UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Engenharia de Alimentos

LEONARDO DO PRADO SILVA

Microbial inactivation by photodynamic treatment: from inactivation kinetics to vegetable applications

Inativação microbiana por tratamento fotodinâmico: da cinética de inativação às aplicações em vegetais

CAMPINAS
2020

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Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Ciência de Alimentos.

Thesis presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for PhD in Food Science.

Orientador: Prof. Dr. Anderson de Souza Sant'Ana
Co-orientador: Prof. Dr. Gilberto Úbida Leite Braga

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELO ALUNO LEONARDO DO PRADO SILVA, ORIENTADO PELO PROF. DR. ANDERSON DE SOUZA SANT'ANA E CO-ORIENTADO PELO PROF. DR. GILBERTO ÚBIDA LEITE BRAGA

CAMPINAS
2020

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

P882m

Prado-Silva, Leonardo do, 1990-
Microbial inactivation by photodynamic treatment : from inactivation kinetics to vegetable applications / Leonardo do Prado Silva. – Campinas, SP : [s.n.], 2020.

Orientador: Anderson de Souza Sant'Ana.
Coorientador: Gilberto Ubida Leite Braga.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Tecnologias emergentes. 2. *Bacillus cereus*. 3. *Alicyclobacillus acidoterrestris*. 4. Deterioração. 5. Patógeno. 6. Fotossensibilização. I. Sant'Ana, Anderson de Souza. II. Braga, Gilberto Ubida Leite. III. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: Inativação microbiana por tratamento fotodinâmico : da cinética de inativação às aplicações em vegetais
Palavras-chave em inglês:
Emerging technologies
Bacillus cereus
Alicyclobacillus acidoterrestris
Spoilage
Pathogen
Photosensitization
Área de concentração: Ciência de Alimentos
Titulação: Doutor em Ciência de Alimentos
Banca examinadora:
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Data de defesa: 05-08-2020
Programa de Pós-Graduação: Ciência de Alimentos

Identificação e informações acadêmicas do(a) aluno(a)
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- Currículo Lattes do autor: http://lattes.cnpq.br/6332876092001571

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Aos meus queridos pais, Mousart *(in memoriam)* e Graça, aos meus irmãos e à minha noiva, Paula, pelo apoio incondicional durante a trajetória.

À Deus, pelo dom da vida e por ser fonte inesgotável de esperança diante das dificuldades.

Ao Professor Anderson pela orientação, confiança, ensinamentos e oportunidades durante a realização deste trabalho. Muito obrigado!

Ao Professor Gilberto pela co-orientação, ensinamentos, auxílio e suporte essenciais para o desenvolvimento deste trabalho.

A todos os colaboradores da Universidade de Aveiro: Profa. Adelaide, Dra. Ana Gomes, Profa. Amparo, Profa. Graça Neves e Mariana pela fundamental contribuição e suporte desprendidos a este trabalho.

Ao meu pai Mousart *(in memoriam)* e minha mãe Graça pelos valiosos princípios de vida, amor e apoio incondicional, sem os quais eu não seria capaz de seguir em frente. Vocês sempre serão os meus maiores exemplos de dedicação, caráter e trabalho árduo!

A meus irmãos Márcio, Marcelo, Ana Paula (Papaula) e Adriano (Padrinho) por transmitirem boas energias e pelo orgulho demonstrado ao irmão caçula.

À minha noiva, Paula, por ser minha companheira de vida e maior incentivadora. Não tenho palavras para descrever o quão importante foi o seu apoio durante este período. Obrigado por tudo, em especial, pela confiança em mim depositada!

À minha Avó Terezinha *(in memoriam)* que sempre será luz e anjo da guarda.

À toda a minha família, em especial à minha Madrinha, Tia Lúcia, Tio Paulinho, Tio Luiz, Tia Ana, Tio Chicão *(in memoriam)*, Tio Tião, Tia Dora *(in memoriam)*, Tia Sônia, Tia Selma, Tio Juninho, Tia Léia, Cláudia, "*Tí*" Nardim, por sempre estarem presentes de alguma forma.

Ao Sr. Itamar, Dona Regina, Laís, Madi e Tia Landa pelo acolhimento à família de vocês, carinho e confiança.

À Dona Maria Rosa (Maricotinha) por ser um exemplo de vida a todos nós e por compartilharmos de uma mesma paixão: Coca-Cola®.

A todos os meus queridos amigos, em especial – Bruna, Henry, Verônica, Marcão, Bia, WJr, João Bosco, Tomoda e Nivaldo pelos momentos de diversão e companheirismo.

Aos companheiros de trabalho do Laboratório de Microbiologia Quantitativa de Alimentos, em especial – Fer, Mari, Ju, Lari, Mayara, Antônio e Syllas pela convivência e dedicação à ciência.

Aos meus amigos de Vittel – Leticia, César, Rony, Etienne, Adélie, Coralie, Christiane, Sylvie, Murielle e Romain pela acolhida e amizade.

A todos os funcionários da FEA pela amizade e auxílios prestados durante o doutorado.

A todos os membros da banca, titulares e suplentes, pela disponibilidade e contribuições ao trabalho.

À Universidade Estadual de Campinas (UNICAMP) e ao Departamento de Ciência de Alimentos da Faculdade de Engenharia de Alimentos pela oportunidade de desenvolvimento profissional e pessoal.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela bolsa de doutorado concedida (número do processo: 140092/2017-0).

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

RESUMO

As bactérias *Bacillus cereus* e *Alicyclobacillus acidoterrestris* são ambas resistentes aos processos tradicionais de inativação térmica com elevado potencial patogênico e deteriorante, respectivamente. O tratamento fotodinâmico antimicrobiano (TFA) é uma alternativa promissora aos métodos convencionais de inativação microbiana. O TFA consiste no uso de um fotossensibilizador (FS) que ao entrar em contato com as células-alvo gera espécies reativas de oxigênio após exposição à luz visível. Inicialmente foi avaliada a eficácia do TFA com o FS fenotiazínico novo azul de metileno (NMB) nas células vegetativas e esporos de *B. cereus*. Inicialmente, a eficácia do TFA foi avaliada pela determinação da concentração inibitória mínima (CIM) do NMB em células vegetativas de 12 cepas diferentes de *B. cereus* através de um conjunto de 96 diodos emissores de luz (LED) vermelha com emissão de 631 nm e irradiância de 24,5 mW/cm^2. Os resultados da CIM permitiram selecionar 4 cepas de *B. cereus* (B3, 436, B63, ATCC 14579) através de uma análise de agrupamento com base na distância Euclidiana. Em seguida, foi realizado um estudo de sobrevivência ao TFA com NMB em três concentrações (5, 50 e 100 µM). Este estudo mostrou que o TFA reduziu a viabilidade tanto das células vegetativas como dos esporos das 4 cepas de *B. cereus* ($P < 0,05$) em todas as fluências aplicadas (0 - 450 J/cm^2). As curvas de inativação de *B. cereus* apresentaram bom ajuste ao modelo de Weibull, o qual foi utilizado para estimar os parâmetros cinéticos δ e *p* de inativação fotodinâmica que correspondem à fluência necessária para a primeira redução decimal e ao parâmetro de curvatura, respectivamente. A cepa ATCC 14579 apresentou em todas as condições $p > 1$, indicando que as células vegetativas e esporos desta cepa foram progressivamente eliminadas pelo TFA. A cepa B3 obteve significativamente ($P < 0,05$) o menor valor de δ (0,51 ± 0,30 J/cm^2), indicando que havia uma população de células vegetativas muito sensível no início do TFA com NMB a 5 µM. No entanto, neste tratamento não foram alcançadas o mínimo de 4 reduções decimais (4D), o que demonstra que uma outra parcela da população foi mais resistente ao longo do TFA. Durante a avaliação do TFA com os esporos de *B. cereus* o NMB a 50 µM foi a única concentração capaz de atingir 4D para todas as cepas. Os resultados deste capítulo demonstraram que houve variabilidade entre as cepas de *B.*

cereus. No capítulo 3 o objetivo foi avaliar o TFA utilizando como FS uma porfirina tetracatiônica (Tetra-Py^+-Me) e o NMB nos esporos de *A. acidoterrestris*. Os experimentos foram conduzidos como descrito a seguir: (*I*) *In vitro,* em tampão fosfato salina (PBS) com os FS Tetra-Py^+-Me e NMB e exposição luminosa através de um LED com emissão de luz branca (400 – 700 nm) e irradiância de 140 mW/cm^2, (*II*) suco de laranja nas mesmas condições anteriores com a adição de iodeto de potássio (KI) como agente potencializador do TFA e (*III*) casca de laranja com apenas o uso do FS Tetra-Py^+-Me com e sem a presença de KI e exposição luminosa através do LED e radiação solar, ambos à 65 mW/cm^2. Os experimentos em PBS com Tetra-Py^+-Me a 10 µM resultaram em 7,3 reduções logarítmicas na viabilidade dos esporos de *A. acidoterrestris* após 5 h de TFA. Contrariamente, os ensaios em PBS com NMB não resultaram em reduções significativas. Em seguida, os resultados obtidos com Tetra-Py^+-Me e NMB a 10 µM em suco de laranja na presença de KI resultaram em 5 reduções logarítmicas com ambos os FS após 10 h de TFA. Posteriormente, a inativação de esporos de *A. acidoterrestris* inoculados artificialmente em cascas de laranja foi realizada com Tetra-Py^+-Me a 10 e 50 µM. Nenhum incremento significativo foi observado pelo uso de KI. A maior concentração de Tetra-Py^+-Me (50 µM) causou 2,8 reduções logarítmicas na viabilidade dos esporos de *A. acidoterrestris* em cascas de laranja após 6 h exposição à radiação solar (65 mW/cm^2). As características colorimétricas e nutricionais do suco de laranja e da casca foram significativamente ($P < 0,05$) influenciadas pela exposição à luz artificial e radiação solar. Os resultados deste estudo sugerem que o TFA é um método potencial para a redução microbiológica de *B. cereus e A. acidoterrestris*. No entanto, novos estudos são necessários para otimizar a aplicação do TFA pela indústria de alimentos.

Palavras-chave: Tecnologias emergentes, *Bacillus cereus*, *Alicyclobacillus acidoterrestris*, Deterioração, Patógeno, Fotossensibilização

ABSTRACT

The bacteria *Bacillus cereus* and *Alicyclobacillus acidoterrestris* are both resistant to conventional thermal microbial inactivation processes with high pathogenic and spoilage potential, respectively. Antimicrobial photodynamic treatment (aPDT) is a promising alternative to conventional microbial inactivation methods. aPDT is based on the use of a photosensitizer (PS) which in contact with the target cells, generate reactive oxygen species (ROS) after exposure to visible light. Initially was evaluated the efficacy of aPDT with the phenothiazinium PS new methylene blue (NMB) on the vegetative cells and spores of *B. cereus*. Initially, the effectiveness of aPDT was assessed by determining the minimum inhibitory concentration (MIC) of NMB on vegetative cells of 12 different strains of *B. cereus* using an array of 96 red light-emitting diodes (LED) with an emission peak of 631 nm and irradiance of 24.5 mW/cm^2. The results of the MIC allowed to select 4 strains of *B. cereus* (B3, 436, B63, ATCC 14579) through a cluster analysis based on the Euclidean distance. Then, a survival study of aPDT with NMB at three concentrations (5, 50, and 100 µM) was performed. This study showed that aPDT reduced the viability of both vegetative cells and spores of the 4 strains of *B. cereus* ($P < 0.05$) at all fluences (0 - 450 J / cm2). *B. cereus* inactivation curves presented a good fit to the Weibull model, which was used to estimate the kinetic parameters δ and *p* of photodynamic inactivation corresponding to the fluency required for the first decimal reduction and the curvature parameter, respectively. The strain ATCC 14579 presented in all conditions $p > 1$, indicating that the vegetative cells and spores of this strain were progressively eliminated by aPDT. The strain B3 obtained significantly ($P < 0.05$) the lowest value of δ (0.51 ± 0.30 J/cm^2), indicating that there was a very sensitive vegetative cell population at the beginning of aPDT with 5 µM NMB. However, in this treatment, a minimum of 4 decimal reductions (4D) was not achieved, which demonstrates that another portion of the population was more resistant throughout the aPDT. During the aPDT with *B. cereus* spores, NMB at 50 µM was the only concentration capable to reach 4D for all strains. The results of this chapter demonstrated that there was variability between *B. cereus* strains. In chapter 3, the objective was to evaluate aPDT using a tetracationic porphyrin (Tetra-Py$^+$-Me) and NMB in the spores of *A. acidoterrestris* as PS. The experiments were

conducted as follows: (I) *In vitro*, in phosphate-buffered saline (PBS) with PS Tetra-Py^+-Me and NMB and light exposure through an LED with white light emission (400 – 700 nm) and irradiance of 140 mW/cm^2, (II) orange juice in the same conditions as before with the addition of potassium iodide (KI) as a potentiator agent of aPDT and (III) orange peel using only the PS Tetra-Py^+-Me in the presence and absence of KI and exposition to LED and solar radiation, both at 65 mW/cm^2. The experiments in PBS with Tetra-Py^+-Me at 10 µM resulted in 7.3 log reductions in the viability of *A. acidoterrestris* spores after 5 h of aPDT. Contrarily, the experiments with NMB did not result in significant reductions. Then, the results obtained with Tetra-Py^+-Me and NMB at 10 µM in orange juice in the presence of KI resulted in 5 log reductions with both PS after 10 h of aPDT. Subsequently, the inactivation of *A. acidoterrestris* spores, artificially inoculated in orange peels, was performed with Tetra-Py^+-Me at 10 and 50 µM. No significant increment was observed for the use of KI. The highest concentration of Tetra-Py^+-Me (50 µM) caused 2.8 log reductions in the viability of *A. acidoterrestris* spores in orange peels after 6 h exposure to solar radiation (65 mW/cm^2). The colorimetric and nutritional characteristics of orange juice and peel were significantly ($P < 0.05$) influenced by exposition to artificial light and solar radiation. The results of this study suggest that aPDT is a potential method for the microbiological reduction of *B. cereus* and *A. acidoterrestris*. However, further studies are required to optimize the application of aPDT in the food industry.

Keywords: Emerging technologies, *Bacillus cereus*, *Alicyclobacillus acidoterrestris*, Spoilage, Pathogen, Photosensitization

Lista de abreviaturas e siglas

ΔE	Total color change
δ	Time for first decimal reduction
$^{\bullet}OH$	Hydroxyl radicals
$^{1}O_2$	Singlet oxygen
$^{3}O_2$	Triplet dioxygen
4D	Four decimal reductions
$ABTS^{\bullet+}$	2,2-Azino-bis(3-ethylbenzothiazoline)-6-sulponic acid
ALA	5-aminolevulinic acid
ANOVA	Analysis of variance
aPDT	Antimicrobial photodynamic treatment
ATCC	American type culture collection
Ca^{2+}	Calcium ions
CDC	Center for disease control and prevention
CFU	Colony forming unit
Chl	Chlorophyllin
CIM	Concentração inibitória mínima
DC	Dark control
DMMB	1,9-dimethylmethylene blue
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-difenil-1-picrilhidrazila
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
DTA	Doença transmitida por alimentos
e^{-}	Electron
EOY	Eosin Y
ERY	Erythrosine
FDA	Food and drug administration
FS	Fotossensibilizador
H_2O_2	Hydrogen peroxide
Hyp	Hypericin
I_2/I_3^{-}	Free molecular iodine

$I_2^{\bullet-}$	Iodine radicals
KI	Potassium iodide
L, a*, b**	Color coordinates
LC	Light control
LED	Light-emitting diode
MB	Methylene blue
Mg^{2+}	Magnesium ions
MIC	Minimal inhibitory concentration
NaBr	Sodium bromide
NaN_3	Sodium azide
NMB	New methylene blue
O_2	Oxygen
$O_2^{\bullet-}$	Superoxide anions
OMS	Organização mundial da saúde
ORAC	Oxygen radical absorbance capacity
p	Curvature parameter
PBS	Phosphate-buffered saline
PDT	Photodynamic therapy
PS	Photosensitizer
RB	Rose bengal
ROS	Reactive oxygen species
SCN^-	Thiocyanate
TBO	Toluidine blue O
Tetra-Py^+-Me	5,10,15,20-tetrakis(1-methylpyridinium-4-yl) porphyrin tetra-iodide
TFA	Tratamento fotodinâmico antimicrobiano
TPC	Total phenolic content
UV	Ultraviolet
WHO	World health organization
YSG-A	Yeast starch glucose agar
YSG-B	Yeast starch glucose broth
ΦΔ	Quantum yield of singlet oxygen

Sumário

Introdução geral

As doenças transmitidas por alimentos (DTAs) são uma das principais preocupações dos órgãos governamentais em relação à saúde pública. A presença de micro-organismos patogênicos em alimentos é responsável pela mortalidade e morbidade que afetam a vida das pessoas, a economia e o desenvolvimento social dos países. De acordo com a Organização Mundial da Saúde (OMS) 1 em cada 10 pessoas (≈ 600 milhões) adoecem após o consumo de alimentos contaminados em todo o mundo. Além disso, aproximadamente 420.000 pessoas morreram em decorrência de DTAs, das quais 125.000 são crianças menores de 5 anos de idade (WHO, 2015). Estima-se que as perdas econômicas em produtividade e tratamento clínico de DTAs ultrapassem US$110 bilhões em países subdesenvolvidos (WORLD BANK, 2018). No entanto, grande parte das DTAs e suas consequências poderiam ser evitadas por meio de boas práticas de higiene em toda a cadeia de produção e consumo de alimentos.

Além das preocupações que envolvem a segurança dos alimentos, outro desafio enfrentado pela humanidade são as perdas de alimentos devido ao desperdício ou deterioração por micro-organismos (ODEYEMI et al., 2020). Um estudo conduzido pela Organização das Nações Unidas para a Alimentação e Agricultura revelou que aproximadamente 1,3 bilhão de tonelada de alimentos para o consumo humano é desperdiçado ou descartado por deterioração (FAO, 2011). Portanto, com o crescente aumento populacional, a demanda por alimentos para o consumo humano também segue o mesmo ritmo de crescimento.

Os micro-organismos estão amplamente distribuídos no ambiente e podem contaminar os alimentos por meio do contato direto com a água e o solo. Dentre os micro-organismos que são encontrados, pode-se destacar a bactéria *Bacillus cereus*, pela frequente associação a surtos de DTAs e deterioração de produtos lácteos (SPANU, 2016). Além disso, também destaca-se o gênero bacteriano *Alicyclobacillus* spp., considerado uma das principais preocupações da indústria de suco de frutas pelo seu elevado potencial de deterioração de produtos ácidos (HIPPCHEN; RÖLL; PORALLA, 1981; ORR et al., 2000).

B. cereus é uma bactéria gram-positiva, formadora de esporos, aeróbia facultativa e mesófila que pode ser frequentemente encontrada no solo e nas fezes de animais (CARLIN, 2011; HEYNDRICKX, 2011). As intoxicações alimentares causadas por *B. cereus* são caracterizadas principalmente por vômitos (toxina emética) e diarreia (enterotoxina) (BOTTONE, 2010). Em produtos lácteos, *B. cereus* pode causar deterioração pela produção de enzimas como lipases, proteinases e fosfolipases que podem desencadear processos de coagulação e alteração de sabor (MEHTA et al., 2019).

As espécies do gênero *Alicyclobacillus* são bactérias Gram-positivas podendo também ser Gram-variáveis, termoacidófilas, formadora de esporos, móveis, aeróbias e caracterizadas pela presença de ácidos graxos ω-alicíclicos como principal componente lipídico da parede celular (TORLAK, 2014; WISOTZKEY et al., 1992; YAMAZAKI; TEDUKA; SHINANO, 1996). Dentre as espécies do gênero, *Alicyclobacillus acidoterrestris* é a espécie mais associada à deterioração desde o primeiro caso descrito, envolvendo suco de maçã (CERNY; HENNLICH; PORALLA, 1984; HU et al., 2020; VAN LUONG et al., 2019). Por meio do contato direto com o solo ou pela poeira, os esporos de *A. acidoterrestris* podem contaminar as frutas usadas na fabricação de suco. Assim, durante o processamento, esses esporos podem sobreviver às etapas de sanitização da superfície das frutas e pasteurização, permanecendo nos produtos finais (OTEIZA et al., 2011; SPINELLI et al., 2009). A deterioração de produtos ácidos como os sucos de frutas pela atividade metabólica de *Alicyclobacillus* é caracterizada por odores desagradáveis provenientes da produção de guaiacol e outros compostos halofenólicos (CHANG; KANG, 2004; SIEGMUND; PÖLLINGER-ZIERLER, 2006). Embora seja visualmente difícil detectar a deterioração pela ausência de produção de gás, a presença de tais compostos causa rejeição pelos consumidores e consequentemente perdas econômicas para as indústrias (SMIT et al., 2011).

Em complemento às políticas públicas de incentivo às boas práticas de higiene e orientações para evitar o desperdício, o processamento industrial contribui para a segurança e a qualidade dos alimentos. Dentre os processamentos estabelecidos pelas indústrias de alimentos, destacam-se os tratamentos térmico e químico. No

entanto, sabe-se que os processos térmicos podem causar efeitos indesejáveis aos alimentos como, por exemplo, perdas na qualidade sensorial e nutricional (UCHIDA; SILVA, 2017). Além disso, o uso de sanitizantes não é considerado ambientalmente correto, além de ser prejudicial aos seres humanos (ÖLMEZ; KRETZSCHMAR, 2009).

Desta forma, pesquisas na área de microbiologia de alimentos buscam por alternativas não térmicas e ambientalmente corretas que sejam capazes de reduzir a contaminação dos alimentos por micro-organismos com a mesma segurança dos métodos tradicionais. Na literatura existem várias tecnologias inovadoras de processamento moderado que foram desenvolvidas para suprir essa necessidade da indústria de alimentos (CEBRIÁN; MAÑAS; CONDÓN, 2016; FERRARIO; GUERRERO, 2018; KIM et al., 2017; REVERTER-CARRIÓN et al., 2018). Até o momento, as tecnologias mais estudadas são as que envolvem o uso da luz pulsada, da radiação UV, de campo elétrico pulsado, de plasma frio e do processamento de alta pressão (BARBA et al., 2017). No entanto, para a maioria das técnicas mencionadas os custos de instalação e manutenção são elevados (BARBA et al., 2017).

Assim, surgiu o interesse dos cientistas por uma técnica antimicrobiana promissora, que já é amplamente usada na área médica, denominada tratamento fotodinâmico antimicrobiano (TFA*)* (WAINWRIGHT et al., 2017). Essa técnica centenária permite que por meio da combinação de luz, oxigênio molecular e um composto fotossensibilizador seja possível matar células-alvos, como as células tumorais e os micro-organismos (JESIONEK; VON TAPPEINER, 1905; RAAB, 1900). A técnica evoluiu grandemente em termos de compostos fotossensibilizadores (FS) e tipos de fontes de luz, na área médica, principalmente para o tratamento de câncer (MANSOORI et al., 2019). O tratamento fotodinâmico destaca-se pela elevada capacidade de produção de espécies reativas de oxigênio, que atuam em múltiplos alvos celulares, como por exemplo, ácidos nucléicos, proteínas e lipídios (HAMBLIN; ABRAHAMSE, 2020). Por conta dessa característica houve o renascimento do tratamento fotodinâmico para a inativação microbiana, particularmente devido ao surgimento de micro-organismos resistentes aos antimicrobianos tradicionais (WAINWRIGHT et al., 2017). Além disso, o uso de compostos FS não tóxicos e de

fontes de luz que emitem no espectro visível, tornam o TFA uma alternativa segura e promissora para o setor de alimentos (PASKEVICIUTE; ZUDYTE; LUKŠIENE, 2019).

Nos últimos 10 anos vários estudos avaliaram os efeitos de diferentes tipos de FS e fontes de luz na redução da contaminação microbiana por meio do TFA (BARTOLOMEU et al., 2016; BEIRÃO et al., 2014; HUANG et al., 2020; LUKSIENE; PASKEVICIUTE, 2011; OLIVEIRA et al., 2009; RODRIGUES et al., 2019; VECCHIO et al., 2015; ŽUDYTE; LUKŠIENE, 2019). No entanto, mais estudos são necessários, principalmente em relação à inativação de micro-organismos termoresistentes como são os casos das bactérias *B. cereus* e *A. acidoterrestris.* Por conta da relevância em relação às DTAs (*B. cereus*) e deterioração de alimentos (*B. cereus* e *A. acidoterrestris*) ambas as bactérias são modelos interessantes para a aplicação do TFA.

Desta forma, no capítulo 1 foi realizada uma revisão de literatura com o objetivo de discutir os avanços mais recentes com TFA no setor agroalimentar. Os principais compostos FS e fontes de luz utilizados em TFA, bem como os mecanismos de inativação, os fatores que podem afetar a eficácia do tratamento e os efeitos de potencialização do TFA foram abordados. Além disso, os principais micro-organismos avaliados em TFA, potenciais aplicações do tratamento no setor agroalimentar e as perspectivas futuras do TFA também foram discutidas.

No capítulo 2, o objetivo do trabalho foi avaliar eficiência do TFA, com exposição à luz vermelha na presença do corante fenotiazínico novo azul de metileno (NMB) como PS, na inativação de células vegetativas e esporos de 12 diferentes cepas de *B. cereus*. Além disso, com os resultados deste capítulo foi possível estimar os parâmetros cinéticos de inativação fotodinâmica por meio do modelo de Weibull para 4 cepas selecionadas de acordo com a resistência ao tratamento.

No capítulo 3, objetivou-se investigar a eficiência do TFA com uma porfirina tetracatiônica (Tetra-Py^{+}-Me) e NMB combinados com exposição à luz branca, na inativação esporos de *A. acidoterrestris* em três diferentes matrizes: PBS, suco de laranja e casca de laranja. O efeito da potencialização do tratamento pela adição de iodeto de potássio (KI) nos experimentos com suco de laranja e casca também foram

avaliados. Por fim, também foi avaliado o impacto do TFA nas características nutricionais e colorimétricas do suco de laranja e da casca após os tratamentos.

Capítulo 1 – Revisão de Literatura

Antimicrobial photodynamic treatment (aPDT) as an innovative technology to control spoilage and pathogenic microorganisms in agrifood products: An updated review.

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Capítulo formatado de acordo com as normas de submissão da revista: ***Food Control***.

Abstract

Antimicrobial photodynamic treatment (aPDT) is a light-based method developed for the inactivation of microorganisms. The advance of oncological diseases has made photodynamic treatment widely used in the treatment of cancer. With the emergence of antifungal and antibiotic-resistant microorganisms, the aPDT has been strongly raised as a promising intervention to this global concern. The treatment involves the combination of visible light, photosensitizer (PS) compounds, and the presence of oxygen resulting in the generation of abundant reactive oxygen species (ROS), which further attack multiple cellular targets of microbial cells and result in their death. The multi-target mechanisms of action triggered by aPDT efficiently prevent the selection of resistant microorganisms. The efficacy of aPDT for microbial inactivation on foods can be affected by several factors such as type and characteristics of the PS, light source features, food surface geometry, environmental aspects, and microbial characteristics. The application of aPDT has spread rapidly in many types of agrifood products and their associated processes, as food cultivation, industrial processing, storage, distribution, and retail. Most of the *in vitro* aPDT studies have shown significant results, including some cases in which up to 8 log CFU/mL reductions was achieved. Strong antimicrobial performance of aPDT was also identified when the investigation was carried out on various food matrixes, including fruits and vegetables (1.5-5.0 log CFU/mL reductions), meat products (0.6-5.0 log CFU/mL reductions), and milk (1.0-7.0 log CFU/mL reductions). This review aims to update information on the advances of aPDT in the food and agriculture sectors, including photosensitizers, source of light, microbial inactivation efficacy, affecting factors, potentiation effects, inactivation mechanisms, possible applications on food and agriculture as well as the future perspectives.

Keywords: Antimicrobial photodynamic treatment, emerging technologies, photosensitization, reactive oxygen species, food safety, surface decontamination, food spoilage

Research Highlights

- Studies revealed the microbial reduction with different photosensitizers (PS)
- Chlorophyllin and curcumin are the most used natural PS
- The aPDT efficiency is dependent on PS formulation and source of light used
- aPDT effect can be enhanced by non-toxic compounds such as potassium iodide
- Studies that evaluate the characteristics of food after photodynamic treatment are still lacking

1. Introduction

Foodborne illness is one of the major global concerns in public health. The contamination of foods with pathogens is responsible for mortality and morbidity that impact on people's lives and countries' economies and in social development (Havelaar et al., 2015). According to the Centers for Disease Control and Prevention of the United States, 3,000 Americans die each year as a result of foodborne illnesses (CDC, 2020a). The latest survey of foodborne outbreaks in Brazil in 2018 registered 597 outbreaks, 8,406 sick people, 916 hospitalizations, and 9 deaths (Saúde, 2019a). Several bacterial (e.g., *Salmonella*, *Clostridium perfringens, Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus,* Shiga toxin-producing *Escherichia coli*), fungal (e.g., *Aspergillus flavus*), viral (e.g., Norovirus, Rotavirus), protozoal (e.g., *Toxoplasma gondii*, *Giardia intestinalis*), and parasites (e.g., *Taenia saginata*, *T. solium*) species can cause illnesses once present in food (WHO, 2015). In Brazil, most foodborne illnesses are caused by bacteria (*E. coli* – 24.0%*, Salmonella* spp. – 11.2%, *S. aureus* – 9.5%*, B. cereus* – 2.6%) and viruses (8.1%) from a total of 2,030 outbreaks identified (Saúde, 2019b). The most common symptoms caused by foodborne pathogens include nausea, vomiting, abdominal pain/discomfort, diarrhea, fever, and lack of appetite (de Freitas Saccol et al., 2016). Food microbial contamination could be avoided through good manufacturing practices, raw material control, and cold chain at the industry and retail level (Jaffee et al., 2019). After four basic steps at home (cleaning, separation, cooking, and cooling), people can be protected from food poisoning (CDC, 2020b). There are still a few cases of human exposure to chemical substances carried by food (0.2 %) from a total of 6,405 cases of chemical intoxication (CIATox, 2018).

Another challenge facing humanity is related to food losses due to spoilage or waste (Odeyemi et al., 2020). The global increase in the human population consequently raises the demand for food as well. A study conducted by the Food and Agriculture Organization of the United Nations revealed that one-third (1.3 billion tons per year) of food production for human consumption is lost due to spoilage or waste (FAO, 2011). For public health reasons, foodborne poisoning received more attention

than food spoilage. However, food spoilage also represents huge economic and reputation losses for the food industry and also means fewer consumable foods for humans (Iulietto et al., 2015). Any undesirable and unacceptable change in food quality due to spoilage can lead to food rejection (Koutsoumanis, 2009). Even with the application of modern preservation techniques, this is a frequent challenge faced by the food and beverage industries (Remenant et al., 2015). The two main issues involved with the inactivation of microorganisms are the antimicrobial resistance and the heat effects on food quality caused by heat treatments. Antimicrobial resistant-microbes can inhabit food, animals, people, and the environment. They can spread easily among animals and food without sanitary measures and inadequate food handling (WHO, 2018). For safety reasons, thermal treatment is the most widely used method for microbial inactivation in the food and beverage industries. The deleterious effects of thermal treatment on food and beverages have been observed, such as sensorial and nutritional losses (Barba et al., 2017). Given the aforementioned problems regarding antimicrobial resistance and undesirable effects of thermal processes, several innovative mild processing technologies have been developed (Cebrián et al., 2016; Ferrario & Guerrero, 2018; Kim & Kang, 2018; Min et al., 2017; Reverter-Carrión et al., 2018). Among them, pulsed light, ultraviolet (UV) light radiation, pulsed electric fields, cold plasma, and high-pressure processing are the most studied techniques so far (Barba et al., 2017).

An innovative method has gained popularity in recent years among the scientific community for microbial inactivation, namely photodynamic therapy (PDT). This is an approved method for cancer treatment by the American Cancer Society (ACS, 2019), and the European Academy of Dermatology and Venereology - EADV (Morton et al., 2013). The advances in this area were transferred to the area of microbiology mainly with the beginning of the era of antimicrobial resistance originating the term antimicrobial photodynamic treatment – aPDT (Wainwright et al., 2017). Although its discovery in 1900 has been reported for the inactivation of the protozoan *Paramecium caudatum* by the combination of sunlight and dyes, its use for the treatment of tumor cells in 1905 preceded the success of the PDT (Jesionek & von Tappeiner, 1905; Raab, 1900). The elementary principle of aPDT depends on the combination of visible

or near-infrared light with a photosensitizer (PS) compounds in the presence of molecular oxygen to generate reactive oxygen species (ROS), leading to cell death. The multiple target action of ROS generated during aPDT reduces the chance of selecting resistant microorganisms. Also, the use of non-toxic PS and harmless visible light makes aPDT a promising alternative for the food applications. At present, aPDT have focused the efforts on the inactivation of a broad spectrum of pathogenic microorganisms as *Salmonella*, *E. coli*, *L. monocytogenes* and *B. cereus* (Josewin et al., 2018; Oliveira et al., 2009; Santos et al., 2020; Vieira et al., 2019).

The current work aims to give a comprehensive report of the most recent advances of aPDT on the food and agriculture sectors. The classifications of PSs and sources of light used for aPDT, as well as the mechanisms of inactivated action including potentiation effects caused by the addition of nontoxic inorganic salts, were discussed in detail. Although the related topic has been reviewed before (Ghate et al., 2019; Lukšiene, 2005; Lukšiene & Brovko, 2013; Lukšiene & Zukauskas, 2009; Silva et al., 2018), a crescent number of studies recently published for agrifood reasons have been observed. Therefore, an update is needed giving the advances in the knowledge regarding aPDT. This review could assist the researchers in the development of aPDT-associated approaches for reducing the food and agriculture microbial threats.

2. Concepts of antimicrobial photodynamic treatment

The basic concept of aPDT is the triggering of photochemical reactions through the combination of visible or near-infrared light at a specific wavelength, a photosensitizer compound, and molecular oxygen (Vieira et a., 2019). Both reactions (Type I and II) presented in Fig. 1 occur after light exposure of the PSs compounds (endogenous or exogenous) which absorb and transfer energy to the molecular oxygen forming the reactive oxygen species (ROS) (Wainwright et al., 2017), such as singlet oxygen ($^1O_2$), hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2). ROS cause damages on microbial cells through the oxidation of essential molecular components such as proteins, lipids, and nucleic acids (Broekgaarden et al., 2015; Vatansever et al., 2013). This multitarget and unspecified mechanism of action make

aPDT an effective alternative for combating multidrug-resistant bacteria (Almeida et al., 2014; Cieplik, et al., 2018a). Also, some studies have investigated the potentiation of aPDT by adding inorganic salts as potassium iodide – KI (Vecchio et al., 2015; Vieira et al., 2018, 2019; Yuan et al., 2020; Zhang et al., 2015), sodium bromide – NaBr (Wu et al., 2016), sodium azide – NaN_3 (Huang et al., 2012), thiocyanate – SCN^- (St Denis et al., 2013), and preliminary studies of sodium nitrite and selenocyanate (Hamblin, 2017).

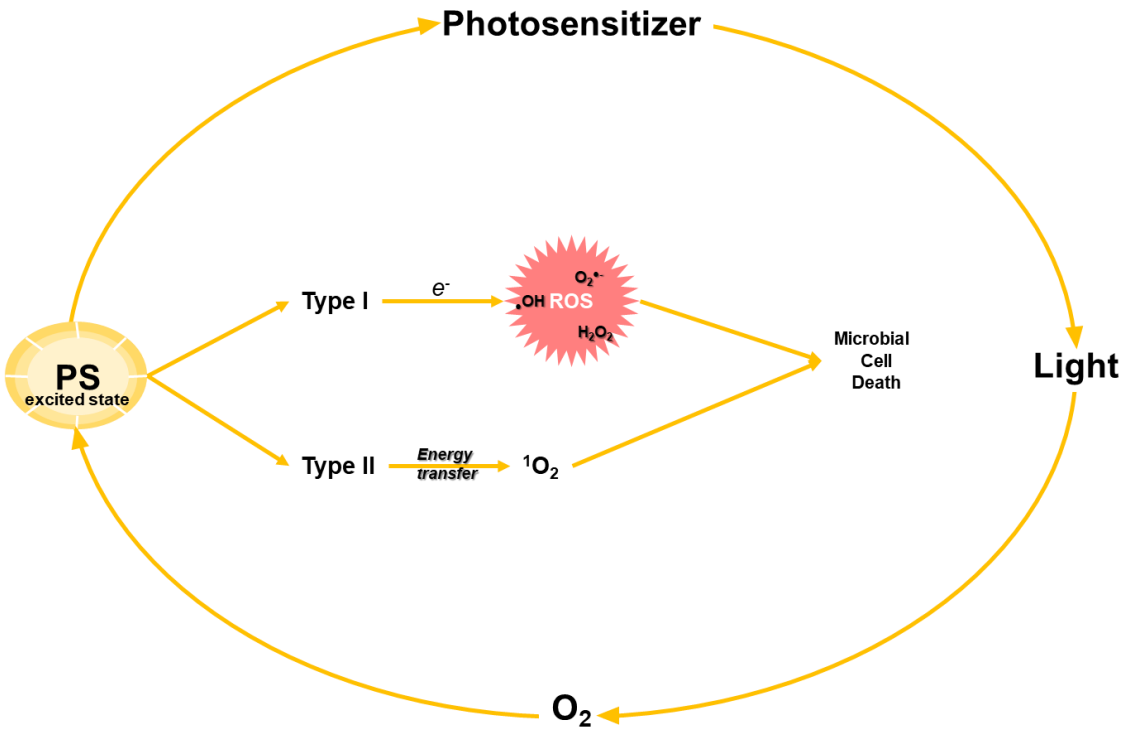


Fig. 1. Process of microbial cell death caused by the generation of reactive oxygen species (ROS) and singlet oxygen ($^1O_2$) after photosensitizer (PS) excitation by light exposure in the presence of oxygen molecules.

2.1. Mechanisms of action

The absorption of light by the PS can follow two alternative pathways, widely known as type I and II. Initially, the PS in the fundamental state can absorb light energy, further moving to an excited singlet state. This state can decay by fluorescence, heat

dissipation or to a longer-lived triplet state (most expected). The formation of the PS triplet state can return to the ground singlet state by phosphorescence or finally allows the interaction with oxygen by the electron transfer (Type I) from excited PS, forming ROS (e.g., $^{\bullet}OH$, $O_2^{\bullet-}$, and H_2O_2); or transfer energy directly to a free oxygen molecule to produce $^1O_2$ in the process (Type II). The microbial cell killing depends on the excited PS characteristics (e.g., lipophilicity, amphiphilicity, ionic charge) which will influence its localization in the cell (e.g., microbial cell walls, lysosomes, mitochondria, lipid membranes, and nucleus) as demonstrated in the Fig. 2 (Brancini et al., 2016; Castano et al., 2004; de Menezes et al., 2014; Menezes et al., 2016; Wainwright et al., 2017). The localization of PS matters because it will only affect the cell structures that are near to the ROS production during aPDT (Castano et al., 2004). The singlet oxygen is considered much more destructive than other ROS, however, the combination of various ROS is desirable to enhance the antimicrobial efficiency (Maisch et al., 2005).

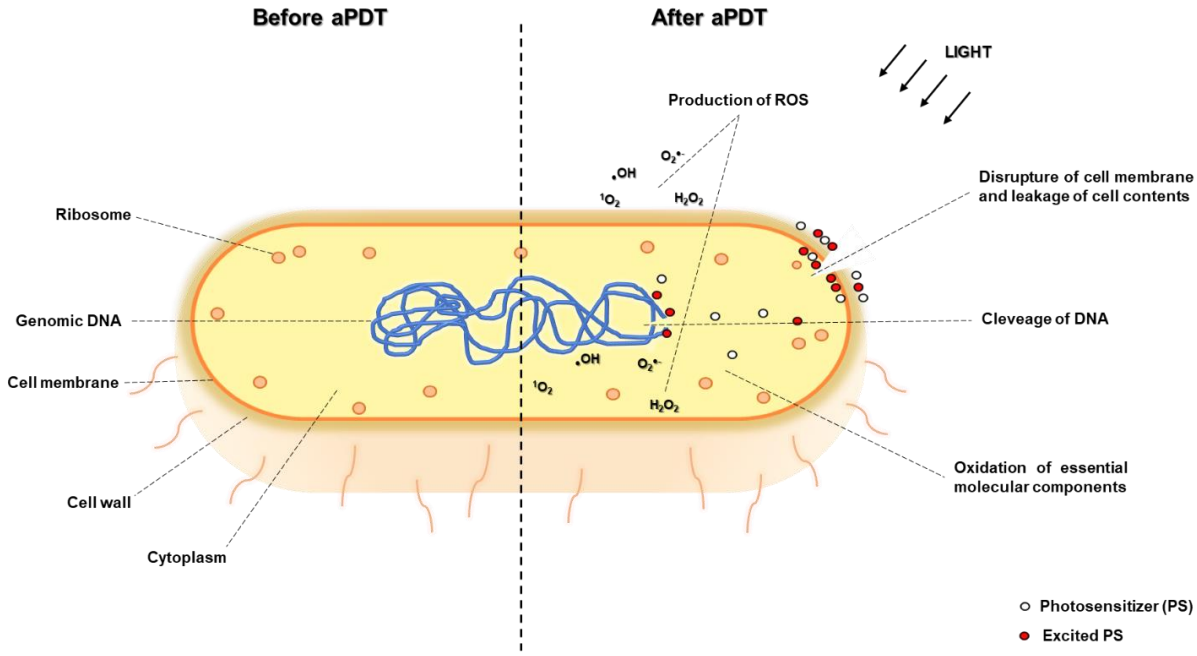


Fig. 2. Illustration of a generic bacterial cell before and after aPDT treatment and the involved mechanisms of photoinactivation.

