Food Research International

Emerging trends in the photodynamic inactivation (PDI) applied to the food decontamination

--Manuscript Draft--

Manuscript Number:	FOODRES-D-20-05635R2
Article Type:	Review Article
Keywords:	Blue LED light; Photodynamic inactivation; Photosensitizer; Curcumin; Porphyrin; Food Sanitation; Antimicrobial activity
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Abstract:	The food and drink manufacturing industry is constantly seeking for alternative sanitation and disinfection systems that may achieve the same antimicrobial efficiency of conventional chemical sanitisers and at the same time be convenient in terms of energy and water savings. A candidate technology for this purpose is the use of light in combination with photosensitisers (PS) to generate a bioactive effect against microbial agents in a process defined as photodynamic inactivation (PDI). This technology can be applied to the food processing of different food matrices to reduce the microbial load of foodborne pathogens such as bacteria, fungi, viruses and protozoa. Also, the PDI can be exploited to increase the shelf-life period of food by inactivation of spoiling microbes. This review analyses new developments in the last five years for PDI systems applied to the food decontamination from foodborne pathogens. The photosensitisation mechanisms and methods are reported to introduce the applied technology against microbial targets in food matrices. Recent blue light emitting diodes (LED) lamp systems for the PDI mediated by endogenous PS are discussed as well PDI technologies with the use of exogenous PS from plant sources such as curcumin and porphyrin-based molecules. The updated overview of the most recent developments in the PDI technology both in wavelengths and employed PS will provide further points of analysis for the advancement of the research on new competitive and effective disinfection systems in the food industry.

HIGHLIGHTS:

- The PDI has been reviewed as an optimised non-thermal food disinfection process
- Food sanitation is achieved by the activation of endogenous PS with blue LED light
- Curcumin and porphyrins are becoming leading PS for the PDI in food matrices
- PDI is a sustainable technology characterised by energy and water cost saving
- PDI is a promising technology among the low-cost non-thermal food sanitation methods

TITLE:

Emerging trends in the photodynamic inactivation (PDI) applied to the food decontamination

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ABSTRACT

The food and drink manufacturing industry is constantly seeking for alternative sanitation and disinfection systems that may achieve the same antimicrobial efficiency of conventional chemical sanitisers and at the same time be convenient in terms of energy and water savings. A candidate technology for this purpose is the use of light in combination with photosensitisers (PS) to generate a bioactive effect against microbial agents in a process defined as photodynamic inactivation (PDI). This technology can be applied to the food processing of different food matrices to reduce the microbial load of foodborne pathogens such as bacteria, fungi, viruses and protozoa. Also, the PDI can be exploited to increase the shelf-life period of food by inactivation of spoiling microbes.

This review analyses new developments in the last five years for PDI systems applied to the food decontamination from foodborne pathogens. The photosensitisation mechanisms and methods are reported to introduce the applied technology against microbial targets in food matrices. Recent blue light emitting diodes (LED) lamp systems for the PDI mediated by endogenous PS are discussed as well PDI technologies with the use of exogenous PS from plant sources such as curcumin and porphyrin-based molecules.

The updated overview of the most recent developments in the PDI technology both in wavelengths and employed PS will provide further points of analysis for the advancement of the research on new competitive and effective disinfection systems in the food industry.

KEYWORDS:

Blue LED light, Photodynamic Inactivation, Photosensitizer, Curcumin, Porphyrin, Food sanitation, Antimicrobial activity

RUNNING TITLE:

PDI in food decontamination

1. Introduction

The development and establishment of new urban environments is driving the consequential new lifestyles towards new habits in food consumption. In this context, the pursuit of new healthy lifestyles is dramatically modifying the social consciousness about the need of more refined qualities in food, including the customer's awareness in microbiological safety of the food product (Bhavya and Umesh Hebbar, 2019).

Biological spoilage given by microbial contamination is one of the main causes determining the annual loss of almost one-third of the food produced in the world. The food loss may be a consequence of quality deviations in any step of the food supply chain, from the production and harvesting processes to the final preparation and customer consumption (FAO, 2019). Common microbial contaminations may occur for food products originating from both plant and animal sources. However, the majority of foodborne poisoning cases originate from contaminations of food matrices deriving from animal sources (Heredia and García, 2018). This may also be due to the route of contaminations, reservoirs and final hosts of several pathogens which have been naturally selected within supply chains, including the overuse and misuse of antibiotics. Consequently, a positive correlation between the extensive use of antibiotics and selection of antibiotic resistant bacteria may be found (Founou et al., 2016).

Foodborne diseases result in detrimental consequences both for economic damages and losses in terms of human lives. The first ever World Health Organisation's (WHO) estimates of foodborne disease revealed that 10% of the world population is affected by foodborne poisoning every year and more than 400,000 deaths are recorded annually (WHO, 2015).

All microorganisms such as viruses, bacteria, fungi and protozoa can be etiological agents of food contaminations that may lead to spoilage or foodborne diseases (Bintsis, 2017; Paterson and Lima, 2017). In order to protect the public health safety and avoid expensive food

recalls, the decontamination of food matrices and sanitisation of food contact surfaces are essential practices within the food industry.

2. Current approaches and challenges in food sanitisation

Decontamination practices are routinely implemented in food processing to inactivate or physically remove microorganisms from different food matrices. The reduction of the microbial viability may result in the extension of the food shelf-life (Ma et al., 2017) as well as in the preservation of the nutritional qualities over the time (Qiu et al., 2019).

The two main categories of food decontamination technologies consist of antimicrobial systems which include the use of (a) chemical and (b) physical processes.

Among the chemical processes, current procedures involve the use of chlorine or peroxyacetic acid based sanitisers. Despite their relative low-cost, these sanitisers may not be effective in achieving sufficient reduction of the microbial load on surfaces containing biofilm or in food matrices with high organic content (Visvalingam and Holley, 2018; Anfruns-Estrada et al., 2019; Hua et al., 2019). Moreover, despite the wide use in different environments and matrices, chlorine-based sanitisers are known to generate a variety of by-products, such as chlorites and trihalomethanes when the sanitiser comes in contact with organic substances (Alves et al., 2014). In some cases these compounds may have a toxic effect to the consumer. This may result in the use of chlorine-based sanitisers specifically with selected food matrices (Paskeviciute et al., 2018). Accordingly, different and more stable chlorine related compounds for sanitation are available on the market, such as the lesser bioactive but more stable chloramines (Wastensson and Eriksson, 2020) and chlorine dioxide. The latter is more efficient than chloramines but its use may result in changes of the organoleptic qualities of the food matrix (Chen, 2017).

In recent years, a dramatic increase in demand for limitations of artificial chemical processing of food led to an interesting expansion of the range of novel technologies aimed in attaining similar magnitudes of food decontamination achieved by common sanitisers. The rationale behind the development of these technologies is based on the need for satisfying the same food safety requirements and integrity of the food quality parameters with a minimally processing strategy. This aspect is of particularly importance in the case of ready-to-eat food for which the variation of organoleptic parameters may drastically affect the quality of the food matrix as well as the customer compliance towards the product (Castro-Ibáñez et al., 2017).

Based on their efficacy and operational simplicity, the first choice technologies for food decontamination involve the use of physical-thermal processes such as dry-heating and steam-heating. Heating processes are still considered the optimal methodologies for the reduction of contamination from viruses, vegetative cells, spores and biofilm. However, the use of heat often causes a reduction in food quality including potential losses in nutritional values and appearance (Impe et al., 2018).

In order to cope with the thermal-damaging effects from dry- and steam- heating, the advent of several physical non-thermal processes brought an alternative variety of strategies in the field of food decontamination.

Irradiation with X-ray, ultrasound-based cavitation, pascalisation with high hydrostatic pressures (HHP), ozonation, pulsed electrified field (PEF) including electrolysed oxidized water, cold plasma and irradiation with ultraviolet (UV) or pulsed light (PL) had been introduced in the food industry (Rahman et al., 2016; Brodowska et al., 2018; Picart-Palmade et al., 2019; Zhang et al., 2019).

The use of these new technologies may also overcome the issues related to the limited antimicrobial efficacy and the chemical hazards resulting from the chemical sanitisation procedure. It follows that the reduction of the chemical hazard may be achieved for both the

operators and the final consumers with the elimination of residual by-products in the final food matrix.

Non-thermal technologies have been introduced for the decontamination of a variety of food matrices from animal and plant sources. It is highly likely that in the future these technologies will be applied as the preferred decontamination processes due to an antimicrobial activity against foodborne pathogens, preserving at the same time the food quality. However, some of these technologies are not yet cost-effective and cannot be feasible for numerous industrial settings. This results in an incomplete implementation of these methodologies within the food industry (Ezeh et al., 2018). Consequently, the development of single selected non-thermal strategies in function of the food matrix may be the best strategy for an effective food decontamination.

3. The use of UV-Visible radiation in the food decontamination

Within the array of non-thermal decontamination strategies, the application of selected wavelengths of the light spectrum for the irradiation of food is a promising technology due to the versatility of commercial lamps, the relative low cost of the technology and the feasibility in an industrial setting.

Until the last two decades, within the UV-Visible (UV-VIS) spectrum only UV wavelengths were considered capable of achieving an antimicrobial effect. UV wavelengths between 250 and 260 nm have a strong bioactivity against all microorganisms through the production of dimers of pyrimidine in the microbial genome which may disrupt the process of the DNA replication (Shibai et al., 2017). A recent study by Huang and Chen showed that the use of water assisted exposure to 254 nm UV-C was able to reduce the contamination from *Salmonella* in simulated wash water up to 6 logs in two minutes of irradiation. This clearly

demonstrated the efficacy of the UV-C in microbial decontamination, however its bioactivity was dramatically affected by the organic content of the substrate (Huang and Chen, 2020). Although UV radiation disinfection as a non-thermal technology for microbial inactivation is able to reduce the microbial load on food surfaces and food-contact surfaces (Cebrián et al., 2016), lower frequencies of the light spectrum such the visible (VIS) light and infrared (IR) light had been recently suggested for their practicality in the reduction of microbial load from the surface of the treated food substrates (Bhavya and Umesh Hebbar, 2017; Aboud et al., 2019). It has been shown that selected VIS light wavelengths are able to excite endogenous porphyrins in the microbial cells (Kumar et al., 2015). Different bacterial and fungal species possess a variety of porphyrin-based compounds (Wang et al., 2017). Porphyrin-based molecules can absorb energy from wavelengths in the range between 400 nm and 430 nm to catalyse the production of reactive oxygen species (ROS) (Kim et al., 2015; Kumar et al., 2015). Indeed, upon irradiation the transition of the porphyrin to a higher energy state may be reverted by relaxation to a ground state coupled with a non-selective energy transfer to organic molecules or molecular oxygen and generate an oxidative power (Ghate et al., 2019). For this reason the VIS light may be taken into consideration in the development of effective antimicrobial strategies through the exposure of food to selected VIS wavelengths.

