LWT - Food Science and Technology 76 (2017) 198–202

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Photodynamic inactivation of foodborne and food spoilage bacteria by curcumin

Camila Benedetti Penha [a], Edineia Bonin [a], Alex Fiori da Silva [b], Noboru Hioka [c], Érica Benassi Zanqueta [d], Tania Ueda Nakamura [d], Benício Alves de Abreu Filho [d], Paula Aline Zanetti Campanerut-Sá [e], Jane Martha Graton Mikcha [e, *]

[a] Programa de Pós-Graduação em Ciência de Alimentos, Universidade Estadual de Maringá, Maringá, PR, Brazil
[b] Programa de Pós-Graduação em Ciências da Saúde, Universidade Estadual de Maringá,Maringá, PR, Brazil
[c] Departamento de Química, Universidade Estadual de Maringá,Maringá, PR, Brazil
[d] Departamento de Ciências Básicas da Saúde, Universidade Estadual de Maringá,Maringá, PR, Brazil
[e] Departamento de Análises Clínicas e Biomedicina, Universidade Estadual de Maringá, Maringá, PR, Brazil

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form
12 July 2016
Accepted 14 July 2016
Available online 16 July 2016

Keywords:
Photoinactivation
Curcumin
LED
Bacteria

ABSTRACT

The present study evaluated the efficacy of photodynamic inactivation (PDI) of foodborne and spoilage bacteria using curcumin and a blue light emitting diode (LED). Curcumin at 75 μM was used to photo-irradiate *Staphylococcus aureus* ATCC 25923, *Aeromonas hydrophila* ATCC 7966, *Salmonella* Typhimurium ATCC 14028, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 at light doses of 139 J/cm^2, 278 J/cm^2 and 417 J/cm^2. The cytotoxicity of curcumin in VERO cells was evaluated in similar conditions to bacterial photoinactivation assay, and the percentage of cell destruction was ≅ 13 ± 0.05%, for all light doses. Curcumin-mediated PDI of *S. aureus* induced a significant reduction of approximately 3.50 log CFU/ml at 139 J/cm^2 and 278 J/cm^2. Full inactivation was observed at 417 J/cm^2. Among Gram negative bacteria, *P. aeruginosa* was the least susceptible to PDI, which counts were not significantly reduced. A significant reduction in *E. coli* counts was observed at 278 J/cm^2, and no viable cells were detected after light exposure at 417 J/cm^2. When photo-irradiated with curcumin at 278 J/cm^2 and 417 J/cm^2, *A. hydrophila* was completely eradicated, while a significant decrease (3.33log CFU/ml) was observed in bacterial counts at 139 J/cm^2. Curcumin in combination with a blue LED is a potential candidate for PDI against Gram positive and Gram negative bacteria.

1. Introduction

Microbial contamination of foods continues to be a major concern for public health, consumers, regulatory agencies and food industries throughout the world. Foodborne pathogens are responsible for numerous illnesses, which affect thousands of people, mostly children, pregnant women, babies, the elderly and people with vulnerable diseases, and can lead to death in many cases (WHO, 2015). The Center for Disease Control and Prevention (CDC) estimates that 48 million people become ill and 3.000 die due to foodborne diseases (CDC, 2015). Spoilage bacteria cause food losses, with a significant economic, social and environmental impact (Lipinski et al., 2013).

Among the strategies used to ensure microbiological safety and food preservation are the use of chemical preservatives, thermal processes, such as pasteurization, other physical methods such as dehydration and irradiation, and new preservation treatments such as high hydrostatic pressure (Gonzalez & Barret, 2010).