The specific inactivation mechanism of aPDT has been explored by several authors and the cell membrane is the major inactivation target in the process (Alves et al.,

2013; Buchovec et al., 2017; Cieplik et al., 2018b; de Menezes et al., 2016; Kim & Yuk, 2017; Tonani et al., 2018). It was revealed that the membrane phospholipids of *Staphylococcus warneri* were affected (e.g., the formation of lipid hydroxides and hydroperoxides) by the aPDT with synthetic cationic porphyrin and artificial white light (Alves et al., 2013). Also, some of these studies highlighted the cell membrane as the main target of ROS action, promoting its disruption and the consequent release of proteins and nucleic acids (Buchovec et al., 2017; Lin et al., 2012). Buchovec et al. (2017) emphasized the role of singlet oxygen decreasing the inactivation of *S.* Typhimurium by aPDT with Na-Chl and LED exposure at 405 nm by adding a singlet oxygen quencher (NaN_3). Moreover, the gene expression evidenced that the process of aPDT can upregulate some relevant genes (*OxyR, GrxA, AhpC, STM0225, AtpC*, *groEL*, *SulA*) related to oxidative, extracytoplasmic, and acidic stress (Buchovec et al., 2017). Another study found interesting results in terms of membrane depolarization and permeability (efflux pump and glucose uptake activity), genomic DNA oxidation, and gene expression of *S.* Saintpaul (LED-resistant) and *S.* Enteritidis (LED-sensitive) strains by 405 nm LED illumination of endogenous PS (coproporphyrin) at 4°C (Kim & Yuk, 2017). The authors also observed an increase of guanine oxidation, complete inhibition of efflux pump activity (due to inhibition of ATPase), and glucose uptake system disorders most likely because of the proximity to endogenous porphyrins. Such mechanisms of action indicate that the aPDT inactivation is highly associated with membrane depolarization and permeability (Cieplik et al., 2018b; de Menezes et al., 206; Kim & Yuk, 2017; Tonani et al., 2018), membrane breakage (Buchovec et al., 2017; Lin et al., 2012), phospholipids rearrangement (Alves et al., 2013) and peroxidation (de Menezes et al., 2016; Tonani et al., 2018). The expression of five genes (*oxyR, recA, rpoS, sodA,* and *soxR*) in non-illuminated and illuminated *S.* Saintpaul and only one gene (*oxyR*) for *S.* Enteritidis cells, indicates that results were more affected by low temperature than LED-illumination (Kim & Yuk, 2017). Such published results are extremely important for a better understanding of the action associated with aPDT on microbial cells, including the development of aPDT resistance. A recent publication reviewed the main factors that can be addressed to the aPDT resistance, including oxidative stress and antibiotic resistance mechanisms

(Kashef & Hamblin, 2017). Nevertheless, the development of resistance or tolerance is highly unlikely given the unspecific characteristic of aPDT-induced cell death by ROS production (Kashef & Hamblin, 2017).

2.2. Potentiation effect of inorganic salts on aPDT

The efficacy of aPDT can be enhanced by adding inorganic salt. The most powerful and versatile is potassium iodide, which has been widely evaluated for the inactivation of bacteria (Huang et al., 2018; Moreira et al., 2020; Vecchio et al., 2015; Vieira et al., 2018, 2019; Yuan et al., 2020; Zhang et al., 2015), fungi (Freire et al., 2016; Zhang et al., 2015) and virus (Vieira et al., 2019). The major antimicrobial inactivation mechanism of potentiation is attributed to the reaction of potassium iodide with singlet oxygen, forming highly oxidant free molecular iodine (I_2/I_3^-), iodine radicals ($I_2^{\bullet -}$), and H_2O_2 (Huang et al., 2017; Vieira et al., 2019). Another potentiation pathway involves the oxidation of 2-electrons of iodide or bromide that occurs during the illumination of titanium dioxide to form hypohalites (Huang et al., 2016; Wu et al., 2016). The third mechanism of aPDT potentiation was discovered, paradoxically, by the addition of NaN_3, which is widely used as a singlet oxygen quencher (Huang et al., 2012). The authors observed that the addition of NaN_3 to the menstruum of bacteria with MB and red-light illumination potentiated the inactivation process, promoting the type I mechanism (electron transfer) rather than acting as a singlet oxygen inhibitor.

2.3.Factors affecting the aPDT efficacy on foods

The efficacy of aPDT can be affected by various elements, including the nature of the microorganisms, PS characteristics, light exposure period, and environmental aspects (Gonzales et al., 2010). Besides these already well-documented factors, some researchers are investigating the impact of some other factors such as liquid food composition and geometry properties of solid foods.

The impact of the milk composition and their interaction with light and PS compound on aPDT efficacy to reduce the viability of Gram-positive and Gram-negative bacteria

was investigated by Galstyan & Dobrindt (2019). It was demonstrated that abundant components in milk as proteins, fat, and free cations (Ca^{2+} and Mg^{2+}) can impair the aPDT antimicrobial efficacy. Such results were confirmed in the experiments where the dilution of milk significantly impacted the bacterial photoinactivation. The authors also detected a significant impact in the stability, generation of ROS, and aggregation of MB as PS compound (Galstyan & Dobrindt, 2019).

Other food properties such as pH and water activity can affect the aPDT efficacy. The influence of pH was investigated on the efficiency of PDT using LEDs, since the acidity of the food can affect the treatment (Ghate et al., 2015). It was demonstrated in this study that acidic and alkaline pH can enhance the effect of aPDT. The findings showed that lower pH (4.5) can assist aPDT against Gram-positive bacteria (L. monocytogenes) due to the absence of the outer membrane structure to protect the permeation of hydrogen ions. In contrast, E. coli and Salmonella representing Gram-negative bacteria were more vulnerable to the treatment at alkaline pH 9.5, most likely due to the saponification of the membrane lipids (Ghate et al., 2015). As the pH of food can vary, the findings of this study may contribute to optimizing the aPDT treatment according to the pH of the food.

The effect of water activity on microbial resistance to the different methods of inactivation has widely recognized from traditional methods to new emerging technologies (Lee et al., 2020). The water content inside the cell is crucial for the microbial survivals (Alvarenga et al., 2018). Also, resistant microbial forms, such as spores of bacteria and fungi, help to survive in low water-activity foods. (e.g., dairy and meat products, cereal and nut products) (Stevenson et al., 2015). However, studies that assess the impact on the aPDT efficacy in different levels of water activity have not be found in the literature, opening new horizons in this sense.

The physical factors (geometry and shadow effects) of solid food may have a significant impact on the aPDT efficacy. The geometry characteristics of solid foods such as fruits and vegetables have been recently investigated by Glueck et al. (2017). The authors tested three types of food surface, namely flat, spherical, and complex, to determine if the efficacy of a curcumin-based aPDT treatment was dependent on superficial food geometry. A significant inactivation of more than 3 log CFU/mL of *E.*

coli were obtained on all flat surface produces, represented by cucumber, tomato and lettuce. Inactivation of 3.7 log CFU/mL and 5 log CFU/mL of *E. coli* were observed on the spherical surface of non-germinated mung beans and fenugreek seeds, respectively, with the use of continuous rotation during the experiment. Only in the complex geometry (multifaceted) represented by mung bean germlings did not reach the minimum of 3 log CFU/mL reduction even with rotation (Glueck et al., 2017). In technologies that are based on exposure to light, there is also a limiting factor known as the shadow effect which is related to the food geometry (Condón-Abanto et al., 2016). However, there are still no studies that have explored this effect in aPDT.

3. Photosensitizers

PSs are chemical compounds that can be natural or synthetic. Also, they can be produced by microorganisms (endogenous) or administered externally (exogenous) (Gábor et al., 2001). The choice of suitable PS is crucial to efficiently reduce microbial loads during aPDT application. The principle of PS selection is based on the source of light with the emission wavelength indicated for the absorption band of the PS to excite the molecule (Hamblin, 2016). The optimization of photodynamic treatment is closely related to the PS as it can increase the efficiency, selectivity, and safety of the treatment (Ghate et al., 2019).

Some microorganisms (especially bacteria and fungi) present the ability of light absorption because they are pigmented by compounds naturally produced inside the cells (Kumar et al., 2015). Some of these compounds, namely porphyrins, are the first generation of PSs classified as heterocyclic macromolecules including protoporphyrin, coproporphyrin, and uroporphyrin (Ghate et al., 2019). This natural pigmentation allows the photoinactivation of the microorganisms only by light exposure without the application of exogenous PS (Demidova & Hamblin, 2004). The natural existing porphyrins can be diverged based on quantity and composition among the species (Kumar et al., 2015). According to these authors, the amount of coproporphyrin is higher in Gram-positive than Gram-negative bacteria. The production of endogenous porphyrins can be induced with the use of PS precursors such as 5-aminolevulinic acid

(ALA) (Le Marc et al., 2009; Lukšiene et al., 2009). ALA is an important component in the heme biosynthesis pathway inside cells, leading to the production of porphyrins endogenously (Fotinos et al., 2008). The strategy of microbial photoinactivation triggering the production of endogenous PS with continuous light exposure is adequate when fast inactivation is not required as in the case of food preservation during its distribution and storage chain (Ghate et al., 2019).

However, microbial inactivation must be effective and fast to prevent the propagation of undesirable microorganisms in food. Most research efforts are focused to screen and evaluate promising candidates of PS to increase the rate of photoinactivation in food matrixes. The ideal PS used for food decontamination should present the following characteristics: (1) absence of toxicity; (2) solubility in non-toxic compound; (3) photostability; (4) microbial cell affinity (positively charged PS can achieve strong adhesion to negatively charged bacterial cell walls); (5) high production of ROS and high quantum yield of singlet oxygen (Φ_{Δ}); and (6) wide spectrum of light absorption (Alves et al., 2009; Cieplik et al., 2014; Ghate et al., 2019; Lukšiene & Brovko, 2013; Lukšiene & Zukauskas, 2009). The absence of toxicity is essential to keep the consumer safe and food without apparent damage. Especially when it is increasingly desired to decrease food additives. In this sense, it is also required that a suitable PS should be easily dissolved in water or any other non-toxic compound to simplify the removal from the food surface. The photostability of the PS is an important issue to be considered since it is desired to be stable when inside the microbial cells for its inactivation. On the other hand, once the PS is free it will decay rapidly, avoiding the persistence in the environment which is a frequent issue with conventional insecticides (de Menezes et al., 2014b; Derosa & Crutchley, 2002). The microbial cell affinity is also desirable to avoid the accumulation of the PS anywhere other than for microbial inactivation (de Menezes et al., 2014a,b; 2016; Rodrigues et al., 2020). Once inside the microbial cell, a good PS should be able to produce high amounts of ROS, especially singlet oxygen species, that are highly cytotoxic (Gonzales et al., 2010; Derosa & Crutchley, 2002).

The exogenous PS used for aPDT can be synthesized or obtained from nature (Table 1). The main synthetic PSs are the porphyrin derivatives (chlorins,

bacteriochlorins, phthalocyanine, tetraphenylporphine, iodide-based porphyrins), ALA (endogenous porphyrin precursor), phenothiazinium dyes (toluidine blue, methylene blue, new methylene blue, 1,9-dimethylmethylene blue), and xanthene dyes (rose bengal, eosin Y, erythrosine) (Bartolomeu et al., 2016; Buchovec et al., 2010; de Menezes et al., 2014b, 2016; Lukšiene & Brovko, 2013; Rodrigues et al., 2012a,b). Most of the porphyrin derivatives are based on the tetrapyrrole nucleus including chlorins, phthalocyanine, bacteriochlorins, phthalocyanine, and iodide-based porphyrins. Their low toxicity in the absence of light and ability to enter into long-lived triplet excited states contributes to its wide uses in aPDT (Demidova & Hamblin, 2004; Lukšiene & Zukauskas, 2009). The phenothiazinium dyes, namely blue dyes, are the most studied PSs against different microbial species (de Menezes et al., 2014; de Menezes et al., 2016; Freire et al., 2016; Tonani et al., 2018; Wainwright et al., 1997; Wainwright, 2004). Their molecular framework, in line with other linear tricyclic heteroaromatics, is ideal for action against nucleic acids, due to their ability to bind to guanosine residues forming 8-hydroxyguanosine causing DNA breakage (Schneider et al., 1990; Wainwright, 1998; Wainwright et al., 2012). Xanthene dyes have been extensively tested as PS to induce microbial photoinactivation due to their low cost, high absorptivity, and abundant singlet oxygen generation (Bonin et al., 2018; Yassunaka et al., 2015). Additionally, some xanthene derivatives have been approved for pharmaceutical and food applications (Santos et al., 2019).

The use of natural PS grows as the demand for natural products increases, following the recent desires of consumers. Some examples of PSs obtained from nature are hypericin (Hyp), coumarins, furocoumarins, chlorophyllin (Chl), curcumin, and riboflavin (vitamin B2) (Bonifácio et al., 2018; Dementavicius et al., 2016; Maisch et al., 2014; Otieno et al., 2020; Paskeviciute et al., 2019). The Hyp extract is a natural pigment obtained from the plant traditionally known as St John's wort (*Hypericum perforatum*) widely used as natural PS and considered one of the most powerful PS in nature (Lukšiene & Zukauskas, 2009). Also, Hyp is free of any toxic or genotoxic effects (Feruszová et al., 2016; Okpanyi et al., 1990). Coumarins and furocoumarins (psoralens) are naturally produced as secondary metabolites in a variety of plant species, in particular of the Umbelliferae, Apiaceae, and Rataceae families (de

Menezes et al., 2014; Fracarolli et al., 2016). Plants produce these compounds to act either via light-dependent or independent mechanisms, as antimicrobials (de Menezes et al., 2014; Fracarolli et al., 2016). Chlorophyllin is a water-soluble derivative of chlorophyll (not water-soluble) frequently used as a food additive (E-140 and E-141) in dietary supplements and cosmetics (López-Carballo et al., 2008). Curcumin is a natural yellow pigment obtained from the rhizome *Curcuma longa* widely used for centuries as a food ingredient and is currently certified as a food additive (E-100). It is also known by its antimicrobial, anti-inflammatory, anti-proliferative, antioxidant, and, more recently, photosensitizing activities (Bonifácio et al., 2018; de Souza et al., 2019; Eigner & Scholz, 1999; Ghosh et al., 2015; Huang et al., 2020). Riboflavin, also known as vitamin B2, is an essential human nutrient (water-soluble) and frequently used in the food industry as a colorant (E-101). It also can produce high amounts of singlet oxygen after light exposure (Baier et al., 2006; Maisch et al., 2014). Also, riboflavin is an important bioproduct photodegraded in the human body when exposed to visible light (Cardoso et al., 2012; Schuyler, 2001), consequently can be considered safe for food applications.

Table 1. Endogenous and exogenous (synthetic and natural) photosensitizers used on aPDT.

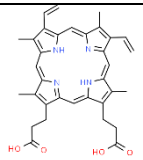
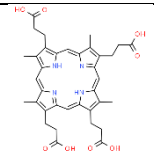
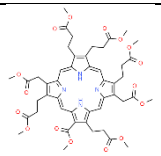
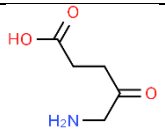
Photosensitizers	Chemical structure	Absorbance (nm)	Quantum yield of $^1O_2$ (Φ_Δ)	Molar absorptivity (L mol^{-1} cm^{-1})	References
Endogenous:					
Protoporphyrin IX	 ChemSpider ID: 10469486	400-650	0.77 (phosphate buffer)	ε_{408nm} = 1.24 x 10^5	Balasubramaniam & Natarajan (1997); Nishimura et al. (2019)
Coproporphyrin III	 ChemSpider ID: 16736509	370-610	0.13 (ethanol) 0.18 (D_2O) 0.41 (D_2O + 2% Triton X-100)	N.A.	Murav'eva et al. (2018)
Uroporphyrin II	 ChemSpider ID: 4575518	546 nm	0.71 (phosphate buffer)	N.A.	Wilkinson et al. (1993)
Exogenous:					
5-aminolevulinic acid	 ChemSpider ID: 134		*Precursor of endogenous porphyrins*		Kennedy & Pottier (1992)

Table 1. Continued

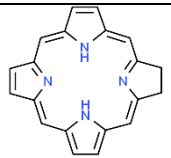
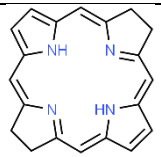
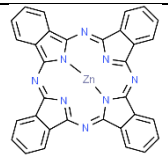
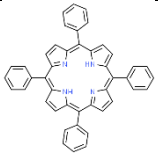
Photosensitizers	Chemical structure	Absorbance (nm)	Quantum yield of $^1O_2$ (Φ_Δ)	Molar absorptivity (L mol^{-1} cm^{-1})	References
Porphyrin derivatives:					
Chlorins	 ChemSpider ID: 58616	650	0.88-0.98 (toluene)	ε_{650nm} = 3.00 x 10^4	Pineiro et al. (2001)
Bacteriochlorins	 ChemSpider ID: 7827701	300-400	0.05 (phosphate buffer) 0.33 (DMPC liposome)	N.A.	Hoebeke & Damoiseau (2002)
Zinc phthalocyanine	 ChemSpider ID: 21169868	675	0.53 (tetrahydrofuran)	ε_{675nm} = 1.58 x 10^5	de Souza et al. (2018)
Tetraphenylporphine	 ChemSpider ID: 10291672	400-650	0.52 (DMSO)	ε_{516nm} = 14.08 x 10^3	da Silva et al. (2008)

Table 1. Continued

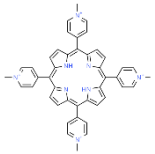
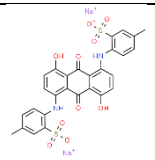
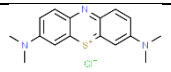
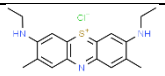
Photosensitizers	Chemical structure	Absorbance (nm)	Quantum yield of $^1O_2$ (Φ_Δ)	Molar absorptivity (L mol^{-1} cm^{-1})	References
5,10,15,20-Tetrakis(1-methylpyridinium-4-yl) porphyrin tetraiodide (Tetra-Py+-Me)	 ChemSpider ID: 4086	600-750	N.A.	ε_{425nm} = 2.69 x 10^5 ε_{516nm} = 1.95 x 10^4 ε_{549nm} = 5.89 x 10^3 ε_{588nm} = 6.92 x 10^3 ε_{642nm} = 2.00 x 10^3	Simões et al. (2016)
Phenothiazinium dyes:					
Toluidine blue O (TBO)	 ChemSpider ID: 69136	600-680 627	0.44 (ethanol)	ε_{627nm} = 7.40 x 10^4	Bacellar et al. (2014)
Methylene blue (MB)	 ChemSpider ID: 5874	600-680 655	0.52 (ethanol)	ε_{655nm} = 9.60 x 10^4	Bacellar et al. (2014; Wilkinson et al. (1993)
New methylene blue N (NMB)	 ChemSpider ID: 16736255	600-680 550-650	N.A.	N.A.	Rodrigues et al. (2012)

Table 1. Continued

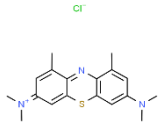
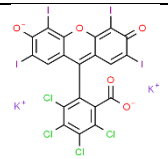
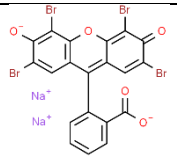
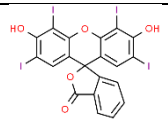
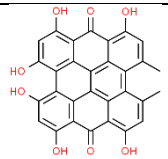
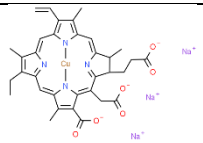
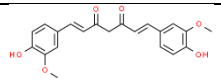
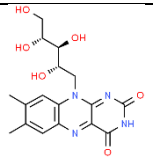
Photosensitizers	Chemical structure	Absorbance (nm)	Quantum yield of $^1O_2$ (Φ_Δ)	Molar absorptivity (L mol^{-1} cm^{-1})	References
1,9-dimethylmethylene blue (DMMB)	 ChemSpider ID: 110430	600-680 651	0.71 (ethanol)	ε_{651nm} = 7.80 x 10^4	Bacellar et al. (2014)
Xanthene dyes:					
Rose Bengal (RB)	 ChemSpider ID: 23773	480-550	0.76 (phosphate buffer)	ε_{543nm} = 10.90 x 10^4	Batistela et al. (2011); Lee & Rodgers (1987)
Eosin Y (EOY)	 ChemSpider ID: 10580	500-600	0.57 (phosphate buffer)	ε_{517nm} = 9.71 x 10^4	Batistela et al. (2011); Gandin et al. (1983)
Erythrosine (ERY)	 ChemSpider ID: 3144	500-600	0.63 (phosphate buffer)	ε_{532nm} = 9.66 x 10^4	Batistela et al. (2011); Gandin et al. (1983)

Table 1. Continued

Photosensitizers	Chemical structure	Absorbance (nm)	Quantum yield of $^1O_2$ (Φ_Δ)	Molar absorptivity (L mol^{-1} cm^{-1})	References
Natural compounds					
Hypericin (Hyp)	 ChemSpider ID: 4444511	630	0.73 (ethanol)	ε_{630nm} = 1.31 x 10^4	Wilkinson et al. (1993)
Chlorophyllin (Chl) + cooper (Obtained from chlorophyll)	 ChemSpider ID: 21781827	400	0.57 (methanol)	ε_{400nm} = 2.08 x 10^4	Uchoa et al. (2015)
Curcumin	 ChemSpider ID: 839564	420	<0.01 (ethanol)	ε_{430nm} = 2.74 x 10^4	Chignell et al. (1994)
Riboflavin (Vitamin B2)	 ChemSpider ID: 431981	470-490	0.54-0.59 (methanol)	ε_{430nm} = 1.16 x 10^4	(Baier et al., 2006; Chacon, McLearie, & Sinclair, 1988)

N.A.: Not available

4. Sources of light

The light sources used in the photodynamic treatment are diverse according to their emission spectrum. The main groups of light sources are the conventional lamps (tungsten-halogen, metal–halide, xenon, etc.) (Calin & Parasca, 2009), light-emitting diodes (LEDs) as the most recent alternative source of light for aPDT (Brancini et al., 2016; Penha et al., 2017; Rodrigues et al., 2012; Silva et al., 2019; Yassunaka et al., 2015), and lasers (light amplification by stimulated emission of radiation) (Gonzales et al., 2010; Rodrigues et al, 2012; Rodrigues et al., 2020). Lasers are extremely efficient alternatives and widely used in clinical treatments (Calin & Parasca, 2009), however, their high cost makes them an impracticable option for the food sector. There are several advantages related to LED usage, such as low driving voltage, lightness, robustness, compactness, shock and vibration resistance, free of toxic compounds (i.e. mercury), the flexibility of assemblage, narrow-band emission, and no residue of undesirable spectral components (Lukšiene & Brovko, 2013). LEDs became more attractive to the food industry (mainly for food production, postharvest storage, and food safety) for its high cost-benefit, inexpensive maintenance, long life, and nonthermal effects (D'Souza et al., 2015). The current choice for LEDs means the delivery of a wide range of emission wavelengths from UVA (350 nm) to near-infrared (1100 nm) and the output power can provide up to 150 mW/cm^2 of irradiance (Brancaleon & Moseley, 2002). Most lamps can burn out abruptly making treatment unfeasible. LEDs can maintain up to 70% of the initial irradiation output flux after 50.000 – 100.000 h of exposure (Lukšiene & Zukauskas, 2009). Recently, an emerging alternative has been the use of sunlight in aPDT against foodborne (Miñán et al., 2015) and plant pathogen (Fracarolli et al., 2016; Gonzales et al., 2017; Jesus et al., 2018) microorganisms, besides clinical purposes (Wiegell et al., 2008). This method is considered efficient, cheap, and environmentally friendly (Yang et al., 2019). As sunlight is a limitless resource, its intelligent use has been increasingly demanded in the field of photodynamic treatment due to its low cost and practicality (Fracarolli et al., 2016; Gonzales et al., 2017). The choice of the appropriate light source is directly

associated to the PS selected for the aPDT. Each PS has a range of maximum absorbance (see Table 1) that can be emitted by several light sources. It is important to note that for maximum treatment efficiency, the emission wavelength must be correlated to the PS absorption spectrum (Lukšiene & Brovko, 2013). This is crucial for the generation of sufficient ROS (Type I) and 1O2 (Type II) to rapidly inactivate microorganisms.

Table 2. Properties of the most frequently used sources of light on aPDT.[1]

Sources of light	Irradiance	Wavelength	Heating	Delivery
Tungsten filament	Up to 250 mW/cm^2	400 – 1100 nm	Yes	Direct
Diode laser	Up to 700 mW/cm^2	600 – 950 nm	Yes	Optical fiber
LED	Up to 150 mW/cm^2	350 – 1100 nm	Yes (low)	Direct

[1]Data extracted from (Wilson & Patterson, 2008) and (Brancaleon & Moseley, 2002)

5. Inactivation efficacy of aPDT on the main foodborne microorganisms

It is widely known that the aPDT has been used to inactivate all classes of microorganism including bacteria (Gram-positive, Gram-negative, vegetative cells and spores), fungi (yeasts and molds), protozoa and viruses. Most of the studies published to date have dedicated the research mainly to photodynamic inactivation of bacteria as demonstrated by a recent comprehensive review focused on food safety (Ghate et al., 2019). Another interesting critical review summarized the studies of photodynamic inactivation of foodborne bacteria in planktonic and biofilm states (Silva et al., 2018). Although bacteria are important foodborne microorganisms, recent studies have shown that most foodborne outbreaks are caused by viruses (Bosch et al., 2018; EFSA, 2015; Painter et al., 2013). However, even the number of studies concerning aPDT of fungi species are higher than foodborne viruses (Ghate et al., 2019), mainly in clinical and agricultural areas (Freire et al., 2016; Gonzales et al., 2017; Rodrigues et al., 2012). The inactivation of protozoan parasites with the aPDT treatment is also

an important investigation field (Barbosa et al., 2020; Sepúlveda et al., 2020). The most relevant studies described in this section are depicted in Table 3 and include only studies that used exogenous PS.

5.1. Bacterial vegetative cells

Most of aPDT studies were focused on the inactivation of bacteria (e.g., antibiotic-resistant, foodborne pathogens, spoilage strains, biofilms, and heat-resistant spores) and this predominance can be attributed to bacterial susceptibility to the treatment and the emergence of multidrug-resistant bacterial species. The efficacy of aPDT on bacteria varies between Gram-positive and Gram -negative species, in all forms, such as vegetative cells, spores, and biofilm (Silva et al., 2018). The majority of the studies are dedicated to foodborne pathogenic bacteria instead of food spoilage bacteria. The significant demand for the control of pathogenic bacteria for health issues is well known; however, the spoilage bacteria also play a fundamental role in the food quality of the industry.

Among the bacterial species frequently evaluated, Gram-negative bacteria are less susceptible than Gram-positive bacteria. In a recent study, the authors confirmed that Gram-negative bacteria (*Aeromonas hydrophila*, *E. coli, S.* Typhimurium, and *Pseudomonas aeruginosa*) required higher concentrations of erythrosine and longer exposure times than Gram-positive bacterium (*S. aureus*) did (Yassunaka et al., 2015). The lower susceptibility of Gram-negative bacteria to aPDT is mainly related to the outer membrane preventing the uptake of anionic and neutral PS (George et al., 2009). The use of positively charged PS (e.g., porphyrin and phenothiazinium derivatives) has been an efficient strategy to achieve significant photoinactivation of Gram-negative species (Alves et al., 2009; Demidova & Hamblin, 2005; Simões et al., 2016). The use of porphyrins (preferably cationic ones) has been extensively applied for a broad-spectrum aPDT (Bartolomeu et al., 2016; Beirão et al., 2014; Moreira et al., 2020; Pereira et al., 2014; Tomé et al., 2004). The main indicators of bacterial photodynamic

efficiency of porphyrin derivatives have been *S. aureus* (Gram-positive; including methicillin-resistant strains) and *E. coli* (Gram-negative). Cells suspensions of six different strains of *S. aureus* were irradiated for 60 min with Tetra-Py^+-Me at 5 µM reaching 5 log CFU/mL of reduction (Bartolomeu et al., 2016). A recent study evaluated the photodynamic efficiency of the cationic porphyrin-imidazole against a bioluminescent *E. coli* strain (Moreira et al., 2020). One of the porphyrin-imidazole derivatives was evaluated in two different concentrations (5 and 20 µM) and reduced the population of *E. coli* of 2.38 and 3.85 log CFU/mL, respectively, after 90 min of irradiation (Moreira et al., 2020). Another study assessed how the outer structures influenced the aPDT process in a diverse spectrum of Gram-positive and Gram-negative bacteria (Pereira et al., 2014). The authors have documented that the susceptibility of each bacterial strain to aPDT with Tetra-Py^+-Me was dependent on bacteria external structures, although Gram-positive bacteria with the complex multi-layered outer structure were still more sensitive to aPDT than Gram-negative bacteria. The authors explained that even multi-layer Gram-positive bacteria are still more porous than gram-negative to the effects of aPDT. The use of porphyrins as PS is still featured in other bacteria of food interest as *B. cereus*, *L. monocytogenes,* and *P. aeruginosa* (Oliveira et al., 2009; Beirão et al., 2014; Romanova et a., 2003).

The use of phenothiazinium dyes (*e.g.* MB, TBO, NMB, DMMB) for photodynamic inactivation of foodborne bacteria have been reported by several authors (Demidova & Hamblin, 2005; Lin et al., 2012; Wainwright et al., 1997; Wainwright et al., 1998; Wu et al., 2009). One of the earliest studies of aPDT with phenothiazinium derivatives against foodborne pathogens tried to inactivate *S. aureus* (including MRSA), *B. cereus*, *E. coli*, *P. aeruginosa*, and *Enterococcus faecalis* using a source of light (350-800 nm) able to give a fluence of 6.3 J/cm^2 after 60-min exposure (Wainwright et al., 1997; Wainwright et al., 1998). The minimum lethal concentrations required for several bacterial species were significantly reduced by light exposure. This study did not provide the results in log reductions. *L. monocytogenes* has also been effectively killed by aPDT with MB excited by a tungsten-halogen lamp with an irradiance of 200

mW/cm^2 (Lin et al., 2012). The viability of *L. monocytogenes* was diminished by about 7 log CFU/mL after 10 min of exposure which corresponded to a fluence of 120 J/cm^2.

Another class of PS frequently used in aPDT processes is the xanthene dyes as erythrosine which is an approved food colorant by the U.S. Food and Drug Administration (FDA, 2020). Several studies have evaluated the photodynamic effects of erythrosine and other xanthene dyes as RB, and EOY (Brovko et al., 2009; Silva et al., 2018; Silva et al., 2019; Yassunaka et al., 2015). Brovko et al. (2009) reported the use of RB at 5 µg/mL against *Bacillus* sp. and *L. monocytogenes* resulting in 5–6 log CFU/mL reductions after 30 min of light exposure (0.81 J/cm^2). However, to achieve the same magnitude of reduction for *E. coli* and *S.* Typhimurium, the concentration of RB had to be increased to 50 µg/mL in the same conditions of illumination. Moreover, the authors also reported that an RB minimum bactericidal concentration of 5 µg/mL for *L. monocytogenes* and 50 µg/mL for *Bacillus* sp. without light exposure. ERY at low concentrations (1 µM) photoactivated by green LED exposure (510 nm) was able to reduce the cell viability of *S. aureus* (4 log reduction) with a fluence of 40 J/cm^2 (Yassunaka et al., 2015). The activity of EOY, another xanthene dye widely used as PS for microbial photosensitization of foodborne bacteria was recently reported by Bonin et al. (2018). The most and less susceptible species were *S. aureus* and *E. coli*, respectively. *S. aureus* population was inactivated by ≈ 6 log CFU/mL with EOY at 5 µM and 5 min of irradiation while *E. coli* was inactivated only by 0.1-0.8 log CFU/mL. *P. aeruginosa*, *B. cereus*, and *S.* Typhimurium presented intermediated resistances with ≈ 6 log CFU/mL reduction at 10 µM EOY for 10 min, 4.3 log CFU/mL reduction at 7.5 µM EOY for 15 min, and 1.7 log CFU/mL reduction at 10 µM EOY for up to 15 min, respectively (Bonin et al., 2018). Approximately 6 log CFU/mL and 7 log CFU/mL reductions of vegetative cells of *S*. Typhimurium and *S*. *aureus* was achieved, respectively with RB at 75 µM and 25 nM, respectively, after only 5 min of illumination (Silva et al., 2019). The maximum concentration tested of ERY (500 nM) was needed to reduce the populations of *S. aureus* to undetectable levels after 5 min, while a reduction of only 2 log CFU/mL of *S.* Typhimurium have been achieved using ERY at 100 µM after 15 min (Silva et al., 2019). Recently, a study used response surface

methodology (RSM) to determine the optimum parameters for inactivation using EOY combined with green LED light (Santos et al., 2020). The authors determined by RSM that the highest inactivation rate (> 4 log CFU/mL) of *S. aureus* was achieved using a fluence of 9.98 J/cm^2 with EOY at 498 nM of concentration.

The use of natural compounds as PS against foodborne bacteria is an interesting strategy of aPDT since compounds like curcumin, chlorophyllin, riboflavin, and hypericin have been approved for use as food additives. aPDT with curcumin as PS and blue LED against Gram-positive and Gram-negative foodborne bacteria has been used by several authors (Huang et al., 2020; Penha et al., 2017). One of these studies evaluated the effects of curcumin at 75 µM in the presence of blue LED (470 nm) on a broad spectrum of foodborne bacteria (Penha et al., 2017). Curcumin-mediated aPDT treatment induced ≈ 6 log CFU/mL reductions of *S. aureus*, *A. hydrophila*, and *E. coli* at fluence of 417 J/cm^2. No reduction was observed for *P. aeruginosa* and only 2.82 log CFU/mL reduction was achieved for *S.* Typhimurium at the same fluence (Penha et al., 2017). Another recent study showed a maximum reduction of 5.94 and 5.91 log CFU/mL for *E. coli* and *S. aureus*, respectively, when treated with 20 µM of curcumin and fluence of 13 J/cm^2 (Bhavya & Hebbar, 2019a). The same authors also evaluated the combinational effect of ultrasound (US) and aPDT with curcumin (50-100 µM) and blue LED (70 J/cm^2) against *E. coli* and *S. aureus* in orange juice (Bhavya & Hebbar, 2019b). However, despite having found an additional effect on the inactivation of *E. coli* by US, the results of this work are still less effective than the previous one using only aPDT. The authors attribute the minimal effect of sonication to the variation in volume of orange juice (Bhavya & Hebbar, 2019b). Photodynamic inactivation of *L. monocytogenes* mediated by edible curcumin at low concentrations and fluence of 0.54 J/cm^2 was reported by Huang et al. (2020). Treatments with curcumin at 0.2 µM and at 1.0 µM reduced the population of vegetative cells by more than 4 log CFU/mL and 8 log CFU/mL, respectively.

The photoactivity of a chlorophyllin derivative when combined with sodium (Na) forms a water-soluble compound, which has been tested against food-borne bacteria

in vitro (Buchovec et al., 2017). In that study, the mechanism of inactivation of *S.* Typhimurium by Na-Chl (15 µM) with LED (405 nm) was evaluated in combination with chitosan (CHS) and high-power pulsed UV light (HPPL). The aPDT process reduced the population of *S.* Typhimurium by only 2.05 log CFU/mL at 46.1 J/cm^2. The combination of CHS with Na-Chl and LED (17.3 J/cm^2) increased the reductions to 7.28 log CFU/mL. Meanwhile, the combination of HPPL at 0.29 J/cm^2 with Na-Chl and LED (46.1 J/cm^2) was able to reduce more than 7 log CFU/mL of *S.* Typhimurium vegetative cells (Buchovec et al., 2017).

Another plant-based PS that has been frequently used in aPDT processes against foodborne bacteria is Hyp. Two studies from the same group evaluated the effectiveness of aPDT with Hyp at 0.1 and 10 µM and LED exposure (9.2 J/cm^2) against *L. monocytogenes*, *S.* Typhimurium, and *B. cereus* (Aponiene, Paskeviciute, Reklaitis, & Lukšiene, 2015; Kairyte, Lapinskas, Gudelis, & Lukšiene, 2012). The inactivation of *L. monocytogenes* after treatment with Hyp at 0.1 µM achieved 7 log CFU/mL, whereas *S.* Typhimurium was reduced by only 1 log CFU/mL with Hyp at 10 µM. However, the authors tested the combination of ADPT with pulsed light at 0.023 J/cm^2 and viability of both species were reduced by 7 log CFU/mL (Kairyte et al., 2012). In the same conditions mentioned above, the authors tested the inactivation of *B. cereus in vitro* and on the surface of fruits and vegetables (Aponiene et al., 2015). Hyp-based photosensitization reduced the population of *B. cereus* by 4.4 log CFU/mL *in vitro* and by 0.77-1.3 log CFU/mL on the surface according to the type of fruit and vegetable.

The micronutrient riboflavin or vitamin B2 is considered very sensitive to UV and visible lights between 420 and 560 nm (Ottaway, 1993). In aPDT, riboflavin is usually excited by visible light to avoid risks related to UV application. Two studies evaluated the viability of *E. coli* after treatment with riboflavin and exposition to blue light (Liang et al., 2013; Liang, Cheng, Yu, & Chen, 2015). DNA was found to be the main target of the photochemical reactions triggered by the presence of riboflavin and its derivatives.

Many studies are reporting some advance in the potentiation effect of non-toxic salt (e.g., potassium iodide; KI) in aPDT as previously cited in this review in section 4.2. It has been shown a significant potentiation effect for at least three different formulations of porphyrin combined with KI against methicillin-resistant *S. aureus* (MRSA) and *E. coli* (Huang et al., 2018; Moreira et al., 2020; Vieira et al., 2019). The authors reported a drastic reduction in the irradiation time (Moreira et al., 2020; Vieira et al., 2019) as well as the inactivation of *E. coli* using an anionic porphyrin which was unable to kill the bacteria in the absence of KI (Huang et al., 2018). aPDT with phenothiazinium dyes (especially MB) has been reported to be potentiated by the addition of inorganic or halide salts such as KI, NaN_3, and SCN^- (Huang et al., 2012; St Denis et al., 2013; Vecchio et al., 2015; Yuan et al., 2020). The most common potentiator is KI, although the addition of NaN_3 and SCN^- has shown interesting and even unexpected results. Huang et al. (2012) observed that the combination between NaN_3–MB and red-light exposure can enhance the inactivation of *S. aureus* and *E. coli* up to 3 log CFU/mL. Interestingly, NaN_3 increased the inactivation of *S. aureus* and *E. coli* even in the absence of oxygen, instead of protection from killing as expected for its capacity to quench singlet oxygen. One year later, St Denis et al. (2013) reported a potentiation effect of SCN^- using 10 µM of MB in the populations of *S. aureus* and *E. coli.* The authors observed that the SCN^- rapidly reacted with singlet oxygen producing both sulfite and cyanide anions, responsible for bacterial death. The addition of KI in MB-based aPDT to potentiate bacterial killing has been explored two years later by Vecchio et al. (2015). The authors observed a consistent increase of red light-mediated bacterial killing of *S. aureus* (4 log CFU/mL) and *E. coli* (2 log CFU/mL). The combination of xanthene dyes and KI photoactivated by green light has been recently evaluated against Gram-negative and Gram-positive bacteria (Santos et al., 2019; Wen et al., 2017). Both studies from different research groups obtained similar and promising results due to the presence of KI.

5.2. Spore forming bacteria and biofilms

The aPDT resistance of the spore forming bacterium *B. cereus* was evaluated in the presence of different porphyrin derivatives (Oliveira et al., 2009). The authors described a reduction of 3.5 log CFU/mL in the viability of the spores using a tricationic porphyrin with a meso-pentafluorophenyl group (Tri-Py$^+$-Me-PF) at 0.5 µM after 4 min of irradiation. The group selected the same PS to investigate the susceptibility of other spore forming *Bacillus* species such as *B. licheniformis*, *B. sphaericus*, and *B. subtilis* as well as *B. cereus* as a model (da Silva et al., 2012). The study demonstrated that more than 3 log CFU/mL reduction in viability was achieved for *B. cereus* and the other species were considered less susceptible to aPDT with Tri-Py$^+$-Me-PF at 10 µM after 4 min of irradiation. Despite the genetic similarity (Helgason et al., 2000), the authors suggest that the reduction in the viability of *B. cereus* should not be considered a surrogate for *B. anthracis* by aPDT. The authors concluded that there was no significant difference in the adhesion of the PS to the spores of *Bacillus* regardless of the structure of the exosporium (with or without glycoprotein layer). (da Silva et al., 2012). To assess the effect of aPDT with porphyrins on *L. monocytogenes*, an important foodborne causative agent of listeriosis, some authors have used *L. innocua* as a surrogate of this pathogen (Bonifácio et al., 2018). The porphyrin tested was the Tetra-Py$^+$-Me at 23.7 mg/L, achieving only 1.1 log CFU/mL reductions of *L. innocua* biofilm. The same research group had carried out studies on the inactivation of *L. innocua* biofilms using a porphyrinic-chitosan antifouling complex during 24 h of irradiation preventing the biofilm progress (Castro et al., 2017). The aPDT with Tetra-Py$^+$-Me at 20 µM and fluence of 64.8 J/cm^2 was able to reduce the viability of *P. aeruginosa* and *S. aureus* by 2.8 and 6.3 log CFU/mL, respectively (Beirão et al., 2014). The authors also reported a reduction of 81% in the polysaccharide content of the *P. aeruginosa* biofilm matrix demonstrating that it can be the primary target of the aPDT process for biofilms eradication.

Demidova and Hamblin (2005) studied the effects of aPDT with phenothiazinium dyes and red light on the survival of spores of different *Bacillus* species. Their findings revealed that *Bacillus* spores were susceptible to aPDT with phenothiazinium dyes at low fluences of red light. It has been shown that in the presence of TBO, DMMB, and

MB (50 µM), the fluence of 40 J/cm^2 was able to achieve 5, > 3, and > 2 log CFU/mL reductions of *B. cereus* spore viability, respectively, while NMB at the same concentration only required 20 J/cm^2 to achieve a reduction of more than 5 log CFU/mL. The team also discovered that the excess of PS, not bound to the spores, can prejudice light delivery and impair inactivation efficiency (Demidova & Hamblin, 2005). The inactivation of *S. aureus*, *P. aeruginosa*, *E. coli*, and *Acinetobacter* sp. biofilms was evaluated using a polyacrylamide (PAA) matrix loaded with MB photoactivated by laser light (Wu et al., 2009). The results showed that the complex PAA-MB damage the cell membrane, proteins, and DNA, killing all the species of bacteria in both vegetative and biofilm forms as determined by plate count and spectrophotometry, respectively (Wu et al., 2009). De Sordi et al. (2015) evaluated the effect of aPDT using 665 nm red laser light (0.24 J/cm^2) on *Clostridium difficile* vegetative cells, biofilm, and germinating spores. They observed that *C. difficile* vegetative cells and biofilm were inactivated at least by 3 log CFU/mL in the presence of MB and other PSs (e.g., chlorin e6) at 100 µM (for vegetative cells) and 1 mM (for biofilm). They also achieved a slight reduction in viability of *C. difficile* spores (~ 1 log CFU/mL), however, it was necessary to induce germination before aPDT (De Sordi et al., 2015). The use of KI in combination with MB was also tested against the formation of *E. faecalis* biofilms (Yuan et al., 2020). It was demonstrated that KI was able to potentiate the aPDT of *E. faecalis* biofilm even in a hypoxic condition and absence of light. Moreover, the presence of KI increases the photobleaching of MB after light exposure which is desirable to reduce tooth staining by MB (Yuan et al., 2020) and could be interesting for food applications.

