.85% (pH 5.0), acidified with citric acid (1M).

2.2 Light Source

The blue LED light system used in the assays consisted of LEDs arranged built to illuminate 96-well plates. The prototype was composed of 20 LEDs (3 W) with an irradiance of 16 mW/cm² and a wavelength of 450 nm. The absolute irradiance of LEDs was evaluated in a Spectroradiometer USB2000 + RAD (Ocean Optics, Winter Park, FL, USA) and the spectral emission was obtained using a spectrofluorometer (Varian Gary Eclipse, San Diego, United States). The maximum light dose (fluency) was 15.0 J/cm², calculated by multiplying irradiance by illumination time.

2.3 Bacterial strains and culture conditions

Staphylococcus aureus ATCC 25923, *Salmonella enterica* serovar Typhimurium ATCC 14028, and *Escherichia coli* ATCC 25922 were used in the study. Bacterial strains were stored at -20ºC (BHI, Difco, Le Pont de Claix France) supplemented with 20% of glycerol (v/v). Before assays, an aliquot of each strain was cultured overnight in BHI at 35ºC. Then, centrifuged at 4500g

for 5 minutes, three-time washed and resuspended in 0.85% saline solution. Cell density was adjusted to 10^7 colonies forming unit per milliliter (CFU/mL) in a Varian Cary-Eclipse spectrophotometer at 580nm for use in the experiments (25).

2.4 Antimicrobial photodynamic therapy

An aliquot of 10µL of inoculum (10^7 CFU/mL) was homogenized with 190µL WSC diluted in acidic saline 0.85% (pH 5.0, acidified with citric acid) at different concentrations in a 96-well microplate and kept in the dark for 10 min. The WSC concentrations used were pre-determined in preliminary studies (data not shown). Five control groups were used: positive control (bacterium inoculum without irradiation – $PS_{(-)}L_{(-)}$), PS control (bacterium inoculum and PS without irradiation – $PS_{(+)}L_{(-)}$), light control (bacterium inoculum exposed to LED – $PS_{(-)}L_{(+)}$), citric acid control (bacterium inoculum in acidic saline without irradiation – $A_{(+)}L_{(-)}$) and citric acid light control (bacterium inoculum in acidic saline exposed to LED – $A_{(+)}L_{(+)}$).

After treatment (Table 1), 100µL of samples and controls were serially diluted in 0.85% saline solution. Then, 10 µL of each dilution were plated on Trypticase Soy Agar (Difco, Le Pont de Claix France) and incubated at 35 ºC for 24 h. The results were expressed as log CFU/mL.

2.5 Optimization of the aPDT using the response surface methodology

Based on the results obtained in aPDT, an experimental design was proposed to determine the optimal conditions for the application of aPDT to lead to an objective and meaningful response. For this, a Rotatable Central Composite Design (RCCD) generated by the software MATLAB R2021b was used to optimize the conditions for photodynamic therapy mediated by WSC against *S. aureus*, *S.* Typhimurium, and *E. coli*. The experimental design proposes eight experiments with four repetitions of the central point performed to evaluate the combined effects of two independent variables, x_1: the irradiation time (minutes) and x_2: the concentrations of WSC (µg/mL) (Table 1).

A second-order polynomial model was used for fitting the experimental data, and its coefficients were obtained by multiple linear regression.

$$\log CFU/mL = b_0 + b_1x_1 + b_{11}x_1^2 + b_2x_2 + b_{22}x_2^2 + b_{12}x_1x_2$$

Where log CFU/mL is the predicted response, x_1 and x_2 are the independent variables in coded values, b_0 is the regression coefficient for the intercept, b_1 and b_2 are the regression coefficients representing the linear effect terms, b_{11} and b_{22} are the quadratic effect terms, and b_{12} is the interaction effect terms.

A t-test was used to analyze the regression coefficients of the mathematical models and excluded those that were not significant ($p > 0.05$). The models were validated through the ANOVA ($\alpha = 0.05$), the coefficient of determinations (R^2), and the adjusted coefficient of determination (R^2_{adj}) were used. The software MATLAB R2021b was used for the data analysis and for generating the response surface plots.

3. Results and Discussion

3.1 Light Doses

In this study, the light-emitting diode (LED) has been applied in aPDT, proving to be an option for activating PS as it is a durable and economical energy source, with a broad spectrum and low heat emission (13, 26). The visible absorption spectrum of WSC and the light-emitting diode potency (P_{LED} emitted) are shown in Figure 1. The literature shows that curcumin in polar solvents the absorption maximum is at ~420nm and in hydrogen bond donor and acceptor solvents are around 430-434 nm (27).

The difference in light spectra emitted by LED ($P_{LED\ emitted}$) and power absorbed by WSC (P_{Abs}) at different PS concentrations is shown in Fig 2. The intensity of the $P_{LED\ emitted}$ was higher than the P_{Abs} spectra at different PS concentrations, and these results demonstrated an adequate match between the PS absorption spectra and the emission of LED.

3.2 Optimization of the photodynamic therapy using the response surface methodology

The combination of blue LED with curcumin has shown good results in aPDT to control foodborne pathogens, and when associated with acids, its demonstrate better PS efficiency,

improving the photoactivity and increasing the susceptibility of bacterial cells to aPDT (14, 19, 20). In addition, the use of RSM made it possible to predict the necessary conditions, PS concentrations and illumination time, to obtain bacterial inhibition with a smaller number of experiments.