3.1 LED Blue light in food sanitation

Incandescence bulbs and fluorescence lamps have now left the place to light-emitting diode (LED) lamps which are now extensively used in agriculture and food processing. The LED technology is based on semiconductors that emit selected ranges of wavelengths within the VIS light spectrum. An important advantage of this technology is the possibility to limit the VIS spectrum to specific wavelengths to obtain monochromatic light (Prasad et al., 2020).

In food decontamination, LED devices become pivotal in interventions where non-thermal technologies are required. LED lamps may be compact, portable and cost-effective (Josewin et al., 2018) because of their low energy consumption and extended life (Ghate et al., 2015).

Several studies have shown that LED lamps emitting wavelengths in the range of 400-460 nm are bioactive against microbes, as the selected spectrum is responsible for the excitation of endogenous photosensitising porphyrin molecules.

The use of blue light with the wavelength of 405 nm has been the subject of investigations for its antimicrobial activity against several foodborne pathogens (Fig. 1). *Salmonella enterica* serovar Typhimurium was found to be more resistant to the 405 nm wavelength than *Bacillus cereus* and *Listeria monocytogenes* (Kumar et al., 2015). It had been suggested that the antimicrobial activity of the 405 nm light might be due to the excitation of the endogenous porphyrins that would trigger the catalysis of the production of intracellular ROS, resulting in cellular oxidation processes and death (Wang et al., 2017). The higher susceptibility of the Gram-positive species to the 405 nm light can be explained by a greater presence of intracellular coproporphyrin in these bacteria (Kumar et al., 2015). Since Gram-negative bacteria possess fewer porphyrins, this would explain the enhanced resistance of *Salmonella enterica* to the 405 nm light exposure. Similar results against the same Gram-positive *B. cereus* and *L. monocytogenes* have been attained, including the observation of a loss of integrity of the bacterial membrane and an increased sensitivity to osmotic stress but with lack of damage to nucleic acids (Kim et al., 2015), thus showing a predominant bioactivity of 405 nm light towards structural components of the cell rather than to the genetic material of the targeted bacteria.

Further studies showed the bioactivity of 400-410 nm light against Gram-negative bacteria on food surfaces. The microbial load of *Campylobacter jejuni* was successfully

reduced on the surface of raw chicken fillets and cutting boards with equal or greater magnitudes of decontamination compared to results previously obtained by chlorine based sanitisers (Haughton et al., 2012). The same *C. jejuni* survival was reduced on chicken skin by 405 nm light exposure as well as on food contact stainless steel surface. However, this light wavelength may show heating issues at high fluences resulting in an antimicrobial effect given by the heat rather than from a photodynamic activity (Gunther et al., 2016). For this reason, additional studies have been conducted in refrigerated and/or controlled temperature conditions. *S. enterica* serovar Enteritidis was inactivated by 405 nm light exposure on the surface of chilled cooked chicken (Kim et al., 2017). The viability of *E. coli* in milk was reduced by using blue monochromatic LED at 406 nm which showed that the maximum microbial reduction can be achieved at shorter wavelengths when the treatment is coupled with higher temperatures. Moreover, the irradiation treatment did not result in any modification of the organoleptic parameters of the processed milk (Srimagal et al., 2016). The survival of *S. enterica* and *L. monocytogenes* was also reduced by 405 nm light exposure on the surface of cantaloupe fruit rinds (Josewin et al., 2018). Furthermore, the same wavelength against planktonic *L. monocytogenes* cells in salmon exudate had been successfully employed for the disinfection of acrylic and stainless steel food contact surface (Li et al., 2018).

A longer wavelength of the blue light at 460 nm was applied for the same purpose of decontamination. However, in this case an increased exposure time was required, resulting in the use of higher fluences (Fig. 2). The viability of *B. cereus, L. monocytogenes*, *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa* and *S. enterica* serovar Typhimurium was successful reduced by the exposure to 460 nm light (Kumar et al., 2017). Also in this case, *S. enterica* showed a better resistance to the inactivation by blue-radiation compared to Gram-positive species. It was reported that the microbial reduction may not be driven only by the intracellular porphyrin content but also by the regulation of metabolic

pathways resulting in up- or down-regulated metabolites (Kumar et al., 2017). The 460 nm light exposure had been suggested also for its bioactive effect against *S. enterica* deposited on fresh cut fruit, however the wavelength manifested a bactericidal effect against *Salmonella* only at low temperatures, whilst at mesophilic temperatures the light exposure had a bacteriostatic effect (Ghate et al., 2017).

By directly comparing the bacterial viability reductions and the use of the two wavelengths of blue light (Fig. 1 and Fig. 2), it is possible to distinguish between the magnitudes of fluence necessary for the bacterial inactivation. For the irradiation at 460 nm, the required fluence was approximately ten times higher than fluence at 405 nm to achieve similar logarithmic reductions against the same bacteria.

4. The use of Photosensitisers (PS) in the photodynamic inactivation (PDI) for food sanitation

As already mentioned, novel LED VIS-light systems have been introduced also in the food industry for food decontamination although their power and efficacy for an antimicrobial effect is still limited when compared to UV wavelengths' bioactivity (Bhavya and Umesh Hebbar, 2019). The shorter VIS wavelengths in the blue region (<500 nm) may still possess enough energy to produce intracellular ROS by excitation of endogenous photosensitising molecules in the target cells. However, the LED VIS-light that have been used in the decontamination of different food matrices, especially fruits and juices, still requires procedures involving extensive exposures for several hours to achieve fluences that are needed for an efficient reduction of the microbial load (Fig. 1 and Fig. 2). To reduce the long exposure periods to light irradiation, which would have also a relevant impact on the food quality, the use of a different light-based technology may be needed to attain similar results in terms of microbial inactivation but with shorter irradiation periods.

The innovative and promising technology of photodynamic inactivation (PDI) appeared in the food sanitation panorama at the interface of chemical and physical decontamination. The PDI technology is characterised by the application of a non-thermal process consisting of the irradiation by light of specific molecules called photosensitisers (PS). The excitation of the PS is coupled with the biological activity of the photosensitisation process. Similarly to UV wavelengths, it has been shown that several wavelengths from the VIS-light in combination with exogenous PS possess an antimicrobial activity (Ogonowska et al., 2018). For this reason, the potential of VIS-light becomes particularly interesting in decontamination systems based on a photosensitisation. The use of light for the activation of molecules as mediators of energy or electron transfer may trigger the production of ROS, resulting in the production of a source of oxidative power against the microbial target (Maisch, 2015). Photosensitisation relies on a catalysis mediated by a physical factor such as light. This process does not show any development of microbial resistance (Al-Asmari et al., 2017; Sabino et al., 2020) even in repeated cycles of non-lethal photosensitisation.

The aim of the process of photosensitisation is to achieve the same levels of microbial inactivation given by the current sanitisation approaches by keeping a low ecological impact of the treatment through limitation of both the environmental and public health hazards. With the advent of high-efficiency monochromatic LEDs in the last decades, this technology is also featured by a low-energy input resulting in a low-cost of operation and thus increasing its eco-compatibility (Nair and Dhoble, 2021).

The recent support of the nanotechnologies has also been recognised as an important factor for the expansion of novel alternatives in food decontamination with the use of bioactive nanoparticles aimed to the disinfection of food (Alves et al., 2014). Oxides of Zinc and Titanium in nanoparticle formulations had been tested and identified for their unique physicochemical properties including their size, stability and limited toxicity towards humans

(Sivakumar et al., 2018; Ziental et al., 2020). Nevertheless, the main interest in the photosensitisation technology applied to the food industry has been directed towards the use of organic molecules which meet better the requirements of a desirable PS for food decontamination. Some of the characteristics are the limited costs for the production of the molecule and the related sanitisation process as well as the absence of toxic residues to the consumer. This can be assured by the use of Generally Recognised As Safe (GRAS) or food-grade molecules with chemical stability during the treatment of the antimicrobial process.

4.1 Mechanism of the photosensitisation process

The physicochemical process of microbial inactivation by photosensitisation is characterised by the interaction between a non-toxic PS molecule and a source of light irradiation in the presence of molecular oxygen (Hamblin, 2016). The outcome of the interaction between light and PS is strictly dependent on oxygen and may follow two types of reaction pathways that may occur simultaneously. In both pathways, a light (photons) absorption by the PS is followed by a photo-oxidation including an electron or hydrogen abstraction (Baptista et al., 2017). A following electron transfer (Type I photosensitisation) or energy transfer (Type II photosensitisation) to the molecular oxygen may then result in the production of ROS (Fig. 3).

In a type I photosensitisation the light wavelength is responsible for the excitation of the PS molecule resulting in a $^{1}PS^{*}$ state which can interact with targeted substrates such as organic molecules during an intersystem crossing to generate a $^{3}PS^{*}$. The transfer of an electron or a proton to the excited substrate may generate radical species that will interact by electron transfer with the molecular oxygen in order to produce the ROS superoxide ($O_2^{\bullet-}$) which after dismutation results in the production of hydrogen peroxide (H_2O_2) (Baptista et al., 2017). The generated ROS H_2O_2 can be degraded by UV exposure, Fenton, Fenton-like or Photo-Fenton

reaction to hydroxyl radical species (•OH) (O'Dowd and Pillai, 2020). Differently, in a type II photosensitisation the excited $^{3}PS^{*}$ can interact with the molecular oxygen by a direct transfer of energy during the relaxation phase to ^{1}PS producing the ROS singlet oxygen ($^{1}O_2$) (Wainwright et al., 2017). Due to the absence of specific bacterial enzymes against the singlet oxygen (Cieplik et al., 2018), this ROS may result in damaging effect to biological macromolecules such as protein crosslinking and aggregation (Marques et al., 2019), oxidation of membrane unsaturated lipids and oxidation of guanine in nucleic acids (Di Mascio et al., 2019).