The application of xanthene derivatives, RB and ERY as PS, in PDT study against *S*. Typhimurium and *S. aureus* biofilm formation using a green LED was performed. (Silva et al., 2019). A total of 8 log CFU/cm^2 reductions of *S. aureus* biofilms required RB at 250 µM and ERY at 500 µM with 30 min of irradiation (Silva et al., 2019). In contrast, the photoinactivation of *S.* Typhimurium biofilms (≥ 3 log CFU/cm^2) only was possible with RB at 50–1000 µM after the same 30 min of irradiation.

The use of natural compounds was also identified against the biofilm formation (Bonifácio et al., 2018; Huang et al., 2020). The reduction of the biofilm formation or structure disorder by curcumin-based aPDT is mainly promoted by DNA damage (Huang et al., 2020). Another study used the spores of *B. atrophaeus* as surrogate of foodborne spore forming bacteria such as *Bacillus* and *Clostridium* to evaluate riboflavin positively charged as PS (Eichner et al., 2015). The spores were incubated with the riboflavin derivatives and photoactivated with blue light at 70 J/cm^2. Depending on the concentration of the PS, the spores were significantly reduced (3.5-4.4 log CFU/mL) after only 10 s of irradiation and effectively killed when immobilized on PET surface (7.0 log CFU/mL) by complete disruption of the coat and the outer membrane (Eichner et al., 2015).

5.3. Yeasts and molds

The presence of fungi species is widespread but unlike bacteria and viruses, the association with outbreaks of foodborne illnesses is rarely attributed to fungal species (Fleet, 2007). Even though the application of aPDT against yeasts and molds is of great relevance for the agrifood and medical sector. In a recent paper it was demonstrated the inactivation of *Candida albicans* and *Candida tropicalis* with diode laser and the compound aluminum phthalocyanine chloride as PS in nanoemulsion (Rodrigues et al., 2019). The authors observed 5 log CFU/mL reduction in the viability of *C. albicans* and 4-5 log CFU/mL reduction in the viability of *C. tropicalis* when the nanoemulsion with PS was photoactivated. Another work also aimed to study the photodynamic effect of phthalocyanines (Pcs) on *C. albicans* (Ozturk et al., 2020). After illumination at 30 and 60 J/cm^2 with ZnPc, the cell viability was reduced by 5 and 2 log CFU/mL, respectively. Another recent study of aPDT using porphyrin derivatives and white light (380-700 nm) investigated its photodynamic effects against *C. albicans*, also in the presence of KI (Vieira et al., 2019). It was demonstrated that a relatively low concentration (0.5 µM) of the porphyrin derivatives in combination with KI and a fluence

of only 6.75 J/cm^2 were enough to achieve 6.7 log CFU/mL reduction of *C. albicans* (Vieira et al., 2019).

Many filamentous fungi have also been investigated for their susceptibility to aPDT. Recent findings on the effect of aPDT using phenothiazinium PSs and red light on the survival of the filamentous fungi *Fusarium keratoplasticum* and *Fusarium moniliforme* have been published (Paziani et al., 2019). aPDT with the PSs MB, NMB, and S137 can efficiently reduce the survival of both species. The susceptibility of the aflatoxin-producing fungi species *Aspergillus flavus* to aPDT was evaluated (Temba et al., 2019). In addition to the microbial reduction, the production of aflatoxin B_1 was lower in the treated maize kernels than in the untreated samples.

5.4. Protozoa and viruses

Even though aPDT for the inactivation of protozoa and viruses do not have the same emphasis as for the inactivation of bacteria and fungi species, its application has presented satisfactory outcomes. It is important to remember that the first experiment of aPDT was conducted precisely with a protozoan species (Raab, 1900). Given the importance of the *Leishmania* and Chagas diseases caused by the infection with protozoa species with apparent resistance to some of the currently used drugs, aPDT has emerged as an alternative treatment. Recently, a study has focused the application of aPDT for the inactivation of *Leishmania amazonensis* (Sepúlveda et al., 2020). The authors investigated the efficacy of titanium dioxide (TiO_2) nanoparticles associated with Zn and Hyp in combination with blue and red light (52.8 J/cm^2). In the tests with infected mice, aPDT was able to reduce the viability of *L. amazonensis* by 43% – 58% (Sepúlveda et al., 2020). Although Chagas disease is considered endemic in Latin American, the constant migration flow is worrying the specialists of public health. As the causative agent of Chagas disease is the protozoan *Trypanosoma cruzi*, the effect of aPDT with phenothiazine derivatives on the protozoan survival was recently investigated (Barbosa et al., 2020). The trypanocidal effect of aPDT with MB and TBO with diode laser illumination (660 nm, 4.2 J/cm^2) was evaluated in infected macrophages. The authors observed that MB at 2.6 µM and TBO at 1.2 µM caused a

reduction of 50% (IC_{50}) in the viability of *T. cruzi* without light exposition. However, the photoactivation of the compounds contributed significantly to the reduction of the IC_{50} values to 1.0 and 0.9 µM for MB and TBO, respectively (Barbosa et al., 2020). Recent studies have shown the parasitical activity of some phenothiazinium dyes, even in the dark (Pereira et al., 2020; Portapilla et al., 2019).

The application of aPDT for virus inactivation is not restricted to only one study, as reported by Ghate et al., (2019). Several studies have been addressed to the inactivation of different viruses, including the use of natural compounds as depicted in a recently published review of the anti-infective properties of curcumin (Praditya et al., 2019). Norovirus (NoV) is reported to be associated with the vast majority of cases of foodborne illnesses in the USA (CDC, 2017). Recent findings of type 1 murine norovirus (MNV-1) inactivation through aPDT using curcumin are available in the literature (Wu et al., 2015; Randazzo et al., 2016). The photoinactivation of MNV-1 was investigated *in vitro* in the presence of curcumin combined with blue light (470 nm) at 3.6 J/cm^2. Inactivation of MNV-1 increased in a dose-dependent manner and achieved more than 3 log PFU/mL reductions at 20 µM of curcumin (Wu et al., 2015). Similarly, other authors also investigated the aPDT efficacy of curcumin and blue light (464-474 nm) against MNV-1 and feline calicivirus (FCV-F9) (Randazzo et al., 2016). The FCV-F9 with almost 5 log $TCID_{50}$/mL of reduction after 30 min was much more susceptible than MNV-1 with only 0.73 log $TCID_{50}$/mL of inactivation after 120 min of treatment (Randazzo et al., 2016). The variability in susceptibility to aPDT among different viruses (bacteriophages MS2 and Qβ, bovine enterovirus type-2 (BEV-2) and MNV-1) was assessed in a recent study using the compound 5, 10,15, 20-tetrakis (1-methyl-4-pyridinio) porphyrin-tetra-*p*-toluene sulfonate (TMPyP) as PS combined with white light (641-661 nm; 230.4 J/cm^2) (Majiya et al., 2018; Majiya et al., 2019). The authors observed approximately 8 log PFU/mL reductions of MS2 and Qβ within only 1 and 8 min of exposure, respectively, followed by 120 min of irradiation to achieve approximately 4 log PFU/mL reductions of MNV-1 and BEV-2. Another recent study of aPDT using porphyrin derivatives and white light (380-700 nm) investigated its effects against a virus (T4-like bacteriophage) including in the presence of KI (Vieira et al., 2019). It was demonstrated that low concentrations (0.1 µM) of the porphyrin

derivatives in combination with KI and a fluence of only 3 J/cm^2 were needed to achieve approximately 8 log PFU/mL reductions of a T4-like phage (Vieira et al., 2019).

Table 3. Overview of *in vitro* studies on aPDT against critical pathogenic and spoilage microorganisms.

Photosensitizer	Microorganisms	Potentiator	Source of light (Wavelength and Irradiance)	Fluence	Log reduction	References
Porphyrin derivatives:						
Porphyrin Tetra-Py^+-Me (5 µM)	*S. aureus* (MRSA)	No	Artificial white light: 380-700 nm 4 mW/cm^2	14.4 J/cm^2	> 5.0 log	Bartolomeu et al. (2016)
Porphyrin derivatives (5 and 20 µM)	*E. coli*	No	Artificial white light: 0.25 mW/cm^2	1.35 J/cm^2	5 µM: 2.38 log; 20 µM: 3.85 log	Moreira et al. (2020)
Porphyrin P3 (0.2 µM) + CHS	*L. innocua*	No	White LED: 400-800 nm 10 mW/cm^2	864 J/cm^2	8.0 log	Castro et al. (2017)
Porphyrin P2 (3 µM) + CHS	*E. coli* (bioluminescent)	No	Artificial white light: 380-700 nm 3 mW/cm^2	16.2 J/cm^2	4.0 log	Castro et al. (2019)
Porphyrin mixture (5 µM)	*S. aureus* (MRSA) *E. coli* (bioluminescent)	Yes, KI (100 mM)	Artificial white light: 380-700 nm 2.5 mW/cm^2	*S. aureus:* 0.75 J/cm^2 *E. coli:* 11.25 J/cm^2	b.d.l	Vieira et a. (2019)
Phenothiazinium dyes:						
MB; TBO; NMB; DMMB (50 µM)	*B. cereus* spores	No	Red light: TBO and DMMN – 635 nm MB and NMB – 660 nm 200 mW/cm^2	TBO, MB and DMMB: 40 J/cm^2; NMB: 20 J/cm^2	TBO: 5 log; MB: > 2 log; DMMB: > 3 log; NMB: > 5 log	Demidova & Hamblin (2005)
MB (100 µM)	*C. difficile* (vegetative cells, biofilm, and spores)	No	Laser light: 665 nm	0.24 J/cm^2	3.0 log	De Sordi et al. (2015)

CHS: Chitosan; b.d.l.: below detection limit

Table 3. Continued

Photosensitizer (concentration)	Microorganisms	Potentiator	Source of light (Wavelength and Irradiance)	Fluence	Log reduction	References
MB (100-200 µM)	*E. coli* *S. aureus*	Yes, NaN_3 (10-100 µM)	Red light: 660 nm 100 mW/cm^2	8 J/cm^2	1-3 log	Huang et al. (2012)
MB (0.4 µM)	*E. faecalis*	Yes, KI (100 mM)	Red light: 660 nm 50 mW/cm^2	6.0 J/cm^2	8.0 log	Yuan et al. (2020)
Xanthene dyes:						
ERY (500 nM) RB (75 µM – 25 nM)	*S. Typhimurium* *S. aureus*	No	Green LED: 510 nm 10 mW/cm2	3.0 J/cm^2	b.d.l	Silva et al. (2019)
EOY (498 nM)	*S. aureus*	No	Green LED: 530 nm 10 mW/cm2	9.98 J/cm^2	> 4.0 log	Santos et al. (2020)
RB (10 µM)	*S. aureus* *E. coli* *P. aeruginosa* *C. albicans*	Yes, KI (100 mM)	Green Light: 540 nm 100 mW/cm2	10-20.0 J/cm^2	> 6.0 log	Wen et al. (2017)
Natural compounds:						
Curcumin (0.2 and 1 µM)	*L. monocytogenes*	No	Blue LED: 455-460 nm 1.8 mW/cm2	0.54 J/cm^2	0.2 µM: > 4.0 log 1 µM: b.d.l	Huang et al. (2020)
Curcumin (75 µM)	*S. aureus* *A. hydrophila* *S. Typhimurium* *E. coli* *P. aeruginosa*	No	Blue LED: 470 nm 1.2 W (1.77 cm2)	417.0 J/cm^2	*S. aureus, A. hydrophila* and *E. coli*: b.d.l; *S.* Typhimurium: 2.82 log *P. aeruginosa*: No reductions	Penha et al. (2017)

b.d.l.: below detection limit

Table 3. Continued

Photosensitizer (concentration)	Microorganisms	Potentiator	Source of light (Wavelength and Irradiance)	Fluence	Log reduction	References
Chl (15 µM) + CHS	*S.* Typhimurium	No	LED: 405 nm 9.6 mW/cm2 HPPL UV light 260 nm	LED + Chl and HPPL: 46.1 J/cm^2 and 0.29 J/cm^2; LED + Chl + CHS: 17.3 J/cm^2	LED + Chl: 2.05 log; LED + Chl + CHS: 7.28 log; LED + Chl + HPPL: 7.5 log	Buchovec et al. (2017)
Hypericin (0.1 µM)	*B. cereus*	No	LED: 585 nm 3.84 mW/cm2	9.2 J/cm^2	4.4 log	Aponiene et al. (2015)
Riboflavin (4 mM)	*B. atrophaeus spores*	No	Blue light: 320-500 nm 7 W/cm2	70.0 J/cm^2	3.5-7.0 log	Eichner et al. (2015)

CHS: Chitosan

6. *Agrifood applications*

The development of environmentally friendly technologies is one of the main objectives of the modern food industry, thus aPDT has been evaluated for a wide application in several sectors of the food industry. According to a recent critical review, most of the studies are focused on fruits, vegetables, and poultry (Ghate et al., 2019). However, the same study notifies the concentrations of studies regarding food-related surface. In the present review, the studies are mainly related to fruits and vegetables, meat products, milk, and phytopathogen inactivation. Interestingly, recent studies from the past two years mostly concentrated on two kinds of plant-based photosensitizers: curcumin (Corrêa et al., 2020; de Oliveira et al., 2018; de Oliveira et al., 2018; Tao et al., 2019; Temba et al., 2019; Tosati et al., 2018) and chlorophyllin (Josewin et al., 2018; Paskeviciute et al., 2018; Paskeviciute et al., 2019; Žudyte & Lukšiene, 2019). The main characteristics and results of these studies are depicted in Table 4.

6.1. Fruits and vegetables

It was demonstrated the bactericidal effect of aPDT with UV-A (320-400 nm) and curcumin on *E. coli* and *L. innocua* cells inoculated on the surface of fresh produce (de Oliveira et al. 2018a, b). Acidified curcumin at 10 mg/L was pulverized by conventional spray-atomization or aerosolization on the inoculated surface of spinach, lettuce and tomatoes before UV-A irradiation (de Oliveira, et al., 2018a). A reduction of 3 log CFU/cm^2 by either conventional spray-atomization or aerosolization in both bacterial populations tested was observed. The aPDT was also applied to prevent cross-contamination of fresh produce from contaminated water of spinach and tomato after washing (de Oliveira, et al., 2018b). A low concentration of curcumin (5 mg/L) was able to inactivate more than 5 log CFU/mL after 10 and 5 min of UV-A light exposure for *E. coli* and *L. innocua*, respectively (de Oliveira, et al., 2018b). The alternative curcumin-based treatment has used 2 µM of PS to inactivate *E. coli* on the surface of apple slices (Tao et al., 2019). Using a fluence of 152 J/cm^2, they observed inactivation of 0.96 log CFU/g in the viability of *E. coli* on the surface of fresh-cut apple (Tao et al., 2019). At

relatively low fluence of 10 J/cm^2 with curcumin at 80 µM, an aPDT process on the surface of apple achieved 2.0 log CFU/mL reductions of *S. aureus* (Corrêa et al., 2020). These findings prove that the structure of Gram-positive bacteria is more susceptible to aPDT.

The aPDT using Chl as PS has been investigated in real food matrices like cantaloupe, cherry tomatoes, basil, and wheat sprouts. Josewin et al. (2018) used cantaloupe rind in their study to evaluate the effectiveness of aPDT with Na-Chl for the inactivation of *L. monocytogenes* and *Salmonella*. Na-Chl at 100 µM in combination with exposure to 405 or 460 nm LED was used to evaluate the effects of photosensitization at 4 and 20 °C, respectively. At both storage temperatures, a fluence of 1,210 J/cm^2 (405 nm; LED) and 5,356 J/cm^2 (460 nm; LED) reached a reduction in cell viability of 3 log CFU/cm^2 of *L. monocytogenes* in both cases whereas the *Salmonella* cell viability was reduced by 1.1 and 3 log CFU/cm^2 for each wavelength, respectively (Josewin et al., 2018). Two aPDT studies were conducted at similar conditions of photosensitization with Chl at 150 µM and fluence of 3 J/cm^2, with cherry tomatoes (Paskeviciute et al., 2018) and basil (Paskeviciute et al., 2019). The viability of *B. cereus* and *L. monocytogenes*, artificially inoculated on the surface of cherry tomatoes, was reduced by 1.5 and 1.6 log CFU/mL, respectively (Paskeviciute et al., 2018). The aPDT in basil was able to reduce the viability of *L. monocytogenes* by 1.6 log CFU/mL (Paskeviciute et al., 2019). A recent study aimed at the decontamination of wheat sprouts by aPDT with Chl at high concentrations of 500 µM combined with LED exposure of 18 J/cm^2 (Žudyte & Lukšiene, 2019). Under such conditions of photosensitization against surface-attached *E. coli* and *Fusarium oxysporum* (plant pathogen) in wheat sprouts, aPDT achieved reduction of 1.5 log CFU/g for both pathogens.

6.2. Meat products

A researcher group attempted to prevent the growth of *S. aureus* and *E. coli* on the surface of cut pieces of beef, chicken, and pork meat samples through aPDT (Corrêa et al., 2020). The aPDT with curcumin at 40 µM and 15 J/cm^2 of UV-C irradiation

reduced the cell viability of *S. aureus* by 1.5, 1.4, and 0.6 log CFU/mL in beef, chicken, and pork, respectively. The reductions of *E. coli* viability in beef, chicken, and pork was 1.0, 1.6, and 1.6 log CFU/mL at 3.9, 3.1, and 7.8 J/cm^2, respectively (Corrêa et al., 2020). The authors emphasized the necessity for additional tests to assess the nutritional and organoleptic effects of the aPDT. Another curcumin-based aPDT was developed to avoid microbial growth on the surface of commercial sausages artificially contaminated with *L. innocua* (Tosati et al., 2018). UV-A photoactivated edible coatings with curcumin (5 mg/L) inactivated more than 5 log CFU/mL of *L. innocua* at a low dose of 0.96 J/cm^2 (Tosati et al., 2018).

6.3. Milk

An interesting study aimed to photoinactivate *Staphylococcus* spp. and *E. coli* vegetative cells was conducted in milk samples with 3.8 and 0.3% fat content (Galstyan & Dobrindt, 2019). The suspensions of Gram-positive *S. aureus, S. hominis*, and *S. warnei*, and Gram-negative *E. coli* isolates were artificially inoculated in diluted milk samples (20%, 60%, and 90%). Firstly, the authors reported that the inactivation of *Staphylococcus* spp. cells by aPDT with MB and silicon phthalocyanine derivative (SiPc) at a higher fluence of 36 J/cm^2 achieved between 2-8 log CFU/mL reduction for both PSs. To better understand the difference among the PSs, a lower fluence of 9 J/cm^2 was tested. The aPDT with SiPc at 10 µM reduced the viability of *Staphylococcus* spp. by over 5 log CFU/mL in milk samples at the dilution rate of 20% and 60%, while MB at the same concentration reduced the viability by only 1 log CFU/mL when the milk content was 20% (Galstyan & Dobrindt, 2019). For the inactivation of *E. coli*, the authors used the higher fluence of 36 J/cm^2 as Gram-negative bacteria are less susceptible than Gram-positive bacteria to aPDT. In the milk samples with 0.3% and 3.8% fat content, the application of SiPc at 50 µM resulted in a reduction of 5-7 log CFU/mL of the *E. coli* loads, while for MB they observed a lower reduction (2-4 log CFU/mL) in the milk samples with 0.3% fat content (Galstyan & Dobrindt, 2019). The authors did not evaluate the organoleptic and sensorial effects of the application of aPDT on milk samples.

6.4. Plant diseases

The aPDT has also been employed to control plant-pathogenic fungi and bacteria species for agricultural purposes avoiding the use of conventional antifungals and bactericides, respectively (de Menezes et al., 2014; de Menezes et al., 2016; Fracarolli et al., 2016; Gonzales et al., 2017; Jesus et al., 2018; Tonani et al., 2018). The excessive use of chemicals is one of the major concerns for the environment. In this way, some research dedicated to studying the effect of aPDT with phenothiazinium dyes and natural plant-produced PSs in combination with artificial and solar radiation has been explored against fungi species (*Colletotrichum* spp., *Fusarium* spp., and *Neoscytalidium dimidiatum*) (de Menezes et al., 2014; Fracarolli et al., 2016; Gonzales et al., 2017; Tonani et al., 2018). All of these studies showed positive results both *in vitro* and *ex vivo* experiments against phytopathogens including some species able to infect humans (*Neoscytalidium* spp.) (Machouart et al., 2013; Tonani et al., 2018). The authors also demonstrated that aPDT did not damage the plant host. An interesting study by Jesus et al. (2018) investigated the effect of aPDT on the control of *Pseudomonas syringae* pv. *actinidiae* (Psa), a phytopathogenic bacterium. The combination of porphyrin Tetra-Py^{+}-Me, artificial light and solar radiation were investigated using artificially contaminated kiwi leaves. Overall, it was demonstrated *in vitro* and *ex vivo* an effective inactivation of Psa without any damage to the kiwi leaves by the authors. However, all cited authors agree that further studies are needed under field conditions to evaluate the environmental impact of this new technique as well as to optimize the parameters of aPDT.

907 **Table 4. Some examples of *ex vivo* APDT studies on the inactivation of microorganisms for agrifood purposes.**

Agrifood applications	Microorganisms	Photosensitizer (concentration)	Source of light (Wavelength and Irradiance)	Fluence	Log reduction	References
Fruit and vegetables:						
Apple	*S. aureus*	Curcumin (80 µM)	LED: 450 nm 55 mW/cm^2	10 J/cm^2	2.0 log	Corrêa et al. (2020)
Spinach and cherry tomatoes	*E. coli* *L. innocua*	Curcumin (5 mg/L)	UV-A lamps: 320-400 nm 3.2 mW/cm^2	*E. coli*: 10 min (1.92 J/cm^2); *L. innocua*: 5 min (0.96 J/cm^2)	> 5.0 log	de Oliveira et al. (2018a)
Cantaloupe rind	*Salmonella* sp. *L. monocytogenes*	Chl (100 µM)	Blue LED 405 nm: 7 mW/cm^2 460 nm: 31 mW/cm^2	405 nm: 1210 J/cm^2 460 nm: 5356 J/cm^2	*L. monocytogenes*: 3 log (405 and 460 nm) *Salmonella:* 3 log – 405 nm and 1.1 log – 460 nm)	Josewin et al. (2018)
Maize kernels	*A. flavus*	Curcumin	Exon Arc lamp: 420 nm	60.0 J/cm^2	N.A. (Reduction of aflatoxin B1)	Temba et al. (2019)
Fresh-cut apples	*E. coli*	Curcumin (2 µM)	LED: 420 nm 298 mW/cm^2	152.0 J/cm^2	0.95 log	Tao et al. (2019)
Spinach, lettuce, and tomato	*E. coli* *L. innocua*	Acidified curcumin (10 mg/L)	UV-A lamps: 320-400 nm 0.68 mW/cm^2	2.0 J/cm^2	3.0 log	de Oliveira et al. (2018b)
Cherry tomatoes and basil	Mesophilic bacteria *B. cereus* *L. monocytogenes*	Chl (150 µM)	LED: 405 nm 10 mW/cm^2	3.0 J/cm^2	Mesophilic: 2.4 log (cherry tomatoes) and 1.3 log (basil); *B. cereus:* 1.5 log (cherry tomatoes); *L. monocytogenes*: 1.6 log (both)	Paskeviciute et al. (2018), (2019)

908 N.A.: Not available

Table 4. Continued

Agrifood applications	Microorganisms	Photosensitizer (concentration)	Source of light (Wavelength and Irradiance)	Fluence	Log reduction	References
Sprouted seeds	Mesophilic bacteria *E. coli* *F. oxysporum*	Chl (500 µM)	LED: 405 nm	18.0 J/cm^2	Mesophilic: 2.5 log; *E. coli*: 1.5 log ; *F. oxysporum*: 1.5 log	Žudyte & Lukšiene (2019)
Meat products:						
Sausage	*L. innocua*	Curcumin edible coating	UV-A lamps: 320-400 nm 3.2 mW/cm^2	0.96 J/cm^2	> 5.0 log	Tosati et al. (2018)
Beef, chicken, and pork.	*S. aureus*	Curcumin (40 µM)	LED: 450 nm 55 mW/cm^2	15 J/cm^2	Beef: 1.5 log; Chicken: 1.4 log; Pork: 0.6 log	Corrêa et al. (2020)
Milk	*S. aureus* *E. coli*	MB and SiPc (50 µM)	Red light 610 nm 10 mW/cm^2	9.0 – 36 J/cm^2	*S. aureus:* MB: 1.0 log (Milk 20%); SiPc: > 5.0 log (Milk 20% and 60%) *E. coli:* MB: 2.0-4.0 logs (Milk 20% / Fat content 0.3%); SiPc: 5.0-7.0 log (Milk 20% / Fat content 0.3% and 3.8%)	Galstyan & Dobrindt, (2019)
Plant diseases:						
Petals and leaves of *Citrus sinensis* Phytopathogen	*C. abscissum*	MB (25-50 µM)	Solar radiation 290-390 nm: 20.1-45.8 W/m^2); Visible light 400-790 nm: 299.7-579.2 W/m^2)	30 min	Petals: 1.9-3.1 log Leaves: > 3.0 log	Gonzales et al. (2017)

Table 4. Continued

Agrifood applications	Microorganisms	Photosensitizer (concentration)	Source of light (Wavelength and Irradiance)	Fluence	Log reduction	References
Phytopathogen	*Neoscytalidium dimidiatum* *N. dimidiatum* var. *hyalinum*	NMB (10-200 µM) S137 (10-25 µM)	Red LED 631 nm 13.89 mW/cm^2	3.0 J/cm^2	NMB: 3-5 log S137: 2.0-3.5 log	Tonani et al. (2018)
Phytopathogen	*C. acutatum* *C. gleosporioides A. nidulans*	NMB (50 µM) S137 (10 µM)	Red LED 634 nm 9.20 mW/cm^2	15.0 J/cm^2	5.0 logs	de Menezes et al. (2014)
Kiwi leaves Phytopathogen	*P. syringae* pv. *actinidiae* (Psa)	Tetra-Py$^+$-Me (50 µM)	Artificial light: 400-800 nm 150 mW/cm^2 Solar radiation: 65 mW/cm^2	90 min	Artificial light: 1.8-4.0 log Solar radiation: 1.5 log	Jesus et al. (2018)

7. Conclusions and future perspectives

The current status of aPDT as an innovative non-thermal technique designed for microbial inactivation has gained increasing popularity in the last years among food scientists. Researchers already know that the photodynamic procedure can inactivate efficiently the most important microorganisms for food and agronomic interests, as depicted in this review. Given the importance of food safety for the industry, aPDT can in some cases still contribute to maintaining the organoleptic/nutritional characteristics of foods. Thus, it is still necessary efforts to evaluated the effects of aPDT on the nutritional and organoleptic features of food. The demand for healthy, fresh, and high-quality food products has increased among consumers. Additionally, food products without preservatives also possess an influence on the consumers in the supermarket. The aPDT with natural PS, such as chlorophyllin and curcumin, has been widely explored and can be an alternative for application in food.

The exploration of endogenous PSs, intrinsically produced by microorganisms, can be particularly interesting in extending the shelf-life during the long-term storage of foods in cold rooms, domestic refrigerators, and even in supermarket shelves. Most of the studies developed for food applications so far are prototypes or methodologies only on the laboratory scale. Despite the excellent results obtained even by the inactivation of resistant forms (biofilms and spores) of foodborne bacteria and fungi species, it is important to consider a scale-up of the technique to make it a reality in the food industry as soon as possible. Some points should be considered to spark the interest of food processors, such as the environmental, food-related, and engineering factors (Ghate et al., 2019). Some of them can be mentioned as the dark period of incubation with PS, the treatment temperature, surface roughness, and the right combination of PS and light source. Photodynamic treatment is a promising technology to control harmful microorganisms and could be used by industries and farms soon.

Acknowledgements

This work was supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Grants #140092/2017-0, #302763/2014-7, #305804/2017-0, 445998, and 307738/2018-3); Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Grant #2016/11386-5) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 001 for the financial support.

Conflict of interest

The authors declare no competing interests.

8. References

ACS. (2019). American Cancer Society. Getting Photodynamic Theraphy. Retrieved April 20, 2020, from https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/radiation/photodynamic-therapy.html

Almeida, J., Tomé, J. P. C., Neves, M. G. P. M. S., Tomé, A. C., Cavaleiro, J. A. S., Cunha, A., Costa, L., Faustino, M.A.F, & Almeida, A. (2014). Photodynamic inactivation of multidrug-resistant bacteria in hospital wastewaters: influence of residual antibiotics. *Photochemical & Photobiological Sciences*, 13(4), 626. https://doi.org/10.1039/c3pp50195g

Alvarenga, V. O., Brancini, G. T. P., Silva, E. K., da Pia, A. K. R., Campagnollo, F. B., Braga, G. Ú. L., Hubinger, M. D., & Sant'Ana, A. S. (2018). Survival variability of 12 strains of *Bacillus cereus* yielded to spray drying of whole milk. *International Journal of Food Microbiology*, 286, 80–89. https://doi.org/10.1016/j.ijfoodmicro.2018.07.020

Alves, E., Costa, L., Carvalho, C. M., Tomé, J. P., Faustino, M. A., Neves, M. G., Tomé, A. C., Cavaleiro, J. A. S., Cunha, Â., & Almeida, A. (2009). Charge effect on the photoinactivation of Gram-negative and Gram-positive bacteria by cationic meso-substituted porphyrins. *BMC Microbiology*, 9(1), 70. https://doi.org/10.1186/1471-2180-9-70

Alves, E., Melo, T., Simões, C., Faustino, M. A. F., Tomé, J. P. C., Neves, M. G. P. M. S., Cavaleiro, J. A. S., Cunha, Â., Gomes, N. C. M., Domingues, P., Domingues, M. R.

M., & Almeida, A. (2013). Photodynamic oxidation of *Staphylococcus warneri* membrane phospholipids: new insights based on lipidomics. *Rapid Communications in Mass Spectrometry*, 27(14), 1607–1618. https://doi.org/10.1002/rcm.6614

Aponiene, K., Paskeviciute, E., Reklaitis, I., & Lukšiene, Ž. (2015). Reduction of microbial contamination of fruits and vegetables by hypericin-based photosensitization: Comparison with other emerging antimicrobial treatments. *Journal of Food Engineering*, 144, 29–35. https://doi.org/10.1016/j.jfoodeng.2014.07.012

Bacellar, I.O.L., Pavani, C., Sales, E.M., Itri, R., Wainwright, M., & Baptista, M.S. (2014). Membrane damage efficiency of phenothiazinium photosensitizers. *Photochemistry and Photobiology*, 90(4), n/a-n/a. https://doi.org/10.1111/php.12264

Baier, J., Maisch, T., Maier, M., Engel, E., Landthaler, M., & Bäumler, W. (2006). Singlet oxygen generation by UVA light exposure of endogenous photosensitizers. *Biophysical Journal*, 91(4), 1452–1459. https://doi.org/10.1529/biophysj.106.082388

Balasubramaniam, E., & Natarajan, P. (1997). Photophysical properties of protoporphyrin IX and thionine covalently attached to macromolecules. *Journal of Photochemistry and Photobiology A*: Chemistry, 103(3), 201–211. https://doi.org/10.1016/S1010-6030(96)04598-4

Barba, F. J., Koubaa, M., Prado-Silva, L., Orlien, V., & Sant'Ana, A. S. (2017). Mild processing applied to the inactivation of the main foodborne bacterial pathogens: A review. *Trends in Food Science & Technology*, 66, 20–35. https://doi.org/10.1016/j.tifs.2017.05.011

Barbosa, A. F. S., Santos, I. P., Santos, G. M. P., Bastos, T. M., Rocha, V. P. C., Meira, C. S., Soares, M. B. P., Pitta, I. R., & Pinheiro, A. L. B. (2020). Anti–*Trypanosoma cruzi* effect of the photodynamic antiparasitic chemotherapy using phenothiazine derivatives as photosensitizers. *Lasers in Medical Science*, 35(1), 79–85. https://doi.org/10.1007/s10103-019-02795-4

Bartolomeu, M., Rocha, S., Cunha, Â., Neves, M. G. P. M. S., Faustino, M. A. F., & Almeida, A. (2016). Effect of photodynamic therapy on the virulence factors of *Staphylococcus aureus*. *Frontiers in Microbiology*, 7, 1–11.

https://doi.org/10.3389/fmicb.2016.00267

Batistela, V. R., Pellosi, D. S., de Souza, F. D., da Costa, W. F., de Oliveira Santin, S. M., de Souza, V. R., Caetano, W., de Oliveira, H. P. M., Scarminio, I. S., & Hioka, N. (2011). pKa determinations of xanthene derivates in aqueous solutions by multivariate analysis applied to UV–Vis spectrophotometric data. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 79(5), 889–897. https://doi.org/10.1016/j.saa.2011.03.027

Beirão, S., Fernandes, S., Coelho, J., Faustino, M. A. F., Tomé, J. P. C., Neves, M. G. P. M. S., Tomé, A. C., Almeida, A., & Cunha, Â. (2014). Photodynamic inactivation of bacterial and yeast biofilms with a cationic porphyrin. *Photochemistry and Photobiology*, 90(6), 1387–1396. https://doi.org/10.1111/php.12331

Bhavya, M. L., & Hebbar, H. U. (2019a). Efficacy of blue LED in microbial inactivation: Effect of photosensitization and process parameters. *International Journal of Food Microbiology*, 290, 296–304. https://doi.org/10.1016/j.ijfoodmicro.2018.10.021

Bhavya, M. L., & Hebbar, H. U. (2019b). Sono-photodynamic inactivation of *Escherichia coli* and *Staphylococcus aureus* in orange juice. *Ultrasonics Sonochemistry*, 57, 108–115. https://doi.org/10.1016/j.ultsonch.2019.05.002

Bonifácio, D., Martins, C., David, B., Lemos, C., Neves, M. G. P. M. S., Almeida, A., Pinto, D. C. G. A., Faustino, M. A. F., & Cunha, Â. (2018). Photodynamic inactivation of *Listeria innocua* biofilms with food-grade photosensitizers: a curcumin-rich extract of *Curcuma longa* vs commercial curcumin. *Journal of Applied Microbiology*, 125(1), 282–294. https://doi.org/10.1111/jam.13767

Bonin, E., Ribeiro, L. H., Favero, M. E., Freitas, C. F. De, Caetano, W., & Hioka, N. (2018). Photodynamic inactivation of foodborne bacteria by eosin Y. *Journal of Applied Microbiology*, 124, 1617–1628. https://doi.org/10.1111/jam.13727

Bosch, A., Gkogka, E., Le Guyader, F. S., Loisy-Hamon, F., Lee, A., van Lieshout, L., Marthi, B., Myrmel, M., Sansom, A., Schultz, A.C., Winkler, A., Zuber, S., & Phister, T. (2018). Foodborne viruses: Detection, risk assessment, and control options in food processing. *International Journal of Food Microbiology*, 285, 110–128.

https://doi.org/10.1016/j.ijfoodmicro.2018.06.001

Brancaleon, L., & Moseley, H. (2002). Laser and non-laser light sources for photodynamic therapy. *Lasers in Medical Science*, 17(3), 173–186. https://doi.org/10.1007/s101030200027

Broekgaarden, M., Weijer, R., van Gulik, T. M., Hamblin, M. R., & Heger, M. (2015). Tumor cell survival pathways activated by photodynamic therapy: a molecular basis for pharmacological inhibition strategies. *Cancer and Metastasis Reviews*, 34(4), 643–690. https://doi.org/10.1007/s10555-015-9588-7

Brovko, L. Y., Meyer, A., Tiwana, A. S., Chen, W., Liu, H., Filipe, C. D. M., & Griffiths, M. W. (2009). Photodynamic treatment: a novel method for sanitation of food handling and food processing surfaces. *Journal of Food Protection*, 72(5), 1020–1024. https://doi.org/10.4315/0362-028x-72.5.1020

Buchovec, I., Lukseviciūtė, V., Kokstaite, R., Labeikyte, D., Kaziukonyte, L., & Luksiene, Z. (2017). Inactivation of Gram (−) bacteria *Salmonella enterica* by chlorophyllin-based photosensitization: Mechanism of action and new strategies to enhance the inactivation efficiency. *Journal of Photochemistry and Photobiology B*: Biology, 172, 1–10. https://doi.org/10.1016/j.jphotobiol.2017.05.008

Buchovec, I., Paskeviciute, E., & Luksiene, Z. (2010). Photosensitization-based inactivation of food pathogen *Listeria monocytogenes* in vitro and on the surface of packaging material. *Journal of Photochemistry and Photobiology B: Biology*, 99(1), 9–14. https://doi.org/10.1016/j.jphotobiol.2010.01.007

Calin, M. A., & Parasca, S. V. (2009). Light sources for photodynamic inactivation of bacteria. *Lasers in Medical Science*, 24(3), 453–460. https://doi.org/10.1007/s10103-008-0588-5

Cardoso, D. R., Libardi, S. H., & Skibsted, L. H. (2012). Riboflavin as a photosensitizer. Effects on human health and food quality. *Food & Function*, 3(5), 487. https://doi.org/10.1039/c2fo10246c

Castano, A. P., Demidova, T. N., & Hamblin, M. R. (2004). Mechanisms in photodynamic therapy: Part one - Photosensitizers, photochemistry and cellular

localization. *Photodiagnosis and Photodynamic Therapy*, 1(4), 279–293. https://doi.org/10.1016/S1572-1000(05)00007-4

Castro, K. A. D. F., Moura, N. M. M., Fernandes, A., Faustino, M. A. F., Simões, M. M. Q., Cavaleiro, J. A. S., Nakagaki, S., Almeida, A., Cunha, Â., Silvestre, A. J. D., Freire, C. S. R., Pinto, R. J. B., & Neves, M. G. P. M. S. (2017). Control of *Listeria innocua* biofilms by biocompatible photodynamic antifouling chitosan based materials. *Dyes and Pigments*, 137, 265–276. https://doi.org/10.1016/j.dyepig.2016.10.020

CDC. (2017). Center for Disease Control and Prevention. Norovirus: Facts for Food Workers. Retrieved April 29, 2020, from https://www.cdc.gov/norovirus/downloads/foodhandlers.pdf

CDC. (2020a). CDC and Food Safety. Retrieved April 17, 2020, from https://www.cdc.gov/foodsafety/cdc-and-food-safety.html

CDC. (2020b). Four steps for food safety. Retrieved April 19, 2020, from https://www.cdc.gov/foodsafety/keep-food-safe.html

Cebrián, G., Mañas, P., & Condón, S. (2016). Comparative resistance of bacterial foodborne pathogens to non-thermal technologies for food preservation. *Frontiers in Microbiology*, 7, 1–17. https://doi.org/10.3389/fmicb.2016.00734

Chacon, J. N., McLearie, J., & Sinclair, R. S. (1988). Singlet oxygen yields and radical contributions in the dye-sensitised photo-oxidation in methanol of esters of polyunsaturated fatty acids (oleic, linoleic, linolenic and arachidonic). *Photochemistry and Photobiology*, 47(5), 647–656. https://doi.org/10.1111/j.1751-1097.1988.tb02760.x

Chignell, C. F., Bilskj, P., Reszka, K. J., Motten, A. G., Sik, R. H., & Dahl, T. A. (1994). Spectral and photochemical properties of curcumin. *Photochemistry and Photobiology*, 59(3), 295–302. https://doi.org/10.1111/j.1751-1097.1994.tb05037.x

CIAtox. Centro de Informação e Assistência Toxicológica – CIATox de Campinas – Faculdade de Ciências Médicas da UNICAMP (2018). Relatório de Atendimentos. Retrieved April 18, 2020, from https://www.fcm.unicamp.br/fcm/sites/default/files/2020/page/relatorio_de_atendimen

tos_ciatox_2018_09jul2020.pdf

Cieplik, F., Tabenski, L., Buchalla, W., & Maisch, T. (2014). Antimicrobial photodynamic therapy for inactivation of biofilms formed by oral key pathogens. *Frontiers in Microbiology*, 5, 1–17. https://doi.org/10.3389/fmicb.2014.00405

Cieplik, F., Deng, D., Crielaard, W., Buchalla, W., Hellwig, E., Al-Ahmad, A., & Maisch, T. (2018a). Antimicrobial photodynamic therapy – what we know and what we don't. *Critical Reviews in Microbiology*, 44(5), 571–589. https://doi.org/10.1080/1040841X.2018.1467876

Cieplik, F., Steinwachs, V.-S., Muehler, D., Hiller, K.-A., Thurnheer, T., Belibasakis, G. N., Buchalla, W., & Maisch, T. (2018b). Phenalen-1-one-mediated antimicrobial photodynamic therapy: antimicrobial efficacy in a periodontal biofilm model and flow cytometric evaluation of cytoplasmic membrane damage. *Frontiers in Microbiology*, 9. https://doi.org/10.3389/fmicb.2018.00688

Condón-Abanto, S., Condón, S., Raso, J., Lyng, J. G., & Álvarez, I. (2016). Inactivation of *Salmonella* Typhimurium and *Lactobacillus plantarum* by UV-C light in flour powder. Innovative *Food Science and Emerging Technologies*, 35, 1–8. https://doi.org/10.1016/j.ifset.2016.03.008

Corrêa, T. Q., Blanco, K. C., Garcia, É. B., Perez, S. M. L., Chianfrone, D. J., Morais, V. S., & Bagnato, V. S. (2020). Effects of ultraviolet light and curcumin-mediated photodynamic inactivation on microbiological food safety: A study in meat and fruit. *Photodiagnosis and Photodynamic Therapy*, 30, 101678. https://doi.org/10.1016/j.pdpdt.2020.101678