For this, previous experiments with different concentrations of PS and times of illumination were performed to determine which parameters would be used in the statistics experimental design (data not shown). The different concentrations of WSC and illumination time established by the experimental design are shown in Table 1.

Staphylococcus aureus counts ranged from 1.88 log CFU/mL to 4.39 log CFU/mL. The highest reduction was observed with 4.41 µg/mL of WSC and an illumination time of 6.77 min (6.8 J/cm²) (Table 1). Dias et al. (19) evaluating the influence of pH on aPDT mediated by curcumin in Pluronic® P123 nanoparticles against *S. aureus*, demonstrated bacterial counts of approximately 2.5 log CFU/mL using 7.80 µmol/L PS at acidic pH with illumination time of 8.75 min (3.80 J/cm²). Dos Santos et al. (18), working with different concentrations of water-soluble salt at neutral pH, observed that 0.75 mg/mL of curcumin showed a high reduction in the counts of *S. aureus* when irradiated for 5 min with blue LED (450 nm; 32.1 mW/cm²; 10 J/cm²). While Mirzahosseinipour et al. (28), working with curcumin (50 µg/mL) and nano curcumin-silica (50 µg/mL) at 10 min irradiation (20 J/cm²) did not observe a significant reduction in the viable count of *S. aureus*.

The complete inhibition of *E. coli* was observed when the bacterial strain was treated with WSC at 13.62 µg/mL and an illumination time of 8.90 min (8.5 J/cm²) (Table 1). Oliveira et al. (20) evaluated the effect of pH (pH 5.0) on the inactivation of *E.* coli, and the combination of 5 µg/mL of curcumin in ethanol exposed to UV-A for 5 min resulted in a reduction of 3.5 log CFU/mL. At neutral pH, Bhavya; Umesh Hebbar (29) reported a reduction of 5.94 log CFU/mL for *E. coli*, when treat with 20 µM of curcumin in ethanol at 1 hour of blue light (13 J/cm²). The full inactivation was observed by Penha et al. (30), with curcumin at 75 µM and 30 min of illumination (139 J/cm²).

The combined treatment of 54.14 µg/mL WSC and illumination time of 13.54 min (13.6 J/cm²) showed the complete inhibition of *S.* Typhimurium (Table 1). Gao et al. (17) evaluated a WSC at 200 ppm at neutral pH and obtained a reduction of 1.8-3.6 log of *Salmonella* spp. strains using 5 min of illumination (32 kJ/m²). The antimicrobial photodynamic therapy effects on *S.* Typhimurium were evaluated by Penha et al. (30), and the authors observed reductions of 1.26, 1.81, and 2.82 log CFU/mL, when treated with 75 µM curcumin in DMSO neutral pH and irradiated for 10, 20, and 30 min, respectively.

Furthermore, it is important to note that when tested alone, the WSC, the light and organics acids, were not able to inhibit bacterial growth.

In the experimental models, coefficients that were not statistically significant ($p > 0.05$) were eliminated (Table 2). However, the coefficients b_1 and b_{11} for *S. aureus* were kept, although they were not significant, to preserve the quality of fit of the experimental design. For *S.* Typhimurium after eliminating the b_{22} and b_{11} coefficients, the b_{12} coefficient became statistically significant ($p = 0.0496$).

The analysis of variance (ANOVA), shown in Table 3, demonstrated that the models were significant. Although, lack of fit was observed (*S. aureus*, $p = 0.0139$, *E. coli*, $p = 0.0117$ and *S.* Typhimurium, $p = 0.0047$), possibly due to the absence of homoscedasticity of the residues, in order words, some experimental points showed high residuals between observed values and predicted values. Possibly the lack of fit of these models was because of the natural variability due to its microbiological experimentation or these exposure factor rangers were too narrow. In the evaluation of the models by ANOVA, the R^2 was considered suitable, although the model indicated that it had a significant lack of fit. This can happen due to the pure error being almost zero, then the Flack of fit/pure error results in a high value, could lead to misinterpretation. Therefore, the ANOVA table must be observed carefully.

The observed values of R^2 of 0.7417, R^2_{adj} of 0.6448, and the root mean square error (RMSE) of 0.4904 for *S. aureus* demonstrated that the model did not present a good predictive capacity as the adjusted R^2 was far from 1. On the other hand, *E. coli* presented R^2 of 0.9401, R^2_{adj} of 0.9176 and RMSE of 0.5150, and *S.* Typhimurium values of R^2 of 0.8688, R^2_{adj} of 0.8196 and

RMSE of 0.4565. So both were considered adequate to predict the photodynamic activity of the WSC with the blue light LED, being the value of R^2_{adj} the measure of the goodness of model's fit, indicates that 91,76% for *E. coli* and 81,96% for *S.* Typhimurium of the total variation is explained by the model. It is important to highlight that the RMSE values obtained were lower than the detection limit of the method, which is 2 log CFU/mL.