4.2 Biological activity of the photosensitisation process

Both typologies of photosensitisation result in an aggressive oxidative action of ROS that may cause several damages to intracellular organic molecules and consequent loss of bio-functionality of the targeted cell. Thus, the final effect of the oxidative stress may lead to severe cell damage, cellular inactivation or cell death and lysis (Al-Asmari et al., 2017). In particular, the damaging effect given by the bioactivity of the photosensitisation process is directly aimed to functional or structural cellular components (Fig. 4 and Fig. 5). The interaction of ROS with the cell envelope of the target may trigger a lipid peroxidation of the membrane fatty acid chains (Kashef and Hamblin, 2017) resulting in the induction of alterative phenomena of the membrane structure such as phase separation and pore formation (Tsubone et al., 2019). Additional damages against protein targets may lead to the shift of the protein isoelectric point (Brancini et al., 2016), protein oxidation and protein cross-linking (Lévy et al., 2019) while ROS damaging DNA may attain the oxidation of the deoxyguanosine (dG) with production of 8-hydroxy-deoxyguanosine (8-OHdG) (Giorgio et al., 2020).

The electrostatic charge of the cell envelope of bacteria may play a role in the partitioning and movement of the PS in the cellular complex. By suspension in the extracellular

environment or surface deposition, the PS biological activity may target the microbial cell wall and/or membrane resulting in a damaging effect to the bacterial surface structures (Aponiene and Luksiene, 2015). Gram-negative bacteria appear less susceptible to negatively net-charged PS because they are usually characterised by a net negative charge on their surface at neutral pH. For these particular bacteria, higher doses of PS or increased power of irradiation are necessary when anionic or neutral PS are used in a photodynamic antimicrobial treatment (Buchovec et al., 2017). Alternatively, the combination of neutral or anionic PS with polycationic permeabilising compounds to vehicle the PS through the external membrane (Nitzan and Pechatnikov, 2011) or the employment of cationic photosensitisers (Aroso et al., 2019) may result in better efficiencies of microbial inactivation of Gram-negative species. The biocidal activity of the PS in a PDI system may also be attained by its intracellular accumulation. The subsequent proximity of the PS to the targeted molecules and structures inside the cell may result in a biological effect of intracellular damage (Ghate et al., 2019).

4.3 Photosensitisation efficacy and variable factors of a PDI system

The efficacy of a PDI intervention is dependent on the presence, variability and inter-function of several physical and chemical parameters. These parameters play a role in the activity of the PS and in determining the potential of the PDI treatment in the food decontamination.

The PDI process can be schematised as a triangular system composed of the three following components: (a) a PS, (b) a food matrix or substrate and (c) a light. Each of these components has variables that may be modified to tailor an optimised PDI treatment for a given food decontamination's scenario (Fig. 6). The physicochemical properties of the PS, especially the light absorbance of the PS (Ghate et al., 2019) and its solubility (Sobotta et al., 2019; Gao and Matthews, 2020), play a relevant role in the level of bioactivity against the microbial target.

In addition, the potential of a PS catalytic cycle as well as its grafting on inert supports may determine its applicability in several PDI cycles (Alves et al., 2014) ensuring the absence of the PS in the final processed food product. The PS's physicochemical properties together with the irradiant light's parameters such as fluence and wavelengths, determine the magnitude of microbial reduction given by the ROS originated from the photosensitising system (Kumar et al., 2015) applied on the food or substrate. The organic content and the limitation in oxygen concentration are the variable chemical properties of the food that are mainly responsible for the decrease in efficacy of the entire PDI system. A high organic content may result in an increased scavenging power for ROS (Alves et al., 2014) while a low concentration of oxygen can cause the complete halt of the photosensitisation reaction, as the PDI is strictly dependent by the presence of molecular oxygen (Baptista et al., 2017; Ghate et al., 2019).

5. Recent applications of photosensitisation in food decontamination

The vast majority of natural PS compounds which have been recently used in novel PDI applications for the food industry are molecules belonging to plant sources or their derivatives (Ghate et al., 2019). Many of these compounds can be excited by VIS-light, in particular in the blue range of the light spectrum such as curcumin and porphyrin-based compounds (chlorophyllin and its derivatives), as well as anthraquinone derivatives such as hypericin.

5.1 Curcumin-based PDI in food systems

Curcumin (diferuloyl methane) is a natural polyphenolic molecule and plant pigment that can be extracted from the powder of the turmeric plant (*Curcuma longa* L.). The compound has a characteristic yellow colour in solution and it is positively charged in acidic range environments (Al-Asmari et al., 2017).

Curcumin is known for its wide antimicrobial activity (Praditya et al., 2019), anti-inflammatory and anti-oxidant power (Hewlings and Kalman, 2017). The antimicrobial activity of the molecule is given by its potential to affect the three-dimensional conformation of the phospholipidic layers in the membrane structures of bacteria (Tyagi et al., 2015) and fungi (Chen et al., 2018). It was suggested that the partitioning of the lipophilic curcumin molecule in the lipid cell membrane of fungal targets is mainly due to the affinity with the aliphatic chains that form the ester bonds with the glycerol (Lee and Lee, 2014). Photosensitised curcumin is known to generate singlet oxygen as main ROS responsible for its antimicrobial effect (Wu et al, 2016). For this reason it has been assumed that the cell envelope of microbial cells may be damaged by the direct interaction with ROS produced by the photo-activated curcumin (Al-Asmari et al., 2017), resulting in a structural damage coupled with cell leakage (Hu et al., 2018).

The European Food Safety Authority (EFSA) defined the curcumin molecule as a non-carcinogenic compound and authorised its use as a food additive in the EU (EFSA, 2010) while the US Food and Drug Administration (FDA) acknowledged curcumin as a GRAS substance (FDA, 2019). However, the recommendation of the WHO about the maximum intake concentration of 200 mg/kg body weight should be followed to meet the current food safety requirements (Liu et al., 2016).

The light-absorption peak of curcumin is between 400 and 500 nm which makes it suitable for excitation with the blue wavelengths of the light below 500 nm. The irradiation may also break down the curcumin molecule thus limiting the presence of the compound in the final decontaminated food product (Corrêa et al., 2020). This feature could be exploited in a PDI food system where the absence of alterations of organoleptic qualities of the food matrix such as the colour may be of particular importance.

The use of curcumin as PS excited by blue light has been clearly displayed in the recent literature about the sanitisation and disinfection of different food matrices, both from plant and animal sources (Fig. 7). Among these studies, photosensitised curcumin had been used on oysters to show the bioaccumulation of the PS compound in the mollusc and assess the antimicrobial power of the PS molecule against a viral surrogate of norovirus (Wu et al., 2015). The shelf-life and quality of the oyster during refrigeration was successfully prolonged using the same PS excited by 470 nm light. The decay of the oyster was slowed down and the food matrix was only minimally oxidised (Liu et al., 2016), thus demonstrating the minimal impact of the PS activity on the organoleptic parameters of the oyster. Curcumin irradiated by blue light had been also tested for the inactivation of sessile and planktonic cells of *Vibrio parahaemoliticus*, which is one of the leading pathogens in seafood mainly because of its multi-drug resistant strains and ability to form biofilm communities (Chen et al., 2020). In the same study, it was demonstrated that the affected targets of the PS bioactivity were the cell wall and the proteins of the bacterial target. PDI using curcumin was able to achieve a complete inactivation of planktonic *V. parahaemoliticus* and up to 90% inactivation of the sessile bacteria including a reduction of the chemical composition of the extracellular polymeric substances of the biofilm (Chen et al., 2020). Upon irradiation by 470 nm the PS produced singlet oxygen which was responsible for the damage of proteins of the outer membrane and genetic material of *V. parahaemoliticus* (Wu et al., 2016).

Curcumin was used for the decontamination of fruit and the extension of the fruit shelf-life. Experiments on date (*Phoenix dactylifera* L.) indicated that sprayed concentrations of curcumin in the range between 1 and 2 mM can even double the shelf-life period of the dates at room temperature and delay the appearance of fungal spoilage when the PS was exposed for few minutes to 420 nm light (Al-Asmari et al., 2018). The same research group also proved the antifungal effect of a PDI using low concentrations of curcumin (< 1 mM) against *Aspergillus*

spp., *Penicillium* spp., *Fusarium oxysporum* and *Candida albicans*, with the last two fungal species as the most susceptible to the PS among the tested species (Al-Asmari et al, 2017). Further studies on PDI with curcumin have been carried out to investigate the antifungal activity of this PS against *Penicillium expansum* in apple fruit (Song et al., 2020) and *Aspergillus flavus* on maize kernels (Temba et al., 2019). Both studies indicated a microbial inhibition of the fungal species when the PS was irradiated with a 420 nm wavelenght.

The antibacterial effect of the PDI using curcumin was recently investigated both on gram-positive and gram-negative bacteria. *E. coli* and *S. aureus* were greatly inhibited by PDI using curcumin both at low and moderate temperature, showing also in this case a mechanism of inactivation based on the production of ROS at an intracellular level which disturbed the membrane integrity and led to morphological modifications in the cell wall (Bhavya and Umesh Hebbar, 2019). *S. aureus* was also inhibited on meat and apple when curcumin was irradiated by 450 nm light (Corrêa et al., 2020). On chicken skin, the irradiation of curcumin with 430 nm light was efficiently employed for an antimicrobial activity against *L. monocytogenes* and *S. enterica*. It was also shown that the treatment did not affect the colour of the chicken skin and achieved similar bioactivity levels against bacteria when compared to standard concentrations of peracetic acid, which is commonly used for the chicken meat disinfection (Gao and Matthews, 2020).

The amount of results from studies about curcumin in PDI food systems clearly suggests the importance of this molecule for its future application to decontamination processes in the food industry, especially for meat-based food products in which curcumin-containing turmeric may be used as a spice (Bonifácio et al., 2018). Given the specific excitation of curcumin in the blue range of the light spectrum, the use of blue LED lamps would also be the most efficient system for this PDI technology with a promising decontamination power, as well as a reduced cost of processing.