D'Souza, C., Yuk, H., Khoo, G. H., & Zhou, W. (2015). Application of light-emitting diodes in food production, postharvest preservation, and microbiological food safety. *Comprehensive Reviews in Food Science and Food Safety*, 14(6), 719–740. https://doi.org/10.1111/1541-4337.12155

da Silva, R. N., Tomé, A. C., Tomé, J. P. C., Neves, M. G. P. M. S., Faustino, M. A. F., Cavaleiro, J. A. S., Oliveira, A., Almeida, A., & Cunha, Â. (2012). Photo-inactivation of Bacillus endospores: inter-specific variability of inactivation efficiency. *Microbiology*

and Immunology, 56(10), 692–699. https://doi.org/10.1111/j.1348-0421.2012.00493.x

de Freitas Saccol, A. L., Serafim, A. L., Hecktheuer, L. H., Medeiros, L. B., & Da Silva, E. A. (2016). Food safety in feeding services: a requirement in Brazil. *Critical Reviews in Food Science and Nutrition*, 56(8), 1363–1369. https://doi.org/10.1080/10408398.2012.691917

de Menezes, H. D., Pereira, A. C., Brancini, G. T. P., de Leão, H. C., Massola Júnior, N. S., Bachmann, L., Wainwright, M., Bastos, J. K., & Braga, G. U. L. (2014a). Furocoumarins and coumarins photoinactivate *Colletotrichum acutatum* and *Aspergillus nidulans* fungi under solar radiation. *Journal of Photochemistry and Photobiology B: Biology*, 131, 74–83. https://doi.org/10.1016/j.jphotobiol.2014.01.008

de Menezes, H. D., Rodrigues, G. B., Teixeira, S. de P., Massola, N. S., Bachmann, L., Wainwright, M., & Braga, G. Ú. L. (2014b). *In vitro* photodynamic inactivation of plant-pathogenic fungi *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* with novel phenothiazinium photosensitizers. *Applied and Environmental Microbiology*, 80(5), 1623–1632. https://doi.org/10.1128/AEM.02788-13

de Menezes, H. D., Tonani, L., Bachmann L., Wainwright M., Braga, G. Ú. L., & Von Zeska Kress, M. R. (2016). Photodynamic treatment with phenothiazinium photosensitizers kills both ungerminated and germinated microconidia of the pathogenic fungi *Fusarium oxysporum*, *Fusarium moniliforme* and *Fusarium solani*. *Journal of Photochemistry and Photobiology B: Biology*, 164,1-12. http://dx.doi.org/10.1016/j.jphotobiol.2016.09.008

de Oliveira, E. F., Tikekar, R., & Nitin, N. (2018a). Combination of aerosolized curcumin and UV-A light for the inactivation of bacteria on fresh produce surfaces. *Food Research International*, 114, 133–139. https://doi.org/10.1016/j.foodres.2018.07.054

de Oliveira, E. F., Tosati, J. V., Tikekar, R. V., Monteiro, A. R., & Nitin, N. (2018b). 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, 86–94. https://doi.org/10.1016/j.postharvbio.2017.11.014

de Sordi, L., Butt, M. A., Pye, H., Kohoutova, D., Mosse, C. A., Yahioglu, G., Stamati, I., Deonarain, M., Battah, S., Ready, D., Allan, E., Mullany, P., & Lovat, L. B. (2015). Development of photodynamic antimicrobial chemotherapy (PACT) for *Clostridium difficile*. *PLOS ONE*, 10(8), e0135039. https://doi.org/10.1371/journal.pone.0135039

de Souza, T., Antonio, F., Zanotto, M., Homem-de-Mello, P., & Ribeiro, A. (2018). Photophysical and photochemical properties and aggregation behavior of phthalocyanine and naphthalocyanine derivatives. *Journal of the Brazilian Chemical Society*, 29(6), 1199–1209. https://doi.org/10.21577/0103-5053.20170215

de Souza, L. M., Inada, N. M., Venturini, F. P., Carmona-Vargas, C. C., Pratavieira, S., de Oliveira, K. T., Kurachi., C., & Bagnato, V. S. (2019). Photolarvicidal effect of curcuminoids from *Curcuma longa Linn*. against *Aedes aegypti larvae*. *Journal of Asia-Pacific Entomology*, 22(1), 151–158. https://doi.org/10.1016/j.aspen.2018.12.016

Dementavicius, D., Lukseviciute, V., Gómez-López, V. M., & Lukšiene, Z. (2016). Application of mathematical models for bacterial inactivation curves using Hypericin-based photosensitization. *Journal of Applied Microbiology*, 120(6), 1492–1500. https://doi.org/10.1111/jam.13127

Demidova, T. N., & Hamblin, M. R. (2004). Photodynamic therapy targeted to pathogens. *International Journal of Immunopathology and Pharmacology*, 17(3), 245–254. https://doi.org/10.1177/039463200401700304

Demidova, T. N., & Hamblin, M. R. (2005). Photodynamic inactivation of Bacillus spores, mediated by phenothiazinium dyes. *Applied and Environmental Microbiology*, 71(11), 6918–6925. https://doi.org/10.1128/AEM.71.11.6918-6925.2005

Derosa, M. C., & Crutchley, R. J. (2002). Photosensitized singlet oxygen and its applications. *Coordination Chemistry Reviews*, 234, 351–371. https://doi.org/10.1016/S0010-8545(02)00034-6

EFSA. (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA Journal*, 13(12). https://doi.org/10.2903/j.efsa.2015.4329

Eichner, A., Gollmer, A., Späth, A., Bäumler, W., Regensburger, J., König, B., &

Maisch, T. (2015). Fast and effective inactivation of *Bacillus atrophaeus* endospores using light-activated derivatives of vitamin B2. *Photochemical & Photobiological Sciences*, 14(2), 387–396. https://doi.org/10.1039/C4PP00285G

Eigner, D., & Scholz, D. (1999). Ferula asa-foetida and *Curcuma longa* in traditional medical treatment and diet in Nepal. *Journal of Ethnopharmacology*, 67(1), 1–6. https://doi.org/10.1016/S0378-8741(98)00234-7

FAO. (2011). Food and Agricultural Organization. Global food losses and food waste – Extent, causes and prevention. Retrieved from http://www.fao.org/3/a-i2697e.pdf

FDA. (2020). Food and Drug Administration. substances added to food (formerly EAFUS). Retrieved April 13, 2020, from https://www.accessdata.fda.gov/scripts/fdcc/?set=FoodSubstances&id=FDCREDNO3

Ferrario, M. I., & Guerrero, S. N. (2018). Inactivation of *Alicyclobacillus acidoterrestris* ATCC 49025 spores in apple juice by pulsed light. Influence of initial contamination and required reduction levels. *Revista Argentina de Microbiologia*, 50(1), 3–11. https://doi.org/10.1016/j.ram.2017.04.002

Feruszová, J., Imreová, P., Bodnárová, K., Ševcovicova, A., Kyzek, S., Chalupa, I., Gálová, E., & Miadoková, E. (2016). Photoactivated hypericin is not genotoxic. *General Physiology and Biophysics*, 35(2), 223–230. https://doi.org/10.4149/gpb_2015045

Fleet, G. H. (2007). Yeasts in foods and beverages: impact on product quality and safety. *Current Opinion in Biotechnology*, 18(2), 170–175. https://doi.org/10.1016/j.copbio.2007.01.010

Fotinos, N., Convert, M., Piffaretti, J.-C., Gurny, R., & Lange, N. (2008). Effects on gram-negative and gram-positive bacteria mediated by 5-aminolevulinic acid and 5-aminolevulinic acid derivatives. *Antimicrobial Agents and Chemotherapy*, 52(4), 1366–1373. https://doi.org/10.1128/AAC.01372-07

Fracarolli, L., Rodrigues, G. B., Pereira, A. C., Massola Júnior, N. S., Silva-Junior, G. J., Bachmann, L., Wainwright, M., Bastos, J. K., & Braga, G. U. L. (2016). Inactivation of plant-pathogenic fungus *Colletotrichum acutatum* with natural plant-produced

photosensitizers under solar radiation. *Journal of Photochemistry and Photobiology B: Biology*, 162, 402–411. https://doi.org/10.1016/j.jphotobiol.2016.07.009

Freire, F., Ferraresi, C., Jorge, A. O. C., & Hamblin, M. R. (2016). Photodynamic therapy of oral *Candida* infection in a mouse model. *Journal of Photochemistry and Photobiology B: Biology*, 159, 161–168. https://doi.org/10.1016/j.jphotobiol.2016.03.049

Gábor, F., Szocs, K., Maillard, P., & Csík, G. (2001). Photobiological activity of exogenous and endogenous porphyrin derivatives in *Escherichia coli* and *Enterococcus hirae* cells. *Radiation and Environmental Biophysics*, 40(2), 145–151. https://doi.org/10.1007/s004110100092

Galstyan, A., & Dobrindt, U. (2019). Determining and unravelling origins of reduced photoinactivation efficacy of bacteria in milk. Journ*al of Photochemistry and Photobiology B: Biology*, 197, 111554. https://doi.org/10.1016/j.jphotobiol.2019.111554

Gandin, E., Lion, Y., & Van de Vorst, A. (1983). Quantum yield of singlet oxygen production by xanthene derivatives. *Photochemistry and Photobiology*, 37(3), 271–278. https://doi.org/10.1111/j.1751-1097.1983.tb04472.x

George, S., Hamblin, M. R., & Kishen, A. (2009). Uptake pathways of anionic and cationic photosensitizers into bacteria. Photochemical & Photobiological Sciences, 8(6), 788. https://doi.org/10.1039/b809624d

Ghate, V., Leong, A. L., Kumar, A., Bang, W. S., Zhou, W., & Yuk, H.-G. (2015). Enhancing the antibacterial effect of 461 and 521 nm light emitting diodes on selected foodborne pathogens in trypticase soy broth by acidic and alkaline pH conditions. *Food Microbiology*, 48, 49–57. https://doi.org/10.1016/j.fm.2014.10.014

Ghate, V. S., Zhou, W., & Yuk, H. G. (2019). Perspectives and trends in the application of photodynamic inactivation for microbiological food safety. *Comprehensive Reviews in Food Science and Food Safety*, 18(2), 402–424. https://doi.org/10.1111/1541-4337.12418

Ghosh, S., Banerjee, S., & Sil, P. C. (2015). The beneficial role of curcumin on

inflammation, diabetes and neurodegenerative disease: A recent update. *Food and Chemical Toxicology*, 83, 111–124. https://doi.org/10.1016/j.fct.2015.05.022

Glueck, M., Schamberger, B., Eckl, P., & Plaetzer, K. (2017). New horizons in microbiological food safety: Photodynamic Decontamination based on a curcumin derivative. *Photochemical & Photobiological Sciences*, 16(12), 1784–1791. https://doi.org/10.1039/C7PP00165G

Gonzales, F. P., da Silva, S. H., Roberts, D. W., & Braga, G. U. L. (2010). Photodynamic inactivation of conidia of the fungi *Metarhizium anisopliae* and *Aspergillus nidulans* with methylene blue and toluidine blue. *Photochemistry and Photobiology*, 86(3): 653-661. https://doi.org/10.1111/j.1751-1097.2009.00689.x

Gonzales, J. C., Brancini, G. T. P., Rodrigues, G. B., Silva-Junior, G. J., Bachmann, L., Wainwright, M., & Braga, G. Ú. L. (2017). Photodynamic inactivation of conidia of the fungus Colletotrichum abscissum on Citrus sinensis plants with methylene blue under solar radiation. *Journal of Photochemistry and Photobiology B: Biology*, 176, 54–61. https://doi.org/10.1016/j.jphotobiol.2017.09.008

Hamblin, M. R. (2016). Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes. *Current Opinion in Microbiology*, 33, 67–73. https://doi.org/10.1016/j.mib.2016.06.008

Hamblin, M. R. (2017). Potentiation of antimicrobial photodynamic inactivation by inorganic salts. *Expert Review of Anti-Infective Therapy*, 15(11), 1059–1069. https://doi.org/10.1080/14787210.2017.1397512

Havelaar, A. H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake, R. J., Praet, N., Bellinger, D. C., de Silva, N. R., Gargouri, N., Speybroeck, N., Cawthorne, A., Mathers, C., Stein, C., Angulo, F. J., Devleesschauwer, B., Adegoke, G. O., Afshari, R., Alasfoor, D., Zeilmaker, M. (2015). World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. *PLoS Medicine*, 12(12), p. 1001923. https://doi.org/10.1371/journal.pmed.1001923

Helgason, E., Økstad, O. A., Caugant, D. A., Johansen, H. A., Fouet, A., Mock, M., Hegna, I., & Kolstø, A. B. (2000). Bacillus anthracis, Bacillus cereus, and *Bacillus*

thuringiensis – One species on the basis of genetic evidence. *Applied and Environmental Microbiology*, 66(6), 2627–2630. https://doi.org/10.1128/AEM.66.6.2627-2630.2000

Hoebeke, M., & Damoiseau, X. (2002). Determination of the singlet oxygen quantum yield of bacteriochlorin a: a comparative study in phosphate buffer and aqueous dispersion of dimiristoyl-l-α-phosphatidylcholine liposomes. *Photochemical & Photobiological Sciences*, 1(4), 283–287. https://doi.org/10.1039/b201081j

Huang, L., St. Denis, T. G., Xuan, Y., Huang, Y.Y., Tanaka, M., Zadlo, A., Sarna, T., & Hamblin, M. R. (2012). Paradoxical potentiation of methylene blue-mediated antimicrobial photodynamic inactivation by sodium azide: Role of ambient oxygen and azide radicals. *Free Radical Biology and Medicine*, 53(11), 2062–2071. https://doi.org/10.1016/j.freeradbiomed.2012.09.006

Huang, Y.Y., Choi, H., Kushida, Y., Bhayana, B., Wang, Y., & Hamblin, M. R. (2016). Broad-spectrum antimicrobial effects of photocatalysis using titanium dioxide nanoparticles are strongly potentiated by addition of potassium iodide. *Antimicrobial Agents and Chemotherapy*, 60(9), 5445–5453. https://doi.org/10.1128/AAC.00980-16

Huang, L., Szewczyk, G., Sarna, T., & Hamblin, M. R. (2017). Potassium iodide potentiates broad-spectrum antimicrobial photodynamic inactivation using photofrin. *ACS Infectious Diseases*, 3(4), 320–328. https://doi.org/10.1021/acsinfecdis.7b00004

Huang, L., El-Hussein, A., Xuan, W., & Hamblin, M. R. (2018). Potentiation by potassium iodide reveals that the anionic porphyrin TPPS4 is a surprisingly effective photosensitizer for antimicrobial photodynamic inactivation. *Journal of Photochemistry and Photobiology B: Biology*, 178, 277–286. https://doi.org/10.1016/j.jphotobiol.2017.10.036

Huang, J., Chen, B., Li, H., Zeng, Q.-H., Wang, J. J., Liu, H., Pan, Y., & Zhao, Y. (2020). Enhanced antibacterial and antibiofilm functions of the curcumin-mediated photodynamic inactivation against *Listeria monocytogenes*. *Food Control*, 108, 106886. https://doi.org/10.1016/j.foodcont.2019.106886

Iulietto, M. F., Sechi, P., Borgogni, E., & Cenci-Goga, B. T. (2015). Meat Spoilage: A

Critical review of a neglected alteration due to ropy slime producing bacteria. *Italian Journal of Animal Science*, 14(3), 4011. https://doi.org/10.4081/ijas.2015.4011

Jaffee, S., Henson, S., Unnevehr, L., Grace, D., & Cassou, E. (2019). The safe food imperative: accelerating progress in low- and middle-income countries. *Agriculture and Food Series. Washington, DC: World Bank*. License: Creative Commons Attribution CC BY 3.0 IGO. https://doi:10.1596/978-1-4648-1345-0

Jesionek, A., & von Tappeiner, H. (1905). Zur behandlung der hautcarcinome mit fluorescierenden stoffen. *Arch Klin Med*, 82 (in German), 223.

Jesus, V., Martins, D., Branco, T., Valério, N., Neves, M. G. P. M. S., Faustino, M. A. F., Reis, L., Barreal, E., Gallego, P. P., & Almeida, A. (2018). An insight into the photodynamic approach versus copper formulations in the control of *Pseudomonas syringae* pv. *Actinidiae* in kiwi plants. *Photochemical and Photobiological Sciences*, 17(2), 180–191. https://doi.org/10.1039/c7pp00300e

Josewin, S. W., Kim, M. J., & Yuk, H. G. (2018). Inactivation of *Listeria monocytogenes* and *Salmonella* spp. on cantaloupe rinds by blue light emitting diodes (LEDs). *Food Microbiology*, 76, 219–225. https://doi.org/10.1016/j.fm.2018.05.012

Kairyte, K., Lapinskas, S., Gudelis, V., & Lukšiene, Ž. (2012). Effective inactivation of food pathogens *Listeria monocytogenes* and *Salmonella enterica* by combined treatment of hypericin-based photosensitization and high power pulsed light. *Journal of Applied Microbiology*, 112(6), 1144–1151. https://doi.org/10.1111/j.1365-2672.2012.05296.x

Kashef, N., & Hamblin, M. R. (2017). Can microbial cells develop resistance to oxidative stress in antimicrobial photodynamic inactivation? Drug Resistance Updates, 31(April), 31–42. https://doi.org/10.1016/j.drup.2017.07.003

Kennedy, J. C., & Pottier, R. H. (1992). Endogenous protoporphyrin IX, a clinically useful photosensitizer for photodynamic therapy. *Journal of Photochemistry and Photobiology B: Biology*, 14(4), 275–292. https://doi.org/10.1016/1011-1344(92)85108-7

Kim, D. K., & Kang, D. H. (2018). UVC LED irradiation effectively inactivates

aerosolized viruses, bacteria, and fungi in a chamber-type air disinfection system. *Applied and Environmental Microbiology*, 84(17), 1–11. https://doi.org/10.1128/AEM.00944-18

Kim, M. J., & Yuk, H.-G. (2017). Antibacterial mechanism of 405-nanometer light-emitting diode against Salmonella at refrigeration temperature. *Applied and Environmental Microbiology*, 83(5), 1–14. https://doi.org/10.1128/AEM.02582-16

Koutsoumanis, K. (2009). Modeling food spoilage in microbial risk assessment. *Journal of Food Protection*, 72(2), 425–427. https://doi.org/10.4315/0362-028x-72.2.425

Kumar, A., Ghate, V., Kim, M., Zhou, W., Hoon, G., & Yuk, H. (2015). Kinetics of bacterial inactivation by 405 nm and 520 nm light emitting diodes and the role of endogenous coproporphyrin on bacterial susceptibility. *Journal of Photochemistry & Photobiology, B: Biology*, 149, 37–44. https://doi.org/10.1016/j.jphotobiol.2015.05.005

Le Marc, Y., Buchovec, I., George, S. M., Baranyi, J., & Lukšiene, Z. (2009). Modelling the photosensitization-based inactivation of *Bacillus cereus*. *Journal of Applied Microbiology*, 107(3), 1006–1011. https://doi.org/10.1111/j.1365-2672.2009.04275.x

Lee, P. C. C., & Rodgers, M. A. J. (1987). Laser flash photokinetic studies of rose bengal sensitized photodynamic interactions of nucleotides and DNA. *Photochemistry and Photobiology*, 45(1), 79–86. https://doi.org/10.1111/j.1751-1097.1987.tb08407.x

Lee, H. S., Park, H. H., & Min, S. C. (2020). Microbial decontamination of red pepper powder using pulsed light plasma. *Journal of Food Engineering*, 284, 110075. https://doi.org/10.1016/j.jfoodeng.2020.110075

Liang, J.-Y., Yuann, J.-M. P., Cheng, C.-W., Jian, H.-L., Lin, C.-C., & Chen, L.-Y. (2013). Blue light induced free radicals from riboflavin on *E. coli* DNA damage. *Journal of Photochemistry and Photobiology B: Biology*, 119, 60–64. https://doi.org/10.1016/j.jphotobiol.2012.12.007

Liang, J. Y., Cheng, C. W., Yu, C. H., & Chen, L. Y. (2015). Investigations of blue light-induced reactive oxygen species from flavin mononucleotide on inactivation of *E. coli*. *Journal of Photochemistry and Photobiology B: Biology*, 143, 82–88. https://doi.org/10.1016/j.jphotobiol.2015.01.005

Lin, S., Hu, J., Tang, S., Wu, X., Chen, Z., & Tang, S. (2012). Photodynamic inactivation of methylene blue and tungsten-halogen lamp light against food pathogen *Listeria monocytogenes. Photochemistry and Photobiology*, 88(4), 985–991. https://doi.org/10.1111/j.1751-1097.2012.01154.x

López-Carballo, G., Hernández-Muñoz, P., Gavara, R., & Ocio, M. J. (2008). Photoactivated chlorophyllin-based gelatin films and coatings to prevent microbial contamination of food products. *International Journal of Food Microbiology*, 126(1–2), 65–70. https://doi.org/10.1016/j.ijfoodmicro.2008.05.002

Lukšiene, Ž. (2005). New approach to inactivation of harmful and pathogenic microorganisms by photosensitization. *Food Technology and Biotechnology*, 43(4), 411–418. https://hrcak.srce.hr/110627

Lukšiene, Ž., & Brovko, L. (2013). Antibacterial photosensitization-based treatment for food safety. *Food Engineering Reviews*, 5(4), 185–199. https://doi.org/10.1007/s12393-013-9070-7

Lukšiene, Ž., Buchovec, I., & Paskeviciute, E. (2009). Inactivation of food pathogen *Bacillus cereus* by photosensitization in vitro and on the surface of packaging material. *Journal of Applied Microbiology*, 107(6), 2037–2046. https://doi.org/10.1111/j.1365-2672.2009.04383.x

Lukšiene, Ž., & Zukauskas, A. (2009). Prospects of photosensitization in control of pathogenic and harmful micro-organisms. *Journal of Applied Microbiology*, 107(5), 1415–1424. https://doi.org/10.1111/j.1365-2672.2009.04341.x

Machouart, M., Menir, P., Helenon, R., Quist, D., & Desbois, N. (2013). *Scytalidium* and scytalidiosis: What's new in 2012? *Journal de Mycologie Médicale*, 23(1), 40–46. https://doi.org/10.1016/j.mycmed.2013.01.002

Maisch, T., Bosl, C., Szeimies, R.-M., Lehn, N., & Abels, C. (2005). Photodynamic effects of novel XF porphyrin derivatives on prokaryotic and eukaryotic cells. *Antimicrobial Agents and Chemotherapy*, 49(4), 1542–1552. https://doi.org/10.1128/AAC.49.4.1542-1552.2005

Maisch, T., Eichner, A., Späth, A., Gollmer, A., König, B., Regensburger, J., & Bäumler,

W. (2014). Fast and effective photodynamic inactivation of multiresistant bacteria by cationic riboflavin derivatives. *PLoS ONE*, 9(12). https://doi.org/10.1371/journal.pone.0111792

Majiya, H., Adeyemi, O. O., Herod, M., Stonehouse, N. J., & Millner, P. (2018). Photodynamic inactivation of non-enveloped RNA viruses. *Journal of Photochemistry and Photobiology B: Biology*, 189, 87–94. https://doi.org/10.1016/j.jphotobiol.2018.10.009

Majiya, H., Chowdhury, K. F., Stonehouse, N. J., & Millner, P. (2019). TMPyP functionalised chitosan membrane for efficient sunlight driven water disinfection. *Journal of Water Process Engineering*, 30, 100475. https://doi.org/10.1016/j.jwpe.2017.08.013

Min, S. C., Roh, S. H., Niemira, B. A., Boyd, G., Sites, J. E., Uknalis, J., & Fan, X. (2017). In-package inhibition of *E. coli* O157:H7 on bulk Romaine lettuce using cold plasma. *Food Microbiology*, 65, 1–6. https://doi.org/10.1016/j.fm.2017.01.010

Miñán, A., Lorente, C., Ipiña, A., Thomas, A. H., Fernández, M. L. de M., & Schilardi, P. L. (2015). Photodynamic inactivation induced by carboxypterin: a novel non-toxic bactericidal strategy against planktonic cells and biofilms of *Staphylococcus aureus. Biofouling*, 31(5), 459–468. https://doi.org/10.1080/08927014.2015.1055731

Moreira, X., Santos, P., Faustino, M. A. F., Raposo, M. M. M., Costa, S. P. G., Moura, N. M. M., Gomes, A. T. P. C., Almeida, A., & Neves, M. G. P. M. S. (2020). An insight into the synthesis of cationic porphyrin-imidazole derivatives and their photodynamic inactivation efficiency against *Escherichia coli. Dyes and Pigments*, 178, 108330. https://doi.org/10.1016/j.dyepig.2020.108330

Morton, C. A., Szeimies, R.-M., Sidoroff, A., & Braathen, L. R. (2013). European guidelines for topical photodynamic therapy part 1: treatment delivery and current indications - actinic keratoses, Bowen's disease, basal cell carcinoma. *Journal of the European Academy of Dermatology and Venereology*, 27(5), 536–544. https://doi.org/10.1111/jdv.12031

Murav'eva, T. D., Dadeko, A. V., Kiselev, V. M., Kris'ko, T. K., Kislyakov, I. M., Kris'ko,

A. V., Starodubtsev, A. M., Bagrov, I. V., Belousova, I. M., & Ponomarev, G. V. (2018). Comparative study of the photophysical properties of low-toxicity photosensitizers based on endogenous porphyrins. *Journal of Optical Technology*, 85(11), 709–721. https://doi.org/10.1364/JOT.85.000709

Nishimura, T., Hara, K., Honda, N., Okazaki, S., Hazama, H., & Awazu, K. (2019). Determination and analysis of singlet oxygen quantum yields of talaporfin sodium, protoporphyrin IX, and lipidated protoporphyrin IX using near-infrared luminescence spectroscopy. *Lasers in Medical Science*. https://doi.org/10.1007/s10103-019-02907-0

Odeyemi, O. A., Alegbeleye, O. O., Strateva, M., & Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety*, 19(2), 311–331. https://doi.org/10.1111/1541-4337.12526

Okpanyi, S. N., Lidzba, H., Scholl, B. C., & Miltenburger, H. G. (1990). Genotoxicity of a standardized Hypericum extract. *Arzneimittelforschung*, 40(8), 851–855.

Oliveira, A., Almeida, A., Carvalho, C. M. B., Tomé, J. P. C., Faustino, M. A. F., Neves, M. G. P. M. S., Tomé, A. C., Cavaleiro, J. A. S., & Cunha, Â. (2009). Porphyrin derivatives as photosensitizers for the inactivation of *Bacillus cereus* endospores. *Journal of Applied Microbiology*, 106(6), 1986–1995. https://doi.org/10.1111/j.1365-2672.2009.04168.x

Otieno, W., Liu, C., Deng, H., Li, J., Zeng, X., & Ji, Y. (2020). Hypocrellin B-mediated photodynamic inactivation of gram-positive antibiotic-resistant bacteria: An *in vitro* study. *Photobiomodulation, Photomedicine, and Laser Surgery*, 38(1), 36–42. https://doi.org/10.1089/photob.2019.4656

Ottaway P.B. (1993) Stability of vitamins in food. In: Ottaway P.B. (eds) *The Technology of Vitamins in Food*. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-2131-0_5

Ozturk, I., Tunçel, A., Yurt, F., Biyiklioglu, Z., Ince, M., & Ocakoglu, K. (2020). Antifungal photodynamic activities of phthalocyanine derivatives on *Candida albicans.*

Photodiagnosis and Photodynamic Therapy, 30, 101715. https://doi.org/10.1016/j.pdpdt.2020.101715

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., & Griffin, P. M. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases*, 19(3), 407–415. https://doi.org/10.3201/eid1903.111866

Paskeviciute, E., Zudyte, B., & Lukšiene, Ž. (2018). Towards better microbial safety of fresh produce: Chlorophyllin-based photosensitization for microbial control of foodborne pathogens on cherry tomatoes. *Journal of Photochemistry and Photobiology B: Biology*, 182, 130–136. https://doi.org/10.1016/j.jphotobiol.2018.04.009

Paskeviciute, E., Zudyte, B., & Lukšiene, Ž. (2019). innovative nonthermal technologies: chlorophyllin and visible light significantly reduce microbial load on basil. *Food Technology and Biotechnology*, 57(1), 126–132. https://doi.org/10.17113/ftb.57.01.19.5816

Paziani, M. H., Tonani, L., de Menezes, H. D., Bachmann, L., Wainwright, M., Braga, G. Ú. L., & von Zeska Kress, M. R. (2019). Antimicrobial photodynamic therapy with phenothiazinium photosensitizers in non-vertebrate model *Galleria mellonella* infected with *Fusarium keratoplasticum* and *Fusarium moniliforme*. *Photodiagnosis and Photodynamic Therapy*, 25, 197–203. https://doi.org/10.1016/j.pdpdt.2018.12.010

Penha, C. B., Bonin, E., da Silva, A. F., Hioka, N., Zanqueta, É. B., Nakamura, T. U., de Abreu Filho, B. A., Campanerut-Sá, P. A. Z., & Mikcha, J. M. G. (2017). Photodynamic inactivation of foodborne and food spoilage bacteria by curcumin. *LWT - Food Science and Technology*, 76, 198–202. https://doi.org/10.1016/j.lwt.2016.07.037

Pereira, L. M., Mota, C. M., Baroni, L., Bronzon da Costa, C. M., Brochi, J. C. V., Wainwright, M., Mineo, T. W. P., Braga, G. Ú. L, & Yatsuda, A. P. (2020). Inhibitory action of phenothiazinium dyes against *Neospora caninum*. *Scientific Reports*, 10(1), 7483. https://doi.org/10.1038/s41598-020-64454-x

Pereira, M. A., Faustino, M. A. F., Tomé, J. P. C., Neves, M. G. P. M. S., Tomé, A. C., Cavaleiro, J. A. S., Cunha, Â., & Almeida, A. (2014). Influence of external bacterial structures on the efficiency of photodynamic inactivation by a cationic porphyrin. *Photochemical & Photobiological Sciences*, 13(4), 680. https://doi.org/10.1039/c3pp50408e

Pineiro, M., Pereira, M. M., d'A Rocha Gonsalves, A. M., Arnaut, L. G., & Formosinho, S. J. (2001). Singlet oxygen quantum yields from halogenated chlorins: potential new photodynamic therapy agents. *Journal of Photochemistry and Photobiology A: Chemistry*, 138(2), 147–157. https://doi.org/10.1016/S1010-6030(00)00382-8

Portapilla, G. B., Pereira, L. M., Bronzon da Costa, C. M., Voltarelli Providello, M., Sampaio Oliveira, P. A., Goulart, A., Anchieta, N. F., Wainwright, M., Braga, G. Ú. L., & Albuquerque, S. (2019). Phenothiazinium dyes are active against *Trypanosoma cruzi in vitro*. *BioMed Research International*, 2019, 1–9. https://doi.org/10.1155/2019/8301569

Praditya, D., Kirchhoff, L., Brüning, J., Rachmawati, H., Steinmann, J., & Steinmann, E. (2019). Anti-infective properties of the golden spice curcumin. *Frontiers in Microbiology*, 10:912. https://doi.org/10.3389/fmicb.2019.00912

Raab, O. (1900). Über die wirkung fluoreszcierender stoffe aus infusorien. *Ztg. Biol.*, 39, 524.

Randazzo, W., Aznar, R., & Sánchez, G. (2016). Curcumin-mediated photodynamic inactivation of norovirus surrogates. *Food and Environmental Virology*, 8(4), 244–250. https://doi.org/10.1007/s12560-016-9255-3

Remenant, B., Jaffrès, E., Dousset, X., Pilet, M.-F., & Zagorec, M. (2015). Bacterial spoilers of food: Behavior, fitness and functional properties. *Food Microbiology*, 45, 45–53. https://doi.org/10.1016/j.fm.2014.03.009

Reverter-Carrión, L., Sauceda-Gálvez, J. N., Codina-Torrella, I., Hernández-Herrero, M. M., Gervilla, R., & Roig-Sagués, A. X. (2018). Inactivation study of *Bacillus subtilis*, *Geobacillus stearothermophilus*, *Alicyclobacillus acidoterrestris* and *Aspergillus niger* spores under Ultra-High Pressure Homogenization, UV-C light and their combination.

Innovative Food Science and Emerging Technologies, 48, 258–264. https://doi.org/10.1016/j.ifset.2018.06.011

Rodrigues, G. B., Ferreira, L. K. S., Wainwright, M., & Braga, G. U. L. (2012a). Susceptibilities of the dermatophytes *Trichophyton mentagrophytes* and *T . rubrum* microconidia to photodynamic antimicrobial chemotherapy with novel phenothiazinium photosensitizers and red light. *Journal of Photochemistry & Photobiology, B: Biology*, 116, 89–94. https://doi.org/10.1016/j.jphotobiol.2012.08.010

Rodrigues, G. B., Primo, F. L., Tedesco, A. C., & Braga, G. U. L. (2012b). *In vitro* photodynamic inactivation of *Cryptococcus neoformans* melanized cells with chloroaluminum phthalocyanine nanoemultision. *Photochemistry and Photobiology*, 88, 440–447. https://doi.org/10.1111/j.1751-1097.2011.01055.x

Rodrigues, G. B., Dias-Baruffi, M., Holman, N., Wainwright, M., & Braga, G. U. L. (2013). *In vitro* photodynamic inactivation of *Candida* species and mouse fibroblasts with phenothiazinium photosensitisers and red light. *Photodiagnostics and Photodynamic Therapy*, 10(2), 141-149. https://doi.org/10.1016/j.pdpdt.2012.11.004

Rodrigues, G. B., Brancini, G. T. P., Pinto, M. R., Primo, F. L., Wainwright, M., Tedesco, A. C., & Braga, G. Ú. L. (2020). Photodynamic inactivation of *Candida albicans* and *Candida tropicalis* with aluminum phthalocyanine chloride nanoemulsion. *Fungal Biology*, 124(5), 297-303. https://doi.org/10.1016/j.funbio.2019.08.004

Romanova, N. A., Brovko, L. Y., Moore, L., Pometun, E., Savitsky, A. P., Ugarova, N. N., & Griffiths, M. W. (2003). Assessment of photodynamic destruction of *Escherichia coli* O157:H7 and *Listeria monocytogenes* by using ATP bioluminescence. *Applied and Environmental Microbiology*, 69(11), 6393–6398. https://doi.org/10.1128/AEM.69.11.6393-6398.2003

Santos, A. R., Batista, A. F. P., Gomes, A. T. P. C., Neves, M. G. P. M. S., Faustino, M. A. F., Almeida, A., Hioka, N., & Mikcha, J. M. G. (2019). The remarkable effect of potassium iodide in eosin and rose bengal photodynamic action against *Salmonella* Typhimurium and *Staphylococcus aureus*. *Antibiotics*, 8(4). https://doi.org/10.3390/antibiotics8040211

Santos, A. R., da Silva, A. F., Batista, A. F. P., Freitas, C. F., Bona, E., Sereia, M. J., Caetano, W., Hioka, N., & Mikcha, J. M. G. (2020). Application of response surface methodology to evaluate photodynamic inactivation mediated by Eosin Y and 530 nm LED against *Staphylococcus aureus*. *Antibiotics*, 9(3), 125. https://doi.org/10.3390/antibiotics9030125

Saúde, Ministério da. (2019a). Surtos de Doenças Transmitidas por Alimentos no Brasil. Informe 2018. SINAN/SVS/Ministério da Sáude. Retrieved April 18, 2020, from https://www.saude.gov.br/images/pdf/2019/maio/17/Apresentacao-Surtos-DTA-Maio-2019.pdf

Saúde, Ministério da. (2019b). Doenças transmitidas por alimentos. Retrieved April 18, 2020, from https://www.saude.gov.br/saude-de-a-z/doencas-transmitidas-por-alimentos

Schneider, J. E., Price, S., Maidt, L., Gutteridge, J. M. C., & Floyd, R. A. (1990). Methylene blue plus light mediates 8-hydroxy 2'-deoxyguanosine formation in DNA preferentially over strand breakage. *Nucleic Acids Research*, 18(3), 631–635. https://doi.org/10.1093/nar/18.3.631

Schuyler, R. (2001). Use of riboflavin for photoinactivation of pathogens in blood components. *Transfusion and Apheresis Science*, 25(3), 189–190. https://doi.org/10.1016/S1473-0502(01)00119-7

Sepúlveda, A. A. L., Arenas Velásquez, A. M., Patiño Linares, I. A., de Almeida, L., Fontana, C. R., Garcia, C., & Graminha, M. A. S. (2020). Efficacy of photodynamic therapy using TiO_2 nanoparticles doped with Zn and hypericin in the treatment of cutaneous Leishmaniasis caused by *Leishmania amazonensis*. *Photodiagnosis and Photodynamic Therapy*, 30, 101676. https://doi.org/10.1016/j.pdpdt.2020.101676

Silva, A. F., Borges, A., Giaouris, E., Mikcha, J. M. G., & Simões, M. (2018). Photodynamic inactivation as an emergent strategy against foodborne pathogenic bacteria in planktonic and sessile states. *Critical Reviews in Microbiology*, 44(6), 667–684. https://doi.org/10.1080/1040841X.2018.1491528

Silva, A. F., Santos, A. R., Trevisan, D. A. C., Bonin, E., Freitas, C. F., Batista, A. F.