The models demonstrated that there was no significant interaction between exposure time and PS concentration for *S. aureus* and *E. coli*. For *S. aureus*, in the slice plot (Figure 3), it was possible to observe a predicted count to 2 log CFU/mL (lowest count) for the time values of 6.3 min (+ 0.75) and concentration of curcumin 5.0 µg/mL (+1.414). Even working with lower concentrations and illumination time than those mentioned studies (18, 19, 28). this study achieved better results in the antimicrobial photodynamic treatment. Based on the surface response model, we decided to test higher concentrations than those evaluated, and the complete inhibition of *S. aureus* was obtained with 7.81 µg/mL curcumin and an illumination time of 5 min (5 J/cm²).

Escherichia coli model showed a quadratic effect of time (b_{11}) with a negative sign. T thus, the response surface has a maximum point for this variable. It is possible to observe a total inhibition when illuminated during 9.51 min (+ 1.23) with a concentration of curcumin 14.74 µg/mL (+ 1.23). The values of illumination time and WSC concentration capable of total inactivation were lower than those found in the literature (20, 29, 30). Oliveira et al. (20) also showed higher efficiency of aPDT in fairly less illumination time, probably due to the action of citric acid on membrane permeability and cell metabolism, promoting penetration of curcumin and greater susceptibility of *E. coli* to the treatment.

For *S.* Typhimurium, none of the quadratic effects was statistically significant (Figure 5). The total inhibition was in the experimental region of maximum concentration (60 µg/mL) and maximum time (15 min), requiring higher concentrations of photosensitizer and illumination time. Confirming that among the Gram-negative bacteria tested, *S.* Typhimurium was less susceptible and required higher concentrations of the PS and/or illumination time. Penha et al. (30) also observed resistance of *Salmonella* Typhimurium to aPDT when compared to *E. coli.*

The present study showed that the combination of WSC in acid pH and blue LED has a great effect in reduced the cell viability of *S. aureus*, *E. coli* and *S.* Typhimurium, even working with lower PS concentrations and irradiation times. The results confirmed that Gram-negative bacteria are more resistant to aPDT than Gram-positive, requiring higher concentrations of PS and illumination time. Gram-negative bacteria have an outer membrane and a thin peptidoglycan layer that act as a physical and functional barrier, while Gram-positive have a thick wall composed of peptidoglycan with a high degree of porosity and permeability (6, 15, 20).

In addition, the effective results with low concentrations and illumination time may be related to the association of WSC with citric acid. According to Zhu et al. (15), citric acid can destroy the integrity of the cell wall and facilitate the entry of PS into the microorganism's cell.

Considering the results obtained, it can be stated that optimization of the photodynamic therapy using the response surface methodology allows making predictions regarding the concentration of WSC and illumination time needed to obtain a successful outcome in aPDT.

4. Conclusion

The results obtained demonstrate a great potential of water-soluble curcumin at acidic pH as a photosensitizer in antimicrobial photodynamic treatment, resulting in the reduction of bacterial growth with low concentrations and illumination times. The model developed provides information on the photoinhibition activity against foodborne pathogens combination of different photosensitizer concentrations and illumination time. This methodology evaluates parameters that can be varied simultaneously, reducing the number of experiments and costs to achieve the good results, being a promising approach to determine the combination of illumination time and concentrations that enhance the action of antimicrobial photodynamic treatment.

Conflict of interests

The authors declare that they have no conflict of interest.

References

[1]. Abdelkarim-Elafifi H, Parada-Avendaño I, Arnabat-Dominguez J. Photodynamic Therapy in Endodontics: A Helpful Tool to Combat Antibiotic Resistance? A Literature Review. Antibiotics [Internet]. 2021 Sep 13;10(9):1106. Available from: https://www.mdpi.com/2079-6382/10/9/1106

[2]. Dos Santos AF, De Almeida DRQ, Terra LF, Baptista MS, Labriola L. Photodynamic therapy in cancer treatment - an update review. J Cancer Metastasis Treat [Internet]. 2019 Mar 29;5(25):1–20. Available from: https://jcmtjournal.com/article/view/3026

[3]. Alves E, Faustino MAF, Neves MGPMS, Cunha Â, Nadais H, Almeida A. Potential applications of porphyrins in photodynamic inactivation beyond the medical scope. J Photochem Photobiol C Photochem Rev [Internet]. 2015 Mar;22:34–57. Available from: http://dx.doi.org/10.1016/j.jphotochemrev.2014.09.003

[4]. Luksiene Z, Brovko L. Antibacterial Photosensitization-Based Treatment for Food Safety. Food Eng Rev [Internet]. 2013 Dec 17;5(4):185–99. Available from: http://link.springer.com/10.1007/s12393-013-9070-7

[5]. Corrêa TQ, Blanco KC, Garcia ÉB, Perez SML, Chianfrone DJ, Morais VS, et al. Effects of ultraviolet light and curcumin-mediated photodynamic inactivation on microbiological food safety: A study in meat and fruit. Photodiagnosis Photodyn Ther [Internet]. 2020 Jun;30:101678. Available from: https://doi.org/10.1016/j.pdpdt.2020.101678

[6]. Galstyan A, Dobrindt U. Determining and unravelling origins of reduced photoinactivation efficacy of bacteria in milk. J Photochem Photobiol B Biol [Internet]. 2019 Aug;197:111554. Available from: https://doi.org/10.1016/j.jphotobiol.2019.111554