5.2 Porphyrin-based PDI in food systems

Porphyrins are a category of natural PS. The main feature of the porphyrin-based PS class is the potential use of different VIS-light wavelengths for the related photosensitising activity which consequently makes this category of PS molecules more feasible for the implementation of multiple PDI systems. Distinctive characteristics of porphyrin-based PS are the low toxicity of the active molecules for the human health (Huang et al, 2015) and the possibility of recycling the PS in water-based processing systems (Liu et al., 2020).

PDI technologies using porphyrin-based PS have also been employed recently in buffers and food systems (Fig. 8).

The use of cationic immobilised porphyrin hybrids excited by the full VIS light spectrum had been carried out against Gram-negative bacteria, showing the antimicrobial efficacy of the PS and the reusability of the molecule in repeated catalytic cycles (Alves and al., 2014). A tetracationic porphyrin was used in combination with the non-toxic potassium iodide (KI) in a full-VIS spectrum PDI against spores from *Alicyclobacillus acidoterrestris* in orange juice. The presence of the inorganic salt enhanced the antimicrobial activity of the PDI technology achieving the destruction of spores which could survive after the use of pasteurisation methods (do Prado-Silva et al., 2020). Also, a Silicon (IV) phtalocyanine (SiPc) derivative was employed for the decontamination of milk from *S. aureus* and *E. coli* using the 610 nm red light irradiation for the PS excitation and generation of singlet oxygen (Galstyan and Dobrindt, 2019). This PDI system was able to inactivate bacteria in a food substrate like milk where light scattering from fat globules and protein micelles may limit the interaction of the light with the PS. Also, milk contains a large amount of organic content that may decrease the bioactivity of the excited PS because of the transfer of energy from the generated ROS to the food matrix rather than to targeted bacteria to be inactivated (Khan et al., 2019).

Within the same category of porphyrin-based PS, chlorophyllin seems to be a promising molecule in the food industry. Chlorophyllin is a food additive (E140) and the molecule is negatively charged in water systems. The PS was shown to be effective against *S. enterica* (Buchovec et al., 2017) and *E. coli* (Aponiene and Luksiene, 2015) when irradiated by 405 nm light. The PDI of targeted bacteria in the presence of curcumin led to the production of singlet oxygen and a bacterial gene expression aimed to the detoxification from ROS. The antimicrobial effect resulted also in the microbial cell leakage of proteins and nucleic acids (Buchovec et al., 2017). The application of the chlorophyllin-based PDI on cherry tomatoes demonstrated an antibacterial activity against the natural microbiota on the tomato skin as well as artificially inoculated *B. cereus* and *L. monocytogenes* (Paskeviciute et al., 2018). The food decontamination system achieved equal or higher levels of microbial reductions than conventional washing technologies, including hypochlorite treatment. The treated tomatoes showed an extended shelf-life with a delay in the microbial growth on the surface as well as no further modification to the nutritional value of the fruits (Paskeviciute et al., 2018).

Alternative chlorophyllin-derived PDI systems were successful in inhibiting microbial growth in food substrates. The recent use of the porphyrin-based pheophorbide in a sodium salt formulation for antifungal studies has shown the efficacy of this derivative from the chlorophyll degradation against *Botrytis cinerea* on tomatoes, by increasing its antifungal bioactivity under full range VIS-light irradiation (Ji et al., 2020). Pheophorbide has no toxic effects to humans and its bioactivity has been already shown in antitumoral and virucidal studies (Saide et al., 2020), thus making it a good candidate as PS in the food industry.

5.3 Anthraquinonic and Xanthene dyes for PDI in food systems

Hypericin is an anthraquinone compound from perforate St John's wort (*Hypericum perforatum* L.). The molecule has lipophilic characteristics which makes its use particularly

difficult in aqueous environment. Photoactivated hypericin showed antimicrobial activity against bacteria, fungi (Alam et al., 2019) and viruses (Chen et al., 2019).

The irradiation of the hypericin-human serum albumin (HSA) complex with a 515 nm wavelength reduced the survival of *S. aureus* when organic content was not present in the sample (Pezzuoli et al., 2018). The use of a polymeric nanoparticle formulation containing hypericin lessened the issue of the low solubility of the PS in water thus attaining a significant bioactivity against bacteria. The formulation was able to reduce the load of planktonic and sessile *S. aureus* cells after irradiation by low power orange light (Malacrida et al., 2020).

Xanthene dyes such as Eosin Y and Rose Bengal are known for their singlet oxygen generated upon excitation by VIS light (Lutkus et al., 2019). The two dyes were both effective against *S. enterica* serovar Typhimurium and S. aureus when irradiated by 530 nm light (Santos et al., 2019). Also, another xanthene dye such as Erythrosine as well as Rose Bengal, when irradiated by green LED lamp was successful in the control of Gram-negative and Gram-positive species both in planktonic and biofilm forms (Silva et al., 2018).

Although their limited use in PDI food systems from the recent literature, these molecules have a significant prospect to be applied in food processing for the decontamination by foodborne pathogens also in light of their food grade status.

6. Conclusions and future perspectives in the development of PDI for the food industry

This review analysed the last five-years period of recent developments in the photosensitisation technology applied to food-based systems. The huge potential of the blue light and endogenous PS for the microbial inactivation of foodborne pathogens as well as the activation of exogenous PS such as curcumin and porphyrin-based PS was shown in interesting alternative technologies to conventional sanitation and disinfection systems. In particular, the

development of low-cost blue LED lamps is making this technology cheaper and thus more approachable by the food industry.

To the best of our knowledge, many of the studies from the literature which contribute to the analysis of the PDI efficacy against foodborne microbes do not evaluate the antimicrobial activity in the food environment context. Also, when the food matrix is considered in the PDI system, a small number of studies consider the use of a variety of food matrices rather than single selected foods. Moreover, the translation of the results from the laboratory setting to a full application in the food industry market is rarely achieved. Thus, it would be useful to initiate PDI studies which are designed around the food matrix rather than the PS, to promote this technology in future applications within the food industry.

The use of PDI non-thermal processes can find application in a variety of food matrices from animal source and plant source to water-based washing systems in the food processing. Most applications are in post-harvest and food processing, but PDI deserves further investigation also as an innovative and cost-effective bacterial and fungicide treatment alternative to conventional phytochemicals in fresh produce production, potentially leading to a new class of organic broad-spectrum compounds with no toxic residues on fruits and vegetables. According to this, a new field of application is in agriculture, where PDI could be introduced in greenhouse horticulture coupled with artificial lighting, aiming to pest-free and zero-residue fresh produce with no toxicity to the consumer. The study of the environmental impact of PDI in agriculture, as well as the proper formulations and dosages, are the future challenges to test the efficacy of PDI both in open field and greenhouse environments, also towards problematic plant pathogens resistant to conventional phytochemicals.

In the next years the development of new PDI systems and their implementation in the food industry are likely to be considered for replacement of conventional sanitisers in many

food matrices processing, maintaining the same or an higher efficiency in the decontamination magnitudes.

ACKNOWLEDGMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHOR CONTRIBUTIONS

Marco Cossu: Methodology, Investigation, Visualisation, Writing - Original draft.

Luigi Ledda: Formal analysis, Data curation, Writing - Review & Editing.

Andrea Cossu: Conceptualization, Methodology, Supervision, Project administration, Writing-original draft, Writing - Review & Editing.

All authors acknowledged their participation to the manuscript and agreed on the final version to be considered for submission.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

FIGURE CAPTIONS

Figure 1. Summary of studies about the antimicrobial activity of 405 nm blue light against Gram-negative and Gram-positive foodborne pathogens. The figure shows the bacterial reductions (Log CFU/mL) achieved by the fluences (J/cm^2) of 405 nm blue light in antimicrobial studies against common foodborne pathogens. The graph and table on the left report the plotted reductions and the food, medium or surface on which the light irradiation was applied against the Gram-negative *Escherichia coli* (■), *Campylobacter jejuni* (●), and *Salmonella enterica* (▲). The graph and table on the right outline the data from studies against the Gram-positive *Staphylococcus aureus* (♦), *Listeria monocytogenes* (+) and *Bacillus cereus* (*).

Figure 2. Summary of studies about the antimicrobial activity of 460 nm blue light against Gram-negative and Gram-positive foodborne pathogens. The figure shows the bacterial reductions (Log CFU/mL) achieved by the fluences (J/cm^2) of 460 nm blue light in antimicrobial studies against common foodborne pathogens. The graph and table on the left report the plotted reductions and the food, medium or surface on which the light irradiation was applied against the Gram-negative *Escherichia coli* (■) and *Salmonella enterica* (▲). The graph and table on the right outline the data from studies against the Gram-positive *Staphylococcus aureus* (♦), *Listeria monocytogenes* (+) and *Bacillus cereus* (*).

Figure 3. Mechanisms of production of reactive oxygen species from the photosensitisation process. The integrated simplified Jablonski diagram shows the energetic states of a photosensitiser (PS) molecule during the type I and type II photosensitisation mechanisms and the origin of reactive oxygen species (ROS). The singlet PS (^{1}PS) at ground

state (S_0) is excited by the irradiation of a specific wavelength of light. The light excitation produces an excited singlet PS ($^1PS^*$) at an excited singlet energetic state (S_1). In a Type I photosensitisation, an electron transfer occurs during the intersystem crossing of ^1PS from S_1 to the excited triplet PS ($^3PS^*$) on the triplet excited state (T_1). The electron originates from a redox reaction of a substrate (S) to a radical substrate ion ($S^{\bullet-}$) and is donated to the triplet molecular oxygen ($^3O_2$) thus generating the ROS superoxide ion ($O_2^{\bullet-}$). The ROS $O_2^{\bullet-}$ can enter a conversion process to originate the ROS hydrogen peroxide (H_2O_2) by spontaneous or enzymatic dismutation and the ROS H_2O_2 may then originate the ROS hydroxyl radical (•OH). In a Type II photosensitisation a transfer of energy to $^3O_2$ occurs with the formation of singlet oxygen ($^1O_2$) during the relaxation phase of the $^3PS^*$ radical from the T_1 to ^1PS on ground state S_0.