P., Hioka, N., Simões, M., & Mikcha, J. M. G. (2019). Xanthene dyes and green LED for the inactivation of foodborne pathogens in planktonic and biofilm states. *Photochemistry and Photobiology*, 95(5), 1230–1238. https://doi.org/10.1111/php.13104

Silva, A. R. da, Pelegrino, A. C., Tedesco, A. C., & Jorge, R. A. (2008). Photodynamic activity of chloro(5,10,15,20-tetraphenylporphyrinato)indium(III). *Journal of the Brazilian Chemical Society*, 19(3), 491–501. https://doi.org/10.1590/S0103-50532008000300017

Simões, C., Gomes, M. C., Neves, M. G. P. M. S., Cunha, A., Tomé, J. P. C., Tomé, A. C., Cavaleiro, J. A. S., Almeida, A., & Faustino, M. A. F. (2016). Photodynamic inactivation of *Escherichia coli* with cationic meso-tetraarylporphyrins - The charge number and charge distribution effects. *Catalysis Today*, 266, 197–204. https://doi.org/10.1016/j.cattod.2015.07.031

St Denis, T. G., Vecchio, D., Zadlo, A., Rineh, A., Sadasivam, M., Avci, P., Huang, L., Kozinska, A., Chandran, R., Sarna, T., & Hamblin, M. R. (2013). Thiocyanate potentiates antimicrobial photodynamic therapy: In situ generation of the sulfur trioxide radical anion by singlet oxygen. *Free Radical Biology and Medicine*, 65, 800–810. https://doi.org/10.1016/j.freeradbiomed.2013.08.162

Stevenson, A., Cray, J. A., Williams, J. P., Santos, R., Sahay, R., Neuenkirchen, N., McClure, C. D., Grant, I. R., Houghton, J. D. R., Quinn, J. P., Timson, D. J., Patil, S. V., Singhal, R. S., Antón, J., Dijksterhuis, J., Hocking, A. D., Lievens, B., Rangel, D. E. N., Voytek, M. A., Gunde-Cimerman, N., Oren, A., Timmis, K. N., McGenity, T. J., & Hallsworth, J. E. (2015). Is there a common water-activity limit for the three domains of life? *The ISME Journal*, 9(6), 1333–1351. https://doi.org/10.1038/ismej.2014.219

Tao, R., Zhang, F., Tang, Q.J, Xu, C.S, Ni, Z. J., & Meng, X.H. (2019). Effects of curcumin-based photodynamic treatment on the storage quality of fresh-cut apples. *Food Chemistry*, 274, 415–421. https://doi.org/10.1016/j.foodchem.2018.08.042

Temba, B. A., Fletcher, M. T., Fox, G. P., Harvey, J., Okoth, S. A., & Sultanbawa, Y. (2019). Curcumin-based photosensitization inactivates *Aspergillus flavus* and reduces aflatoxin B1 in maize kernels. *Food Microbiology*, 82, 82–88.

https://doi.org/10.1016/j.fm.2018.12.013

Tomé, J. P. C., Neves, M. G. P. M. S., Tomé, A. C., Cavaleiro, J. A. S., Soncin, M., Magaraggia, M., Ferro, S., & Jori, G. (2004). Synthesis and antibacterial activity of new Poly-*S*-lysine−Porphyrin conjugates. *Journal of Medicinal Chemistry*, 47(26), 6649–6652. https://doi.org/10.1021/jm040802v

Tonani, L., Morosini, N. S., Dantas de Menezes, H., Nadaletto Bonifácio da Silva, M. E., Wainwright, M., Leite Braga, G. Ú., & von Zeska Kress, M. R. (2018). *In vitro* susceptibilities of *Neoscytalidium* spp. sequence types to antifungal agents and antimicrobial photodynamic treatment with phenothiazinium photosensitizers. *Fungal Biology*, 122(6), 436–448. https://doi.org/10.1016/j.funbio.2017.08.009

Tosati, J. V., de Oliveira, E. F., Oliveira, J. V., Nitin, N., & Monteiro, A. R. (2018). Light-activated antimicrobial activity of turmeric residue edible coatings against cross-contamination of *Listeria innocua* on sausages. *Food Control*, 84, 177–185. https://doi.org/10.1016/j.foodcont.2017.07.026

Uchoa, A. F., Konopko, A. M., & Baptista, M. S. (2015). Chlorophyllin derivatives as photosensitizers: synthesis and photodynamic properties. *Journal of the Brazilian Chemical Society*, 26(12), 2615–2622. https://doi.org/10.5935/0103-5053.20150290

Vatansever, F., de Melo, W.C.M.A., Avci, P., Vecchio, D., Sadasivam, M., Gupta, A., Chandran, R., Karimi, M., Parizotto, N.A, Yin, R., Tegos, G.P., & Hamblin, M.R. (2013). Antimicrobial strategies centered around reactive oxygen species – bactericidal antibiotics, photodynamic therapy, and beyond. *FEMS Microbiology Reviews*, 37(6), 955–989. https://doi.org/10.1111/1574-6976.12026

Vecchio, D., Gupta, A., Huang, L., Landi, G., Avci, P., Rodas, A., & Hamblin, M. R. (2015). Bacterial photodynamic inactivation mediated by methylene blue and red light is enhanced by synergistic effect of potassium iodide. *Antimicrobial Agents and Chemotherapy*, 59(9), 5203–5212. https://doi.org/10.1128/AAC.00019-15

Vieira, C., Gomes, A. T. P. C., Mesquita, M. Q., Moura, N. M. M., Neves, G. P. M. S., Faustino, A. F., & Almeida, A. (2018). An insight into the potentiation effect of potassium iodide on APDT efficacy. *Frontiers in Microbiology*, 9, 1–16.

https://doi.org/10.3389/fmicb.2018.02665

Vieira, C., Santos, A., Mesquita, M. Q., Gomes, A. T. P. C., Neves, M. G. P. M. S., Faustino, M. A. F., & Almeida, A. (2019). Advances in aPDT based on the combination of a porphyrinic formulation with potassium iodide: Effectiveness on bacteria and fungi planktonic/biofilm forms and viruses. *Journal of Porphyrins and Phthalocyanines*, 23(4–5), 534–545. https://doi.org/10.1142/S1088424619500408

Wainwright, M. (2004). Photoinactivation of viruses. *Photochemical & Photobiological Sciences*, 3(5), 406. https://doi.org/10.1039/b311903n

Wainwright, M., Phoenix, D. A., Marland, J., Wareing, D. R. A., & Bolton, F. J. (1997). A study of photobactericidal activity in the phenothiazinium series. *FEMS Immunology & Medical Microbiology*, 19(1), 75–80. https://doi.org/10.1111/j.1574-695X.1997.tb01074.x

Wainwright, M. (1998). Photodynamic antimicrobial chemotherapy (PACT). The *Journal of Antimicrobial Chemotherapy*, 42(1), 13–28. https://doi.org/10.1093/jac/42.1.13

Wainwright, M, Phoenix, D. A., Laycock, S. L., Wareing, D. R. A., & Wright, P. A. (1998). Photobactericidal activity of phenothiazinium dyes against methicillin-resistant strains of *Staphylococcus aureus. FEMS Microbiology Letters*, 160(2), 177–181. https://doi.org/10.1111/j.1574-6968.1998.tb12908.x

Wainwright, Mark, Maisch, T., Nonell, S., Plaetzer, K., Almeida, A., Tegos, G. P., & Hamblin, M. R. (2017). Photoantimicrobials—are we afraid of the light? *The Lancet Infectious Diseases*, 17(2), e49–e55. https://doi.org/10.1016/S1473-3099(16)30268-7

Wainwright, Mark, Smalley, H., Scully, O., & Lotfipour, E. (2012). Comparative photodynamic evaluation of new phenothiazinium derivatives against *Propionibacterium acnes*. *Photochemistry and Photobiology*, 88(3), 523–526. https://doi.org/10.1111/j.1751-1097.2011.01021.x

Wen, X., Zhang, X., Szewczyk, G., El-Hussein, A., Huang, Y.-Y., Sarna, T., & Hamblin, M. R. (2017). Potassium iodide potentiates antimicrobial photodynamic inactivation mediated by rose bengal in *in vitro* and *in vivo* studies. *Antimicrobial Agents and*

Chemotherapy, 61(7), 1–15. https:// doi:10.1128/AAC.00467-17

WHO. (2015). World Health Organization. WHO estimates of the global burden of foodborne diseases. Retrieved May 25, 2020, from https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf?sequence=1

WHO. (2018). World Health Organization. Antimicrobial resistance. Retrieved April 19, 2020, from https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

Wiegell, S. R., Hædersdal, M., Philipsen, P. A., Eriksen, P., Enk, C. D., & Wulf, H. C. (2008). Continuous activation of PpIX by daylight is as effective as and less painful than conventional photodynamic therapy for actinic keratoses; a randomized, controlled, single-blinded study. *British Journal of Dermatology*, 158(4), 740–746. https://doi.org/10.1111/j.1365-2133.2008.08450.x

Wilkinson, F., Helman, W. P., & Ross, A. B. (1993). Quantum yields for the photosensitized formation of the lowest electronically excited singlet state of molecular oxygen in solution. *Journal of Physical and Chemical*, 22(1), 113–262. https://doi.org/10.1063/1.555934

Wilson, B. C., & Patterson, M. S. (2008). The physics, biophysics and technology of photodynamic therapy. *Physics in Medicine and Biology*, 53(9), R61–R109. https://doi.org/10.1088/0031-9155/53/9/R01

Wu, J., Xu, H., Tang, W., Kopelman, R., Philbert, M. A., & Xi, C. (2009). Eradication of bacteria in suspension and biofilms using methylene blue-loaded dynamic nanoplatforms. *Antimicrobial Agents and Chemotherapy*, 53(7), 3042–3048. https://doi.org/10.1128/AAC.01604-08

Wu, J., Hou, W., Cao, B., Zuo, T., Xue, C., Leung, A. W., Xu, C., & Tang, Q. J. (2015). Virucidal efficacy of treatment with photodynamically activated curcumin on murine norovirus bio-accumulated in oysters. *Photodiagnosis and Photodynamic Therapy*, 12(3), 385–392. https://doi.org/10.1016/j.pdpdt.2015.06.005

Wu, X., Huang, Y.-Y., Kushida, Y., Bhayana, B., & Hamblin, M. R. (2016). Broad-spectrum antimicrobial photocatalysis mediated by titanium dioxide and UVA is

potentiated by addition of bromide ion via formation of hypobromite. *Free Radical Biology and Medicine*, 95, 74–81. https://doi.org/10.1016/j.freeradbiomed.2016.03.012

Yang, M., Yang, T., & Mao, C. (2019). Enhancement of photodynamic cancer therapy by physical and chemical factors. *Angewandte Chemie*, 58(40), 14066–14080. https://doi.org/10.1002/anie.201814098

Yassunaka, N. N., de Freitas, C. F., Rabello, B. R., Santos, P. R., Caetano, W., Hioka, N., Nakamura, T. U., de Abreu Filho, B. A., & Mikcha, J. M. G. (2015). Photodynamic inactivation mediated by erythrosine and its derivatives on foodborne pathogens and spoilage bacteria. *Current Microbiology*. https://doi.org/10.1007/s00284-015-0827-5

Yuan, L., Lyu, P., Huang, Y. Y., Du, N., Qi, W., Hamblin, M. R., & Wang, Y. (2020). Potassium iodide enhances the photobactericidal effect of methylene blue on *Enterococcus faecalis* as planktonic cells and as biofilm infection in teeth. *Journal of Photochemistry and Photobiology B: Biology*, 203, 111730. https://doi.org/10.1016/j.jphotobiol.2019.111730

Zhang, Y., Dai, T., Wang, M., Vecchio, D., Chiang, L. Y., & Hamblin, M. R. (2015). Potentiation of antimicrobial photodynamic inactivation mediated by a cationic fullerene by added iodide: *In vitro* and *in vivo* studies. *Nanomedicine*, 10(4), 603–614. https://doi.org/10.2217/nnm.14.131

Žudyte, B., & Lukšiene, Ž. (2019). Toward better microbial safety of wheat sprouts: Chlorophyllin-based photosensitization of seeds. Photochemical and Photobiological Sciences, 18(10), 2521–2530. https://doi.org/10.1039/c9pp00157c

Capítulo 2 – Inactivation kinetics of Bacillus cereus vegetative cells and spores from different sources by antimicrobial photodynamic treatment (aPDT)

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Capítulo formatado de acordo com as normas de submissão da revista: ***LWT – Food Science and Technology***.

Abstract

Antimicrobial photodynamic treatment (aPDT) is a promising alternative to conventional thermal inactivation methods. This study aimed to evaluate the inactivation of *Bacillus cereus* vegetative cells and spores by aPDT with new methylene blue (NMB) as photosensitizer (PS) using red light. The efficacy of aPDT was determined, initially by minimal inhibitory concentration (MIC) of NMB at different concentrations and fluences. Cluster analysis from the results of MIC grouped *B. cereus* strains according to their aPDT resistance. Then, four strains (B63, B3, 436, and ATCC 14579) were selected for a survival study of aPDT with NMB in three concentrations (5, 50, and 100 µM). The viability of *B. cereus* vegetative cells and spores was reduced in all tested fluences. The strain ATCC 14579 (vegetative cells and spores) was the most susceptible to aPDT at relatively low fluence (25 J/cm^2). The Weibull model presented a good fit for the inactivation data estimating the kinetic parameters δ (first decimal reduction) and *p* (shape parameter). This study contributes to the knowledge on the behavior of different strains of *B. cereus* regarding an emerging method of microbial inactivation. The variability of inactivation among strains will allow the development of more reliable processes.

Keywords: Emerging technology, Nonthermal treatment, Pathogen, Spoilage, Phenothiazine dye

Research Highlights

- Antimicrobial photodynamic treatment (aPDT) for inactivation of *B. cereus* is effective
- Cluster analysis grouped strains according to aPDT resistance
- Variability among the strains of the same origin was observed
- The Weibull model was able to estimate the inactivation kinetic parameters of aPDT

1. Introduction

The contamination of foods with pathogens is responsible for mortality and morbidity that impact on people's lives, countries' economies, and in social development (WHO, 2015). According to the Center for Disease Control and Prevention (CDC) from the United States, 1 in 6 Americans get sick from food poisoning each year (CDC, 2020). *Bacillus cereus* is a Gram-positive spore-forming rod-shaped bacterium commonly found in soil environments and associated with foodborne illnesses and food spoilage (Spanu, 2016). Food poisonings caused by *B. cereus* is mainly characterized by vomiting (emetic toxin) and diarrhea (enterotoxin) (Bottone, 2010). In dairy products, *B. cereus* can also cause food spoilage by the production of lipase, proteinase, and phospholipases that causes off-flavour, coagulation, and bitterness (Heyndrickx, 2011; Mehta, Metzger, Hassan, Nelson, & Patel, 2019; Spanu, 2016).

Food industries seek for nonthermal and environmentally friendly alternatives to reduce or avoid the contamination of the products and, consequently, to diminish foodborne diseases. Antimicrobial photodynamic treatment (aPDT) is a promising technology that can effectively reduce microbial counts from the surface of foods and related materials (Gonzales et al., 2017; Luksiene, Buchovec, & Paskeviciute, 2009).

Mostly, the microbial inactivation is obtained through thermal and chemical treatment and controlled by good manufacturing, transportation, and storage practices. Even though conventional thermal methodologies are effective, it is known that they cause undesirable effects as losses in the sensory and nutritional quality of food (Barba et al., 2017; Uchida & Silva, 2017). Besides that, the use of chemical products as sanitizers is not considered environmentally friendly also being harmful to humans (Ölmez & Kretzschmar, 2009).

The mechanism of action of aPDT is based on the combination of three nontoxic components: visible light, oxygen and a photosensitizer (PS). Visible light with the appropriate wavelength excites the PS molecule to a high-energy electronic state. This excited state reacts with the oxygen molecules nearby producing reactive oxygen species (ROS) such as singlet oxygen, superoxide, and radicals (Demidova &

Hamblin, 2005). These cytotoxic compounds produced can oxidize many biomolecules, such as proteins, lipids, and nucleic acids causing cell death (Almeida, Faustino, & Tomé, 2015; Brancini et al., 2016; Tonani et al., 2018; Žudyte & Lukšiene, 2019).

aPDT has been known since the mid-1900s as a treatment capable to reduce microbial counts after light exposition in the presence of dyes (Wainwright, 1998). Some studies have investigated the effect of different classes of PS as well light source on the reduction of microbial contamination (Buchovec, Vaitonis, & Luksiene, 2009; Demidova & Hamblin, 2005; Le Marc, Buchovec, George, Baranyi, & Luksiene, 2009; Oliveira et al., 2009). It has been demonstrated the efficiency of aPDT with phenothiazinium dyes, such as toluidine blue O (TBO), methylene blue (MB) and new methylene blue (NMB), against *B*. *cereus* spores and vegetative cells (Demidova & Hamblin, 2005). Therefore, *B. cereus* comprises an interesting microorganism to be employed as a model for aPDT.

The current work aimed to evaluate the efficiency of aPDT using NMB and red light against *B. cereus* vegetative cells and spores from different sources. Also, this study also estimated the aPDT inactivation kinetics for both forms of selected strains using the Weibull model. Also, when possible the fluence for 4D (fluence needed for four decimal reductions) was calculated.

2. Material and methods

2.1 Bacterial strains, cultivation and preparation of spore suspensions

All strains of *B. cereus* (n = 12) used in this study were obtained from Fundação Oswaldo Cruz (Rio de Janeiro, Brazil). The strains used were isolated from the following sources: ATCC 14579 – ***"Standard strain"***; 432 and 436 – ***"Chocolate"***; 511, 512, 540 – ***"Dairy products"***; B3 and B94 – ***"Milk"***; B18 – ***"Starch"***; B51 – ***"Meat"***; B63 – ***"Ready meal"***; and B86 – ***"Corn flour"***. Cells were grown in nutrient broth (Kasvi, Italy) at 30 °C for 48 h, after centrifugation (Sorvall Legend XTR, Thermo Fisher Scientific, Waltham, MI, USA) the pure cells were stored at – 80 °C (Revco EXF, Thermo Fisher Scientific, Marietta, OH, USA) in cryotubes with 20% (w/w) glycerol until further use.

The spore suspensions were prepared according to Pflug (1999) and confirmed by Alvarenga et al., 2018. Briefly, roux bottles were filled with approximately 200 mL of nutrient agar (Kasvi, Italy) supplemented with manganese sulfate (10 ppm) (Synth, Diadema, Brazil). After inoculation, the roux bottles were incubated at 30 °C for 30 days. The sporulation progress was frequently microscopically observed with malachite green staining to evaluate the spore formation. After such a period of incubation, the suspensions were gently collected scraping the agar surface with sterile deionized water followed by centrifugation (1500 x *g* for 20 min at 4 °C). After five rounds of centrifugation, the spore suspensions were resuspended in sterile deionized water and heat-shocked (80 °C for 30 min) to kill any vegetative cells and enumerate the initial concentration of the spores and, finally, stored at – 20 °C until further use.

2.2 Photosensitizer

The phenothiazinium dye new methylene blue N zinc chloride double salt (NMB; Fig. 1) was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). A stock solution at the concentration of 500 µM was prepared in phosphate-buffered saline (PBS; pH

7.4) and stored in the dark at −20 °C. Dilutions were prepared with PBS on the same day of the experiments.

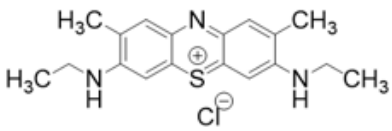

Figure 1. Chemical structure of the new methylene blue (NMB) used as photosensitizer in the study.

2.3 Light source

Light was provided by an in-house-made array of 96 red light-emitting diodes (red LED96) with an emission peak at 631 nm. The measured irradiance from 400 to 700 nm was 24.50 mW/cm^2. Light measurements were performed according to de Menezes et al., 2016 using a cosine-corrected irradiance probe (CC-3-UV, Ocean Optics, Dunedin, FL, USA) screwed onto the end of an optical fiber coupled to an USB4000 spectroradiometer (Ocean Optics, Dunedin, FL, USA). Light intensity was measured inside the well to reduce external interference. The choice of this light source was based on previous results obtained by the application of phenothiazinium photosensitizers and red light (Rodrigues et al 2012). The emission spectrum for the light source can be seen in a previous publication (Rodrigues et al 2012).

The fluences provided by the red LED96 calculated by the Eq. 1:

$$\mathbf{f} = \mathbf{I} \times \mathbf{t} \qquad Eq.(1)$$

where, $\mathbf{f}$ = fluence in J/cm^2, $\mathbf{I}$ = irradiance in W/cm^2, and $\mathbf{t}$ = time of irradiation in s.

2.4 Antimicrobial photodynamic treatment

2.4.1 Evaluation of aPDT efficacies on vegetative cells of *B. cereus* based on the photosensitizer minimum inhibitory concentration (MIC)

The efficacy of aPDT with NMB combined with different fluences on vegetative cells of 12 strains of *B. cereus* was evaluated by determining the MIC of the PS at each fluence as previously described (Rodrigues, Ferreira, Wainwright, & Braga, 2012). Briefly, 50 µL of the bacterial cell suspension (≈ 10^5 CFU/mL) and 50 µL of NMB were added to each well of sterile 96-well flat-bottomed plates (NEST Biotechnology, China). The final concentrations of NMB were 0, 1, 2.5, 5, 10, 12.5, 25, 50, 75, 100, and 200 µM. Plates were kept in the dark for 30 min at 30 °C and exposed to light fluences of 4.41 (3 min), 8.82 (6 min), 13.23 (9 min), and 22.05 J/cm^2 (15 min) using the red LED96 array as the light source (irradiance of 24.5 mW/cm^2) or kept in the dark. The light and dark controls were performed to determine the effects of the light and NMB separately. After the exposures, 100 µL of nutrient broth (Kasvi, Italy) was added to each well followed the incubation of the plates at 30 °C for 48 h. MIC was considered the lowest PS concentration, for each fluence, which inhibited the visible growth. Two independent experiments were performed in quadruplicate.

2.4.2 aPDT resistance of vegetative cells and spores of selected *B. cereus* strains

Vegetative cells from selected *B. cereus* strains (B3, B63,436, and ATCC 14579) were grown overnight in nutrient broth (Kasvi, Italy) at 30 °C and cell concentration was adjusted to $10^{6–8}$ CFU/mL in PBS using a McFarland turbidimeter (MS Tecnopon, Brazil). Spore suspensions of the selected strains at 10^7 spores/mL were prepared as previously described. Experiments were performed in 12-well flat-bottomed plates (NEST Biotechnology, China). Five mL of cell or spore suspensions and the solution of NMB were added to each well. The final concentrations of NMB were 5, 50, and 100 µM, which were selected based on previous MIC experiments.

Plates were kept in the dark for 30 min at 30 °C before light exposition (24.5 mW/cm^2). Vegetative cells and spores were illuminated for up to 120 min and 300 min, respectively. Aliquots of 100 µL were serial diluted and the counts (CFU/mL) were determined by drop-plating onto nutrient agar (Kasvi, Italy) followed by overnight incubation at 30 °C. Three independent experiments were performed.

2.5 Modelling of aPDT inactivation data

Survival curves were obtained by plotting the logarithmical population counts (log CFU/mL or spores/mL) versus the fluence (J/cm^2). The inactivation data were analyzed by GInaFiT Excel® add-in according to Geeraerd, Valdramidis, & Van Impe, 2005.

The Weibull model (Mafart, Couvert, Gaillard, & Leguerinel, 2002) was used with modifications according to Izquier & Gómez-López, 2011 (Eq. 2):

$$log_{10}N(t) = log_{10}N(0) - \left(\frac{f}{\delta}\right)^p \qquad Eq\ (2)$$

where *N*(t) (CFU/mL or spores/mL) is the number of survivors at referred time, *N*(0) (CFU/mL or spores/mL) is the initial population of vegetative cells or spores, *f* is the fluence (J/cm^2), δ (J/cm^2) is the fluence needed for the first decimal reduction, and *p* is the shape parameter (dimensionless) (Mafart et al., 2002).

2.6 Statistical analysis

All graphics and statistics were made using GraphPad Prism 6 (GraphPad Software, USA). To evaluate the differences between the conditions tested the data were submitted to analysis of variance (ANOVA) followed by post-hoc Tukey test. *P* values of <0.05 were considered significant. A cluster analysis was performed using Ward's algorithm of the ape package in software R, determining the Euclidean distance (Alvarenga et al., 2018).

3. Results and discussion

Given the importance of *B. cereus* commonly associated with foodborne illnesses, being also able to spoil food products (Mehta et al., 2019) in the present study were evaluated 12 strains from different sources for their resistance to aPDT. The efficacy of aPDT was firstly evaluated determining the MIC for NMB at different fluences (4.41, 8.82, 13.23, and 22.05 J/cm^2). Exposure to red light in the absence of the PS did not inhibit the growth of any strain independent of the fluence used (data not shown). As well as the dark control of the NMB at concentrations up to 200 µM did not inhibit the growth of any strain. The MIC of the NMB for all the fluences is depicted in Table 1. Only after 6 min of exposure corresponding to 8.82 J/cm^2 was possible to determine the MIC for all strains. The MIC varied among strains and for most of them was possible to observe a decrease while fluence increases. The strain B63 was less susceptible to all fluences. The strains 432, B3, and B86 showed the same survival at fluences 4.41, 8.82, and 22.05 J/cm^2. These strains survived in 200, 50, and 10 µM of NMB in the respective fluences. The strains B18, B51, B94, and ATCC 14579 (standard) presented the same MIC (25 µM) at 8.82 J/cm^2.

Table 1. Minimal inhibitory concentration of the NMB at concentrations from 0 to 200 µM illuminated by an array of 96 red light-emitting diodes (LED) at different fluences (J/cm^2).

Strains	Source	Fluences (J/cm^2)				
		(Dark control)	4.41	8.82	13.23	22.05
432	Chocolate	>200	200	50	25	10
436		>200	>200	75	25	10
511	Dairy products	>200	>200	200	25	10
512		>200	>200	100	25	75
540		>200	75	25	10	5
B3	Milk	>200	200	50	50	10
B94		>200	200	25	12.5	75
B18	Starch	>200	200	25	25	5
B51	Meat	>200	200	25	10	10
B63	Ready meal	>200	200	200	100	200
B86	Corn flour	>200	200	50	50	10
ATCC 14579	Standard	>200	200	25	75	12.5

According to the cluster analysis of such strains, two main groups could be observed based on their MIC (Fig. 2). The strains B63 (ready meal) and 511 (dairy products) were considered the less susceptible strains. These strains survived in a concentration of 200 µM at fluences of 22.05 and 8.82 J/cm^2, respectively. The second group is formed by four subgroups. In the first subgroup (less susceptible) was composed by the strains B3 (milk), B86 (corn flour), and ATCC 14579 (standard strain) from different sources. These strains survived in a concentration of 200 µM at a fluence of 4.41 J/cm^2. The second subgroup, composed by the strains 432 and 436 (chocolate) presenting the same MIC of 200 and 25 µM using fluences of 4.41 and 13.23 J/cm^2, respectively. The third subgroup, composed of the strains B18 (starch), B51(meat), and 540 (dairy products) presented the same MIC of 25 µM at fluences of 8.82 J/cm^2. The fourth subgroup, B94 (milk) and 512 (dairy products) composed by the strains were considered the most susceptible. These strains survived in concentrations of 25 and 12.5 µM, respectively, at a fluence of 13.23 J/cm^2. The heterogeneity of the strains with different levels of aPDT resistance revealed a significative variability among the strains. This behavior was previously described by Alvarenga et al., 2018 with the same strains.

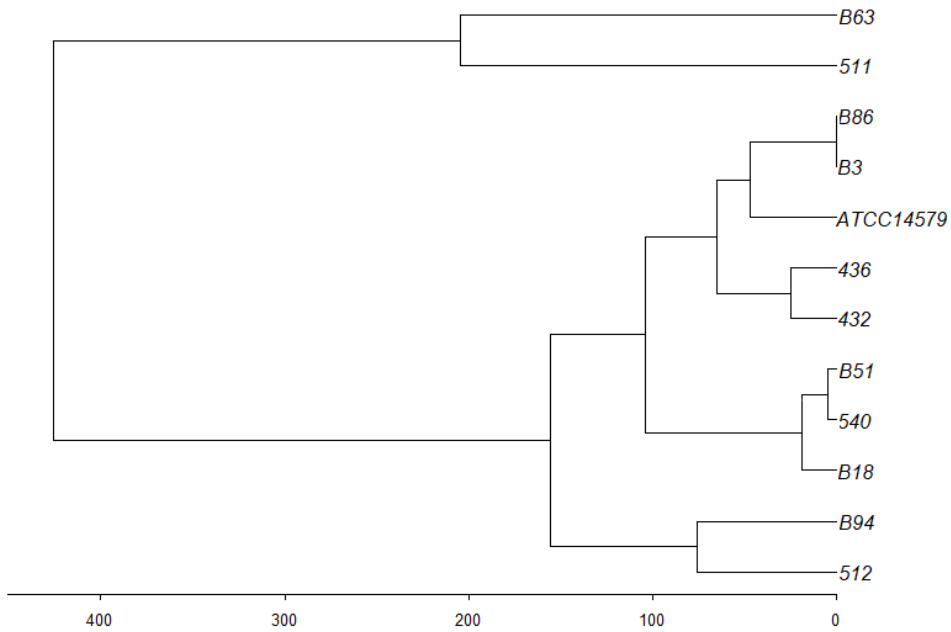

Figure 2. Cluster analysis based on the MIC of *B. cereus* vegetative cells.

From the MIC results and the cluster analysis, 4 strains (B3, 436, B63, ATCC 14579) were selected according to their aPDT resistance. For such strains, the aPDT with NMB and red light was conducted with vegetative cells and spores. The inactivation curves are presented as the log (N/N_0) of each strain, to avoid small variation in the initial populations, as a function of the fluence (J/cm^2).

As presented in Fig. 3, Fig. 4, the inactivation data of *B. cereus* vegetative cells and spores did not follow a log-linear inactivation in most cases. Therefore, the Weibull model with modifications (Izquier & Gómez-López, 2011) was used to fit inactivation data and estimate the aPDT inactivation kinetic parameters. From the Weibull model, it is possible to determine the *δ*-value which was initially described by Mafart et al., (2002) as the time for first decimal reduction and adapted by Izquier & Gómez-López (2011) as the fluence (J/cm^2) needed for the first decimal reduction. Also, the Weibull model provides the *p*-value (shape parameter) which contributes to the understanding of microbial behavior. The R^2 obtained were higher than 0.87, indicating a good fit of the Weibull model to the data.

Fig. 3 shows the inactivation curves of the strains *B. cereus* vegetative cells submitted to aPDT. As expected, the less susceptible strain (B63; Fig. 3B) showed the lowest reduction in the viability at concentrations of 50 and 100 µM compared to the other strains ($P < 0.05$). At the same concentrations (50 and 100 µM), strains B3 (Fig. 3A), 436 (Fig. 3C), and ATCC 14579 (Fig. 3D) showed 4 and 5 log CFU/mL reductions when exposed to fluences of 73.50 and 88.20 J/cm^2, respectively. For the most susceptible strain (ATCC 14579), it was only necessary 29.40 J/cm^2, corresponding to 20 min of exposure to light, and 5 µM of NMB to achieve around 4 log CFU/mL reductions in cell viability. In previous studies, the viability of *B. cereus* was reduced by 4.4 log CFU/mL reductions after aPDT with the PS hypericin and approximately 40 min of light exposure (9.2 J/cm^2) (Aponiene, Paskeviciute, Reklaitis, & Luksiene, 2015). The use of 5-aminolevulinic acid (ALA) as a precursor of endogenous PSs at 3 and 7.5 mmol/L reduced the viability of *B. cereus* from 4 to 6 log CFU/mL reductions (Le Marc et al., 2009). According to the authors, the efficiency of the aPDT was dependent on the fluence in agreement with the observed by the present study.

The spore inactivation required higher fluences than vegetative cells, which is expected given the high resistance structure, namely exosporium, developed during the sporulation process (Gerhardt & Ribi, 1964; Sanchez-Salas, Setlow, Zhang, Li, & Setlow, 2011). Nevertheless, during the spore inactivation of the most sensitive strain (ATCC 14579; Fig. 4D), it was necessary the same fluence as for vegetative cells (29.40 J/cm^2) to achieve around 4 log spores/mL reductions. However, the concentration required of NMB was higher (50 µM) than used for vegetative cells. A small difference between vegetative cells and spores of *B. cereus* in terms of PS concentration was previously cited by Demidova & Hamblin, 2005. The same authors also discussed the differential sensitivity of spores from*, B. subtilis, B. atrophaeus, and B. megaterium* (Demidova & Hamblin, 2005). A huge difference in terms of aPDT resistance was detected between *B. cereus/B. thuringiensis* and *B. megaterium* which the authors attribute to a structural difference in the spores. The exosporium present in the species *B. cereus/B. thuringiensis* and not in the *B. megaterium* can contribute to the accumulation of PS, allowing the diffusion of the dye inside the spore (Demidova & Hamblin, 2005).

The strains B3, B63, and 436 (Fig. 4 A-B-C, respectively) presented similar inactivation curves at all NMB concentrations and fluences. Such less susceptible behavior of these strains was also detected during the spray drying process, where the strain B63 was classified with intermediate resistance and the strains 436 and B3 as less susceptible to the process (Alvarenga et al., 2018). The same authors explored the differences in heat stress tolerance among different *B. cereus* strains at the molecular level with proteomic analysis. The results indicated that the observed variability between *B. cereus* strains could be related to spore coat protein expression (Alvarenga et al., 2018). So far there are no studies in the literature that can explain the variability between *B. cereus* strains related to aPDT which presents different inactivation mechanisms compared to thermal processes.

Photoinactivation of *B. cereus* spores mediated by ALA-based, porphyrin derivatives, phenothiazinium dyes, and hypericin-based PS showed significant inactivation results from approximately 2.5 to 6.0 log CFU/mL reductions (Dementavicius, Lukseviciute, Gómez-López, & Luksiene, 2016; Demidova & Hamblin,

2005; Luksiene et al., 2009; Oliveira et al., 2009). TBO is a phenothiazinium dye frequently used for PDT of bacteria and fungi (Demidova & Hamblin, 2005; Gonzales, Da Silva, Roberts, & Braga, 2010; Mahmoudi, Pourhajibagher, Alikhani, & Bahador, 2019). Since TBO had presented promising results of *B. cereus* spores inactivation, other types of phenothiazinium dyes were evaluated including NMB (Demidova & Hamblin, 2005). In that study, photoinactivation of *B. cereus* spores mediated by NMB at 50 µM achieved more than 5 log CFU/mL reductions with half of the fluence used with TBO (40 J/cm^2) (Demidova & Hamblin, 2005). It is necessary to consider the time of incubation of the PS of 3 h opposed with 30 min in our study. In addition, it is important to highlight the age of the spores significantly different with 30 days in our study and 3 days declared by Demidova & Hamblin (2005). Such discrepancy in the spore maturation may explain the difference between the present study and the literature (Dementavicius et al., 2016; Demidova & Hamblin, 2005; Luksiene et al., 2009; Oliveira et al., 2009).

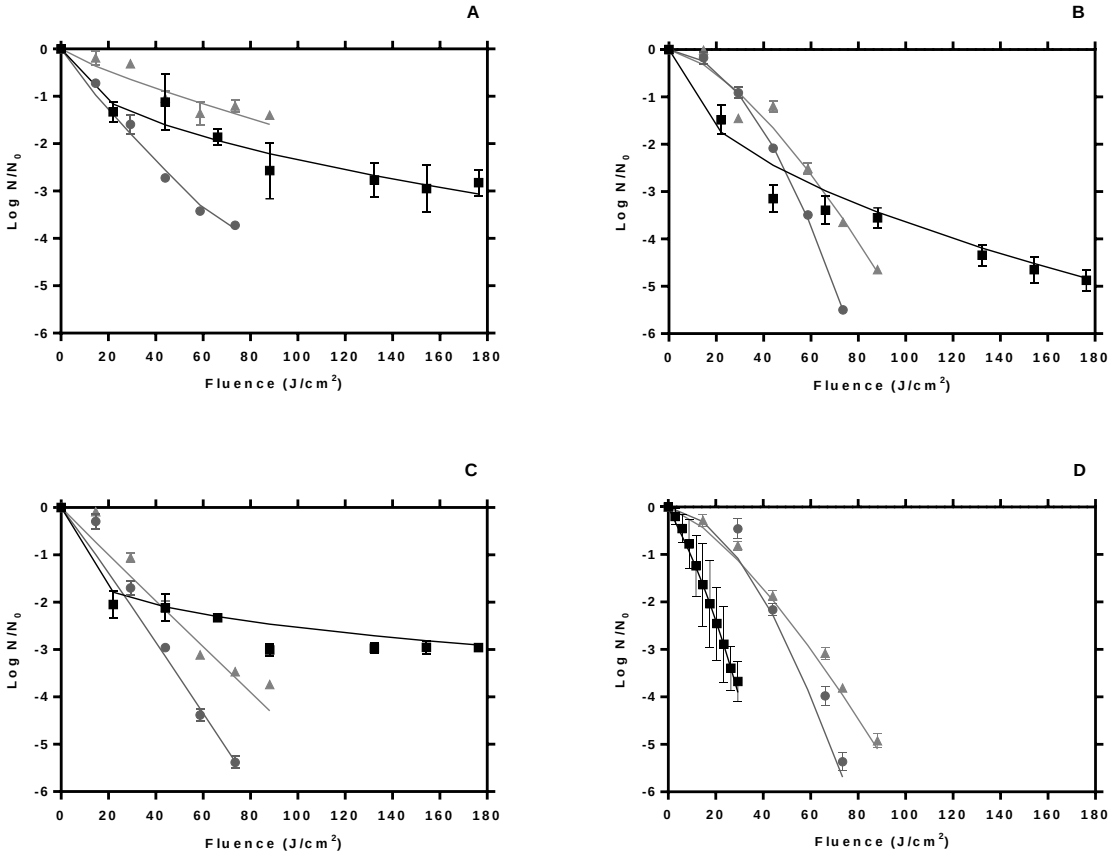

Figure 3. aPDT inactivation curves of the vegetative cells of different strains of *B. cereus* – B63 (A), 436 (B), B3 (C), and ATCC 14579 (D) – in the presence of NMB at concentrations of ■ 5, ● 50, and ▲100 μM and different fluences (J/cm^2). All data correspond to three independent experiments. —: estimated curve by the Weibull model. Error bars represent the standard deviation (SD) of three independent experiments and in some cases are hidden by the symbols.

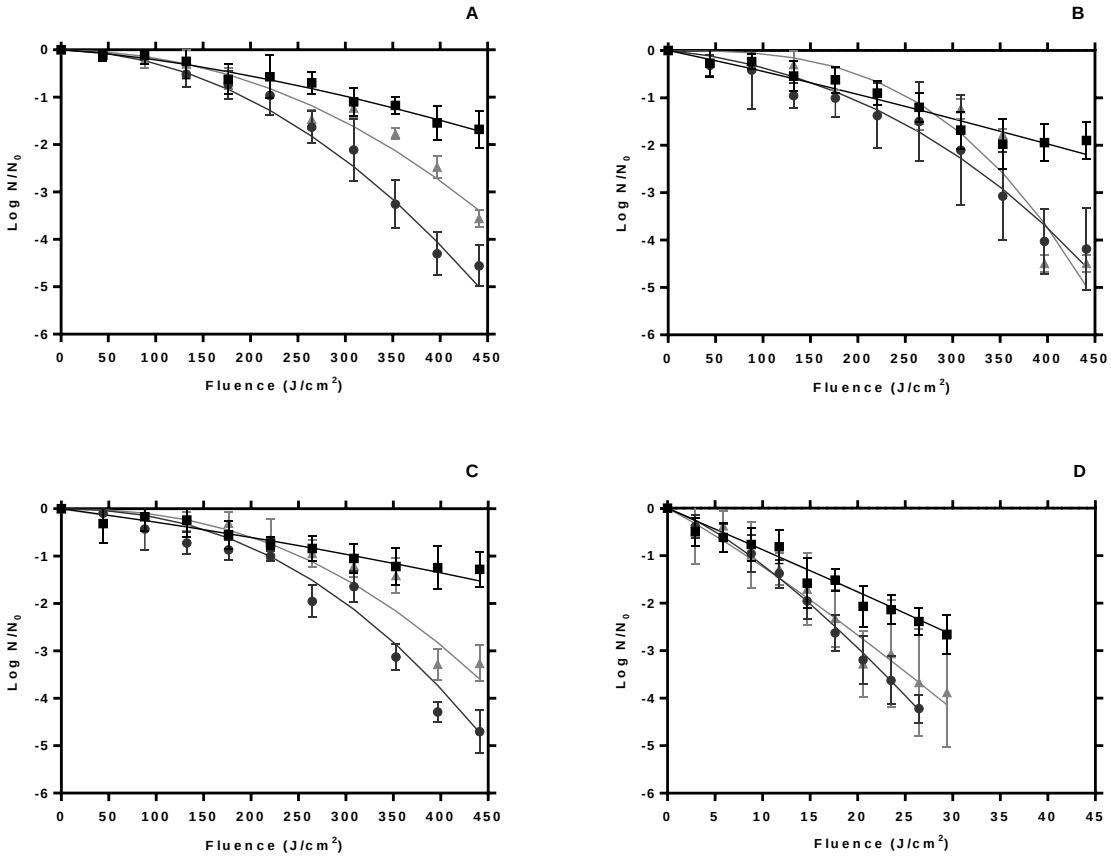

Figure 4. aPDT inactivation curves of the spores of different strains of *B. cereus* – B63 (A), 436 (B), B3 (C), and ATCC 14579 (D) – in the presence of NMB at concentrations of ■ 5, ● 50, and ▲100 μM and different fluences (J/cm^2). All data correspond to three independent experiments. —: estimated curve by the Weibull model. Error bars represent the standard deviation (SD) of three independent experiments and in some cases are hidden by the symbols.

Table 2 and 3 gives the fitted photodynamic inactivation kinetics parameters (*δ* and *p*-values) of vegetative cells and spores of the *B. cereus* strains using the Weibull model. As depicted in Table 2, the highest and smallest δ value (J/cm^2) was found for the strain B63 (50.24 ± 5.21) and B3 (0.51 ± 0.30) with NMB at 100 and 5 μM, respectively ($P < 0.05$). The highest δ values of the strains 436 and ATCC 14579 did not differ significantly at 50 and 100 μM ($P < 0.05$). Most of the lowest δ values were observed with NMB at 5 μM suggesting that the minimum concentration of NBMN binds

quickly to the cell membrane causing fast inactivation. However, according to the *p* values presented in Table 2, the strains B3, B63, and 436 become less affected as the fluence increase with NMB 5 µM. This is confirmed by Fig. 3-A-B-C which clearly shows an upward concavity ($p < 1$) of the curves. The only strain with $p > 1$ (downward concavity) for all concentrations of NMB was the ATCC 14579, which indicates that the cells are progressively killed in all conditions. The Weibull model was able to describe the photoinactivation mediated by ALA of *B. cereus* spores with linear, downward, and upward concavity shapes of the inactivation curves (Le Marc et al., 2009). In that study, the authors suggest that the incubation time and concentration of PS impact the *p*-value increasing the inactivation in the first minutes although remaining cells become less affected (Le Marc et al., 2009).

In all conditions tested for *B. cereus* spores, *p* values were > 1 (Table 3). The downward concavity ($p > 1$) indicates that the microorganisms become increasingly damaged (Van Boekel, 2002). Such behavior can be confirmed by the δ values in all conditions of the *B. cereus* spore inactivation, except in the case of the strain ATCC 14579. Among the strains B3, B63, and 436 the smallest and highest δ values (196.23 ± 32.27 and 296.88 ± 66.86) were observed for B63 and B3 with NMB at 50 and 5 µM, respectively. δ values for the strain ATCC 14579 were significantly different in all conditions ($P > 0.05$). Also, the fluence of four decimal reductions (4D) for all strains tested to come up to 400 J/cm^2 at 50 µM of NMB, except for the strain ATCC 14579 with 25 J/cm^2 at 50 and 100 µM.

Table 2. Photoinactivation kinetic parameters of aPDT obtained from Weibull modeling for *B. cereus* vegetative cells.

Strains	NMB (µM)	δ (mean ± SD) (J/cm^2)	*p*-value (mean ± SD)	4D (mean ± SD) (J/cm^2)	R^2
B3	5	0.51 ± 0.30$^{c\ C}$	0.21 ± 0.06$^{c\ B}$	ND	0.96
	50	14.81 ± 0.62$^{b\ C}$	1.05 ± 0.02$^{a\ C}$	56.01 ± 1.45$^{b\ D}$	0.98
	100	20.17 ± 1.15$^{a\ C}$	0.99 ± 0.03$^{b\ C}$	82.61 ± 1.33$^{a\ A}$	0.96
B63	5	16.01 ± 5.15$^{b\ A}$	0.46 ± 0.09$^{b\ B}$	ND	0.90
	50	15.32 ± 1.36$^{b\ C}$	0.89 ± 0.05$^{a\ B}$	72.76 ± 0.46 ^{D}	0.98
	100	50.24 ± 5.21$^{a\ A}$	0.83 ± 0.13$^{a\ D}$	ND	0.87
436	5	7.67 ± 3.75$^{b\ B}$	0.49 ± 0.06$^{c\ B}$	121.42 ± 14.82$^{a\ A}$	0.96
	50	30.50 ± 0.29$^{a\ A}$	1.94 ± 0.01$^{a\ A}$	62.60 ± 0.55$^{c\ B}$	0.99
	100	31.62 ± 1.16$^{a\ B}$	1.51 ± 0.04$^{b\ A}$	79.38 ± 0.92$^{b\ B}$	0.97
ATCC 14579	5	10.54 ± 5.53$^{b\ AB}$	1.40 ± 0.57$^{a\ A}$	26.46 ± 0.00$^{c\ B}$	0.99
	50	28.21 ± 1.70$^{a\ B}$	1.84 ± 0.11$^{a\ A}$	60.39 ± 1.18$^{b\ C}$	0.98
	100	27.05 ± 1.62$^{a\ B}$	1.38 ± 0.08$^{a\ B}$	74.68 ± 0.72$^{a\ C}$	0.99

δ: fluence needed for the first decimal reduction
p-value: shape parameter (dimensionless)
4D: fluence needed to achieve four log reductions
R^2: determination coefficient
Different lowercase letters in the same column within each strain (B3, B63, 436, and ATCC 14579) indicate significant difference in inactivation kinetic parameters ($P < 0.05$) according to one-way ANOVA followed by post-hoc Tukey test
Different uppercase letters in the same column within each concentration of NMB (5, 50, and 100 µM) indicate significant difference in inactivation kinetic parameters ($P < 0.05$) according to one-way ANOVA followed by post-hoc Tukey test

Table 3. Photoinactivation kinetic parameters of aPDT obtained from Weibull modeling of *B. cereus* spores.