[7]. Josewin SW, Kim M-J, Yuk H-G. Inactivation of Listeria monocytogenes and Salmonella spp. on cantaloupe rinds by blue light emitting diodes (LEDs). Food Microbiol [Internet]. 2018 Dec;76:219–25. Available from: https://doi.org/10.1016/j.fm.2018.05.012

[8]. Centers for Disease Control and Prevention. Foodborne Germs and Illnesses [Internet]. 2020. Available from: https://www.cdc.gov/foodsafety/foodborne-germs.html

[9]. Lin Y, Hu J, Li S, Hamzah SS, Jiang H, Zhou A, et al. Curcumin-Based Photodynamic Sterilization for Preservation of Fresh-Cut Hami Melon. Molecules [Internet]. 2019 Jun 27;24(13):2374. Available from: https://www.mdpi.com/1420-3049/24/13/2374

[10]. Glueck M, Schamberger B, Eckl P, Plaetzer K. New horizons in microbiological food safety:

Photodynamic Decontamination based on a curcumin derivative. Photochem Photobiol Sci [Internet]. 2017;16(12):1784–91. Available from: http://xlink.rsc.org/?DOI=C7PP00165G

[11]. do Prado-Silva L, Brancini GTP, Braga GÚL, Liao X, Ding T, Sant'Ana AS. Antimicrobial photodynamic treatment (aPDT) as an innovative technology to control spoilage and pathogenic microorganisms in agri-food products: An updated review. Food Control [Internet]. 2022 Feb;132:108527. Available from: https://doi.org/10.1016/j.foodcont.2021.108527

[12]. Polat E, Kang K. Natural Photosensitizers in Antimicrobial Photodynamic Therapy. Biomedicines [Internet]. 2021 May 21;9(6):584. Available from: https://www.mdpi.com/2227-9059/9/6/584

[13]. Cieplik F, Deng D, Crielaard W, Buchalla W, Hellwig E, Al-Ahmad A, et al. Antimicrobial photodynamic therapy – what we know and what we don't. Crit Rev Microbiol [Internet]. 2018 Sep 3;44(5):571–89. Available from: https://doi.org/10.1080/1040841X.2018.1467876

[14]. Wang Z, Jia Y, Li W, Zhang M. Antimicrobial photodynamic inactivation with curcumin against Staphylococcus saprophyticus, in vitro and on fresh dough sheet. LWT [Internet]. 2021 Jul;147:111567. Available from: https://doi.org/10.1016/j.lwt.2021.111567

[15]. Zhu S, Song Y, Pei J, Xue F, Cui X, Xiong X, et al. The application of photodynamic inactivation to microorganisms in food. Food Chem X [Internet]. 2021 Dec;12:100150. Available from: https://doi.org/10.1016/j.fochx.2021.100150

[16]. Mangolim CS, Moriwaki C, Nogueira AC, Sato F, Baesso ML, Neto AM, et al. Curcumin–β-cyclodextrin inclusion complex: Stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application. Food Chem [Internet]. 2014 Jun;153:361–70. Available from: http://dx.doi.org/10.1016/j.foodchem.2013.12.067

[17]. Gao J, Matthews KR. Effects of the photosensitizer curcumin in inactivating foodborne pathogens on chicken skin. Food Control [Internet]. 2020 Mar;109:106959. Available from: https://doi.org/10.1016/j.foodcont.2019.106959

[18]. dos Santos RF, Campos BS, Rego Filho F de AMG, Moraes JDO, Albuquerque ALI, da Silva MCD, et al. Photodynamic inactivation of S. aureus with a water-soluble curcumin salt and an application to cheese decontamination. Photochem Photobiol Sci [Internet].

2019;18(11):2707–16. Available from: http://xlink.rsc.org/?DOI=C9PP00196D

[19]. Dias VHC, Malacrida AM, dos Santos AR, Batista AFP, Campanerut-Sá PAZ, Braga G, et al. pH interferes in photoinhibitory activity of curcumin nanoencapsulated with pluronic® P123 against Staphylococcus aureus. Photodiagnosis Photodyn Ther [Internet]. 2021 Mar;33:102085. Available from: https://doi.org/10.1016/j.pdpdt.2020.102085

[20]. de Oliveira EF, Tosati J V, Tikekar R V, Monteiro AR, Nitin N. Antimicrobial activity of curcumin in combination with light against Escherichia coli O157:H7 and Listeria innocua : Applications for fresh produce sanitation. Postharvest Biol Technol [Internet]. 2018 Mar;137:86–94. Available from: https://doi.org/10.1016/j.postharvbio.2017.11.014

[21]. Amaral LS, Azevedo EB, Perussi JR. The response surface methodology speeds up the search for optimal parameters in the photoinactivation of E. coli by photodynamic therapy. Photodiagnosis Photodyn Ther [Internet]. 2018 Jun;22:26–33. Available from: https://doi.org/10.1016/j.pdpdt.2018.02.020

[22]. Santos AR, Silva AF, Freitas CF, Silva M V., Bona E, Nakamura C V., et al. Response surface methodology can be used to predict photoinactivation of foodborne pathogens using Rose Bengal excited by 530 nm LED. J Food Saf [Internet]. 2020 Feb 19;40(1):e12736. Available from: https://onlinelibrary.wiley.com/doi/10.1111/jfs.12736

[23]. Calado V, Montgomery DC. Planejamento de Experimentos usando Statistica. Rio de Janeiro: E-papers; 2003.