Figure 4. Biological damages to the microbial cell membrane lipids and DNA caused by the photodynamic inactivation (PDI) process. The presence of an exogenous photosensitiser (ExoPS) in the extracellular environment or in contact with the cell envelope of the microbial target may originate reactive oxygen species (ROS) upon a photosensitisation operated by a light irradiation (**A1**). At the cell membrane level, the ROS hydroxyl radical (•OH) may be responsible for the initiation of a lipid peroxidation of the unsaturated aliphatic chains of the membrane phospholipids producing a lipid radical that reacts with molecular oxygen to form a lipid peroxyl radical (**A2**). At intracellular level, an endogenous photosensitiser (EndoPS) or an absorbed ExoPS may be triggered upon light irradiation to generate intracellular ROS (**B1**). The photodynamic inactivation (PDI) by intracellular ROS may be directed against the membrane as well as the chromosomal or extra-chromosomal DNA following a DNA oxidation. The nucleic acid is mainly oxidised by the ROS hydroxyl radical (•OH) resulting in

the hydroxylation of the C8 of the deoxyguanosine (dG) to 8-hydroxy-deoxyguanosine (8-OHdG) which tautomerises to 8-Oxo-deoxyguanosine (8-Oxo-dG) at intracellular pH (**B2**).

Figure 5. Biological damages to the microbial cell proteins caused by the photodynamic inactivation (PDI) process. At intracellular level, an endogenous photosensitiser (EndoPS) or an absorbed exogenous photosensitiser (ExoPS) may be triggered upon light irradiation to generate intracellular reactive oxygen species (ROS) and induce protein oxidation (**A1**). ROS may produce cleavage of the peptide bond and fragmentation of the protein backbone by proton abstraction caused by the ROS hydroxyl radical (•OH) (**A2**) or carbonylation of the side chains of the amino acid (aa) residues by the ROS hydrogen peroxide (H_2O_2) (**A3**). Sulphur-containing amino acid residues such as cysteine may be directly oxidised to cystine by the ROS •OH or superoxide ion ($O_2^{\bullet-}$) (**A4**) or by the ROS singlet oxygen ($^1O_2$) resulting in a correspondent zwitterionic peroxide **(A5)**. Alternatively, cysteine can be oxidised by the ROS H_2O_2 to S-Hydroxycysteine which can react with a second cysteine resulting in the formation of cystine (**A6**). Methionine may be oxidised by the ROS H_2O_2 or •OH to methionine sulfoxide and methionine sulfone (**A7**) or by the ROS ($^1O_2$) to a zwitterionic peroxide **(A8)**. A histidine residue may be oxidised by the ROS $^1O_2$ giving the correspondent endoperoxides which decompose to hydrated imidazolones. The opening of the imidalozone ring may then result in the production of poorly defined amides **(A9)**. The residue of the aromatic amino acid tryptophan may be oxidised by the ROS $^1O_2$ to either a tryptophan hydroperoxide or a tryptophan dioxetane **(A10)** followed by a decomposition to N-formylkynurenine and kynurenine **(A11)**. Alternatively the tryptophan hydroperoxide may react with an α-amino group resulting in a ring closure and the formation of a 3α-hydroperoxypyrroloindole residue **(A12)**. When the amino acid residue of tyrosine is oxidised by the ROS $^1O_2$, a tyrosine

endoperoxide may be formed before the following opening of the ring to give an hydroperoxide that is decomposed to the corresponding alcohol group **(A13)**.

Figure 6. Variable factors determining the efficacy of a photodynamic inactivation (PDI) system. The physical-chemical properties of the photosensitiser and the food, substrate or medium as well as the characteristics of the irradiant light determine the efficacy a PDI system. Each of the three components include sub-categorical parameters that may increase or decrease the efficacy of the photosensitisation process against the microbial target.

Figure 7. Overview of studies about the use of curcumin in photodynamic inactivation (PDI) systems against common foodborne pathogens. Bacterial reductions (Log CFU/mL) of *Vibrio parahaemolyticus* (●), *Escherichia coli* (■), *Salmonella enterica* (▲), *Listeria monocytogenes* (+) and *Staphylococcus aureus* (♦) are plotted in function of the fluences (J/cm^2) of the wavelengths applied to the food, medium or surface of the antimicrobial studies. Data are additionally clustered in function of the irradiating wavelength (nm) of the PDI system used which is reported on the top of each cluster. The table on the right shows the concentration of curcumin (μM) and the food, medium or surface on which the PDI by curcumin photosensitisation was accomplished.

Figure 8. Overview of studies about the use of porphyrin-based compounds in photodynamic inactivation (PDI) systems against common foodborne pathogens. Bacterial reductions (Log CFU/mL) of *Escherichia coli* (■), *Salmonella enterica* (▲), *Listeria monocytogenes* (+), *Staphylococcus aureus* (♦) and *Bacillus cereus* (*) are plotted in function of the fluences (J/cm^2) of the wavelengths applied to the food, medium or surface of the antimicrobial studies. Data are additionally clustered in function of the irradiating wavelengths

(nm) of the PDI systems used which are reported on the visible light spectrum on the top of the graph. The table on the right shows the porphyrin-related photosensitisers and their concentrations used on the PDI studies, as well as the food, medium or surface on which the photosensitisation was carried out.

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FIGURE 1 - 405 nm photosensitisation rev2

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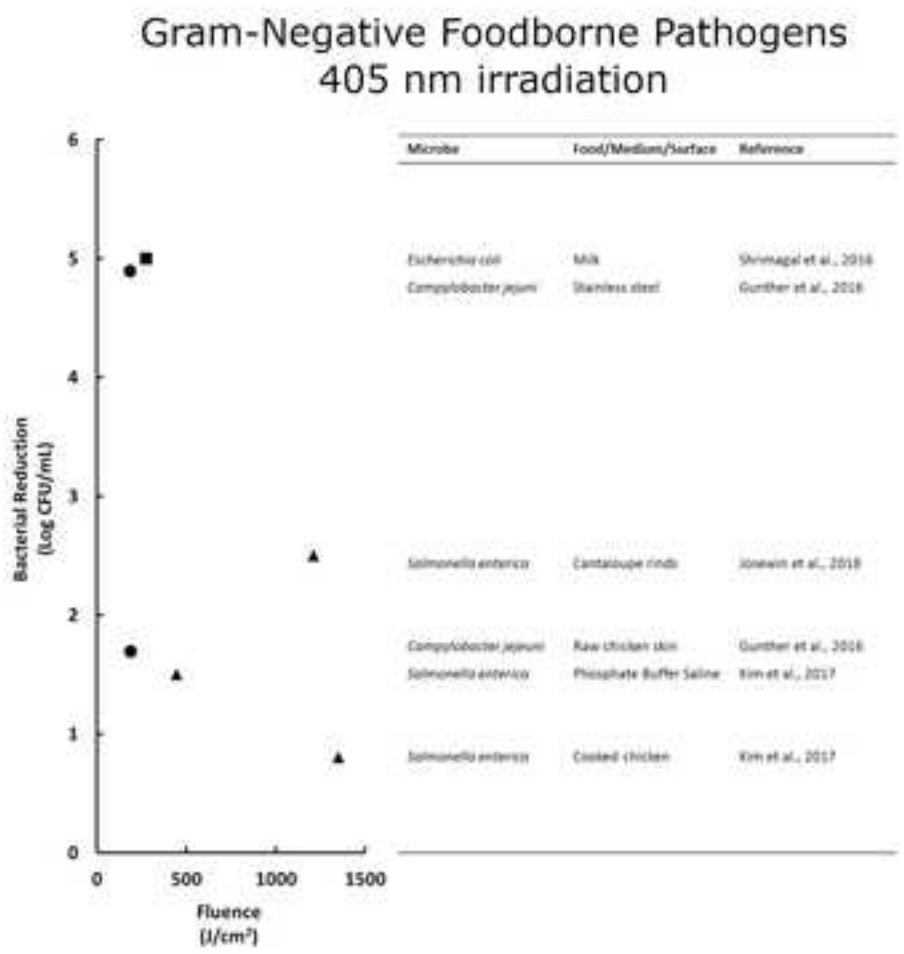