Photodynamic inactivation (PDI) is a new and promising strategy to eradicate microorganisms such as Gram positive and Gram negative bacteria, yeasts, molds, viruses and parasites (Alves et al., 2015). This technique is based on the use of photosensitizers (PSs) activated by an appropriate wavelength light (Jiang, Leung, Hua, Rao, & Xu, 2014). Several light sources such as lasers, LEDs and

* Corresponding author. Departamento de Análises Clínicas e Biomedicina, Universidade Estadual de Maringá, Avenida Colombo, 5790, Z07, Maringá, 87020-900, PR, Brazil.
E-mail addresses: camilabenedetti@gmail.com (C.B. Penha), edineiabonin42@hotmail.com (E. Bonin), alex_skiba@hotmail.com (A.F. da Silva), nhioka@uem.br (N. Hioka), erica_b_zanqueta@hotmail.com (É.B. Zanqueta), tunakamura@uem.br (T.U. Nakamura), baafilho@uem.br (B.A. de Abreu Filho), paulacampanerut@gmail.com (P.A.Z. Campanerut-Sá), jmgmikcha@uem.br (J.M.G. Mikcha).

http://dx.doi.org/10.1016/j.lwt.2016.07.037
0023-6438/

halogen lamps are currently used (Nagata et al., 2012). LEDs have been used as alternative light sources due to their low cost, wider emission bands, easy of use and greater flexibility in irradiation time (Costa et al., 2011).

The toxicity of PDI comprises the drug excitation phases, encouraging the production of reactive oxygen species (ROS) and leads to cell death (Alisson, Mota, Bagnato, & Sibata, 2008; Chatterjee, Fong, & Zhang, 2008). This free radical hinders microbial resistance as it interacts in many cell structures such as lipid membranes, proteins and nucleid acids (Konopka & Goslinski, 2007).

The advantages of PDI are that no toxic chemicals are generated, the only energy required is the light source, there is a low probability of triggering the development of resistance in microorganisms and it can be potential applied in several areas: hospital, dental, industrial and environmental (Alves et al., 2015; Luksiene & Brovko, 2013).

Different PSs, including porphyrins, phthalocyanines, chlorophyllin and xanthene dyes have been tested against microorganisms. These are fundamentally defined as agents that produce singlet oxygen following light stimulation (Alisson, Mota, Bagnato, & Sibata, 2008). Among naturally occurring PSs, curcumin is a yellow pigment isolated from *Curcuma longa* (Soria-Lozano et al., 2015), and has been used as a spice since ancient times (Rao & Khanum, 2016). Among its many biological activities are its antioxidant (Rao & Khanum, 2016; Singh et al., 2010), antimicrobial (Arutselvi, Balasaravanan, Ponmurugan, Saranji, & Suresh, 2012), anti-HIV (Rao & Khanum, 2016) anti-inflammatory and anticancer (Sharma, Gescher, & Steward, 2005) properties. Curcumin absorbs blue light in an absorption spectrum range of 400–500 nm, and it can be used as a potential natural photosensitizer (Jiang et al., 2014; Soria-Lozano et al., 2015).

The use of curcumin-mediated photosensitization has been reported against a range of bacteria and fungi, such as *Staphylococcus aureus* (Jiang et al., 2014), *Staphylococcus epidermidis* (Hegge, Bruzell, Kristensen, & Tønnesen, 2012) *Enterococcus faecalis* (Frota et al., 2015; Haukvik, Bruzell, Kristensen, & Tønnesen, 2009), *Streptococcus mutans* (Manoil et al., 2014; Paschoal et al., 2013; Soria-Lozano et al., 2015), *Streptococcus intermedius* (Haukvik et al., 2009) *Lactobacillus* spp. (Bulit et al., 2014), *Escherichia coli* (Haukvik et al., 2009), *Candida* spp. (Andrade et al., 2013; Dovigo et al., 2011; Soria-Lozano et al., 2015). and *Aspergillus flavus* (Temba, Fletcher, Fox, Harvey, & Sultanbawa, 2016). Thus, the aim of this study was to evaluate antimicrobial photodynamic activity *in vitro* against pathogenic and spoilage bacteria using curcumin as a photosensitizer.