[24]. Bhattacharya S. Central composite design for response surface methodology and its application in pharmacy. In: IntechOpen, editor. Response Surface Methodology in Engineering Science. 2021.

[25]. Yassunaka NN, de Freitas CF, Rabello BR, Santos PR, Caetano W, Hioka N, et al. Photodynamic Inactivation Mediated by Erythrosine and its Derivatives on Foodborne Pathogens and Spoilage Bacteria. Curr Microbiol [Internet]. 2015 Aug 30;71(2):243–51. Available from: http://link.springer.com/10.1007/s00284-015-0827-5

[26]. Aurum FS, Nguyen LT. Efficacy of photoactivated curcumin to decontaminate food surfaces under blue light emitting diode. J Food Process Eng [Internet]. 2019 May 4;42(3):e12988. Available from: https://onlinelibrary.wiley.com/doi/10.1111/jfpe.12988

[27]. Priyadarsini KI. Photophysics, photochemistry and photobiology of curcumin: Studies from organic solutions, bio-mimetics and living cells. J Photochem Photobiol C Photochem

Rev [Internet]. 2009 Jun;10(2):81–95. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1389556709000331

[28]. Mirzahosseinipour M, Khorsandi K, Hosseinzadeh R, Ghazaeian M, Shahidi FK. Antimicrobial photodynamic and wound healing activity of curcumin encapsulated in silica nanoparticles. Photodiagnosis Photodyn Ther [Internet]. 2020 Mar;29:101639. Available from: https://doi.org/10.1016/j.pdpdt.2019.101639

[29]. Bhavya ML, Umesh Hebbar H. Efficacy of blue LED in microbial inactivation: Effect of photosensitization and process parameters. Int J Food Microbiol [Internet]. 2019 Feb;290:296–304. Available from: https://doi.org/10.1016/j.ijfoodmicro.2018.10.021

[30]. Penha CB, Bonin E, da Silva AF, Hioka N, Zanqueta ÉB, Nakamura TU, et al. Photodynamic inactivation of foodborne and food spoilage bacteria by curcumin. LWT - Food Sci Technol [Internet]. 2017 Mar;76:198–202. Available from: https://linkinghub.elsevier.com/retrieve/pii/S002364381630439X

Table 1. Experimental design with coded and real values was used to evaluate of influence of two independent variables (irradiation time and water-soluble curcumin concentrations) on *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* Typhimurium cells viability (log CFU/mL).

Experiments	Coded values		*Staphylococcus aureus*			*Escherichia coli*			*Salmonella* Typhimurium		
			Real values			Real values			Real values		
	x_1	x_2	Time (min)	Concentration (µg/mL)	Cell viability (log CFU/mL) **	Time (min)	Concentration (µg/mL)	Cell viability (log CFU/mL) **	Time (min)	Concentration (µg/mL)	Cell viability (log CFU/mL) **
Control (PS-L-)*	-	-	0	0	7.04	0	0	7.48	0	0	7.24
1	-1	-1	3.23	1.59	4.92	3.60	3.96	6.34	6.46	25.86	4.15
2	-1	1	3.23	4.41	3.07	3.60	13.62	3.35	6.46	54.14	2.63
3	1	-1	6.77	1.59	4.39	8.90	3.96	4.44	13.54	25.86	3.63
4	1	1	6.77	4.41	1.88	8.90	13.62	0.00	13.54	54.14	0.00
5	-1.41421	0	2.50	3.00	3.62	2.50	8.79	4.38	5.00	40.00	3.30
6	1.41421	0	7.50	3.00	2.84	10.00	8.79	1.74	15.00	40.00	2.49
7	0	-1.41421	5.00	1.00	3.44	6.25	1.95	6.61	10.00	20.00	3.23
8	0	1.41421	5.00	5.00	2.48	6.25	15.63	3.08	10.00	60.00	1.35
9	0	0	5.00	3.00	2.98	6.25	8.79	4.18	10.00	40.00	2.73
10	0	0	5.00	3.00	2.72	6.25	8.79	4.48	10.00	40.00	2.58
11	0	0	5.00	3.00	2.86	6.25	8.79	4.26	10.00	40.00	2.55
12	0	0	5.00	3.00	3.00	6.25	8.79	4.34	10.00	40.00	2.65

*Control containing only bacterium inoculum in saline without irradiation. **Mean value observed for two genuine repetitions.