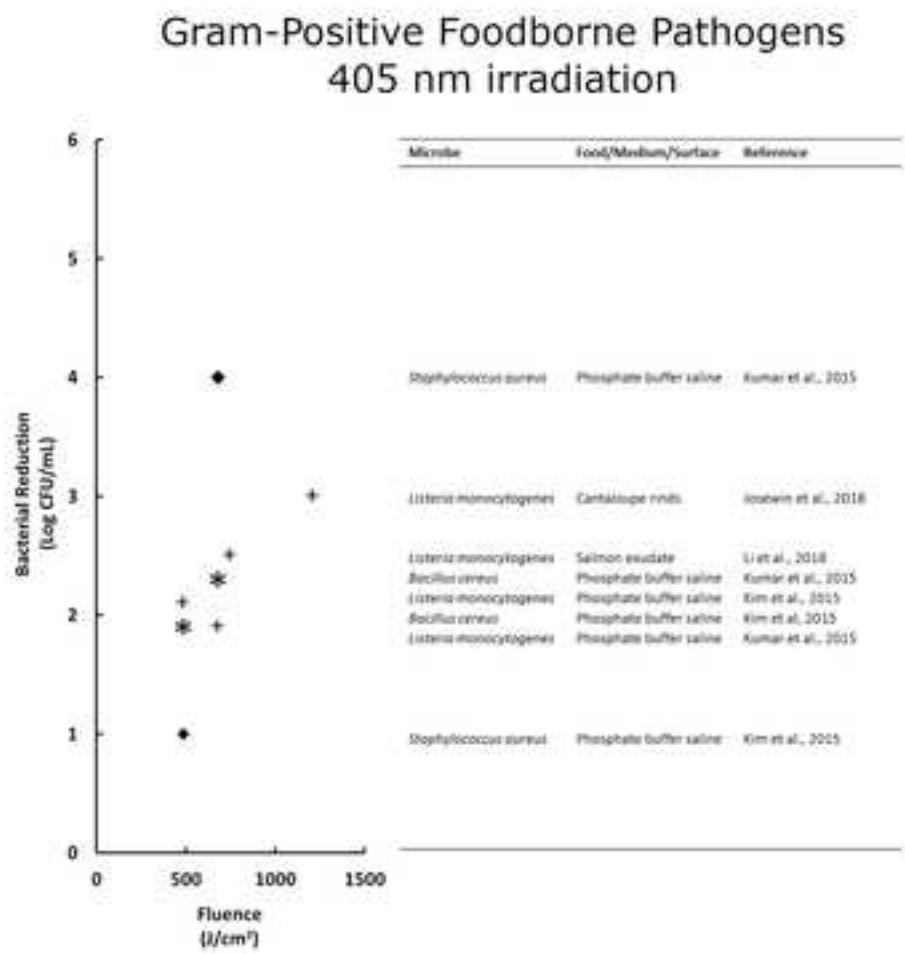

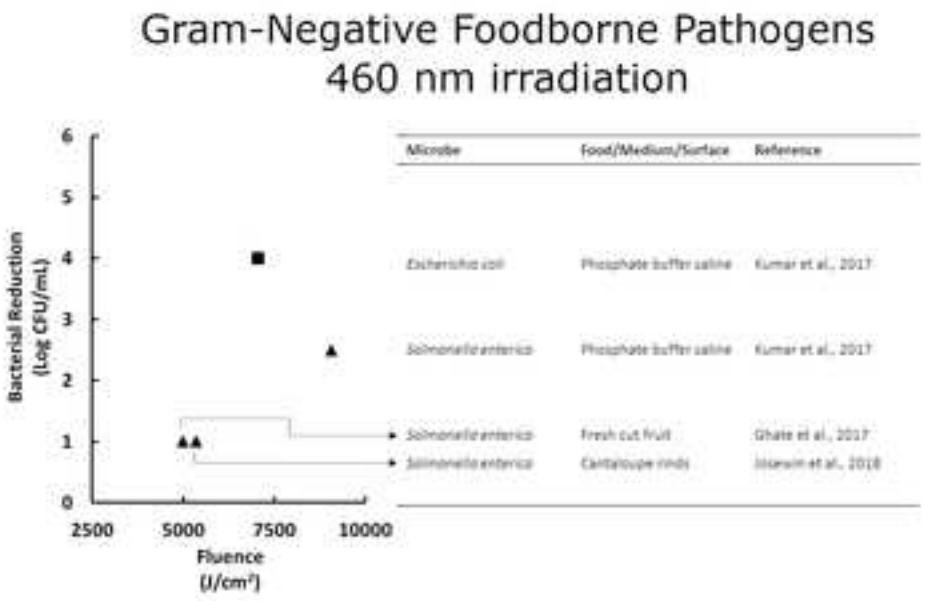

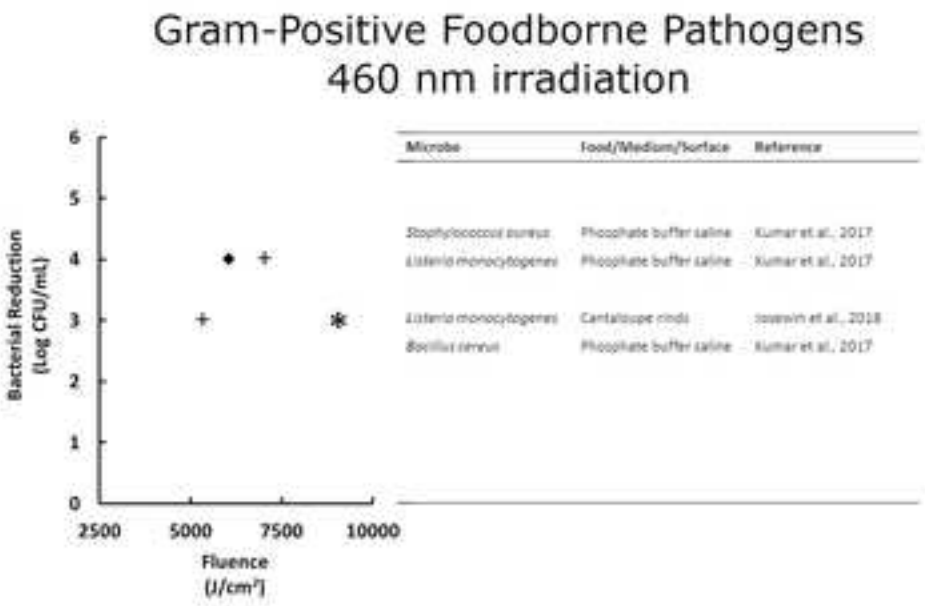

FIGURE 3 - Mechanism photosensitisation rev2

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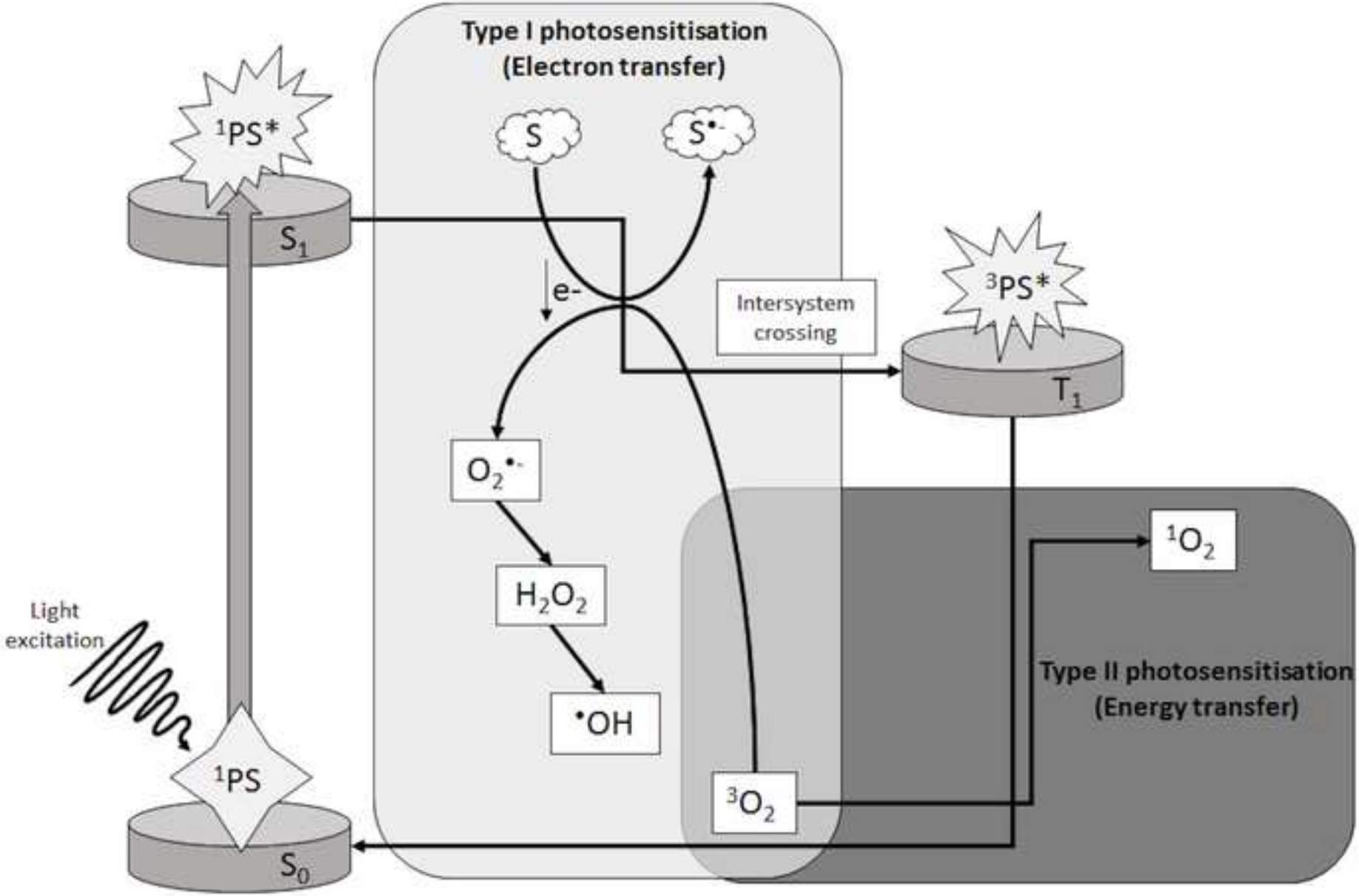