Strains	NMB (µM)	δ (mean ± SD) (J/cm^2)	*p*-value (mean ± SD)	4D (mean ± SD) (J/cm^2)	R^2
B3	5	296.88 ± 66.86$^{a\ A}$	1.25 ± 0.37$^{b\ AB}$	ND	0.94
	50	219.61 ± 12.05$^{b\ A}$	2.24 ± 0.27$^{a\ A}$	413.07 ± 10.10 ^{A}	0.95
	100	251.67 ± 11.42$^{ab\ A}$	2.29 ± 0.20$^{a\ B}$	ND	0.91
B63	5	309.07 ± 56.83$^{a\ A}$	1.50 ± 0.32$^{b\ A}$	ND	0.88
	50	196.23 ± 32.27$^{b\ A}$	2.02 ± 0.38$^{a\ A}$	395.92 ± 18.55 ^{A}	0.98
	100	243.54 ± 27.57$^{b\ A}$	2.10 ± 0.39$^{a\ B}$	ND	0.91
436	5	222.57 ± 42.78$^{a\ B}$	1.09 ± 0.18$^{c\ B}$	ND	0.89
	50	202.66 ± 69.15$^{a\ A}$	2.13 ± 0.89$^{b\ A}$	400.05 ± 36.01$^{a\ A}$	0.94
	100	255.20 ± 14.10$^{a\ A}$	2.95 ± 0.32$^{a\ A}$	411.13 ± 1.93$^{a\ A}$	0.90
ATCC 14579	5	11.57 ± 2.50$^{a\ C}$	1.04 ± 0.18$^{b\ B}$	ND	0.92
	50	8.87 ± 1.70$^{b\ B}$	1.33 ± 0.20$^{a\ B}$	24.96 ± 1.15$^{a\ B}$	0.98
	100	8.56 ± 1.59$^{b\ B}$	1.11 ± 0.21$^{ab\ C}$	25.09 ± 0.70$^{a\ B}$	0.93

δ: fluence needed for the first decimal reduction
p-value: shape parameter (dimensionless)
4D: fluence needed to achieve four log reductions
R^2: determination coefficient
Different lowercase letters in the same column within each strain (B3, B63, 436, and ATCC 14579) indicate significant difference in inactivation kinetic parameters ($P < 0.05$) according to one-way ANOVA followed by post-hoc Tukey test
Different uppercase letters in the same column within each concentration of NMB (5, 50, and 100 µM) indicate significant difference in inactivation kinetic parameters ($P < 0.05$) according to one-way ANOVA followed by post-hoc Tukey test

The spore inactivation kinetic parameters (especially, δ value) of the strain ATCC 14579 (Table 3) confirm that this microorganism has low resistance to the aPDT compared to the other strains.

This study evaluated for the first time the effect of aPDT against *B. cereus* strains from different sources. The use of aPDT with NMB and the red light was able to reduce *B. cereus* in both forms (vegetative cells and spores). The variability among strains of *B. cereus* represents a major challenge for food safety. Also, the results of this study indicated that the strain ATCC 14579, widely used as the standard for thermal processing, was the most susceptible to aPDT. Further studies are needed to explain how the variability among strains of the same species occurs. The Weibull model successfully described a non-log-linear photoinactivation of vegetative cells and spores of *B. cereus* as well as the estimation of the kinetic parameters. The mathematical modelling can be a useful tool to determine the ideal conditions for photodynamic inactivation and assist a feasible implementation of aPDT by the industry.

Acknowledgment

This work was supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Grants #140092/2017-0, #302763/2014-7, and #305804/2017-0); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 001 for the financial support.

Conflict of interest

The authors declare no competing interests.

4. References

Almeida, A., Faustino, M. A. F., & Tomé, J. P. C. (2015). Photodynamic inactivation of bacteria: finding the effective targets. *Future Medicinal Chemistry*, *7*(10), 1221–1224. https://doi.org/10.4155/fmc.15.59

Alvarenga, V. O., Brancini, G. T. P., Silva, E. K., da Pia, A. K. R., Campagnollo, F. B., Braga, G. Ú. L., Hubinger, M. D., & Sant'Ana, A. S. (2018). Survival variability of 12 strains of *Bacillus cereus* yielded to spray drying of whole milk. *International Journal of Food Microbiology*, *286*, 80–89. https://doi.org/10.1016/j.ijfoodmicro.2018.07.020

Aponiene, K., Paskeviciute, E., Reklaitis, I., & Luksiene, Z. (2015). Reduction of microbial contamination of fruits and vegetables by hypericin-based photosensitization: Comparison with other emerging antimicrobial treatments. *Journal of Food Engineering*, *144*, 29–35. https://doi.org/10.1016/j.jfoodeng.2014.07.012

Barba, F. J., Koubaa, M., Prado-Silva, L., Orlien, V., & Sant'Ana, A. S. (2017). Mild processing applied to the inactivation of the main foodborne bacterial pathogens: A review. *Trends in Food Science & Technology, 66*, 20–35. https://doi.org/10.1016/j.tifs.2017.05.011

Bottone, E. J. (2010). *Bacillus cereus*, a volatile human pathogen. *Clinical Microbiology Reviews*, *23*(2), 382–398. https://doi.org/10.1128/CMR.00073-09

Brancini, G. T. P., Rodrigues, G. B., Rambaldi, M. D. S. L., Izumi, C., Yatsuda, A. P., Wainwright, M., Rosa, J.C., & Braga, G. Ú. L. (2016). The effects of photodynamic treatment with new methylene blue N on the: *Candida albicans* proteome. *Photochemical and Photobiological Sciences*, *15*(12), 1503–1513. https://doi.org/10.1039/c6pp00257a

Buchovec, I., Vaitonis, Z., & Luksiene, Z. (2009). Novel approach to control *Salmonella enterica* by modern biophotonic technology: Photosensitization. *Journal of Applied Microbiology*, *106*(3), 748–754. https://doi.org/10.1111/j.1365-2672.2008.03993.x

CDC. (2020). Food safety. Center for Disease Control and Prevention. Retrieved February 23, 2020, from https://www.cdc.gov/foodsafety/index.html

de Menezes, H. D., Tonani, L., Bachmann, L., Wainwright, M., Braga, G. Ú. L., & von

Zeska Kress, M. R. (2016). Photodynamic treatment with phenothiazinium photosensitizers kills both ungerminated and germinated microconidia of the pathogenic fungi *Fusarium oxysporum*, *Fusarium moniliforme* and *Fusarium solani*. *Journal of Photochemistry and Photobiology B: Biology*, *164*, 1–12. https://doi.org/10.1016/j.jphotobiol.2016.09.008

Dementavicius, D., Lukseviciute, V., Gómez-López, V. M., & Luksiene, Z. (2016). Application of mathematical models for bacterial inactivation curves using Hypericin-based photosensitization. *Journal of Applied Microbiology*, *120*(6), 1492–1500. https://doi.org/10.1111/jam.13127

Demidova, T. N., & Hamblin, M. R. (2005). Photodynamic inactivation of *Bacillus* spores, mediated by phenothiazinium dyes. *Applied and Environmental Microbiology*, *71*(11), 6918–6925. https://doi.org/10.1128/AEM.71.11.6918

Geeraerd, A. H., Valdramidis, V. P., & Van Impe, J. F. (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, *102*(1), 95–105. https://doi.org/10.1016/j.ijfoodmicro.2004.11.038

Gerhardt, P., & Ribi, E. (1964). Ultrastructure Of The Exosporium Enveloping Spores Of *Bacillus cereus*. Journal of Bacteriology, 88(6), 1774–1789. https://doi.org/10.1128/jb.88.6.1774-1789.1964

Gonzales, F. P., Da Silva, S. H., Roberts, D. W., & Braga, G. U. L. (2010). Photodynamic inactivation of conidia of the fungi *Metarhizium anisopliae* and *Aspergillus nidulans* with methylene blue and toluidine blue. *Photochemistry and Photobiology*, *86*(3), 653–661. https://doi.org/10.1111/j.1751-1097.2009.00689.x

Gonzales, J. C., Brancini, G. T. P., Rodrigues, G. B., Silva-Junior, G. J., Bachmann, L., Wainwright, M., & Braga, G. Ú. L. (2017). Photodynamic inactivation of conidia of the fungus *Colletotrichum abscissum* on *Citrus sinensis* plants with methylene blue under solar radiation. *Journal of Photochemistry and Photobiology B: Biology*, *176*, 54–61. https://doi.org/10.1016/j.jphotobiol.2017.09.008

Heyndrickx, M. (2011). The importance of endospore-forming bacteria originating from soil for contamination of industrial food processing. *Applied and Environmental Soil Science*, *2011*, 1–11. https://doi.org/10.1155/2011/561975

Izquier, A., & Gómez-López, V. M. (2011). Modeling the pulsed light inactivation of microorganisms naturally occurring on vegetable substrates. *Food Microbiology*, *28*(6), 1170–1174. https://doi.org/10.1016/j.fm.2011.03.010

Le Marc, Y., Buchovec, I., George, S. M., Baranyi, J., & Luksiene, Z. (2009). Modelling the photosensitization-based inactivation of *Bacillus cereus*. *Journal of Applied Microbiology*, *107*(3), 1006–1011. https://doi.org/10.1111/j.1365-2672.2009.04275.x

Luksiene, Z., Buchovec, I., & Paskeviciute, E. (2009). Inactivation of food pathogen *Bacillus cereus* by photosensitization in vitro and on the surface of packaging material. *Journal of Applied Microbiology*, *107*(6), 2037–2046. https://doi.org/10.1111/j.1365-2672.2009.04383.x

Mafart, P., Couvert, O., Gaillard, S., & Leguerinel, I. (2002). On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. *International Journal of Food Microbiology*, *72*, 107–113. https://doi.org/10.17660/ActaHortic.2001.566.11

Mahmoudi, H., Pourhajibagher, M., Alikhani, M. Y., & Bahador, A. (2019). The effect of antimicrobial photodynamic therapy on the expression of biofilm associated genes in *Staphylococcus aureus* strains isolated from wound infections in burn patients. *Photodiagnosis and Photodynamic Therapy*, *25*, 406–413. https://doi.org/10.1016/j.pdpdt.2019.01.028

Mehta, D. S., Metzger, L. E., Hassan, A. N., Nelson, B. K., & Patel, H. A. (2019). The ability of spore formers to degrade milk proteins, fat, phospholipids, common stabilizers, and exopolysaccharides. *Journal of Dairy Science*, *102*(12), 10799–10813. https://doi.org/10.3168/jds.2019-16623

Oliveira, A., Almeida, A., Carvalho, C. M. B., Tomé, J. P. C., Faustino, M. A. F., Neves, M. G. P. M. S., … Cunha, A. (2009). Porphyrin derivatives as photosensitizers for the inactivation of Bacillus cereus endospores. *Journal of Applied Microbiology*, *106*(6), 1986–1995. https://doi.org/10.1111/j.1365-2672.2009.04168.x

Ölmez, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact.

LWT - Food Science and Technology, *42*(3), 686–693. https://doi.org/10.1016/j.lwt.2008.08.001

Rodrigues, G. B., Ferreira, L. K. S., Wainwright, M., & Braga, G. U. L. (2012). Journal of Photochemistry and Photobiology B : Biology Susceptibilities of the dermatophytes Trichophyton mentagrophytes and T . rubrum microconidia to photodynamic antimicrobial chemotherapy with novel phenothiazinium photosensitizers and red light. *Journal of Photochemistry & Photobiology, B: Biology*, *116*, 89–94. https://doi.org/10.1016/j.jphotobiol.2012.08.010

Sanchez-Salas, J. L., Setlow, B., Zhang, P., Li, Y. Q., & Setlow, P. (2011). Maturation of released spores is necessary for acquisition of full spore heat resistance during Bacillus subtilis sporulation. *Applied and Environmental Microbiology*, *77*(19), 6746–6754. https://doi.org/10.1128/AEM.05031-11

Spanu, C. (2016). Sporeforming bacterial pathogens in ready-to-eat dairy products. In *Food Hygiene and Toxicology in Ready-to-Eat Foods*. https://doi.org/10.1016/B978-0-12-801916-0.00015-7

Tonani, L., Morosini, N. S., Dantas de Menezes, H., Nadaletto Bonifácio da Silva, M. E., Wainwright, M., Leite Braga, G. Ú., & Regina von Zeska Kress, M. (2018). In vitro susceptibilities of Neoscytalidium spp. sequence types to antifungal agents and antimicrobial photodynamic treatment with phenothiazinium photosensitizers. *Fungal Biology*, *122*(6), 436–448. https://doi.org/10.1016/j.funbio.2017.08.009

Uchida, R., & Silva, F. V. M. (2017). *Alicyclobacillus acidoterrestris* spore inactivation by high pressure combined with mild heat: Modeling the effects of temperature and soluble solids. *Food Control*, *73*, 426–432. https://doi.org/10.1016/j.foodcont.2016.08.034

Van Boekel, M. A. J. S. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, *74*(1–2), 139–159. https://doi.org/10.1016/S0168-1605(01)00742-5

Wainwright, M. (1998). Photodynamic antimicrobial chemotherapy (PACT). *Journal of Antimicrobial Chemotherapy*, *42*(1), 13–28. https://doi.org/10.1093/jac/42.1.13

WHO. (2015). World Health Organization. WHO estimates of the global burden of foodborne diseases. Retrieved July 25, 2020, from https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf?sequence=1

Žudyte, B., & Lukšiene, Ž. (2019). Toward better microbial safety of wheat sprouts: Chlorophyllin-based photosensitization of seeds. *Photochemical and Photobiological Sciences*, *18*(10), 2521–2530. https://doi.org/10.1039/c9pp00157c

Capítulo 3 - Antimicrobial photodynamic treatment as an alternative approach for Alicyclobacillus acidoterrestris inactivation

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Original research paper published in the ***International Journal of Food Microbiology – Elsevier.***

https://doi.org/10.1016/j.ijfoodmicro.2020.108803

Abstract

Alicyclobacillus acidoterrestris is a cause of major concern for the orange juice industry due to its thermal and chemical resistance, as well as its spoilage potential. *A. acidoterrestris* spoilage of orange juice is due to off-flavor taints from guaiacol production and some halophenols. The present study aimed to evaluate the effectiveness of antimicrobial photodynamic treatment (aPDT) as an emerging technology to inactivate the spores of *A. acidoterrestris*. The aPDT efficiency towards *A. acidoterrestris* was evaluated using as photosensitizers the tetracationic porphyrin (Tetra-Py^+-Me) and the phenothiazinium dye new methylene blue (NMB) in combination with white light-emitting diode (LED; 400-740 nm, 65-140 mW/cm^2). The spores of *A. acidoterrestris* were cultured on YSG agar plates (pH 3.7 ± 0.1) at 45 °C for 28 days and submitted to the aPDT with Tetra-Py^+-Me and NMB at 10 µM in phosphate-buffered saline (PBS) in combination with white light (140 mW/cm^2). The use of Tetra-Py^+-Me at 10 µM resulted in a 7.3 ± 0.04 log reduction of the viability of *A. acidoterrestris* spores. No reductions in the viability of this bacterium were observed with NMB at 10 µM. Then, the aPDT with Tetra-Py^+-Me and NMB at 10 µM in orange juice (UHT; pH 3.9; 11 °Brix) alone and combined with potassium iodide (KI) was evaluated. The presence of KI was able to potentiate the aPDT process in orange juice, promoting the inactivation of 5 log CFU/mL of *A. acidoterrestris* spores after 10 h of white light exposition (140 mW/cm^2). However, in the absence of KI, both photosensitizers did not promote a significant reduction in the spore viability. The inactivation of *A. acidoterrestris* spores artificially inoculated in orange peels (10^5 spores/mL) was also assessed using Tetra-Py^+-Me at 10 and 50 µM in the presence and absence of KI in combination with white light (65 mW/cm^2). No significant reductions were observed ($p < 0.05$) when Tetra-Py^+-Me was used at 10 µM, however at the highest concentration (50 µM) a significant spore reduction (≈ 2.8 log CFU/mL reductions) in orange peels was observed after 6 h of sunlight exposition (65 mW/cm^2). Although the color, total phenolic content (TPC), and antioxidant capacity of orange juice and peel (only color evaluation) seem to have been affected by light exposition, the impact on the visual and nutritional characteristics of the products remains

inconclusive so far. Besides that, the results found suggest that aPDT can be a potential method for the reduction of *A. acidoterrestris* spores on orange groves.

Key-words: aPDT; Emerging technologies; Sporeforming bacteria; Orange juice; Food spoilage; Decontamination

Research Highlights

- *Alicyclobacillus acidoterrestris* (AA) inactivation by antimicrobial photodynamic treatment (aPDT)
- AA inactivation using aPDT using two photosensitizers (PS): Tetra-Py^+-Me and NMB
- aPDT+Tetra-Py^+-Me caused 7 log CFU/mL reductions of AA under *in vitro* conditions
- aPDT+Tetra-Py^+-Me+NMB+KI caused 5 log CFU/mL reductions of AA in orange juice
- aPDT+Tetra-Py^+-Me in sunlight caused 2.8 log CFU/mL reductions of AA in orange peel

1. Introduction

With over three-quarters of global orange juice exports, Brazil is the largest producer of this important commodity (USDA, 2020), demonstrating the importance of this juice for the Brazilian economy. Given this, sustaining the market requires high-quality standards, including those related to the microbiological quality of orange juice.

Several microorganisms may represent challenges for the fruit juices industries. Among critical microorganisms impacting the microbiological quality of orange juice, spore-forming bacteria such as *Alicyclobacillus* spp. stand out. As other microbial contaminants, *Alicyclobacillus* spp. is found in the soil (Albuquerque et al., 2000; Groenewald et al., 2008, 2009; Hippchen et al., 1981; Sawaki, 2007). This bacterium is gram-variable, spore-forming, and thermo-acidophilic bacteria that emerged as spoilage bacterium of fruit juices in the 90s (Wisotzkey et al., 1992). *Alicyclobacillus* spp. spoilage of acidic products is characterized by off-flavor taints from guaiacol production and some halophenols without gas production (Siegmund and Pöllinger-Zierler, 2006). Although it is visually difficult to detect the spoilage, these compounds change the organoleptic characteristics of the products causing rejection by consumers and, consequently, economic losses for the industries (Orr and Beuchat, 2000). Through direct contact or dust, spores of *Alicyclobacillus* spp. can contaminate the peel of fruits used for juice making. Counts of this bacterium of 10^2 CFU per Kg of fruits collected directly from the trees have been reported (ABECitrus, 1999). Once this contamination occurs, the spores can survive fruit decontamination and heat treatment steps, potentially causing spoilage of finished products (Oteiza et al., 2011).

The juice industry widely uses pasteurization (≈ 95 °C) as a microbial inactivation method (Silva et al., 2015). However, depending on the pasteurization conditions, survival, and even stimulation of germination of *Alicyclobacillus* spp. spores may occur, likely resulting in juice spoilage (Gouws et al., 2005; Groenewald et al., 2008). Intense thermal treatments might also modify fruit juices' quality, nutritional, and sensorial characteristics (Evelyn et al., 2016; Uchida and Silva, 2017). Given these facts, alternative strategies to reduce the contamination in fruit surface immediately before juice extraction have been proposed (Lee et al., 2004, 2010), such as fruit washing

with disinfectants, *e.g.*, peracetic acid (Friedrich et al., 2009; Osopale et al., 2017). Despite this, this approach seems not to work effectively as spoilage problems caused by *Alicyclobacillus* spp. are frequently reported (Pornpukdeewattana et al., 2020). Additionally, *Alicyclobacillus* spp. spores have also been found in ingredients derived from fruit processing, *e.g.* flavorings (Oteiza et al., 2014), reinforcing that demand for strategies to reduce contamination before fruit processing.

Technologies with potential for fruit peel decontamination must be inexpensive, easy to apply on a large scale, and environmentally friendly. In this way, antimicrobial photodynamic treatment (aPDT) is a light-based technology with the potential for fruit peel decontamination. The antimicrobial effect of aPDT comprises the combination of three non-toxic components *per se*: a photosensitizer (PS), visible light, and molecular oxygen (Deng et al., 2016; Luksiene and Paskeviciute, 2011; Martins et al., 2018; Wainwright et al., 2017). The PS is considered one of the most critical factors of this technique and typically takes non-toxic dyes in the absence of light, like phenothiazines and porphyrins (Maisch, 2009; Oliveira et al., 2009; Wainwright et al., 2017). The activation of an appropriate PS with visible light in the presence of triplet dioxygen ($^3O_2$), allows the generation of reactive oxygen species (ROS), like singlet oxygen ($^1O_2$), responsible by the oxidation of microbial targets leading the cell death; these interactions can kill microorganisms through damage in cellular components such as nucleic acid bases (guanine and thymine), amino acids (cysteine, histidine, and tryptophan), proteins, and lipids (Almeida et al., 2015; Brancini et al., 2016; de Menezes et al., 2016; Wainwright et al., 2017; Zudyte and Luksiene, 2019).

The efficiency of aPDT using phenothiazines and porphyrins as PS against pathogenic and spoilage microorganisms (de Menezes et al., 2016; Gonzales et al., 2017; Oliveira et al., 2009) and their biofilms (Beirão et al., 2014; Castro et al., 2017; Vieira et al., 2019) have been reported. However, aPDT can be more effective against bacterial spores (negatively charged) if the PS used are positively charged porphyrins (Carvalho et al., 2007; Costa et al., 2008; Minnock et al., 2000; Oliveira et al., 2009; Tomé et al., 2007). Also, to enhance aPDT efficiency, inorganic salts such as potassium iodide (KI) have been proposed (Freire et al., 2016; Huang et al., 2018a; Santos et al., 2019; Vecchio et al., 2015; Wen et al., 2017; Yuan et al., 2020; Zhang et

al., 2015). KI has low toxicity and an accepted chemical for medical studies (Hamblin, 2017).

Therefore, the objective of the present study was to evaluate the effectiveness of aPDT against *A. acidoterrestris* spores in three matrices: phosphate buffer (PBS), orange juice, and orange peel. The treatments were performed in the presence of the tetracationic porphyrin 5,10,15,20-tetrakis(1-methylpyridinium-4-yl) porphyrin tetra-iodide (Tetra-Py^+-Me) or of new methylene blue (NMB; a phenothiazinium dye) using a white light-emitting diode (LED) to activate the PSs. The combined effect of aPDT and KI in orange juice and peel contaminated with *A. acidoterrestris* spores was also assessed. Lastly, the impact on nutritional and colorimetric characteristics of orange juice and peel after the aPDT was also determined.

2. *Materials and Methods*

2.1. Spore cultivation and growth conditions

The strain *A. acidoterrestris* (DSM2498) used in this study was obtained from the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures. The growth of viable cells was performed in two Petri plates containing yeast starch glucose agar (YSG-A; in g/L: yeast extract – 2.0 [Kasvi, Italy]; soluble starch – 2.0 [Merck, Germany]; glucose – 1.0 [Sigma, USA]; pH 3.7 ± 0.1), followed by incubation at 45 °C for up to 3 days. The cells obtained were added to 10 mL of YSG broth (YSG-B) (formulated as YSG-A without agar; pH 3.7 ± 0.1) and incubated at 45 °C for 24 h. One mL from the grown broth was spread on YSG-A Petri plates (pH 3.7 ± 0.1) supplemented with manganese sulfate at 10 ppm (Merck, Germany). After inoculation, the plates were incubated at 45 °C for 28 days as previously described (Pflug, 1999) with modifications. The sporulation progress was frequently verified through malachite green staining. After incubation, the cell mass was gently collected scraping the agar surface with sterile deionized water followed by centrifugation (1500 × *g* for 20 min at 4 °C). After five rounds of centrifugation, the spore suspension was resuspended in sterile deionized water and heat-shocked (80 °C for 10 min) to kill any vegetative cells and enumerate the initial concentration of the spores and, finally, stored at – 80 °C (Ultra Freezer MDF U73V; Sanyo, Japan) until further use. The concentration of the spore suspension was 2.0×10^8 spores/mL.

2.2. Photosensitizers

The photosensitizers (PSs; Figure 1) used in this study were: 5,10,15,20-tetrakis(1-methylpyridinium-4-yl) porphyrin tetraiodide (Tetra-Py^+-Me) and the phenothiazinium dye new methylene blue N (NMB) which were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). The porphyrin was prepared according to previously reported in the literature, and the purity was confirmed by thin-layer chromatography and ^{1}H NMR spectroscopy (Simões et al., 2016). For Tetra-Py^+-Me, a stock solution at 500 μM in dimethyl sulfoxide (DMSO) was prepared while for NMB, the stock solution at the same

concentration was prepared in phosphate-buffered saline (PBS; pH 7.4). Before each experiment, the PSs stock solutions were sonicated for 30 min at room temperature (Ultrasonic Bath, Nahita, China).

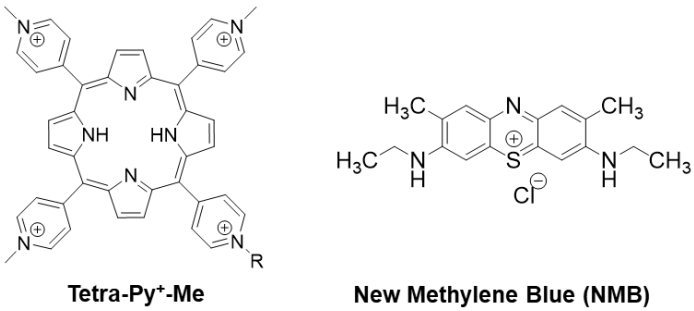

Figure 1. Chemical structures of the PSs used in this study.

2.3. Light source

The irradiations during the aPDT assays were performed in the presence of a white light-emitting diode (LED; Inspire, China) at a light irradiance of 140 mW/cm^2 or 65 mW/cm^2 and a wavelength range between 400 and 740 nm (Figure 2). The aPDT experiments were also carried out under solar radiation. Samples were exposed to natural solar radiation during sunny days at light irradiances ranging from 25 to 65 mW/cm^2. Exposures were conducted during the summer of 2019 in the Faculty of Food Engineering of the University of Campinas – São Paulo, Brazil *(22°49'13.2168'' S, 47°4'3.3924'' W)*. All light measurements were performed using an energy meter (FieldMaxII-TOP; Coherent, USA) combined with a high-sensitivity thermopile sensor (PowerSens – PS19Q; Coherent, USA) as previously detailed (Martins et al., 2018).

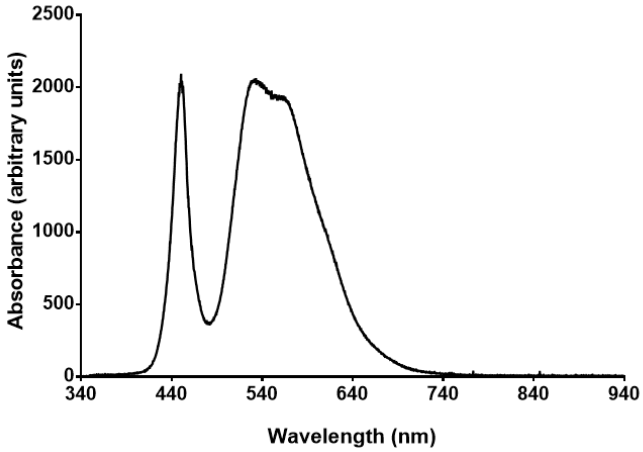

Figure 2. The spectrum of absorbance of the white light-emitting diode (LED).

2.4. Antimicrobial photodynamic treatments

2.4.1. *In vitro* photoinactivation of *A. acidoterrestris* spores

Experiments were performed in sterile 12-well flat-bottomed plates (NEST Biotechnology, China), containing a volume of each PSs to obtain a final concentration of 10 μM (Tetra-Py^+-Me and NMB). The wells were inoculated with *A. acidoterrestris* suspension of spores to achieve a final concentration of 10^7 spores/mL. Plates were kept in the dark for 30 min at 25 °C under agitation (100 rpm) and then exposed for up to 6 h using the white light (140 mW/cm^2). Light (LC) and dark controls (DC) were prepared in parallel to determine the effects of the light and PS separately, respectively. After the exposures, samples of 100 μL were taken every hour and submitted to heat shock (80 °C for 10 min) to stimulate the spore germination (Ferrario and Guerrero, 2018; Prado et al., 2019). The counts of *A. acidoterrestris* spores were determined by pour plating the serial diluted samples onto YSG-A (pH 3.7 ± 0.1), following incubation at 45 °C for 72 h. The counts of *A. acidoterrestris* were performed in duplicate and expressed as spores/mL. At least three independent experiments were performed for each condition.

2.4.2. Photoinactivation of *A. acidoterrestris* spores in orange juice

The experiments were performed with orange juice (UHT) purchased from a local market in Aveiro – Portugal. The pH and °Brix values of the juice extracted from the orange were measured using a pH meter (EDGE, Hanna Instruments, USA) and a refractometer (PAL-1, Atago, Japan), respectively. The experiments were conducted as previously described for PBS experiments (section 2.4.1) with some modifications: *(i)* the PSs were tested in the absence and presence of KI (100 mM); *(ii)* the plates were irradiated for up to 10 h using the white light provided by the LED at a light irradiance of 140 mW/cm^2; and *(iii)* the LC and DC controls were prepared in the presence of KI (100 mM). The concentration of *A. acidoterrestris* spores in orange juice was 10^5 spores/mL.

2.4.3. Photoinactivation of *A. acidoterrestris* spores on orange peel

The experiments were performed with orange peels portions from fresh oranges purchased from a local market in Aveiro – Portugal, under different light sources: an artificial light source (white light) and sunlight, both at light irradiance of 65 mW/cm^2.

The orange peel portions (Figure 3) were cleaned with 70% alcohol and exposed to ultraviolet (UV) irradiation for 15 min inside a laminar flow cabinet, before each experiment. The orange peel portions were placed on sterile 12-well flat-bottomed plates (NEST Biotechnology, China). The suspension of spores of *A. acidoterrestris* and Tetra-Py$^+$-Me were spread superficially to each orange peel portion to obtain a final concentration of 10^6 – 10^7 spores/mL. Firstly, the aPDT with Tetra-Py$^+$-Me at 10 µM in the presence and absence of KI was assessed. A higher concentration of Tetra-Py$^+$-Me (50 µM) without the addition of KI has also evaluated accordingly to previous studies (Martins et al., 2018). One orange peel sample was used for each time. The orange peels were kept in the dark 30 min for pre-incubation. Light (LC) and dark (DC) controls were carried out simultaneously to the treatment. Sample and LC were irradiated for 6 h while DC was kept in the dark. Three orange peel portions (sample, LC, and DC) were washed in beaker glass with 5 mL of PBS with agitation (100 rpm)

for 30 min in the dark before and after exposure. After washing, *A. acidoterrestris* spores counts in the orange peels portions was performed as previously described in the section 2.4.1.

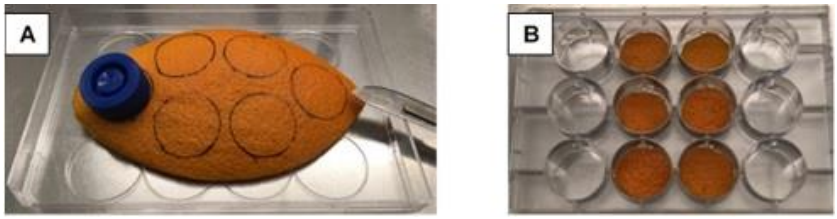

Figure 3. Representation of the orange peel (**A**) and the cuts in the 12-well plate (**B**).

2.5. Colorimetric analysis

Orange juice and peel color coordinates (*L*, a*, b**) were measured using a spectrophotometer (Color Quest II, Hunter Lab, USA) according to Fundo et al., 2019 with adaptations. The equipment was calibrated before every experiment with a blank calibration tile. A total of 50 mL of orange juice in a glass colorimeter cell or an orange peel portion (Ø ≈ 2.1 cm) were appropriately placed in the equipment. Three readings were performed for each sample, always with the same experimental conditions. The color change was expressed by the coordinates *L*, a*,* and *b** according to the Hunter Lab color scale. The parameter *L** is responsible for the whiteness value from black (0) to white (100). The chromaticity from green (-) to red (+) and from blue (-) to yellow (+) is related to the parameter *a** and *b**, respectively. Total color change (Δ*E*) was calculated according to Eq. (1) Furthermore, it is used to evaluate the color changes of untreated and light-exposed samples (Ihns et al., 2011). Higher ΔE values indicate a more pronounced color change (Fundo et al., 2019).

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \qquad Eq.1$$

In equation 1, the number “0” is the initial color values from untreated juice samples.

2.6. Total phenolics content and antioxidant capacity of orange juice after aPDT.

2.6.1. Total phenolic content

The total phenolic content of orange juice after aPDT was measured by the Folin-Ciocalteu method, according to Ainsworth and Gillespie (2007), with minor modification. Aqueous dilutions of orange juice (500 μL) were mixed with 10-fold diluted Folin-Ciocalteu reagent (2.5 mL). After 5 min, 7.5% sodium carbonate solution (2.0 mL) was added, and the absorbance was measured at 750 nm in a spectrophotometer (DU-640TM, Beckman-Coulter, CA, USA). The total phenolic content was quantified using a standard curve of gallic acid, ranging from 15 to 300 µg/mL. Results were expressed as µg of gallic acid equivalents (GAE) per mL of orange juice.

2.6.2. Antioxidant capacity

2.6.2.1. DPPH scavenging activity

2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH) radical-scavenging activity was measured according to Brand-Williams et al., 1995 method, with modifications. Briefly, 50 µL of ethanolic dilutions of orange juice was mixed with 250 µL of DPPH solution (0.004% w/v) in transparent 96-well microplate (Costar, Cambridge, MA, USA). After 30 min reaction, the absorbance was measured at 517 nm in a microplate reader (NOVOstar BMG Labtech®, Offenburg, Germany). Antioxidant capacity was determined using a standard curve of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ranging from 12.5 to 250 µM. Results were expressed as µg of Trolox equivalent per mL of orange juice.

2.6.2.2. $ABTS^{\bullet+}$ scavenging capacity

The 2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) scavenging capacity was determined, according to Le et al., (2007). The solution of $ABTS^{\bullet+}$ was prepared by reacting 5 mL of aqueous ABTS solution (7 mM) with 88 µL of potassium persulfate (140 mM) and incubated in the dark at room temperature for 16 h. The assay was performed by mixing 200 µL of the diluted orange juice and 1000 µL of $ABTS^{\bullet+}$ solution. After 6 min of reaction, the solution absorbance was measured at 734 nm in a spectrophotometer (DU-640™, Beckman-Coulter, CA, USA). Antioxidant capacity was determined using a standard curve of Trolox, ranging from 10 to 250 µM. Results were expressed as µg of Trolox equivalent per mL of orange juice.

2.6.2.3. Oxygen Radical Absorbance Capacity (ORAC)

The Oxygen Radical Absorbance Capacity (ORAC) assay was performed according to Dávalos et al. (2004) and Prior et al. (2003). The reaction was carried out in phosphate buffer (75 mM, pH 7.4), at 37 °C, using fluorescein as a fluorescent probe and 2,2'-azobis(2-methylpropionamidine) dihydrochloride (APPH) as a free radical generator. The diluted orange juice (20 µL) was added to a black-walled 96-well microplate, followed by the addition of fluorescein (120 µL, 70 mM) and AAPH (60 µL, 12 mM). The fluorescence was monitored every 60 s cycle, for 80 cycles, in a microplate reader, at 485 nm of excitation and 520 nm of emission. Results were determined by the difference of the areas under fluorescein decay curves of samples and blank. A calibration curve using Trolox (15 to 1500 µM) was obtained, and the results were expressed as µg of Trolox equivalent per mL of orange juice.

2.7. Statistical analysis

All graphics and data analyses were performed using GraphPad Prism 6 (GraphPad Software, USA). The statistical difference between treatments was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test and *t*-test at 95% of significance. A value of $p < 0.05$ was considered significant. At least three independent experiments were performed for each condition.

3. Results

3.1. aPDT of *Alicyclobacillus* spores

3.1.1. *In vitro* photoinactivation of *A. acidoterrestris* spores

The effect of aPDT with Tetra-Py$^+$-Me and NMB on the viability of *A. acidoterrestris* spores are shown in figure 4. The spores were incubated in the dark with the PSs (10 µM) in PBS for 30 min before exposition to artificial white light (140 mW/cm^2). The inactivation curves were expressed in terms of logarithmic spore viability per milliliter of PBS (log spores/mL) after heat-shock. Exposure only to the artificial white light source (LC) did not reduce the viability of *A. acidoterrestris* spores. In the absence of light (dark control), both PSs at 10 µM also did not affect the viability of the spores. aPDT with the porphyrin Tetra-Py$^+$-Me (at 10 µM) was able to deliver 7 log CFU/mL reductions ($p < 0.05$) of the spores after 5 h of light exposition. No significant spore viability reduction was observed after aPDT with NMB at 10 µM (Figure 4; $p > 0.05$). Additionally, photodegradation of the NMB in the course of aPDT treatment has been observed through the experiment (data not shown).

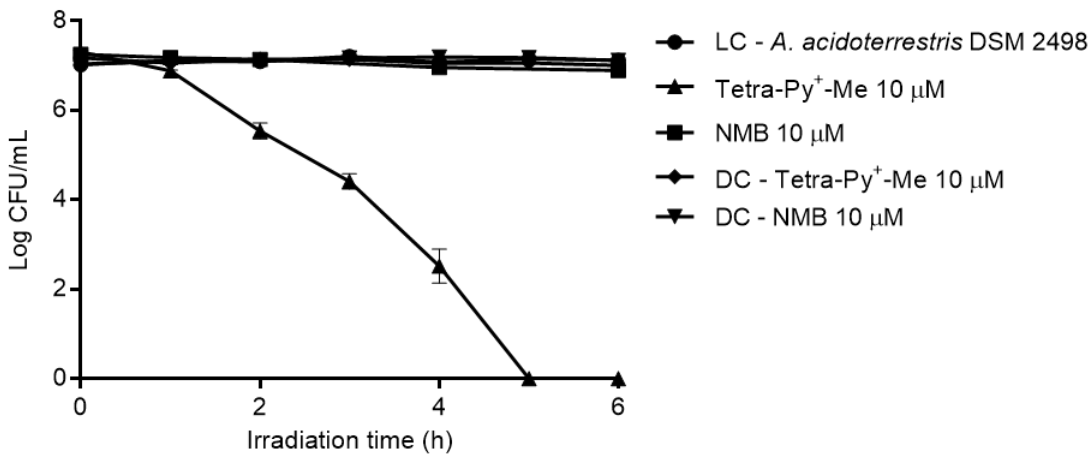

Figure 4. Inactivation curve of *A. acidoterrestris* spores in PBS after aPDT with Tetra-Py$^+$-Me and NMB at 10 µM using white LED (400-740 nm) at an irradiance of 140 mW/cm^2 for up to 6 h. DC: Dark Control; LC: Light Control. Error bars represent the

standard deviation (SD) of three independent experiments and in some cases are hidden by the symbols.

3.1.2. Photoinactivation of *A. acidoterrestris* spores in orange juice

Figure 5 shows the aPDT inactivation of *A. acidoterrestris* spores artificially inoculated in orange juice (pH 3.9; 11 °Brix) in the presence and the absence of KI. Also, in this case, the light (LC) and dark (DC) controls in the presence of KI did not affect the viability of *A. acidoterrestris* spores. The results showed no reduction in spore's viability when the aPDT assays were performed in the presence of Tetra-Py^+-Me or NMB at 10 µM in the absence of KI. It is worth to mention that, the photodegradation of NMB in the absence of KI was detected in the orange juice experiments, as observed in the previous trials in PBS. The inactivation of *A. acidoterrestris* spores in orange juice by aPDT was only observed with Tetra-Py^+-Me and NMB in the presence of KI. The treatment of orange juice inoculated with *A. acidoterrestris* spores resulted in 5 log CFU/mL after 10 h of light exposure in the presence of each PS combined with KI (Figure 5).

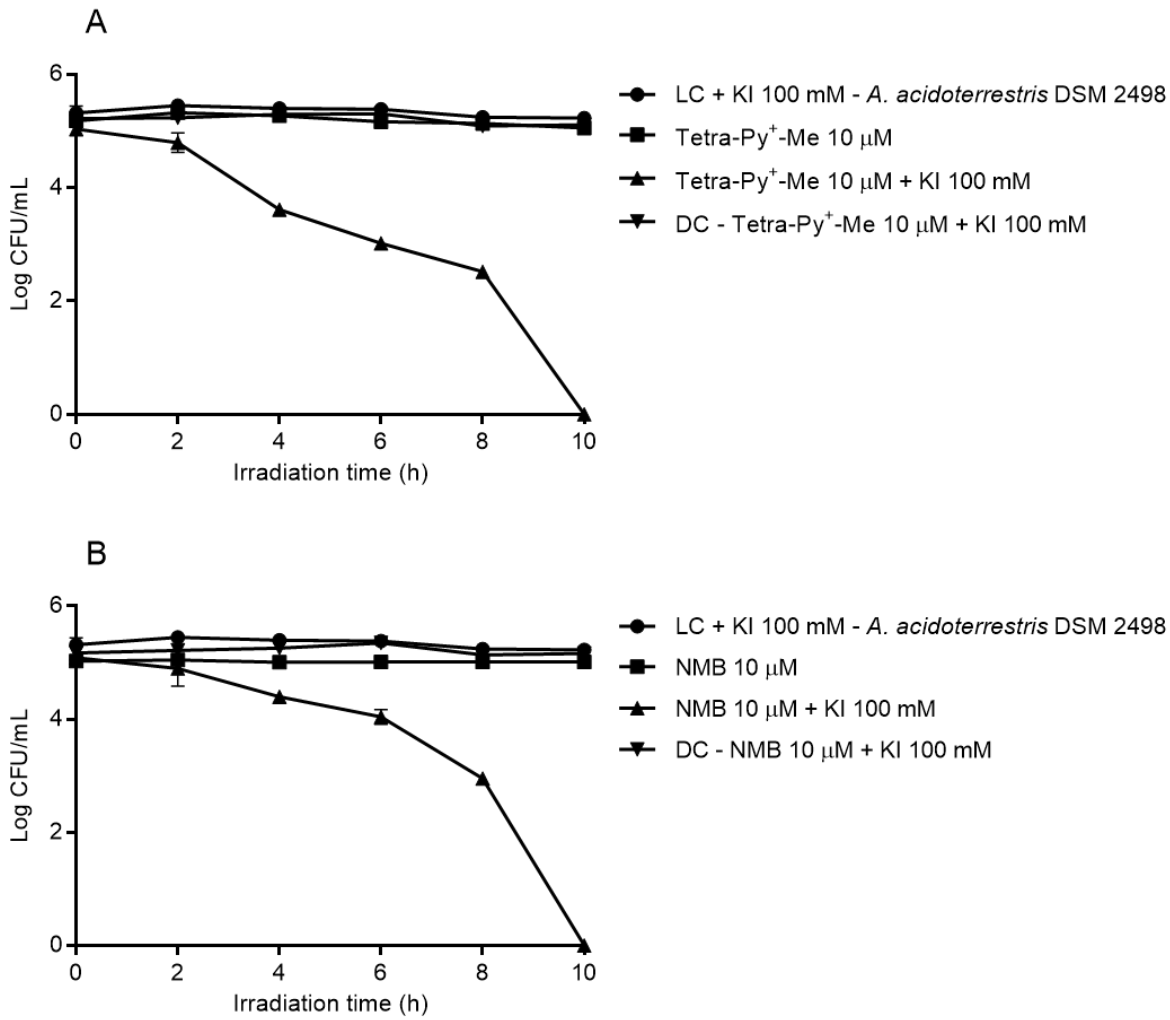

Figure 5. Inactivation curves of *A. acidoterrestris* spores artificially inoculated in orange juice during aPDT with Tetra-Py^+-Me (A) and NMB (B) at 10 µM with or without KI using white LED (400-740 nm) at an irradiance of 140 mW/cm^2 for 10 h. DC: Dark Control with KI; LC: Light Control with KI. Error bars represent the SD of three independent experiments and in some cases are hidden by the symbols.