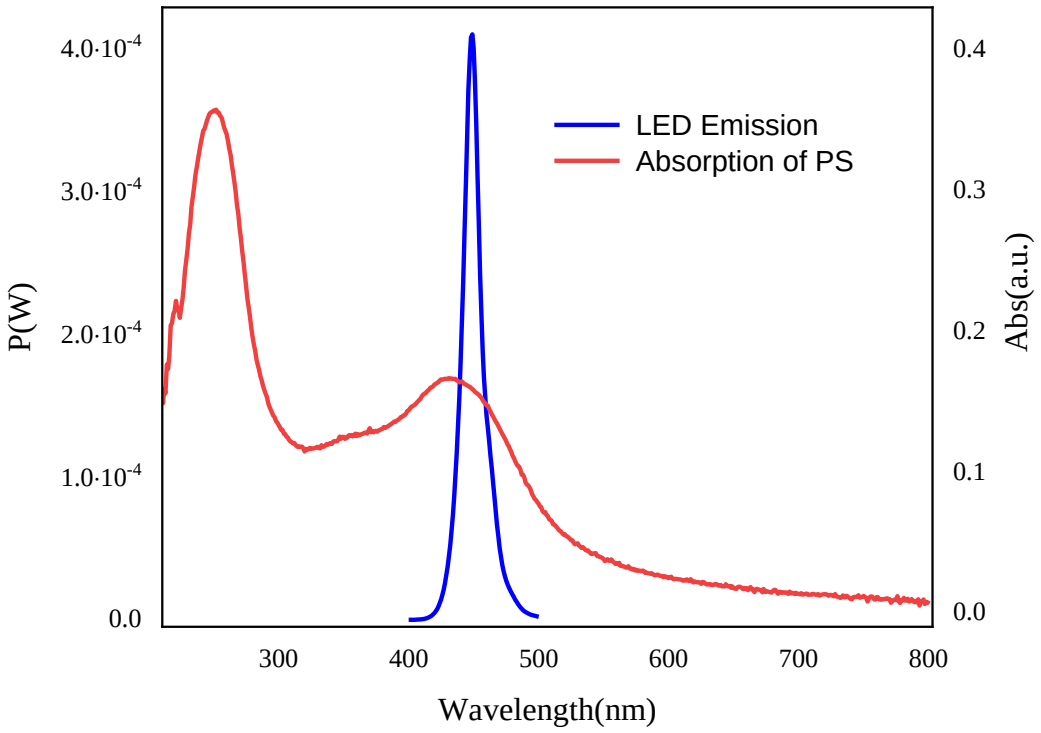

Figure 1. Light-emitting diode emitted potency ($P_{LED\ emitted}$) and PS electronic absorption spectra.

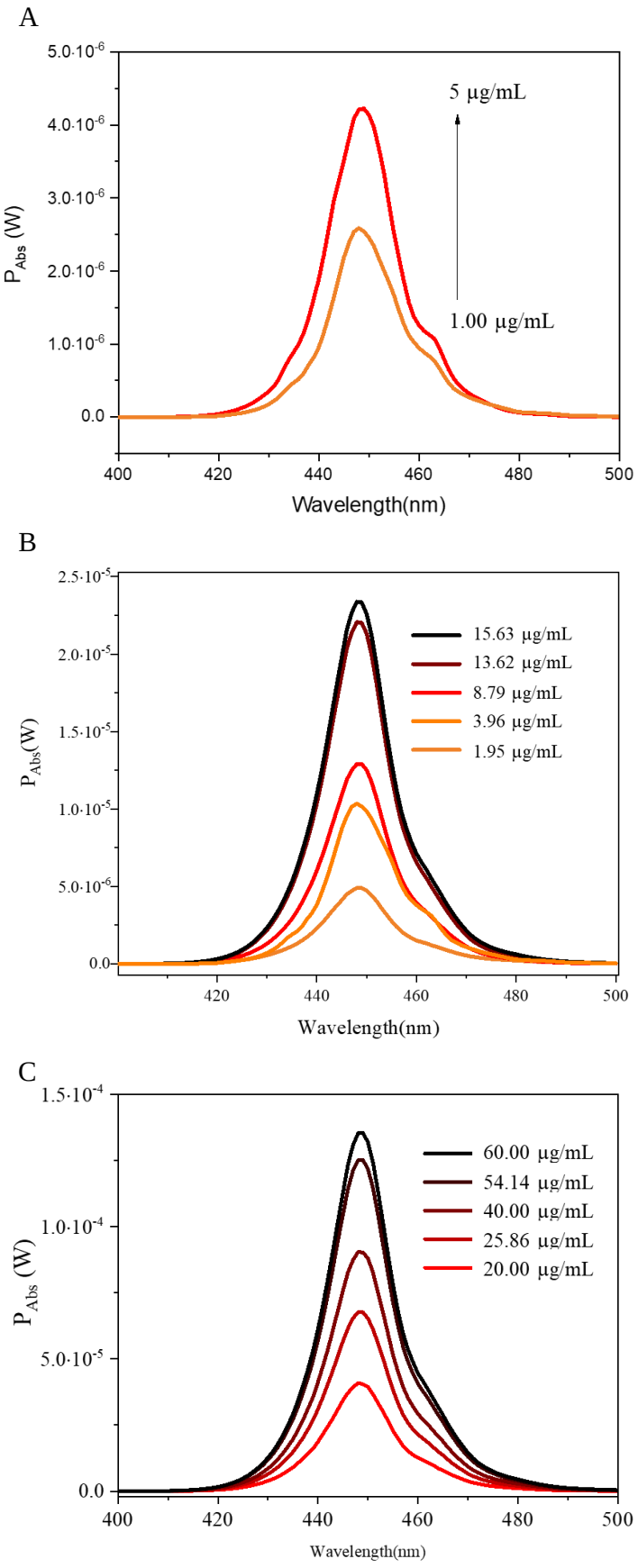

Figure 2. Spectra of light emitted by LED and power absorbed by WSC at different concentrations for *Staphylococcus aureus* (A), *Escherichia coli* (B) *Salmonella* Typhimurium (C).

Table 2. Regression coefficients of the full quadratic model to predict the effects of photodynamic therapy against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* Typhimurium.

Coefficient*	***Staphylococcus aureus***		***Escherichia coli***		***Salmonella* Typhimurium**	
	Regression coefficient	**p-*value***	**Regression coefficient**	**p-*value***	**Regression coefficient**	**p-*value***
b_0	2.89	<0.0001	4.32	<0.0001	2.63	<0.0001
b_1	-0.35	0.1070	-1.12	0.0006	-0.54	0.0172
b_{11}	0.29	0.2167	-0.73	0.0090	0.14	0.4830
b_2	-0.71	0.0085	-1.55	0.0001	-0.98	0.0010
b_{22}	0.15	0.4917	0.16	0.4391	-0.17	0.4042
b_{12}	-0.16	0.5542	-0.36	0.1884	-0.53	0.0639

* b_0: intercept; b_1: linear coefficient of illumination time; b_{11}: quadratic coefficient of illumination time; b_2: linear coefficient of PS concentration; b_{22}: quadratic coefficient of PS concentration; b_{12}: interaction coefficient between illumination time and PS concentration.

Table 3. Analyses of variance for the experimental design

Source of variation	*Staphylococcus aureus*					*Escherichia coli*					*Salmonella* Typhimurium				
	Sum of square	**df**	**Mean square**	**F- ratio**	***p-value***	**Sum of square**	**df**	**Mean square**	**F- ratio**	***p-value***	**Sum of square**	**df**	**Mean square**	**F- ratio**	***p-value***
Model	5.5230	3	1.8410	7.6553	0.0098	33.2732	3	11.0911	41.8195	$3.1003x10^{-5}$	11.0415	3	3.6805	17.6607	0.0007
Residual	1.9239	8	0.2405			2.1217	8	0.2652			1.6672	8	0.2084		
Lack of fit	1.8739	5	0.3748	22.4866	0.0139	2.0726	5	0.4145	25.3271	0.0117	1.6463	5	0.3293	47.2621	0.0047
Pure error	0.0500	3	0.0167			0.0491	3	0.0164			0.0209	3	0.0070		
Total	7.4469	11				35.3949	11				12.7087	11			

Coefficient of determination (R^2): *S. aureus* = 0.7417;*E. coli* = 0.9401 and *S.* Typhimurium = 0.8688

Adjusted coefficient of determination (R^2_{adj}): *S. aureus* = 0.6448;*E. coli* = 0.9176 and *S.* Typhimurium = 0.8196

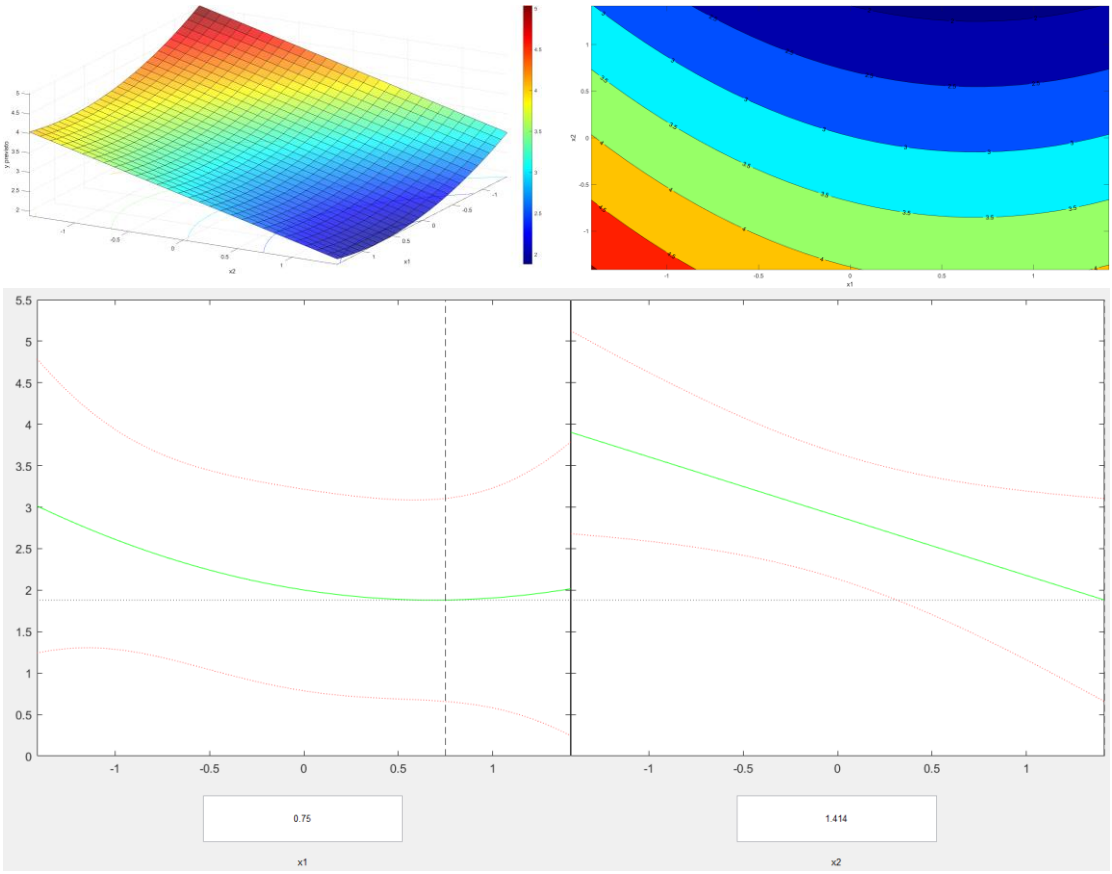

Figure 3. Response surface, contour plot, and slice plot for *Staphylococcus aureus*. The values of independent variables are coded.

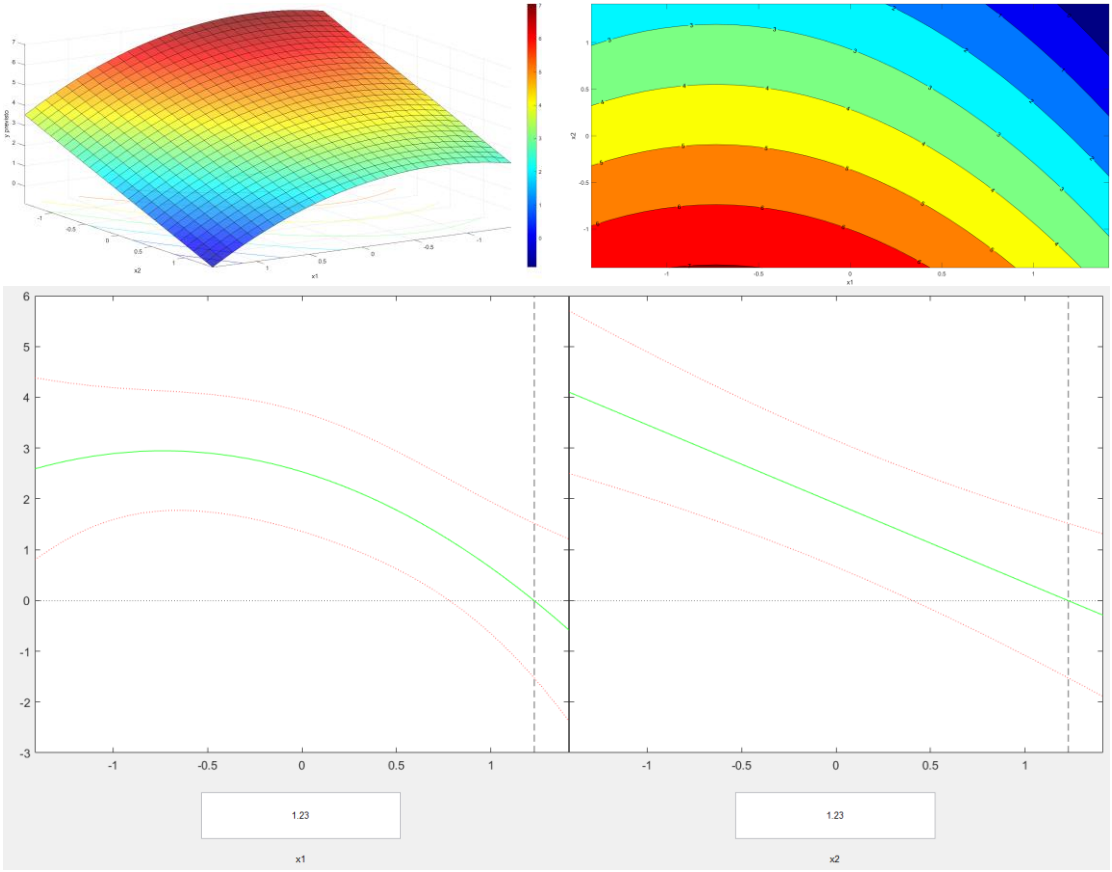

Figure 4. Response surface, contour plot, and slice plot for *Escherichia coli*. The values of independent variables are coded.

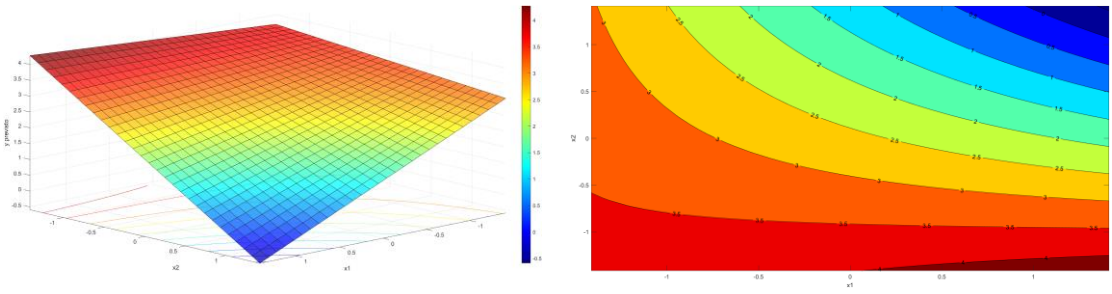

Figure 5. Response surface and contour plot for *Salmonella* Typhimurium. The values of independent variables are coded.