FIGURE 4 - Damages Photosensitisation Lipid DNA rev2

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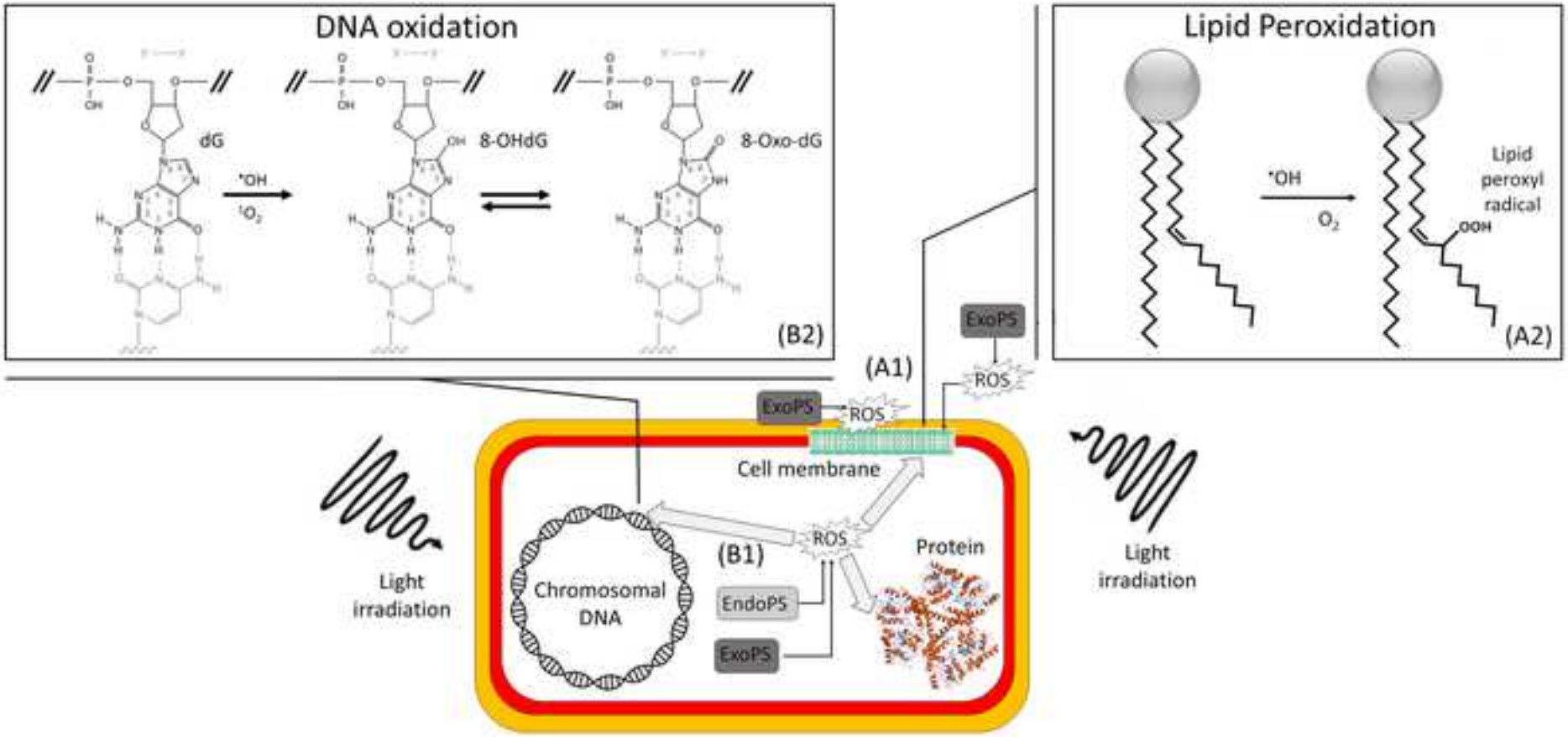

FIGURE 5 - Damages Photosensitisation Proteins rev2

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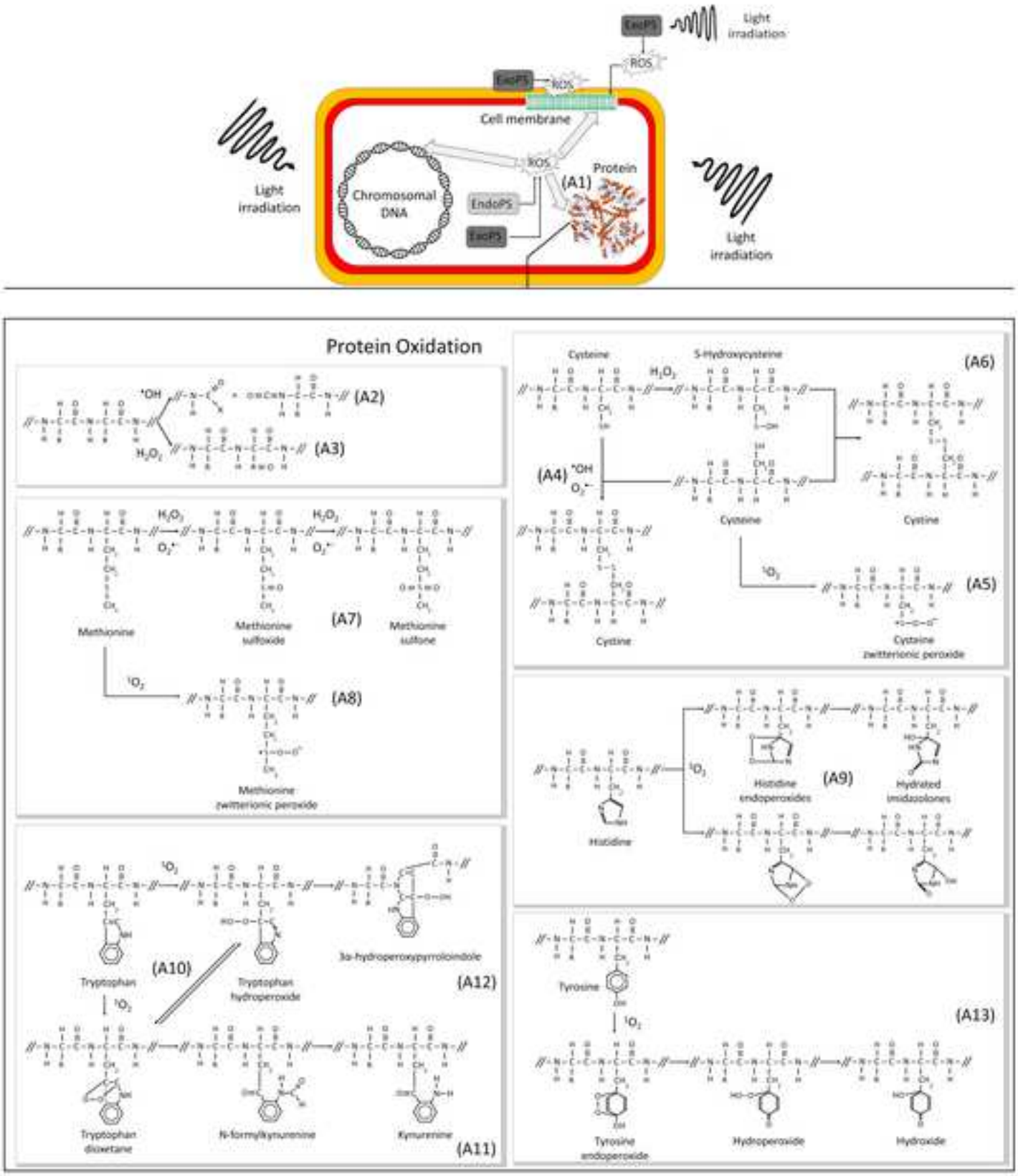