3.1.3. Photoinactivation of *A. acidoterrestris* spores on orange peel

Two concentrations of Tetra-Py+-Me (10 and 50 µM) were used to evaluate the photoinactivation A. acidoterrestris spores on the surface of orange peels. The higher concentration of this PS was used due to the complexity of the matrix and the condition of the experiment (without agitation) and also based on previous studies (Jesus et al., 2018; Martins et al., 2018). Since in the experiments with PBS and orange juice, it was observed the photodegradation of NMB, this PS was not included in these assays. These studies were conducted using artificial white light and solar radiation at 65 mW/cm2 (Figure 6A and Figure 6B, respectively).

The results achieved with white light had shown that, in the absence of light (DC), Tetra-Py^+-Me at 50 µM did not significantly affect the viability of *A. acidoterrestris* spores on orange peel (Figure 6A). However, 1.3 and 0.9 log CFU/mL reductions ($p < 0.05$) were achieved with treatments at 10 and 50 µM of Tetra-Py^+-Me, respectively. Moreover, in this study, no evidence of the KI potentiation effect was observed.

The assays with Tetra-Py^+-Me at 10 and 50 µM were also done under sunlight irradiation aiming to assess a possible application of aPDT in orange groves. Thus, orange peels artificially contaminated with *A. acidoterrestris* spores were exposed to aPDT with Tetra-Py^+-Me at 50 µM using sunlight (at an irradiance of 65 mW/cm^2) as a light source for 6 h. The results are presented in figure 6B and showed that treatment only with the PS (DC) did not reduce the viability of the spores; however, exposures to solar radiation for 6 h reduced the viability of *A. acidoterrestris* spores by 2.8 log CFU/mL ($p < 0.05$) with Tetra-Py^+-Me at 50 µM. A slight significant reduction in spore viability by 0.3 and 0.7 log CFU/mL were observed in aPDT with Tetra-Py^+-Me at 10 µM and LC, respectively (Figure 6B, $p < 0.05$).

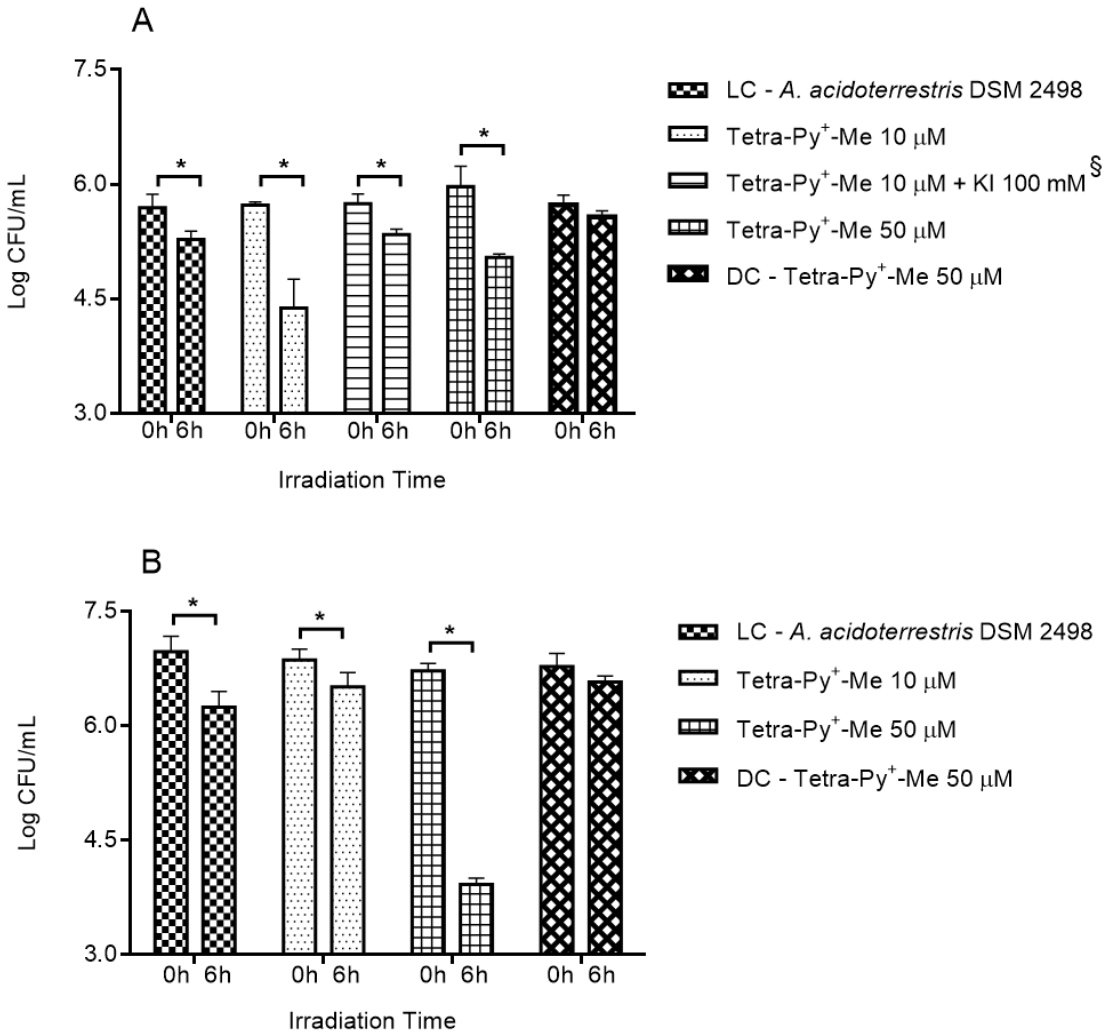

Figure 6. aPDT of *A. acidoterrestris* spores artificially inoculated in orange peels by Tetra-Py^+-Me at 10 and 50 μM using white light (A; 400-740 nm) and sunlight (B) exposition at an irradiance of 65 mW/cm^2 for 6 h. DC: Dark Control; LC: Light Control. Error bars represent the SD of three independent experiments and in some cases are hidden by the symbols. *Significantly different according to t-test ($p < 0.05$). §KI was tested with Tetra-Py^+-Me at 10 μM only in white light exposures.

3.2. Colorimetric analysis

The effects of exposure to light, in the absence of PS, are presented in Table 1. The presence of both PS visually altered the characteristics of the juice (data not shown). Moreover, our results clearly showed a significant change in the color coordinates (*L*, a*, b**) for orange juice and peel after only light exposure for 6 and 10 h. ΔE was calculated to assess the magnitude of the orange juice (20.9 ± 0.314) and peel color changes after white light (8.31 ± 1.25) and sunlight (23.4 ± 0.281) exposures.

Table 1. Estimated color parameters for untreated and light-exposed orange juice and peel during 10 and 6 hours, respectively.

Sample	Treatment	*L**	*a**	*b**	$\Delta E^{\dagger}$
Orange juice	Untreated	42.0 ± 0.535^{b}	2.17 ± 0.064^{a}	21.1 ± 0.488^{a}	-
	White light	48.7 ± 0.217^{a}	-3.31 ± 0.021^{b}	1.98 ± 0.021^{b}	20.9 ± 0.314
Orange peel	Untreated	61.0 ± 0.467^{b}	-1.74 ± 1.21^{b}	52.7 ± 0.022^{b}	-
	White light	66.0 ± 0.380^{a}	3.11 ± 0.280^{a}	57.2 ± 0.353^{a}	8.31 ± 1.25
	Untreated	59.7 ± 0.137^{b}	-4.53 ± 0.299^{b}	52.4 ± 0.615^{a}	-
	Sunlight	61.6 ± 0.038^{a}	12.8 ± 0.686^{a}	36.9 ± 1.46^{b}	23.4 ± 0.281

† ΔE, total color change.

The values are average ± SD. For a given parameter, values with different letters differ significantly ($p < 0.05$)

3.3. Phenolic content and antioxidant capacity of orange juice exposed to light

The effect of exposure to light on total phenolic content (TPC) and antioxidant capacity of orange juice are presented in figure 7. Results clearly show that there is a significant decrease of 3.25 µg GAE/mL in TPC of light exposed orange juice compared to the control (Figure 7-A; $p < 0.05$). The antioxidant capacity of the juice was also significantly reduced in terms of DPPH and ABTS scavenging activity, whereas no significant alteration was detected by the ORAC assay (Figure 7-B; $p > 0.05$).

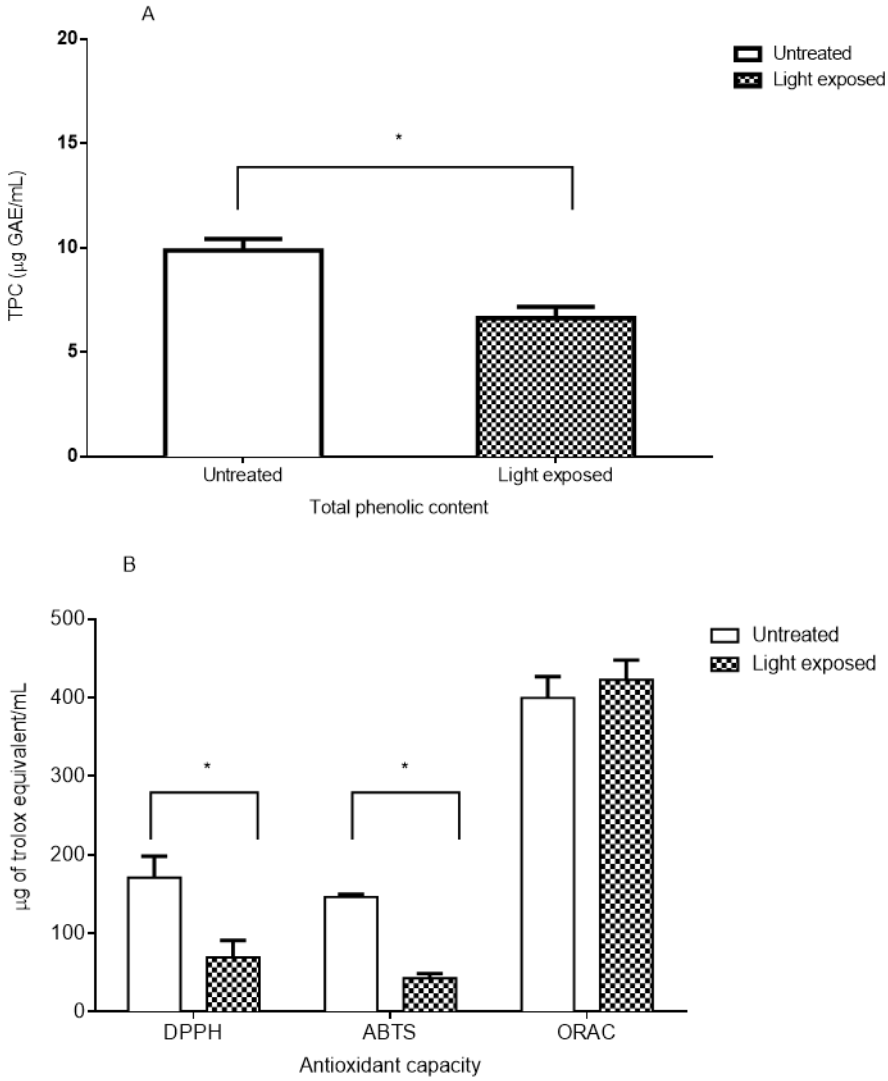

Figure 7. Effect of light exposition using white LED (400-740 nm) on the total phenolic content (A) and antioxidant capacity (B) in orange juice.

4. Discussion

In the present study, the inactivation efficiency of aPDT towards *A. acidoterrestris* spores using Tetra-Py$^+$-Me and NMB as PSs have been evaluated. As far as the author's best knowledge, this is the first study to demonstrate the application of aPDT towards inactivation of *Alicyclobacillus* spp. spores. This study was initially performed *in vitro* (PBS) under artificial white light irradiation using both PSs at 10 µM. This concentration was chosen based on previous studies which demonstrated its effectiveness against *B. cereus* spores (Oliveira et al., 2009) and prevented the aggregation of the PSs (Fernández-Pérez et al., 2019). The maximum efficiency of *A. acidoterrestris* spores inactivation was achieved with Tetra-Py$^+$-Me at 10 µM (Figure 4). On the contrary, no photoinactivation of *A. acidoterrestris* spores using NMB at the same concentration was observed in PBS (Figure 4). The observed photodegradation of NMB during the long light exposures may be one of the reasons for the low efficiency of aPDT with this PS. The photodegradation of phenothiazine dyes may occur during single oxygen production after exposure to visible light (Nassar et al., 2019).

Previous studies have already demonstrated the effectiveness of aPDT to reduce the load of viruses (3-8 log PFU/mL), bacteria (3-8 log CFU/mL) and fungi (0.8-5 log CFU/mL) (Beirão et al., 2014; Castro et al., 2017; da Silva et al., 2012; de Menezes et al., 2016; Freire et al., 2016; Huang et al., 2018b; Oliveira et al., 2009; Santos et al., 2019; Sousa et al., 2019; Vieira et al., 2019; Wen et al., 2017; Wu et al., 2015; Yuan et al., 2020). For instance, aPDT mediated by Tetra-Py$^+$-Me caused from 3 to 6 log CFU/mL reductions of single-species and mixed biofilms of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* (Beirão et al., 2014). Biofilms are known as structures of persistence of microorganisms in food processing environments (Galié et al., 2018). When in biofilms, microbial inactivation becomes harder (Bridier et al., 2011). Therefore, the efficiency of aPDT for inactivation of microorganisms in biofilms may suggest this technology may also be effective on spore-forming bacteria inactivation. For instance, studies involving porphyrin derivatives have demonstrated that aPDT using Tetra-Py$^+$-Me as PS led to up 3.5 log CFU/mL reductions of *Bacillus cereus* spores (Oliveira et al., 2009). Therefore, given

the effectiveness of aPDT towards *A. acidoterrestris* inactivation *in vitro* conditions, further tests were conducted in orange juice.

Given the complexity of the orange juice compared to PBS (*e.g.* turbidity), the presence and absence of KI was assessed, since it was expected that the photodynamic effect would be reduced (Sousa et al., 2019). The aPDT treatment with Tetra-Py^+-Me and NMB at 10 µM in the presence of KI in orange juice resulted in up to 5 log CFU/mL of *A. acidoterrestris* spores (Figure 5).

Since the use of KI as a potentiator agent of the aPDT effect was introduced (Vecchio et al., 2015; Zhang et al., 2015), several studies have been conducted to confirm such action on different microorganisms and microbial structures (Freire et al., 2016; Huang et al., 2018a; Vieira et al., 2018, 2019; Wen et al., 2017). In these reports, the authors concluded that KI potentiates the photodynamic effects in most cases, increasing the inactivation of microorganisms for up to 6 log CFU/mL reductions. According to these authors, this potentiation is due to the reaction of KI with $^1O_2$ affording the production of longer-lived reactive species as free iodine and triiodide (I_2 / I_3^-) and also the production of short-lived species such as reactive iodine radicals ($I_2^{\bullet-}$). The production of these species contributes to the remaining microbial inactivation after aPDT (Vieira et al., 2019). In the current study the potentiation effect of KI for both PSs in the inactivation of *A. acidoterrestris* spores was observed since no reduction was detected in the absence of KI (Figure 5). The inefficiency of the aPDT with the PSs in the absence of KI may be explained by the presence of organic compounds in the orange juice. The ROS and $^1O_2$ are preferentially acting on the oxidation of organic compounds of the beverage. In the presence of KI, $^1O_2$ is consumed to form the iodine species that are capable of destroying *A. acidoterrestris* spores.

Even though there is a lack of studies on the use of aPDT for the inactivation of *A. acidoterrestris* in fruit juices, other light-based technologies have been employed with that purpose. For instance, UV-C radiation has been found to cause a reduction (log CFU/mL) in *A. acidoterrestris* spores of 2 in apple juice and 5.5 in grape juice by Baysal et al. (2013), 5 in apple juice by Tremarin et al. (2017) and 4.7 in melon juice by Fundo et al. (2019). The differences in inactivation efficiency observed amongst the different

juices may be explained by the difference in their turbidity, which may impact on light penetration. For instance, the higher turbidity of apple juice is known to limit light penetration, resulting in overall lower number of decimal reductions of *A. acidoterrestris* spores. On the other hand, the lower turbidity of grape juice facilitates the penetration of light and the inactivation of *A. acidoterrestris* spores (Baysal et al., 2013). These findings highlight that aPDT can also deliver up ≈ 5 log CFU/mL reductions of *A. acidoterrestris* spores under *in vitro* conditions, despite the much higher intensity (140 mW/cm^2) and exposure time to light (10 h) employed. Furthermore, the similar number of decimal reductions of *A. acidoterrestris* spores obtained by aPDT (Figure 4) and UV-C (Baysal et al., 2013; Fundo et al., 2019; Tremarin et al., 2017), despite the difference in the age (and resistance) of spores used (28 days and 2-10 days, respectively), highlight the potentials of this technology for the inactivation of this bacterium. Despite the longer times to reach similar number of decimal reductions, another advantage of using visible light sources (such in aPDT) is that aPDT does not present risks to the operator's health (Guerrero-Beltrán and Barbosa-Cánovas, 2004). Nonetheless, aPDT efficiency and reduction of exposure time can be further enhanced by the combination of non-toxic compounds with the PS.

The use of aPDT as an inactivation method for pathogenic and spoilage microorganisms in fruit juices is rare. However, recently, the combination of ultrasound (US) and aPDT for inactivation of *Escherichia coli* and *S. aureus* in orange juice has been evaluated (Bhavya and Hebbar, 2019). Despite the significant number of decimal reductions of *E. coli* and *S. aureus*, 4.2 and 2.3 log CFU/mL, respectively, a negative impact on the antioxidant capacity has been reported (Bhavya and Hebbar, 2019), corroborating the findings of the current study (Figure 7).

The assessment of the light exposures impact on the quality of orange juice was also investigated in order to gain insights into the effects on the color of the juice. The results of this study demonstrated that there was an alteration in all color parameters (*L*, a*, b**) of the orange juice after 10 h of white light (LED) exposures, as represented by the ΔE in Table 1. In fact, these analyses were performed aiming to assess the effects of the exposure times to light deemed necessary to result in a certain inactivation of *A. acidoterrestris* spores. Despite this, it has been reported that even a

short exposure time (20 min) of melon juice to UV-C radiation resulted in a significant alteration in the *L** coordinate (Fundo et al., 2019). This slight change in the *L** coordinate was linked with the high concentrations of pigments present in the melon juice resulting in a masking effect on the ΔE (Taze et al., 2015). Nevertheless, the impact of aPDT in terms of color varies in the literature depending on the fruit matrix (Bhavya and Hebbar, 2019; Kim et al., 2017; Tao et al., 2019). Whereas a slight change on the color of orange juice was observed with curcumin and blue light (Bhavya and Hebbar, 2019), a positive effect on fresh-cut apples by the use of the same PS (Tao et al., 2019). Contrarily, the absence of the PS allowed change in *L*, a*, b** parameters of fresh-cut papaya (Kim et al., 2017). Previous studies also evaluated the impact of aPDT on the antioxidant profile of strawberries, apricots, plumes, cauliflowers, and orange juice (Aponiene et al., 2015; Luksiene and Paskeviciute, 2011). Although such studies did not show any adverse effect on total phenolic content or antioxidant capacity after only 30 min of light exposure. However, none of these studies have applied the aPDT for long period of light exposure in liquid substrate as in the current study.

Given the results obtained from the orange juice experiments, the photoinactivation of *A. acidoterrestris* spores on the surface of orange peels was performed. Remarkably, aPDT caused a reduction ($p < 0.05$) of *A. acidoterrestris* spores (≈ 2.8 log CFU/mL) on orange peels when carried out under solar radiation. In other studies, aPDT with LED-light resulted in 1.8 log CFU/mL, 0.95 log CFU/g and 0.6-0.7 log CFU/g reductions of *L. monocytogenes* in artificially inoculated on the surface of strawberries (Luksiene & Pakeuviciute, 2011), *E. coli* on apple slices (Tao et al., 2019) and of *B. cereus* on the surface of apricots, cauliflowers, and plums (Aponiene et al., 2015), respectively. However, very high reduction (> 6 log CFU/mL) of *Pseudomonas syringae* pv. *actinidiae* has been reported on kiwifruit leaves submitted to aPDT treatment done under solar radiation for 2 cycles of 90 min (Martins et al., 2018). This finding reinforces that solar radiation seems to be crucial in enhancing the inactivation efficiency of aPDT with PSs. The higher efficiency of aPDT with sunlight for inactivation of *A. acidoterrestris* spores on orange peel than aPDT with white light (LED) can be explained by antimicrobial properties of each source of light. If on one hand, white light

(LED) contains only the visible spectrum, which is known to not present antimicrobial effects (Santos et al., 2020), on the other hand, the full-spectrum of solar radiation contains UV rays with known antimicrobial activity (Dias et al., 2018).

It is known that the counts of *Alicyclobacillus* spp. in fruits in the trees may reach up to 10^2 spores/Kg (ABECitrus, 1999). As a result, a step of fruit disinfection is currently used to reduce *Alicyclobacillus* spp. spores loads at the beginning of fruit processing. Nonetheless, this step is not able to deliver more than 2 log CFU/mL reductions of *Alicyclobacillus* spp. spores (Orr and Beuchat, 2000). Thus, counts of *Alicyclobacillus* spp. spores in fruit products after processing of up to 10^2-10^3 spores/mL have been reported (ABECitrus, 1999). Consequently, this level of contamination is enough for this bacterium to germinate, grow and further cause juice spoilage if storage conditions are apropriate leading to severe losses (Spinelli et al., 2009), highlighting that controling strategies to reduce the counts of this bacterium prior to processing are needed. Therefore, the ≈ 3 log CFU/mL reductions of *A. acidoterrestris* spores on orange peels caused by aPDT under solar radiation comprises a remarkable finding. This technology could be applied in practice directly in the fruits in the trees exposed to abundant sunlight. As such, it emerges as a feasible strategy to reduce the counts of *Alicyclobacillus* spp. spores prior to fruit processing. As such aPDT under solar radiation may contribute to the overall reduction of *Alicyclobacillus* spp. spores throughout the production chain required for the production of shelf-stable orange juice and derived beverages. Besides, with further refinements and enhancements in the inactivation efficiency, aPDT under solar radiation could favor the decrease in the use/concentration of chemical sanitizers. In practice, this technology would allow the reduction of costs as well as contribute with an environmental friendly processing. The current work is the first to evaluate the potential of aPDT as an emerging technology towards inactivation of *A. acidoterrestris* spores.

Acknowledgements

This work was supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Grants: #140092/2017-0, #302763/2014-7, and #305804/2017-0); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 001 for the financial support. Thanks are due for the financial support to CESAM Research Unit (UID/AMB/50017/2019 and UIDB/50017/2020+UIDP/50017/2020), QOPNA Research Unit (FCT UID/QUI/00062/2019) and LAQV-REQUIMTE Research Unit (UIDB/50006/2020), to FCT/MCTES through national funds, and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020.

Conflict of interest

The authors declare no competing interests.

5. References

ABECitrus, 1999. Acidothermophilic sporeforming bacteria (ATSB) in orange juices: Detection methods, ecology, and involvement in the deterioration of fruit juices. Assoc. Bras. dos Export. Cítricos. URL http://www.citrusbr.com.br/exportadores-citricos/sobre-citrusbr/imagens/ATSB_Abecitrus.pdf (accessed 5.15.20).

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc. 2, 875–877. https://doi.org/10.1038/nprot.2007.102

Albuquerque, L., Rainey, F.A., Chung, A.P., Sunna, A., Nobre, M.F., Grote, R., Antranikian, G., Da Costa, M.S., 2000. Alicyclobacillus hesperidum sp. nov. and a related genomic species from solfataric soils of Sao Miguel in the Azores. Int. J. Syst. Evol. Microbiol. 50, 451–457. https://doi.org/10.1099/00207713-50-2-451

Almeida, A., Faustino, M.A.F., Tomé, J.P.C., 2015. Photodynamic inactivation of bacteria: finding the effective targets. Future Med. Chem. 7, 1221–1224.

https://doi.org/10.4155/fmc.15.59

Aponiene, K., Paskeviciute, E., Reklaitis, I., Luksiene, Z., 2015. Reduction of microbial contamination of fruits and vegetables by hypericin-based photosensitization: Comparison with other emerging antimicrobial treatments. J. Food Eng. 144, 29–35. https://doi.org/10.1016/j.jfoodeng.2014.07.012

Baysal, A.H., Molva, C., Unluturk, S., 2013. UV-C light inactivation and modeling kinetics of Alicyclobacillus acidoterrestris spores in white grape and apple juices. Int. J. Food Microbiol. 166, 494–498. https://doi.org/10.1016/j.ijfoodmicro.2013.08.015

Beirão, S., Fernandes, S., Coelho, J., Faustino, M.A.F., Tomé, J.P.C., Neves, M.G.P.M.S., Tomé, A.C., Almeida, A., Cunha, A., 2014. Photodynamic inactivation of bacterial and yeast biofilms with a cationic porphyrin. Photochem. Photobiol. 90, 1387–1396. https://doi.org/10.1111/php.12331

Bhavya, M.L., Hebbar, H.U., 2019. Sono-photodynamic inactivation of Escherichia coli and Staphylococcus aureus in orange juice. Ultrason. Sonochem. 57, 108–115. https://doi.org/10.1016/j.ultsonch.2019.05.002

Brancini, G.T.P., Rodrigues, G.B., Rambaldi, M.D.S.L., Izumi, C., Yatsuda, A.P., Wainwright, M., Rosa, J.C., Braga, G.Ú.L., 2016. The effects of photodynamic treatment with new methylene blue N on the Candida albicans proteome. Photochem. Photobiol. Sci. 15, 1503–1513. https://doi.org/10.1039/C6PP00257A

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Bridier, A., Briandet, R., Thomas, V., Dubois-Brissonnet, F., 2011. Resistance of bacterial biofilms to disinfectants: a review. Biofouling 27, 1017–1032. https://doi.org/10.1080/08927014.2011.626899

Carvalho, C.M.B., Gomes, A.T.P.C., Fernandes, S.C.D., Prata, A.C.B., Almeida, M.A., Cunha, M.A., Tomé, J.P.C., Faustino, M.A.F., Neves, M.G.P.M.S., Tomé, A.C., Cavaleiro, J.A.S., Lin, Z., Rainho, J.P., Rocha, J., 2007. Photoinactivation of bacteria in wastewater by porphyrins: Bacterial β-galactosidase activity and leucine-uptake as

methods to monitor the process. J. Photochem. Photobiol. B Biol. 88, 112–118. https://doi.org/10.1016/j.jphotobiol.2007.04.015

Castro, K.A.D.F., Moura, N.M.M., Fernandes, A., Faustino, M.A.F., Simões, M.M.Q., Cavaleiro, J.A.S., Nakagaki, S., Almeida, A., Cunha, Â., Silvestre, A.J.D., Freire, C.S.R., Pinto, R.J.B., Neves, M. da G.P.M.S., 2017. Control of Listeria innocua biofilms by biocompatible photodynamic antifouling chitosan based materials. Dye. Pigment. 137, 265–276. https://doi.org/10.1016/j.dyepig.2016.10.020

Costa, L., Alves, E., Carvalho, C.M.B., Tomé, J.P.C., Faustino, M.A.F., Neves, M.G.P.M.S., Tomé, A.C., Cavaleiro, J.A.S., Cunha, Â., Almeida, A., 2008. Sewage bacteriophage photoinactivation by cationic porphyrins: A study of charge effect. Photochem. Photobiol. Sci. 7, 415–422. https://doi.org/10.1039/b712749a

da Silva, R.N., Tomé, A.C., Tomé, J.P.C., Neves, M.G.P.M.S., Faustino, M.A.F., Cavaleiro, J.A.S., Oliveira, A., Almeida, A., Cunha, Â., 2012. Photo-inactivation of Bacillus endospores: inter-specific variability of inactivation efficiency. Microbiol. Immunol. 56, 692–699. https://doi.org/10.1111/j.1348-0421.2012.00493.x

Dávalos, A., Gómez-Cordovés, C., Bartolomé, B., 2004. extending applicability of the oxygen radical absorbance capacity (ORAC−Fluorescein) assay. J. Agric. Food Chem. 52, 48–54. https://doi.org/10.1021/jf0305231

de Menezes, H.D., Tonani, L., Bachmann, L., Wainwright, M., Braga, G.Ú.L., von Zeska Kress, M.R., 2016. Photodynamic treatment with phenothiazinium photosensitizers kills both ungerminated and germinated microconidia of the pathogenic fungi Fusarium oxysporum, Fusarium moniliforme and Fusarium solani. J. Photochem. Photobiol. B Biol. 164, 1–12. https://doi.org/10.1016/j.jphotobiol.2016.09.008

Deng, X., Tang, S., Wu, Q., Tian, J., Riley, W.W., Chen, Z., 2016. Inactivation of Vibrio parahaemolyticus by antimicrobial photodynamic technology using methylene blue. J. Sci. Food Agric. 96, 1601–1608. https://doi.org/10.1002/jsfa.7261

Dias, L.P., Araújo, C.A.S., Pupin, B., Ferreira, P.C., Braga, G.Ú.L., Rangel, D.E.N., 2018. The Xenon Test Chamber Q-SUN® for testing realistic tolerances of fungi

exposed to simulated full spectrum solar radiation. Fungal Biol. 122, 592–601. https://doi.org/10.1016/j.funbio.2018.01.003

Evelyn, Kim, H.J., Silva, F.V.M., 2016. Modeling the inactivation of Neosartorya fischeri ascospores in apple juice by high pressure, power ultrasound and thermal processing. Food Control 59, 530–537. https://doi.org/10.1016/j.foodcont.2015.06.033

Fernández-Pérez, A., Valdés-Solís, T., Marbán, G., 2019. Visible light spectroscopic analysis of Methylene Blue in water; the resonance virtual equilibrium hypothesis. Dye. Pigment. 161, 448–456. https://doi.org/10.1016/j.dyepig.2018.09.083

Ferrario, M.I., Guerrero, S.N., 2018. Inactivation of Alicyclobacillus acidoterrestris ATCC 49025 spores in apple juice by pulsed light. Influence of initial contamination and required reduction levels. Rev. Argent. Microbiol. 50, 3–11. https://doi.org/10.1016/j.ram.2017.04.002

Freire, F., Ferraresi, C., Jorge, A.O.C., Hamblin, M.R., 2016. Photodynamic therapy of oral Candida infection in a mouse model. J. Photochem. Photobiol. B Biol. 159, 161–168. https://doi.org/10.1016/j.jphotobiol.2016.03.049

Friedrich, L.M., Goodrich-Schneider, R., Parish, M.E., Danyluk, M.D., 2009. Mitigation of Alicyclobacillus spp. spores on food contact surfaces with aqueous chlorine dioxide and hypochlorite. Food Microbiol. 26, 936–941. https://doi.org/10.1016/j.fm.2009.06.011

Fundo, J.F., Miller, F.A., Mandro, G.F., Tremarin, A., Brandão, T.R.S., Silva, C.L.M., 2019. UV-C light processing of Cantaloupe melon juice: Evaluation of the impact on microbiological, and some quality characteristics, during refrigerated storage. LWT - Food Sci. Technol. 103, 247–252. https://doi.org/10.1016/j.lwt.2019.01.025

Galié, S., García-Gutiérrez, C., Miguélez, E.M., Villar, C.J., Lombó, F., 2018. Biofilms in the food industry: health aspects and control methods. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00898

Gonzales, J.C., Brancini, G.T.P., Rodrigues, G.B., Silva-Junior, G.J., Bachmann, L., Wainwright, M., Braga, G.Ú.L., 2017. Photodynamic inactivation of conidia of the fungus Colletotrichum abscissum on Citrus sinensis plants with methylene blue under

solar radiation. J. Photochem. Photobiol. B Biol. 176, 54–61. https://doi.org/10.1016/j.jphotobiol.2017.09.008

Gouws, P. A., Gie, L., Pretorius, A., Dhansay, N., 2005. Isolation and identification of Alicyclobacillus acidocaldarius by 16S rDNA from mango juice and concentrate. Int. J. Food Sci. Technol. 40, 789–792. https://doi.org/10.1111/j.1365-2621.2005.01006.x

Groenewald, W.H., Gouws, P.A., Witthuhn, R.C., 2008. Isolation and identification of species of Alicyclobacillus from orchard soil in the Western Cape, South Africa. Extremophiles 12, 159–163. https://doi.org/10.1007/s00792-007-0112-z

Groenewald, W.H., Gouws, P.A., Witthuhn, R.C., 2009. Isolation, identification and typification of Alicyclobacillus acidoterrestris and Alicyclobacillus acidocaldarius strains from orchard soil and the fruit processing environment in South Africa. Food Microbiol. 26, 71–76. https://doi.org/10.1016/j.fm.2008.07.008

Guerrero-Beltrán, J.A., Barbosa-Cánovas, G. V., 2004. Review: Advantages and limitations on processing foods by UV light. Food Sci. Technol. Int. 10, 137–147. https://doi.org/10.1177/1082013204044359

Hamblin, M.R., 2017. Potentiation of antimicrobial photodynamic inactivation by inorganic salts. Expert Rev. Anti. Infect. Ther. 15, 1059–1069. https://doi.org/10.1080/14787210.2017.1397512

Hippchen, B., Alfred, R., Poralla, K., 1981. Occurrence in soil of thermo-acidophilic bacilli possessing ω-cyclohexane fatty acids and hopanoids. Arch. Microbiol. 129, 53–55.

Huang, L., El-Hussein, A., Xuan, W., Hamblin, M.R., 2018a. Potentiation by potassium iodide reveals that the anionic porphyrin TPPS4 is a surprisingly effective photosensitizer for antimicrobial photodynamic inactivation. J. Photochem. Photobiol. B Biol. 178, 277–286. https://doi.org/10.1016/j.jphotobiol.2017.10.036

Huang, Y.Y., Wintner, A., Seed, P.C., Brauns, T., Gelfand, J.A., Hamblin, M.R., 2018b. Antimicrobial photodynamic therapy mediated by methylene blue and potassium iodide to treat urinary tract infection in a female rat model. Sci. Rep. 8, 1–9. https://doi.org/10.1038/s41598-018-25365-0

Ihns, R., Diamante, L.M., Savage, G.P., Vanhanen, L., 2011. Effect of temperature on the drying characteristics, colour, antioxidant and beta-carotene contents of two apricot varieties. Int. J. Food Sci. Technol. 46, 275–283. https://doi.org/10.1111/j.1365-2621.2010.02506.x

Jesus, V., Martins, D., Branco, T., Valério, N., Neves, M.G.P.M.S., Faustino, M.A.F., Reis, L., Barreal, E., Gallego, P.P., Almeida, A., 2018. An insight into the photodynamic approach: Versus copper formulations in the control of Pseudomonas syringae pv. Actinidiae in kiwi plants. Photochem. Photobiol. Sci. 17, 180–191. https://doi.org/10.1039/c7pp00300e

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

Le, K., Chiu, F., Ng, K., 2007. Identification and quantification of antioxidants in Fructus lycii. Food Chem. 105, 353–363. https://doi.org/10.1016/J.FOODCHEM.2006.11.063

Lee, S.-Y., Gray, P.M., Dougherty, R.H., Kang, D.-H., 2004. The use of chlorine dioxide to control Alicyclobacillus acidoterrestris spores in aqueous suspension and on apples. Int. J. Food Microbiol. 92, 121–127. https://doi.org/10.1016/j.ijfoodmicro.2003.09.003

Lee, S.-Y., Ryu, S.-R., Kang, D.-H., 2010. Treatment with chlorous acid to inhibit spores of Alicyclobacillus acidoterrestris in aqueous suspension and on apples. Lett. Appl. Microbiol. no-no. https://doi.org/10.1111/j.1472-765X.2010.02874.x

Luksiene, Z., Paskeviciute, E., 2011. Novel approach to the microbial decontamination of strawberries: Chlorophyllin-based photosensitization. J. Appl. Microbiol. 110, 1274–1283. https://doi.org/10.1111/j.1365-2672.2011.04986.x

Maisch, T., 2009. A new strategy to destroy antibiotic resistant microorganisms: antimicrobial photodynamic treatment. Mini-Reviews Med. Chem. 9, 974–983. https://doi.org/10.2174/138955709788681582

Martins, D., Mesquita, M.Q., Neves, M.G.P.M.S., Faustino, M.A.F., Reis, L., Figueira,

E., Almeida, A., 2018. Photoinactivation of Pseudomonas syringae pv. actinidiae in kiwifruit plants by cationic porphyrins. Planta 248, 409–421. https://doi.org/10.1007/s00425-018-2913-y

Minnock, A., Vernon, D.I., Schofield, J., Griffiths, J., Parish, J.H., Brown, S.B., 2000. Mechanism of uptake of a cationic water-soluble pyridinium zinc phthalocyanine across the outer membrane of Escherichia coli. Antimicrob. Agents Chemother. 44, 522–527. https://doi.org/10.1128/AAC.44.3.522-527.2000

Nassar, S.J.M., Wills, C., Harriman, A., 2019. Inhibition of the photobleaching of methylene blue by association with urea. ChemPhotoChem 3, 1042–1049. https://doi.org/10.1002/cptc.201900141

Oliveira, A., Almeida, A., Carvalho, C.M.B., Tomé, J.P.C., Faustino, M.A.F., Neves, M.G.P.M.S., Tomé, A.C., Cavaleiro, J.A.S., Cunha, A., 2009. Porphyrin derivatives as photosensitizers for the inactivation of Bacillus cereus endospores. J. Appl. Microbiol. 106, 1986–1995. https://doi.org/10.1111/j.1365-2672.2009.04168.x

Orr, R.V., Beuchat, L.R., 2000. Efficacy of disinfectants in killing spores of Alicyclobacillus acidotorrestris and performance of media for supporting colony development by survivors. J. Food Prot. 63, 1117–1122. https://doi.org/10.4315/0362-028X-63.8.1117

Osopale, B.A., Witthuhn, C.R., Albertyn, J., Oguntoyinbo, F.A., 2017. Inhibitory spectrum of diverse guaiacol-producing Alicyclobacillus acidoterrestris by poly dimethyl ammonium chloride disinfectant. LWT - Food Sci. Technol. 84, 241–247. https://doi.org/10.1016/j.lwt.2017.05.052

Oteiza, J.M., Ares, G., Sant'Ana, A.S., Soto, S., Giannuzzi, L., 2011. Use of a multivariate approach to assess the incidence of Alicyclobacillus spp. in concentrate fruit juices marketed in Argentina: Results of a 14-year survey. Int. J. Food Microbiol. 151, 229–234. https://doi.org/10.1016/j.ijfoodmicro.2011.09.004

Oteiza, J.M., Soto, S., Alvarenga, V.O., Sant'Ana, A.S., Giannuzzi, L., 2014. Flavorings as new sources of contamination by deteriogenic Alicyclobacillus of fruit juices and beverages. Int. J. Food Microbiol. 172, 119–124.

https://doi.org/10.1016/j.ijfoodmicro.2013.12.007

Pflug, I.J., 1999. Microbiology and engineering of sterilization process., 10th ed. University of Minnesota, Minneapolis, MN.

Pornpukdeewattana, S., Jindaprasert, A., Massa, S., 2020. Alicyclobacillus spoilage and control - a review. Crit. Rev. Food Sci. Nutr. 60, 108–122. https://doi.org/10.1080/10408398.2018.1516190

Prado, D.B. do, Szczerepa, M.M. dos A., Capeloto, O.A., Astrath, N.G.C., Santos, N.C.A. dos, Previdelli, I.T.S., Nakamura, C.V., Mikcha, J.M.G., Abreu Filho, B.A. de, 2019. Effect of ultraviolet (UV-C) radiation on spores and biofilms of Alicyclobacillus spp. in industrialized orange juice. Int. J. Food Microbiol. 305. https://doi.org/10.1016/j.ijfoodmicro.2019.108238

Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., Jacob, R., 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (orac fl) of plasma and other biological and food samples. J. Agric. Food Chem. 51, 3273–3279. https://doi.org/10.1021/jf0262256

Santos, A.R., Batista, A.F.P., Gomes, A.T.P.C., Neves, M. G.P.M.S., Faustino, M.A.F., Almeida, A., Hioka, N., Mikcha, J.M.G., 2019. The remarkable effect of potassium iodide in eosin and rose bengal photodynamic action against Salmonella Typhimurium and Staphylococcus aureus. Antibiotics 8. https://doi.org/10.3390/antibiotics8040211

Santos, A.R., da Silva, A.F., Batista, A.F.P., Freitas, C.F., Bona, E., Sereia, M.J., Caetano, W., Hioka, N., Mikcha, J.M.G., 2020. Application of response surface methodology to evaluate photodynamic inactivation mediated by eosin y and 530 nm led against Staphylococcus aureus. Antibiotics 9, 125. https://doi.org/10.3390/antibiotics9030125

Sawaki, T., 2007. Introduction, in: Yokota, A., Fujii, T., Goto, K. (Eds.), Alicyclobacillus: Thermophilic acidophilic bacilli. Springer, Japan, Tokyo, pp. 1–5. https://doi.org/10.1007/978-4-431-69850-0_1

Siegmund, B., Pöllinger-Zierler, B., 2006. Odor thresholds of microbially induced off-

flavor compounds in apple juice. J. Agric. Food Chem. 54, 5984–5989. https://doi.org/10.1021/jf060602n

Silva, L.P., Gonzales-Barron, U., Cadavez, V., Sant'Ana, A.S., 2015. Modeling the effects of temperature and pH on the resistance of Alicyclobacillus acidoterrestris in conventional heat-treated fruit beverages through a meta-analysis approach. Food Microbiol. 46, 541–552. https://doi.org/10.1016/j.fm.2014.09.019

Simões, C., Gomes, M.C., Neves, M.G.P.M.S., Cunha, A., Tomé, J.P.C., Tomé, A.C., Cavaleiro, J.A.S., Almeida, A., Faustino, M.A.F., 2016. Photodynamic inactivation of Escherichia coli with cationic meso-tetraarylporphyrins - The charge number and charge distribution effects. Catal. Today 266, 197–204. https://doi.org/10.1016/j.cattod.2015.07.031

Sousa, V., Gomes, A.T.P.C., Freitas, A., Faustino, M.A.F., Neves, M.G.P.M.S., Almeida, A., 2019. Photodynamic Inactivation of Candida albicans in Blood Plasma and Whole Blood. Antibiotics 8, 221. https://doi.org/10.3390/antibiotics8040221

Spinelli, A.C.N.F., Sant'Ana, A.S., Rodrigues-Junior, S., Massaguer, P.R., 2009. Influence of different filling, cooling, and storage conditions on the growth of Alicyclobacillus acidoterrestris CRA7152 in orange juice. Appl. Environ. Microbiol. 75, 7409–7416. https://doi.org/10.1128/AEM.01400-09

Tao, R., Zhang, F., Tang, Q. juan, Xu, C. shan, Ni, Z.J., Meng, X. hong, 2019. Effects of curcumin-based photodynamic treatment on the storage quality of fresh-cut apples. Food Chem. 274, 415–421. https://doi.org/10.1016/j.foodchem.2018.08.042

Taze, B.H., Unluturk, S., Buzrul, S., Alpas, H., 2015. The impact of UV-C irradiation on spoilage microorganisms and colour of orange juice. J. Food Sci. Technol. 52, 1000–1007. https://doi.org/10.1007/s13197-013-1095-7

Tomé, J.P.C., Silva, E.M.P., Pereira, A.M.V.M., Alonso, C.M.A., Faustino, M.A.F., Neves, M.G.P.M.S., Tomé, A.C., Cavaleiro, J.A.S., Tavares, S.A.P., Duarte, R.R., Caeiro, M.F., Valdeira, M.L., 2007. Synthesis of neutral and cationic tripyridylporphyrin-D-galactose conjugates and the photoinactivation of HSV-1. Bioorganic Med. Chem. 15, 4705–4713. https://doi.org/10.1016/j.bmc.2007.05.005

Tremarin, A., Brandão, T.R.S., Silva, C.L.M., 2017. Inactivation kinetics of Alicyclobacillus acidoterrestris in apple juice submitted to ultraviolet radiation. Food Control 73, 18–23. https://doi.org/10.1016/j.foodcont.2016.07.008

Uchida, R., Silva, F.V.M., 2017. Alicyclobacillus acidoterrestris spore inactivation by high pressure combined with mild heat: Modeling the effects of temperature and soluble solids. Food Control 73, 426–432. https://doi.org/10.1016/j.foodcont.2016.08.034

USDA, 2020. United States Department of Agriculture. Foreign Agricultural Service. Citrus: World Markets and Trade. January. https://www.fas.usda.gov/data/citrus-world-markets-and-trade (accessed 4.23.20).

Vecchio, D., Gupta, A., Huang, L., Landi, G., Avci, P., Rodas, A., Hamblin, M.R., 2015. Bacterial photodynamic inactivation mediated by methylene blue and red light is enhanced by synergistic effect of potassium iodide. Antimicrob. Agents Chemother. 59, 5203–5212. https://doi.org/10.1128/AAC.00019-15

Vieira, C., Gomes, A.T.P.C., Mesquita, M.Q., Moura, N.M.M., Neves, M.G.P.M.S., Faustino, A.F., Almeida, A., 2018. An insight into the potentiation effect of potassium iodide on APDT efficacy. Front. Microbiol. 9, 1–16. https://doi.org/10.3389/fmicb.2018.02665

Vieira, C., Santos, A., Mesquita, M.Q., Gomes, A.T.P.C., Neves, M.G.P.M.S., Faustino, M.A.F., Almeida, A., 2019. Advances in aPDT based on the combination of a porphyrinic formulation with potassium iodide: Effectiveness on bacteria and fungi planktonic/biofilm forms and viruses. J. Porphyr. Phthalocyanines 23, 534–545. https://doi.org/10.1142/S1088424619500408

Wainwright, M., Maisch, T., Nonell, S., Plaetzer, K., Almeida, A., Tegos, G.P., Hamblin, M.R., 2017. Photoantimicrobials—are we afraid of the light? Lancet Infect. Dis. 17, e49–e55. https://doi.org/10.1016/S1473-3099(16)30268-7

Wen, X., Zhang, X., Szewczyk, G., El-Hussein, A., Huang, Y.-Y., Sarna, T., Hamblin, M.R., 2017. Potassium iodide potentiates antimicrobial photodynamic inactivation mediated by rose bengal in in vitro and in vivo studies. Antimicrob. Agents Chemother.

61, 1–15.

Wisotzkey, J.D., Jurtshuk, P., Fox, G.E., Deinhard, G., Poralla, K., 1992. Comparative sequence analyses on the 16S rRNA (rDNA) of Bacillus acidocaldarius, Bacillus acidoterrestris, and Bacillus cycloheptanicus and proposal for creation of a new genus, Alicyclobacillus gen. nov. Int. J. Syst. Bacteriol. 42, 263–269. https://doi.org/10.1099/00207713-42-2-263

Wu, J., Hou, W., Cao, B., Zuo, T., Xue, C., Leung, A.W., Xu, C., Tang, Q.J., 2015. Virucidal efficacy of treatment with photodynamically activated curcumin on murine norovirus bio-accumulated in oysters. Photodiagnosis Photodyn. Ther. 12, 385–392. https://doi.org/10.1016/j.pdpdt.2015.06.005

Yuan, L., Lyu, P., Huang, Y.Y., Du, N., Qi, W., Hamblin, M.R., Wang, Y., 2020. Potassium iodide enhances the photobactericidal effect of methylene blue on Enterococcus faecalis as planktonic cells and as biofilm infection in teeth. J. Photochem. Photobiol. B Biol. 203, 111730. https://doi.org/10.1016/j.jphotobiol.2019.111730

Zhang, Y., Dai, T., Wang, M., Vecchio, D., Chiang, L.Y., Hamblin, M.R., 2015. Potentiation of antimicrobial photodynamic inactivation mediated by a cationic fullerene by added iodide: in vitro and in vivo studies. Nanomedicine 10, 603–614. https://doi.org/10.2217/nnm.14.131

Zudyte, B., Luksiene, Z., 2019. Toward better microbial safety of wheat sprouts: Chlorophyllin-based photosensitization of seeds. Photochem. Photobiol. Sci. 18, 2521–2530. https://doi.org/10.1039/c9pp00157

Discussão geral

A partir da revisão de literatura disponível no capítulo 1 observou-se uma grande quantidade de estudos envolvendo a inativação fotodinâmica de células vegetativas e biofilmes de origem bacteriana (HUANG et al., 2020; SANTOS et al., 2019; SILVA et al., 2019; VIEIRA et al., 2019; YASSUNAKA et al., 2015). Assim, neste estudo uma maior atenção foi direcionada para a avaliação dos efeitos de aPDT em esporos de *B. cereus* e *A. acidoterrestris*.

No capítulo 2 foram avaliadas 12 diferentes cepas de *B. cereus* em relação aos efeitos do TFA com NMB e um aparato de luz vermelha (Figura 1 – A). A partir dos resultados da análise de concentração mínima inibitória (MIC) ao NMB, foi possível determinar a resistência das cepas de *B. cereus* ao tratamento fotodinâmico. Embora algumas cepas fossem da mesma origem essa condição não determinou a resistência ao processo fotodinâmico. A formação de quatro grupos de diferentes níveis de resistência à TFA revelou uma importante variabilidade entre as cepas estudadas. Este comportamento também foi observado durante o processo de secagem de leite por *spray drying* com as mesmas cepas utilizadas neste estudo (ALVARENGA et al., 2018) e confirmado entre cepas de *B. subtilis* por outro estudo (DEN BESTEN et al., 2017).

Desta forma, uma cepa de *B. cereus* de cada grupo foi selecionada de acordo com a sua resistência (B63 > 436 > B3 > ATCC 14579) para a determinação dos parâmetros cinéticos de inativação fotodinâmica. O processo de TFA na presença de NMB combinado com luz vermelha foi avaliado em células vegetativas e esporos de cada uma das cepas. Os resultados demonstraram que a inativação fotodinâmica das células vegetativas e esporos das 4 cepas selecionadas não obedeceu a uma cinética de inativação linear. Assim, o modelo de Weibull (não-linear) foi usado para ajustar os dados observados e estimar os parâmetros cinéticos de fotoinativação. O modelo de Weibull já havia sido usado para descrever a inativação fotodinâmica de células vegetativas de *B. cereus* (LE MARC et al., 2009). Outro estudo também investigou os efeitos de TFA na viabilidade celular de *B. cereus* e observou que a eficiência do

tratamento era dependente da fluência aplicada (APONIENE et al., 2015). O mesmo comportamento também foi observado neste estudo.

Como era esperado, a inativação fotodinâmica dos esporos exigiu doses de luz mais elevadas do que as células vegetativas. A estrutura da capa dos esporos, principalmente de esporos envelhecidos, elevou a resistência térmica e ao hipoclorito de sódio de *B. subtilis* em estudos anteriores (SANCHEZ-SALAS et al., 2011). Entretanto, a cepa mais sensível identificada neste estudo (ATCC 14579) apresentou resultados semelhantes entre células vegetativas e esporos. A sensibilidade entre células vegetativas e esporos de *B. cereus* foi comparada anteriormente, onde os esporos foram ligeiramente mais resistentes do que as células vegetativas usando compostos fenotiazínicos (DEMIDOVA; HAMBLIN, 2005).

Curiosamente, os valores mais baixos de δ (J/cm^2) para as células vegetativa foram observados na presença de NMB à 5 µM, sugerindo que nesta concentração a primeira redução decimal ocorreu mais rápido do que em concentrações mais elevadas. No entanto, de acordo com os valores de *p* apresentados na Tabela 2 do Capítulo 2, as cepas B63, 436 e B3 tornam-se resistentes à medida que a fluência aumenta com NMB 5 µM. Este comportamento confirmou-se pelas curvas de inativação côncavas ($p < 1$) apresentadas nas figuras 3-A-B-C. A única cepa com curvas de inativação convexas ($p > 1$) para todas as concentrações de NMB foi a ATCC 14579, indicando que essa cepa foi progressivamente inativada em todas as condições.

Em todas as condições testadas para esporos de *B. cereus*, os valores de *p* foram maiores do que 1 (Tabela 3; Capítulo 1). Curvas de inativação convexas ($p > 1$) indicam que os micro-organismos se tornam cada vez mais susceptíveis ao tratamento aplicado (VAN BOEKEL, 2002). Desta forma, observou-se que os esporos de *B. cereus* forma progressivamente inativados ao longo do tempo. Além disso, a fluência necessária para atingir 4 reduções decimais (4D) para todas as cepas testadas chegou a 400 J/cm^2 e 50 µM de NMB, exceto para a cepa mais susceptível ATCC 14579 com 25 J/cm^2 a 50 e 100 µM.

No capítulo 3, a eficiência de tratamento fotodinâmico para a inativação de esporos de *A. acidoterrestris* usando Tetra-Py^+-Me e NMB foi investigada. Até onde

se sabe, este é o primeiro estudo que demonstrou a aplicação de TFA em esporos de *Alicyclobacillus* sp. Este estudo foi realizado inicialmente *in vitro* (PBS) para ambos os FS a 10 µM de concentração combinados com luz branca artificial do tipo LED (Figura 1 – B). O tratamento fotodinâmico com a porfirina Tetra-Py^{+}-Me (10 µM) foi capaz de reduzir em 7 ciclos logarítmicos a população de esporos de *A. acidoterrestris* após 5 horas de exposição à luz. Entretanto, não foi observada fotoinativação dos esporos de *A. acidoterrestris* utilizando NMB na mesma concentração (Figura 4; Capítulo 3). Este resultado, pode ser atribuído ao processo de degradação ou fotobranqueamento de corantes fenotiazínicos já observado em estudos anteriores pela reação com o oxigênio singleto (GALSTYAN; DOBRINDT, 2019; NASSAR; WILLS; HARRIMAN, 2019).

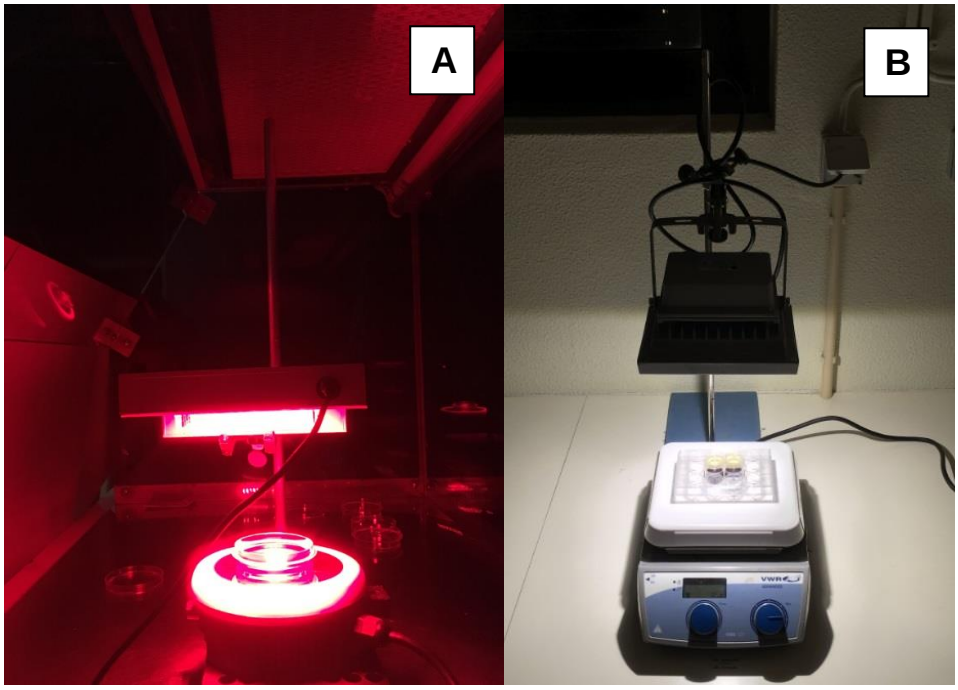

Figura 1. Aparato de 96 LEDs emissores de luz vermelha (400-700 nm; máximo: 631 nm; **A**) e aparato de LED emissor de luz branca (400-700 nm; máximo: ~ 440 nm e 540 nm; **B**)

Estudos anteriores já haviam demonstrado a eficiência do tratamento fotodinâmico em reduzir a carga de bactérias e vírus (3-8 ciclos logarítmicos) e fungos (0,8-5 ciclos logarítmicos) (BEIRÃO et al., 2014; DE MENEZES et al., 2016; VIEIRA et al., 2019; YUAN et al., 2020). Por exemplo, a porfirina Tetra-Py^{+}-Me também causou de 3 a 6 reduções logarítmicas durante a fotoinativação de biofilmes (mono- e multi-

espécies) de *Staphylococcus aureus*, *Pseudomonas aeruginosa* e *Candida albicans* (BEIRÃO et al., 2014). A formação de biofilmes em ambientes de processamento de alimentos dificulta a inativação microbiana já que são estruturas que aderem fortemente à uma determinada superfície (BRIDIER et al., 2011; GALIÉ et al., 2018). Portanto, a eficiência da inativação fotodinâmica de microrganismos em biofilmes pode sugerir que essa tecnologia também pode ser eficaz na inativação de bactérias formadoras de esporos. Por exemplo, estudos envolvendo derivados de porfirinas já demonstraram que o uso de Tetra-Py^{+}-Me como FS reduziu em até 3,5 ciclos logarítmicos as populações de esporos de *B. cereus* (OLIVEIRA et al., 2009).

A partir dos resultados *in vitro* de inativação fotodinâmica dos esporos de *A. acidoterrestris*, outros testes foram realizados em matrizes reais de alimentos como o suco de laranja. O uso do suco de laranja justifica-se pelos impactos negativos causados pela presença da bactéria *Alicyclobacillus* neste alimento, descritos anteriormente neste trabalho.

Dada a complexidade do suco de laranja em relação ao PBS (por exemplo, turbidez), a presença e a ausência de iodeto de potássio (KI) foram avaliadas, pois era esperado que o efeito fotodinâmico fosse reduzido (SOUSA et al., 2019). O tratamento TFA com Tetra-Py^{+}-Me e NMB (10 µM) na presença de KI no suco de laranja resultou em até 5 reduções logarítmicas de esporos de *A. acidoterrestris* (Figura 5).

Desde que foi introduzido o uso de KI como agente potenciador do efeito fotodinâmico (VECCHIO et al., 2015; ZHANG et al., 2015), vários estudos foram conduzidos para confirmar essa ação em diferentes micro-organismos e estruturas microbianas (FREIRE et al., 2016; HUANG et al., 2018; VIEIRA et al., 2018, 2019; WEN et al., 2017). Nestes estudos, os autores concluíram que o KI potencializa os efeitos fotodinâmicos na maioria dos casos, aumentando a inativação de microrganismos em até 6 reduções logarítmicas.

Embora nenhum relato de estudo sobre o uso de TFA para a inativação de *A. acidoterrestris* em bebidas tenha sido identificado, outras tecnologias baseadas em exposição à luz foram utilizadas com esse objetivo. Por exemplo, verificou-se que a radiação UV-C foi capaz de causar entre 2 – 5,5 reduções logarítmicas na população

de esporos de *A. acidoterrestris* em sucos de maçã, uva e melão (BAYSAL; MOLVA; UNLUTURK, 2013; FUNDO et al., 2019; TREMARIN; BRANDÃO; SILVA, 2017). As diferenças de eficiência de inativação observadas entre os diferentes sucos podem ser explicadas pelo grau de turbidez de cada suco, que pode influenciar a penetração da luz.

O número semelhante de reduções decimais de esporos de *A. acidoterrestris* obtidas pelo presente estudo (Figura 4 e 5) e UV-C (Baysal et al., 2013; Fundo et al., 2019; Tremarin et al., 2017), apesar da diferença na idade dos esporos utilizados (28 e 2-10 dias, respectivamente), destacam os potenciais do tratamento fotodinâmico para a inativação dessa bactéria. Apesar do tempo mais longo para atingir um número semelhante de reduções decimais, outra vantagem do uso de fontes de luz visível (como no TFA) é que o tratamento fotodinâmico não apresenta riscos à saúde do operador (GUERRERO-BELTRÁN; BARBOSA-CÁNOVAS, 2004). No entanto, a eficiência do TFA e a redução do tempo de exposição podem ser aprimoradas pela combinação de compostos não tóxicos com o FS.

Como parte do processo de avaliação do impacto de TFA nas características do suco de laranja, também foram avaliados os efeitos na cor do suco. Os resultados demonstraram que houve alteração em todos os parâmetros de cor (*L*, a*, b**) do suco de laranja após 10 h de tratamento. Entretanto, estudos recentes mostraram que até mesmo períodos curtos (20 min) de exposição à radiação UV-C, foi observada uma alteração significativa na coordenada *L** em suco de melão (FUNDO et al., 2019). Além disso, o impacto da exposição à UV-C e LED em termos de alteração de cor diferem na literatura (BHAVYA; HEBBAR, 2019; TAO et al., 2019). Estudos anteriores também avaliaram o impacto da TFA na qualidade nutricional de morangos, damascos, ameixas, couve-flor e suco de laranja (APONIENE et al., 2015; BHAVYA; HEBBAR, 2019; LUKSIENE; PASKEVICIUTE, 2011). Embora esses estudos não tenham demonstrado nenhum efeito adverso significante na capacidade antioxidante do alimento, nenhum desses estudos aplicou o TFA por um período tão longo ou avaliou a inativação de esporos bacterianos como o presente estudo.

A partir dos resultados obtidos nos experimentos com suco de laranja, avaliou-se a aplicação do tratamento fotodinâmico superficialmente em laranjas artificialmente

contaminadas com esporos de *A. acidoterrestris*. Os resultados mostraram uma diminuição significativa de esporos (≈ 2,8 reduções logarítmicas) durante o tratamento sob luz solar. Este é um resultado importante, visto que a contaminação de *Alicyclobacillus* spp. é de até 10^2-10^3 esporos/mL em produtos finais (ABECITRUS, 1999; OTEIZA et al., 2011). Portanto, os resultados de inativação de esporos de *A. acidoterrestris* em cascas de laranja obtidas pelo tratamento fotodinâmico sob radiação solar constituem um notável avanço tecnológico. Essa tecnologia pode ser aplicada na prática diretamente nos frutos das árvores expostas à luz solar abundante. Como tal, surge como uma estratégia viável para reduzir as contagens de esporos de *Alicyclobacillus* spp. antes do processamento de frutas.

Conclusão geral

Neste estudo foram avaliadas duas das principais espécies bacterianas envolvidas em processos de DTAs e deterioração de alimentos e bebidas. Por meio de uma nova abordagem de inativação microbiana voltada para o setor agroalimentar, foi possível determinar parâmetros cinéticos de inativação e a aplicação tecnológica em matrizes reais de alimentos.

Inicialmente nos ensaios de CIM com as 12 cepas de *B. cereus* foi observado que existe, inclusive, variabilidade entre cepas da mesma origem em relação ao processo de inativação fotodinâmica. Este fato é extremamente relevante visto que os processos industriais estabelecem parâmetros de inativação referentes a uma determinada espécie microbiana. As cepas de *B. cereus* utilizadas nos estudos subsequentes, como mencionado anteriormente, são oriundas de diferentes origens como refeições prontas (B63), chocolate (436) e leite (B3), além de uma cepa padrão (ATCC 14579). Tais cepas podem causar doenças e deterioração em produtos lácteos. O uso de TFA mediado por NMB e luz vermelha foi capaz de reduzir as populações de células vegetativas e esporos de *B. cereus*. O uso da modelagem matemática por meio do modelo de Weibull foi capaz de descrever o comportamento não-linear das curvas de inativação, bem como estimar os parâmetros cinéticos de inativação fotodinâmica. Essa ferramenta poderá contribuir com a otimização de ensaios de TFA como, por exemplo, determinando as concentrações de FS e tempo de exposição à luz.

Assim como foi observado no capítulo 2 que o processo de TFA inativou células vegetativas e esporos de *B. cereus*, o capítulo 3 elucidou a inativação de esporos de *A. acidoterrestris* em diferentes matrizes. Além disso, este estudo confirmou o efeito potencializador do KI em processos fotodinâmicos e o uso da radiação solar, sugerindo que a técnica poderia ser aplicada em campos de plantações. Comprovadamente TFA trata-se de uma tecnologia emergente que poderá em um futuro próximo substituir métodos tradicionais, como é o caso da pasteurização e sanitização. No entanto, existem gargalos que podem afetar o processo de aplicação tecnológica da TFA na indústria de alimentos, como por exemplo, efeitos indesejáveis

de alteração de cor e redução do valor nutricional dos alimentos. A otimização do processo de TFA, como o uso de FS e fontes de luz que não alterem o valor nutricional bem como as características organolépticas dos alimentos surgem como uma próspera tendência para trabalhos futuros.

Referências

ABECITRUS. Acidothermophilic sporeforming bacteria (ATSB) in orange juices: Detection methods, ecology, and involvement in the deterioration of fruit juices. Disponível em: <http://www.citrusbr.com.br/exportadores-citricos/sobre-citrusbr/imagens/ATSB_Abecitrus.pdf>. Acesso em: 15 maio. 2020.

ALVARENGA, V. O. et al. Survival variability of 12 strains of *Bacillus cereus* yielded to spray drying of whole milk. **International Journal of Food Microbiology**, v. 286, p. 80–89, 2018.

APONIENE, K. et al. Reduction of microbial contamination of fruits and vegetables by hypericin-based photosensitization: Comparison with other emerging antimicrobial treatments. **Journal of Food Engineering**, v. 144, p. 29–35, 2015.

BARBA, F. J. et al. Mild processing applied to the inactivation of the main foodborne bacterial pathogens: A review. **Trends in Food Science & Technology**, v. 66, p. 20–35, 2017.

BARTOLOMEU, M. et al. Effect of photodynamic therapy on the virulence factors of *Staphylococcus aureus*. **Frontiers in Microbiology**, v. 7, p. 1–11, 2016.

BAYSAL, A. H.; MOLVA, C.; UNLUTURK, S. UV-C light inactivation and modeling kinetics of *Alicyclobacillus acidoterrestris* spores in white grape and apple juices. **International Journal of Food Microbiology**, v. 166, n. 3, p. 494–498, 2013.

BEIRÃO, S. et al. Photodynamic inactivation of bacterial and yeast biofilms with a cationic porphyrin. **Photochemistry and Photobiology**, v. 90, n. 6, p. 1387–1396, 2014.

BHAVYA, M. L.; HEBBAR, H. U. Sono-photodynamic inactivation of *Escherichia coli* and *Staphylococcus aureus* in orange juice. **Ultrasonics sonochemistry**, v. 57, n. February, p. 108–115, 2019.

BOTTONE, E. J. *Bacillus cereus*, a volatile human pathogen. **Clinical Microbiology Reviews**, v. 23, n. 2, p. 382–398, 2010.

BRIDIER, A. et al. Resistance of bacterial biofilms to disinfectants: a review.

Biofouling, v. 27, n. 9, p. 1017–1032, 2011.

CARLIN, F. Origin of bacterial spores contaminating foods. **Food Microbiology**, v. 28, n. 2, p. 177–182, 2011.

CEBRIÁN, G.; MAÑAS, P.; CONDÓN, S. Comparative resistance of bacterial foodborne pathogens to non-thermal technologies for food preservation. **Frontiers in Microbiology**, v. 7, p. 1–17, 2016.

CERNY, G.; HENNLICH, W.; PORALLA, K. Fruchtsaftverderb durch Bacillen: Isolierung und charakterisierung des verderbserregers. **Zeitschrift für Lebensmittel-Untersuchung und -Forschung**, v. 179, n. 3, p. 224–227, 1984.

CHANG, S.-S.; KANG, D.-H. *Alicyclobacillus* spp. in the fruit juice industry: history, characteristics, and current isolation/detection procedures. **Critical reviews in microbiology**, v. 30, n. 2, p. 55–74, 2004.

DE MENEZES, H. D. et al. Photodynamic treatment with phenothiazinium photosensitizers kills both ungerminated and germinated microconidia of the pathogenic fungi *Fusarium oxysporum*, *Fusarium moniliforme* and *Fusarium solani*. **Journal of Photochemistry and Photobiology B: Biology**, v. 164, p. 1–12, 2016.

DEMIDOVA, T. N.; HAMBLIN, M. R. Photodynamic inactivation of *Bacillus* spores, mediated by phenothiazinium dyes. **Applied and Environmental Microbiology**, v. 71, n. 11, p. 6918–6925, 2005.

DEN BESTEN, H. M. W. et al. Microbial variability in growth and heat resistance of a pathogen and a spoiler: All variabilities are equal but some are more equal than others. **International Journal of Food Microbiology**, v. 240, p. 24–31, 2017.

FAO. Food and Agricultural Organization. Global food losses and food waste – Extent, causes and prevention. Disponível em: <http://www.fao.org/3/a-i2697e.pdf>. Acesso em: 18 abr. 2020.

FERRARIO, M. I.; GUERRERO, S. N. Inactivation of *Alicyclobacillus acidoterrestris* ATCC 49025 spores in apple juice by pulsed light. Influence of initial contamination and required reduction levels. **Revista Argentina de Microbiologia**, v. 50, n. 1, p. 3–11, 2018.

FREIRE, F. et al. Photodynamic therapy of oral *Candida* infection in a mouse model. **Journal of Photochemistry and Photobiology B: Biology**, v. 159, p. 161–168, 2016.

FUNDO, J. F. et al. UV-C light processing of Cantaloupe melon juice: Evaluation of the impact on microbiological, and some quality characteristics, during refrigerated storage. **LWT - Food Science and Technology**, v. 103, p. 247–252, 2019.

GALIÉ, S. et al. Biofilms in the food industry: Health aspects and control methods. **Frontiers in Microbiology**, v. 9, n. 898, 2018.

GALSTYAN, A.; DOBRINDT, U. Determining and unravelling origins of reduced photoinactivation efficacy of bacteria in milk. **Journal of Photochemistry and Photobiology B: Biology**, v. 197, p. 111554, 2019.

GUERRERO-BELTRÁN, J. A.; BARBOSA-CÁNOVAS, G. V. Review: Advantages and limitations on processing foods by UV light. **Food Science and Technology International**, v. 10, n. 3, p. 137–147, 2004.

HAMBLIN, M. R.; ABRAHAMSE, H. Oxygen-independent antimicrobial photoinactivation: Type III photochemical mechanism? **Antibiotics**, v. 9, n. 53, 2020.

HEYNDRICKX, M. The importance of endospore-forming bacteria originating from soil for contamination of industrial food processing. **Applied and Environmental Soil Science**, v. 2011, p. 1–11, 2011.

HIPPCHEN, B.; RÖLL, A.; PORALLA, K. Occurrence in soil of thermo-acidophilic bacilli possessing ω-cyclohexane fatty acids and hopanoids. **Archives of Microbiology**, v. 129, p. 53–55, 1981.

HU, X. et al. Factors affecting *Alicyclobacillus acidoterrestris* growth and guaiacol production and controlling apple juice spoilage by lauric arginate and ϵ-polylysine. **LWT**, v. 119, p. 108883, fev. 2020.

HUANG, J. et al. Enhanced antibacterial and antibiofilm functions of the curcumin-mediated photodynamic inactivation against Listeria monocytogenes. **Food Control**, v. 108, n. July 2019, p. 106886, fev. 2020.

HUANG, L. et al. Potentiation by potassium iodide reveals that the anionic porphyrin TPPS4 is a surprisingly effective photosensitizer for antimicrobial photodynamic inactivation. **Journal of Photochemistry and Photobiology B: Biology**, v. 178, n. August 2017, p. 277–286, jan. 2018.

JESIONEK, A.; VON TAPPEINER, H. Zur behandlung der hautcarcinome mit fluorescierenden stoffen. **Arch Klin Med**, v. 82, n. (in German), p. 223, 1905.

KIM, M.-J. et al. Antibacterial effect of 405±5nm light emitting diode illumination against Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella on the surface of fresh-cut mango and its influence on fruit quality. **International Journal of Food Microbiology**, v. 244, p. 82–89, 2017.

LE MARC, Y. et al. Modelling the photosensitization-based inactivation of Bacillus cereus. **Journal of Applied Microbiology**, v. 107, n. 3, p. 1006–1011, 2009.

LUKSIENE, Z.; PASKEVICIUTE, E. Novel approach to the microbial decontamination of strawberries: chlorophyllin-based photosensitization. **Journal of applied microbiology**, v. 110, n. 5, p. 1274–83, maio 2011.

MANSOORI, B. et al. Photodynamic therapy for cancer: Role of natural products. **Photodiagnosis and Photodynamic Therapy**, v. 26, p. 395–404, 2019.

MEHTA, D. S. et al. The ability of spore formers to degrade milk proteins, fat, phospholipids, common stabilizers, and exopolysaccharides. **Journal of Dairy Science**, v. 102, n. 12, p. 10799–10813, 2019.

NASSAR, S. J. M.; WILLS, C.; HARRIMAN, A. Inhibition of the Photobleaching of Methylene Blue by Association with Urea. **ChemPhotoChem**, v. 3, n. 10, p. 1042–1049, 10 out. 2019.

ODEYEMI, O. A. et al. Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. **Comprehensive Reviews in Food Science and Food Safety**, v. 19, n. 2, p. 311–331, 2020.

OLIVEIRA, A. et al. Porphyrin derivatives as photosensitizers for the inactivation of Bacillus cereus endospores. **Journal of Applied Microbiology**, v. 106, n. 6, p. 1986–1995, jun. 2009.

ÖLMEZ, H.; KRETZSCHMAR, U. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. **LWT - Food Science and Technology**, v. 42, n. 3, p. 686–693, 2009.

ORR, R. V. et al. Detection of guaiacol produced by Alicyclobacillus acidoterrestris in apple juice by sensory and chromatographic analyses, and comparison with spore and vegetative cell populations. **Journal of Food Protection**, v. 63, n. 11, p. 1517–1522, 2000.

OTEIZA, J. M. et al. Use of a multivariate approach to assess the incidence of Alicyclobacillus spp. in concentrate fruit juices marketed in Argentina: Results of a 14-year survey. **International Journal of Food Microbiology**, v. 151, n. 2, p. 229–234, dez. 2011.

PASKEVICIUTE, E.; ZUDYTE, B.; LUKŠIENE, Ž. Innovative Nonthermal Technologies: Chlorophyllin and Visible Light Significantly Reduce Microbial Load on Basil. **Food technology and biotechnology**, v. 57, n. 1, p. 126–132, mar. 2019.

RAAB, O. Über die Wirkung fluoreszcierender Stoffe aus Infusorien. **Ztg. Biol.**, v. 39, n. (in German), p. 524, 1900.

REVERTER-CARRIÓN, L. et al. Inactivation study of Bacillus subtilis, Geobacillus stearothermophilus, Alicyclobacillus acidoterrestris and Aspergillus niger spores under Ultra-High Pressure Homogenization, UV-C light and their combination. **Innovative Food Science and Emerging Technologies**, v. 48, n. June 2017, p. 258–264, 2018.

RODRIGUES, G. B. et al. Photodynamic inactivation of Candida albicans and Candida tropicalis with aluminum phthalocyanine chloride nanoemulsion. **Fungal Biology**, n. xxxx, p. 4–10, 2019.

SANCHEZ-SALAS, J. L. et al. Maturation of released spores is necessary for acquisition of full spore heat resistance during Bacillus subtilis sporulation. **Applied and Environmental Microbiology**, v. 77, n. 19, p. 6746–6754, 2011.

SANTOS, A. R. et al. The remarkable effect of potassium iodide in eosin and rose bengal photodynamic action against salmonella typhimurium and staphylococcus aureus. **Antibiotics**, v. 8, n. 4, 2019.

SIEGMUND, B.; PÖLLINGER-ZIERLER, B. Odor thresholds of microbially induced off-flavor compounds in apple juice. **Journal of Agricultural and Food Chemistry**, v. 54, n. 16, p. 5984–5989, 2006.

SILVA, A. F. et al. Xanthene Dyes and Green <scp>LED</scp> for the Inactivation of Foodborne Pathogens in Planktonic and Biofilm States. **Photochemistry and Photobiology**, v. 95, n. 5, p. 1230–1238, 16 set. 2019.

SMIT, Y. et al. Alicyclobacillus spoilage and isolation – A review. **Food Microbiology**, v. 28, n. 3, p. 331–349, maio 2011.

SOUSA, V. et al. Photodynamic Inactivation of Candida albicans in Blood Plasma and Whole Blood. **Antibiotics**, v. 8, n. 4, p. 221, 13 nov. 2019.

SPANU, C. **Sporeforming bacterial pathogens in ready-to-eat dairy products**. [s.l.] Elsevier Inc., 2016.

SPINELLI, A. C. N. F. et al. Influence of different filling, cooling, and storage conditions on the growth of Alicyclobacillus acidoterrestris CRA7152 in orange juice. **Applied and Environmental Microbiology**, v. 75, n. 23, p. 7409–7416, 2009.

TAO, R. et al. Effects of curcumin-based photodynamic treatment on the storage quality of fresh-cut apples. **Food Chemistry**, v. 274, n. April 2018, p. 415–421, 2019.

TORLAK, E. Inactivation of Alicyclobacillus acidoterrestris spores in aqueous suspension and on apples by neutral electrolyzed water. **International Journal of Food Microbiology**, v. 185, p. 69–72, ago. 2014.

TREMARIN, A.; BRANDÃO, T. R. S.; SILVA, C. L. M. Inactivation kinetics of Alicyclobacillus acidoterrestris in apple juice submitted to ultraviolet radiation. **Food Control**, v. 73, p. 18–23, 2017.

UCHIDA, R.; SILVA, F. V. M. Alicyclobacillus acidoterrestris spore inactivation by high pressure combined with mild heat: Modeling the effects of temperature and soluble solids. **Food Control**, v. 73, p. 426–432, 2017.

VAN BOEKEL, M. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. **International Journal of Food Microbiology**, v. 74, n. 1–

2, p. 139–159, 25 mar. 2002.

VAN LUONG, T. S. et al. Diversity and guaiacol production of Alicyclobacillus spp. from fruit juice and fruit-based beverages. **International Journal of Food Microbiology**, v. 311, n. August, p. 108314, 2019.

VECCHIO, D. et al. Bacterial photodynamic inactivation mediated by methylene blue and red light is enhanced by synergistic effect of potassium iodide. **Antimicrobial Agents and Chemotherapy**, v. 59, n. 9, p. 5203–5212, 2015.

VIEIRA, C. et al. An insight into the potentiation effect of potassium iodide on APDT efficacy. **Frontiers in Microbiology**, v. 9, n. NOV, p. 1–16, 2018.

VIEIRA, C. et al. Advances in aPDT based on the combination of a porphyrinic formulation with potassium iodide: Effectiveness on bacteria and fungi planktonic/biofilm forms and viruses. **Journal of Porphyrins and Phthalocyanines**, v. 23, n. 4–5, p. 534–545, 2019.

WAINWRIGHT, M. et al. Photoantimicrobials—are we afraid of the light? **The Lancet Infectious Diseases**, v. 17, n. 2, p. e49–e55, 2017.

WEN, X. et al. Potassium Iodide Potentiates Antimicrobial Photodynamic Inactivation Mediated by Rose Bengal in In Vitro and In Vivo Studies. **Antimicrobial Agents and Chemotherapy**, v. 61, n. 7, p. 1–15, 2017.

WHO. **World Health Organization. WHO estimates of the global burden of foodborne diseases**. [s.l: s.n.]. Disponível em: <https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf?sequence=1>.

WISOTZKEY, J. D. et al. Comparative Sequence Analyses on the 16S rRNA (rDNA) of Bacillus acidocaldarius, Bacillus acidoterrestris, and Bacillus cycloheptanicus and Proposal for Creation of a New Genus, Alicyclobacillus gen. nov. **International Journal of Systematic Bacteriology**, v. 42, n. 2, p. 263–269, 1 abr. 1992.

WORLD BANK. **The Safe Food Imperative: Accelerating Progress in Low- and Middle-Income Countries**. Disponível em: <https://openknowledge.worldbank.org/bitstream/handle/10986/30568/97814648134

50.pdf?sequence=6&isAllowed=y>. Acesso em: 24 abr. 2020.

YAMAZAKI, K.; TEDUKA, H.; SHINANO, H. Isolation and Identification of Alicyclobacillus acidoterrestris from Acidic Beverages. **Bioscience, Biotechnology, and Biochemistry**, v. 60, n. 3, p. 543–545, 12 jan. 1996.

YASSUNAKA, N. N. et al. Photodynamic Inactivation Mediated by Erythrosine and its Derivatives on Foodborne Pathogens and Spoilage Bacteria. **Current Microbiology**, v. 71, n. 2, p. 243–251, 2015.

YUAN, L. et al. Potassium iodide enhances the photobactericidal effect of methylene blue on Enterococcus faecalis as planktonic cells and as biofilm infection in teeth. **Journal of Photochemistry and Photobiology B: Biology**, v. 203, n. December 2019, p. 111730, 2020.

ZHANG, Y. et al. Potentiation of antimicrobial photodynamic inactivation mediated by a cationic fullerene by added iodide: In vitro and in vivo studies. **Nanomedicine**, v. 10, n. 4, p. 603–614, 2015.

ŽUDYTE, B.; LUKŠIENE, Ž. Toward better microbial safety of wheat sprouts: Chlorophyllin-based photosensitization of seeds. **Photochemical and Photobiological Sciences**, v. 18, n. 10, p. 2521–2530, 2019.

Anexos

Anexo 1. Confirmação de aceite de publicação de artigo original referente ao Capítulo 3 entítulado: “Antimicrobial photodynamic treatment as an alternative approach for *Alicyclobacillus acidoterrestris* inactivation”.

16/09/2020 Fwd: Your Submission - prado.leonardo27@gmail.com - Gmail

De: **Luca Cocolin** <eesserver@eesmail.elsevier.com>
Date: seg., 27 de jul. de 2020 às 08:11
Subject: Your Submission
To: <and@unicamp.br>
Cc: <ubarron@ipb.pt>

Ms. Ref. No.: FOOD-D-20-00511R1
Title: Antimicrobial photodynamic treatment as an alternative approach for Alicyclobacillus acidoterrestris inactivation
International Journal of Food Microbiology

Dear Anderson,

I am pleased to inform you that your paper "Antimicrobial photodynamic treatment as an alternative approach for Alicyclobacillus acidoterrestris inactivation" has been accepted for publication in International Journal of Food Microbiology.

Your accepted manuscript will now be transferred to our production department and work will begin on creation of the proof. If we need any additional information to create the proof, we will let you know. If not, you will be contacted again in the next few days with a request to approve the proof and to complete a number of online forms that are required for publication.

Further information on the handling of your manuscript as well as the scheduled time of publication may be obtained at: http://authors.elsevier.com

Yours sincerely,

Luca Cocolin, Ph.D
Editor In Chief
International Journal of Food Microbiology

https://mail.google.com/mail/u/0/?tab=wm&ogbl#search/ijfm/FMfcgxwJXCCfTXsslZjDZXDDBvRPmljr 1/1

Anexo 2. Comprovante de cadastro de acesso ao Patrimônio Genético/CTA no Sisgen.

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº A276CB6

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A276CB6**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Bacillus cereus

Título da Atividade:	**Inativação microbiana por tratamento fotodinâmico: da cinética de inativação à aplicações em vegetais**

Equipe

Leonardo do Prado Silva	**UNICAMP**
Anderson de Souza Sant'Ana	**UNICAMP**

Data do Cadastro:	**22/06/2020 11:33:49**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **11:35** de **22/06/2020**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**