2. Materials and methods

2.1. Photosensitizer

The curcumin to be used as a photosensitizer was purchased commercially from Sigma (USA). Fig. 1 shows the chemical structure of curcumin. A stock solution was prepared in dimethyl sulfoxide (DMSO) at 1 mM and stored in the dark at −20 °C. The working solution was diluted with phosphate buffered saline (PBS) to obtain a concentration of 75 μM for use in photodynamic therapy experiments.

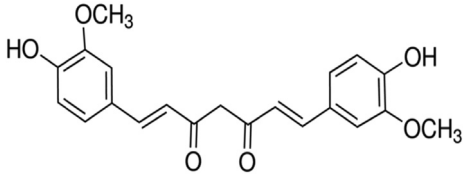

Fig. 1. Schematic representation of curcumin. Source Pillegi et al.(2013).

2.2. Light source

The light source used was blue LED (λ_{max} = 470 nm), and the emission spectra were measured by a Varian-Cary Eclipse spectrofluorometer. The light doses were calculated as described by Yassunaka et al. (2015), considering an irradiated area of 1.77 cm^2, exposure time of 10, 20 and 30 min and LED potency of 1.2 W.

2.3. Cytotoxicity assay

The cytotoxicity of curcumin was evaluated according to Skehan et al. (1990) using VERO cells (ATCC CCL − 81). The cells were cultured in plastic culture flasks (TPP) containing Dulbecco's Modified Eagle Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (SFB − Gibco) and 50 μg/ml gentamicin at 37 °C in a humid atmosphere (Fischer Scientific model Isotemp) containing 5% CO_2. VERO cells at a cellular density of 2.5 × 10^4 cells/100μl/well were inoculated into 96 well tissue culture plates and incubated for 24 h to obtain a confluent monolayer. The cells were then washed with PBS and exposed to serial decimal dilutions of curcumin (1000–0.01 μg/ml) for 72 h. Afterward, the cells were washed with PBS, fixed with 10% trichloroacetic acid for one hour and stained with 0.4% sulforhodamine B for 30 min. The plates were washed with 1% acetic acid to remove excess dye and color revelation occurred through the addition of 10 mM Tris Base. The absorbance of each well was read at 530 nm using a microplate enzyme-linked immunosorbent assay reader. Control cells were prepared without the addition of curcumin. The absorbance of the treated wells was compared with the control cells and the CC_{50} (cytotoxic concentration that reduced cell viability to 50%) was calculated. The experiment was performed in triplicate.

The cytotoxicity of curcumin exposed to LED, under the same conditions used in photoinactivation assay, was also evaluated, as described above. Curcumin at 75 μM was added to VERO cell cultures, incubated in the dark for 10 min and LED irradiated for 10, 20 and 30 min. The control cells received only irradiation blue light. The experiment was performed in triplicate.

2.4. Bacterial strains and culture conditions

The Gram positive bacterium *Staphylococcus aureus* ATCC 25923 and the Gram negative bacteria *Aeromonas hydrophila* ATCC 7966; *Escherichia coli* ATCC 25922; *Salmonella enterica* serotype Typhimurium ATCC 14028 and *Pseudomonas aeruginosa* ATCC 27853 were used in the experiments.

The bacterial strains were stored at −20 °C in brain heart infusion broth (BHI; Difco) containing 20% (vol/vol) glycerol, in the Laboratory of Food Microbiology of the State University of Maringá, UEM.

2.5. Photoinactivation assay

Bacterial strains were cultured in Brain Heart Infusion broth (BHI, DifcoTM) at 37 °C for 24 h and plated on agar plates specific for each bacterium. Enteric Hektoen Agar (Difco, Becton Dickinson, Sparks, MD, USA) was used for *S.* Typhimurium, *A. hydrophila* and *P. aeruginosa*; Eosin Methylene Blue Agar (Difco, Becton Dickinson, Sparks, MD, USA) was used for *E. coli*, and Baird Parker Agar (Difco, Becton Dickinson, Sparks, MD, USA) was used for *S. aureus*. After incubation at 37 °C the colonies were transferred to 5 ml of BHI and incubated at 37 °C overnight. The cultures were harvested by