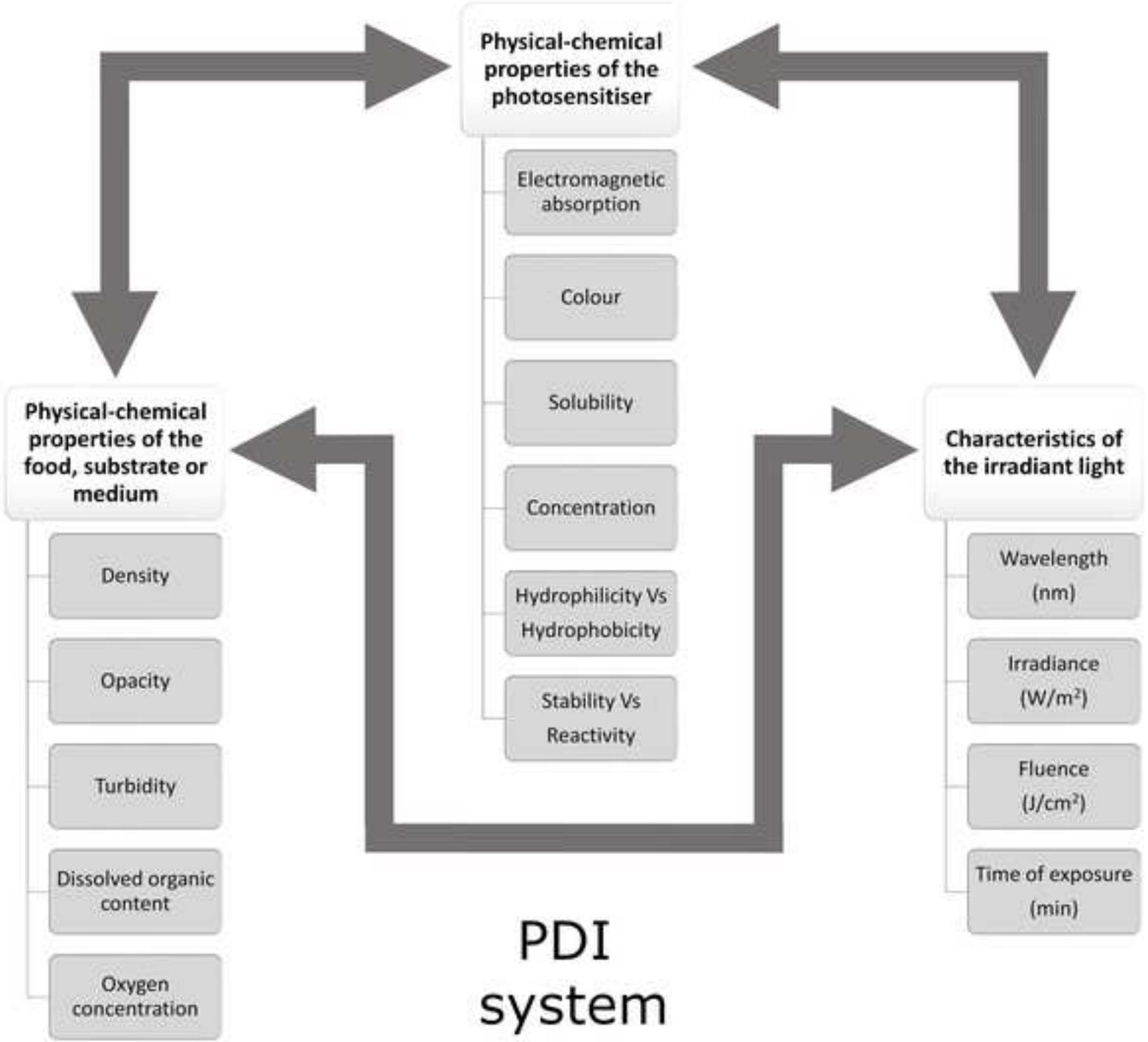

FIGURE 7 - Curcumin photosensitisation

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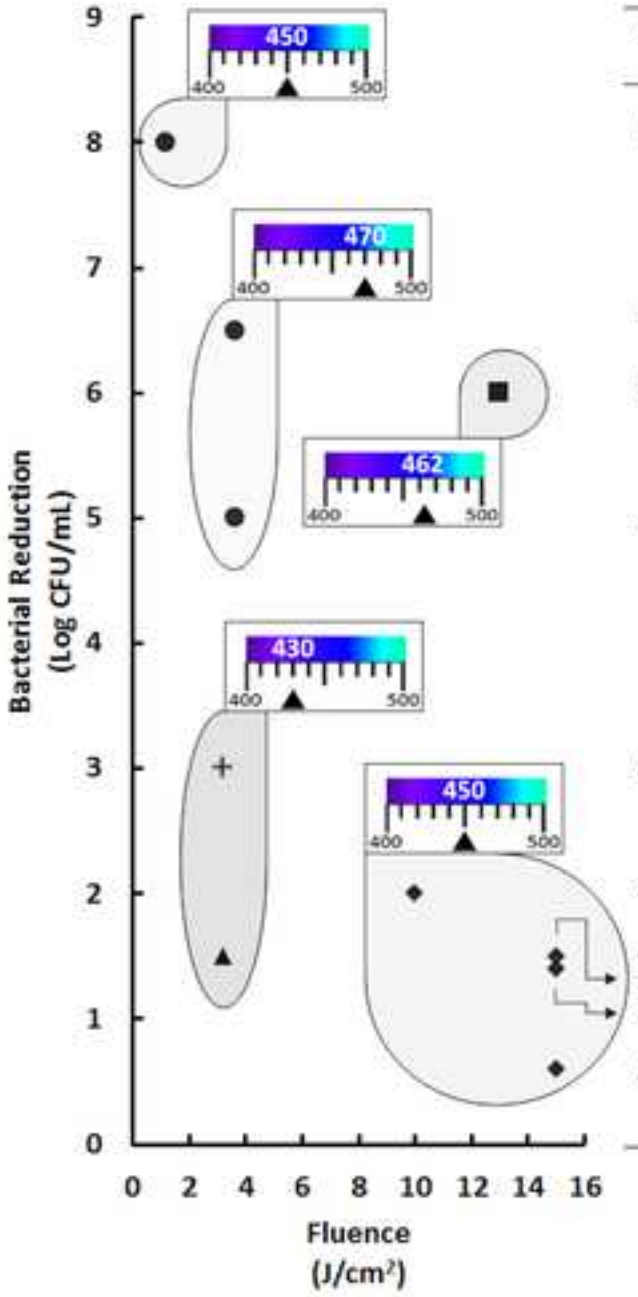
Curcumin Photosensitisation - Foodborne Pathogens



Microbe	Curcumin concentration (μM)	Food/Medium/Surface	Reference
Vibrio parahaemolyticus	1	Phosphate buffer saline	Chen et al., 2020
Vibrio parahaemolyticus	10	Phosphate buffer saline	Wu et al., 2016
Escherichia coli	20	Maximum recovery diluent	Bhavya and Umesh Hebbar, 2019
Vibrio parahaemolyticus	10	Oyster	Wu et al., 2016
Listeria monocytogenes	814	Chicken skin	Gao and Matthews, 2020
Staphylococcus aureus	80	Apple	Corrêa et al., 2020
Salmonella enterica	814	Chicken skin	Gao and Matthews, 2020
Staphylococcus aureus	40	Beef	Corrêa et al., 2020
Staphylococcus aureus	40	Chicken	Corrêa et al., 2020
Staphylococcus aureus	40	Pork	Corrêa et al., 2020

FIGURE 8 - Phorphyrin based photosensitisation

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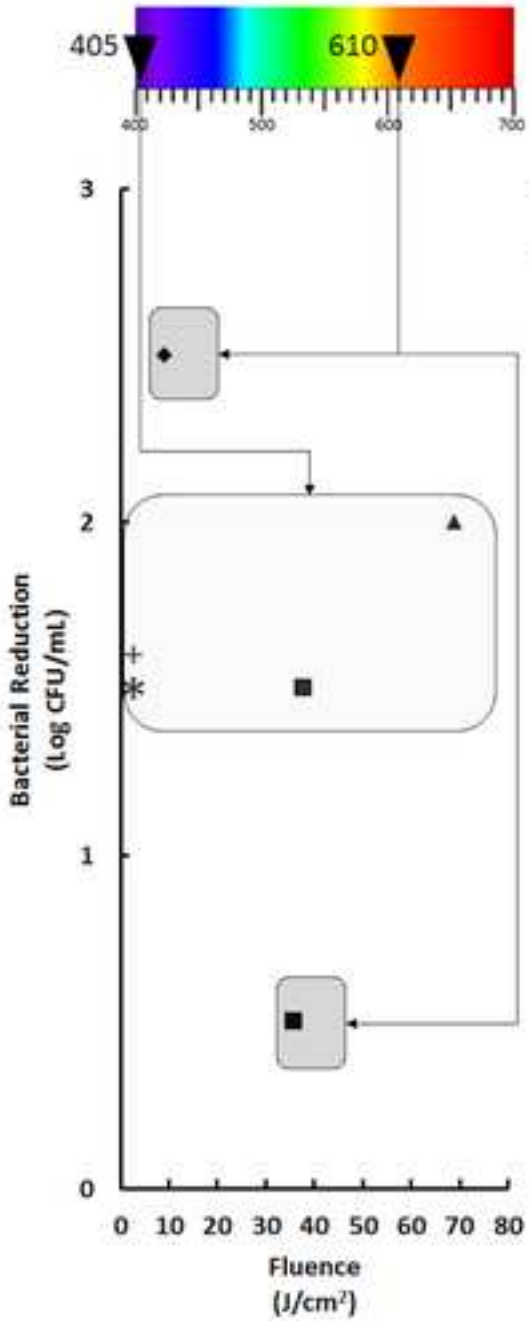
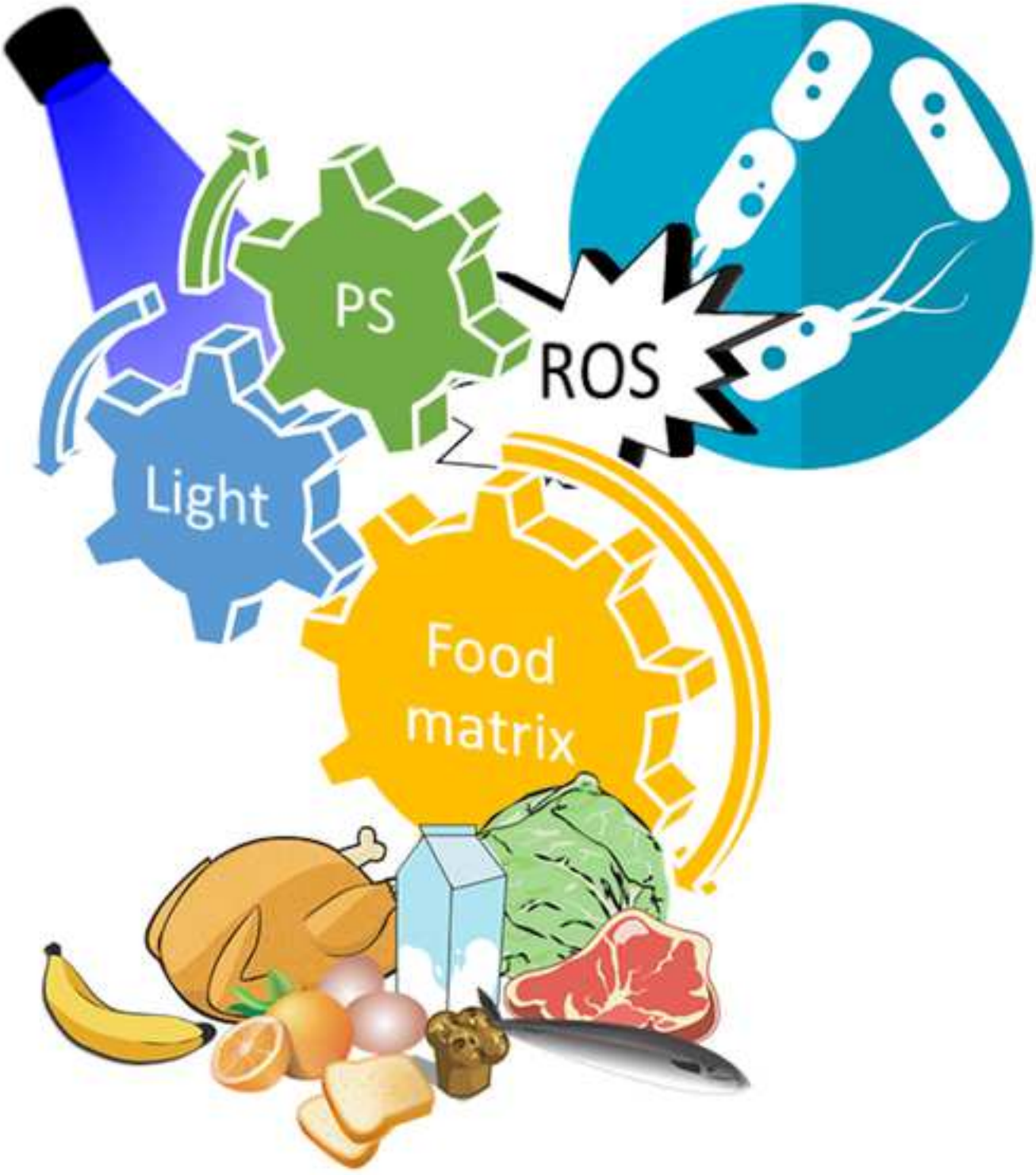
Porphyrin-based Photosensitisers - Foodborne Pathogens



Microbe	Photosensitiser	Photosensitiser concentration (μM)	Food/Medium/Surface	Reference
Staphylococcus aureus	Phthalocyanine	10	Milk	Galstyan and Dobrindt, 2019
Salmonella enterica	Chlorophyllin	15	Saline	Buchovec et al., 2017
Listeria monocytogenes	Chlorophyllin	150	Cherry tomatoes	Paskeviciute et al., 2018
Bacillus cereus	Chlorophyllin	150	Cherry tomatoes	Paskeviciute et al., 2018
Escherichia coli	Chlorophyllin	15	Saline	Aponiene and Luksiene, 2015
Escherichia coli	Phthalocyanine	10	Milk	Galstyan and Dobrindt, 2019



Declaration of interests

☒ The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests which may be considered as potential competing interests: