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# The application of photodynamic inactivation to microorganisms in food

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ABSTRACT

Nowadays, food safety issues have drawn increased attention due to the continual occurrence of infectious diseases caused by foodborne pathogens, which is an important factor causing food safety hazard. Meanwhile, the emergence of an increasing number of antibiotic-resistant pathogens is a worrisome phenomenon. Therefore, it is imperative to find new technologies with low-cost to inactivate pathogenic microorganisms and prevent cross-contamination. Compared with traditional preservatives, photodynamic inactivation (PDI) has emerged as a novel and promising strategy to eliminate foodborne pathogens with advantages such as non-toxic and low microbial resistance, which also meets the demand of current consumers for green treatment. Over the past few years, reports of using this technology for food safety have increased rapidly. This review summarizes recent progresses in the development of photodynamic inactivation of foodborne microorganisms. The mechanisms, factors influencing PDI and the application of different photosensitizers (PSs) in different food substrates are reviewed.

## Introduction

Food safety has become a hot issue of global concern, and accidents caused by various foodborne pathogens have occurred frequently. At present, the sterilization methods in food industry can be broadly divided into thermal sterilization and non-thermal sterilization technology. Thermal sterilization technology has a long history with wide application and the broad-spectrum sterilization ability, but its energy consumption is high and could cause food to lose the original nutrition and flavor under high temperatures. New non-thermal sterilization technologies such as antibiotics, ultra-high voltage pulse electric field, irradiation, high hydrostatic pressure and high-intensity ultrasound are also constantly emerging (Li & Farid, 2016; Wang et al., 2021). However, the overuse of antibiotics has led to bacterial resistance, which makes antibiotic sterilization technology phased out. The application of other non-thermal sterilization technologies, such as irradiation and pulse electric field were limited because of expensive equipment and large energy consumption. Moreover, non-thermal technologies such as high-intensity ultrasound could generate free radicals, denature proteins, lead to hotspots and release metal ions (Su & Cavaco-Paulo, 2021; Xue et al., 2021), while higher doses of ionizing radiation may cause slight changes in the color of beef, pork, and poultry.

Photodynamic inactivation is a technique that relies on the occurrence of non-thermal photophysical and photochemical reactions, requiring light and PSs in the presence of oxygen (Penha et al., 2017). It works on the principle that PSs can be activated in certain wavelengths to produce reactive oxygen species (ROS) with strong oxidation, so as to inactivate malignant cells and pathogenic microorganisms (Jiang et al., 2014). The advantages of PDI are that it does not produce toxic chemicals, the only energy required is the light source and the possibility of causing microbial resistance is low due to its multi-target nature (Costa et al., 2011), hence it has been widely applied in the fields of medicine (Jiang et al., 2020), dentistry (Rocha et al., 2020) and environment (Luksiene & Brovko, 2013). Since the beginning of the 21st century, researchers have begun to explore the possibility of using PDI as a novel strategy for food decontamination in a non-thermal way to achieve food safety. In recent years, attempts to use PDI in foods have become increasingly popular, and many researchers have devoted themselves to studying the effects of PDI on food-borne microorganisms, including inactivation mechanisms and influencing factors, as well as the efficacy of PDI in food substrates. So far, its application has been mainly hindered by changes in sensory properties of foods after introducing. This review will provide reference on the potential use of PDI as a novel environmentally friendly non-thermal sterilization technology.

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## Mechanism of PDI

Photosensitizer (PS) refers to a kind of chemical substance that can absorb photons in photochemical reactions and transfer energy to specific molecules to enable them participate in chemical reactions (Jiang et al., 2013) Photodynamic reaction is a series of physical, chemical and biological processes driven by ultraviolet light (200–380 nm), visible light (380–760 nm) or near-infrared light (760 nm-0.002 mm) and triggered by the de-excitation of excited photosensitizers in biological tissues. There are two main types of PDI reactions (Alves et al, 2015):

(1) As shown in Fig. 1, under illumination, PS in ground state ($^1PS^0$) can absorb photons and turn into excited singlet state ($^1PS^*$). Then, part of the $^1PS^*$ may lose energy through fluorescence emission and return to its ground state, while the rest of it can be successfully converted to the long-lived triplet state ($^3PS^*$) through an intersystem crossing (ISC), which was required by the PDI process. Afterwards, part of the $^3PS^*$ may decay to ground state by phosphorescent emission, while the rest may react with the surrounding organic matters and ground state oxygen to produce ROS (Type I), such as hydrogen peroxide, superoxide anion, hydroxyl radical and singlet oxygen, which are able to inactivate target cells (Chatterjee, Fong, & Zhang, 2008). In this process, PS may be consumed and cannot be regenerated.

(2) The second type of PDI reaction is that the resulting $^3PS^*$ can react with the triplet oxygen molecule ($^3O_2$) to generate singlet oxygen ($^1O_2$), and then the active $^1O_2$ deoxidizes the substrate (Type II). During this process, the PS will not be destroyed or consumed, the molecule will return to its ground state to participate in the next cycle of reaction (Chatterjee, Fong, & Zhang, 2008). Since these two processes may occur simultaneously in a typical PDI reaction, singlet oxygen plays a prevalent role in the molecular processes initiated by PDI.

ROS produced in type I and type II reactions has high chemical reactivity, which can interact with a variety of components in cells, such as proteins, lipids and nucleic acids. Some organic like acids, alcohols, amines, carbohydrates, nitrogen heterocyclic molecular, hormone, pyrrole, vitamins, and cytokines are also sensitive to singlet oxygen, thereby causes cell necrosis or apoptosis (Konopka & Goslinski, 2007; Allison et al., 2008). However, the research to date has tended to focus on the application rather than elucidating the mechanism of PDI, especially for foodborne microorganisms.

Since PDI is a dynamic process of multi-factor interactions, involving many disciplines such as tissue optics, photo physics, photochemistry and cell biology. At present, it is still difficult to clarify its action rules or give a quantitative description of the photodynamic reaction process as well as the interactions among the photodynamic components.

## Factors influencing PDI

PDI has three essential elements: light, oxygen and PS, all of which are indispensable. Therefore, the selection of these three elements also determines the ultimate sterilization efficiency of PDI. Each of these variables can be regulated. Light is usually the easiest to adjust, oxygen is the most difficult to control, and PS is the most critical factor in the

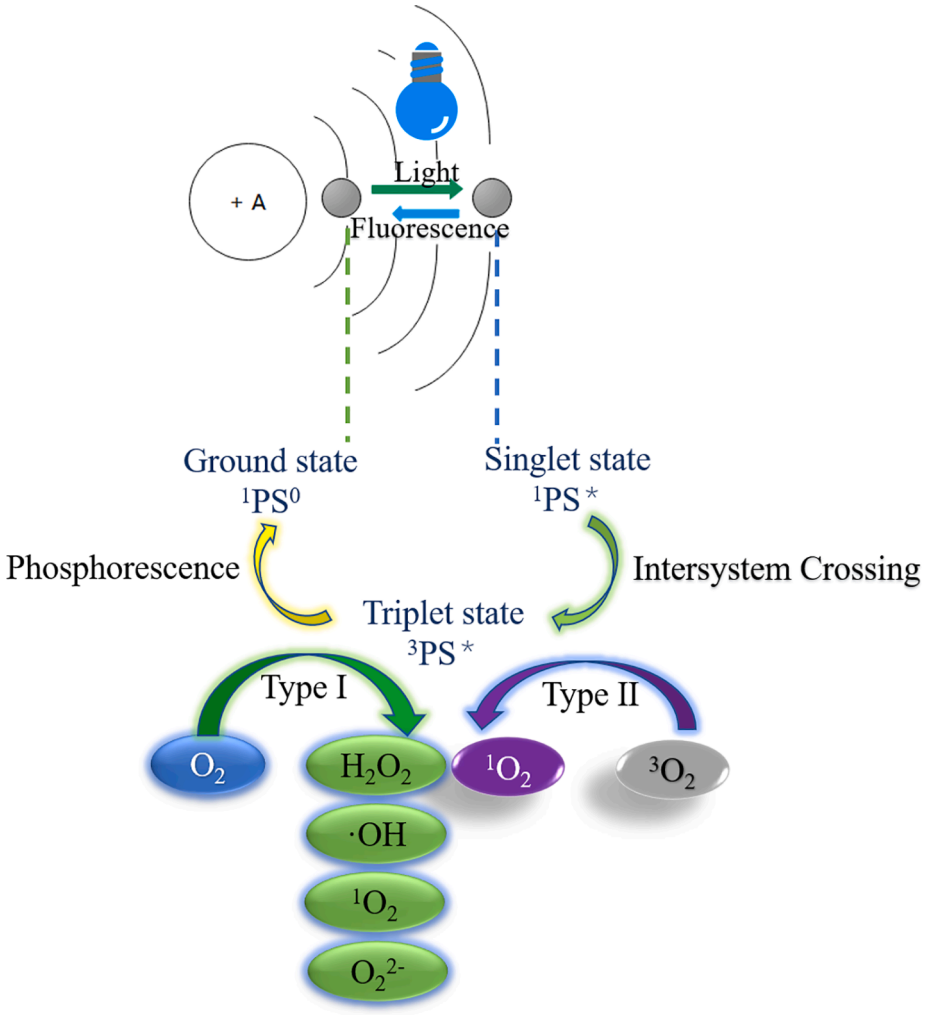


**Fig. 1.** Schematic diagram of possible optical processes during PDI.

process of PDI. For this reason, many researchers are focusing on finding suitable PSs that are safer and more effective to apply.

However, considering only these three basic components does not guarantee the effect of PDI on food substrates, as many other relevant factors will also have influences, such as the classes of food substrates, environmental conditions and process constraints. Each influencing factors must be taken into account to determine whether PDI can be effectively used in practical applications. This section will analyze the importance of all these factors and their potential impacts.

*Light sources*

Several light sources such as light-emitting diodes (LEDs), lasers and halogen lamps are currently used to provide specific wavelengths. LED is a semiconductor device with very narrow emission wavelength range compared with other traditional visible light sources (Kumar et al., 2017), hence it can be used as monochromatic wavelength. Furthermore, it is widely used due to its low energy consumption and high durability.

The wavelength of light is an important factor affecting PDI, which was chosen according to the structure and absorption peak of PS applied in the PDI process (Nowis et al., 2005). Generally speaking, PDI is more effective when given longer exposure time. In this case, two wavelengths are important: the absorption wavelength of PS and the emission wavelength of the light source. In general experiments, these two wavelengths should be set as the same as possible to maximize the effect of PS. Therefore, a narrow light source with an emission spectrum that overlaps as much as possible with the absorption spectrum of the PS should be used. Although using broad spectrum light is not efficient from a photodynamic point of view, it is usually desirable because most of the light for daily use is broad-band, both sunlight and ambient white light.

Ultraviolet light with wavelength<280 nm is deemed to be bactericidal to most types of microorganisms. Bacterial and fungal cells contain porphyrins, a kind of endogenous PS, which also contains protoporphyrin and uroporphyrin. Kumar et al. (2015) pointed out that the sensitivity of different bacteria to light sources is related to the porphyrin components in the bacteria. This discovery suggested that one of the reasons for the inactivation of bacteria after exposure to ultraviolet light is PDI caused by endogenous PS.

Ultraviolet light can be divided into UVA (315–400 nm), UVB (280–315 nm) and UVC (200–280 nm). Ha et al. (2016) treated *E. coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* inoculated on sliced cheese through packaging with UVC, and suggested that the ultimate death of microbes was due to light damage and conformational changes of the nucleic acids and proteins in cells after absorbing UVC radiation. UVA is known to be less effective at killing bacteria than UVB or UVC. Probst-Rüd et al. (2017) found that UVA radiation mainly induced sublethal effects, such as oxidative stress, protein damage, growth delay and energy metabolism reduction, which could damage cells rather than kill them. In this case, damaged cells can recover and functionalize normally under appropriate conditions, which should be avoided in experiments and applications. The main bactericidal mechanism of UVB is due to the direct absorption of light by DNA and its subsequent damage. To a lesser extent, UVB also induces protein oxidative damages.

*Temperature*

It is known that bacterial cells can change the ratio of saturated fatty acids to unsaturated fatty acids in the membranes in response to changes in ambient temperature to maintain membrane fluidity. Therefore, bacteria exposed to lower temperatures during illumination may be more susceptible to damage due to the increased unsaturated fatty acids in the membrane.

Ghate et al. (2013) studied the bacteriostatic effect of bacterial cultures irradiated with LED in the visible region of the electromagnetic spectrum (461, 521, 642 nm) at 20, 15 and 10℃. The study found that the antibacterial effect of PDI on 461 nm and 521 nm LEDs was better at the temperature 10℃ than at 20℃, while no antibacterial effect was observed at any temperature under 642 nm. Therefore, the results showed that the antibacterial effect of LEDs is highly dependent on wavelength and illumination temperature. This study showed that under the appropriate wavelength of light, combination with freezing is a promising method to serve as a new food preservation technology. Kumar et al. (2016) observed that 405 nm LEDs at 4 ℃ and 10 ℃ were more suitable for inactivating *S. aureus* cells than 25 ℃. The authors suggested that bacterial cells may have a greater metabolic burden at room temperature and the high temperature accelerates the rate of cytotoxic response.

*Acidity*

The acidity of food may also affect the efficacy of exogenous PSs. Most natural foods are acidic, especially fruits. However, they have a wide range of pH values. Watermelons, for example, are weakly acidic (pH = 5.5), while strawberries are more acidic (pH = 3–4). Ghate et al. (2013) have reported that *E. coli* and *S. typhimurium* are more susceptible to LED irradiation under alkaline conditions than under acidic conditions. This might be the result of the dissolution of proteins and the saponification of membrane lipids by hydroxyl ions weakening the cell membranes, which is easier to be damaged by ROS once weakened by high pH level. Another possible reason is the change in lipid composition of bacterial membrane caused by the pH of the environment. According to Yuk and Marshall (2004), using hydrochloric acid to lower the pH value increased the proportion of saturated fatty acids in the membrane, and using sodium hydroxide to increase the pH value resulted in an increase in the proportion of unsaturated fatty acids in the lipid composition of the *E. coli* membrane. Studies have found that unsaturated fatty acids are more sensitive to ROS than saturated fatty acids. Therefore, a higher proportion of unsaturated fatty acids enhanced the toxic effect of ROS on cells, thus improving the antibacterial effect.

More importantly, the acids that play a major role in pH also vary from fruit to fruit. For example, the main acid in grapes is tartaric acid, while the acid in strawberries and citrus is mainly citric acid, and these acids have different sizes and polarities, which affect the ability of PSs to penetrate cell membranes. Ghate et al. (2015) investigated the effects of different organic compounds (citric acid, malic acid and lactic acid) at the same pH (4.5) on four foodborne pathogens (*E. coli, S. typhimurium, L. monocytogenes* and *S. aureus*) under the irradiation of 461 nm. Among all three organic acids increased the antimicrobial efficacy of the LED against the tested foodborne pathogens, lactic acid was the most effective one, followed by citric acid and malic acid. This may be because lactic acid has the highest concentration of undissociated molecules among the three acids and produces the largest intracellular effect. Furthermore, lactic acid and citric acid can destroy the integrity of cell wall, which is more conducive for PS to enter the cell to functionalize. Consequently, the organic acids in food may determine the success of the sensitization of exogenous PSs to bacterial cells.

*Buffer agent*

Since the pH of the environment will affect bacteria and the efficacy of PSs, neutralizing buffer solutions are necessary in the experiment to resist pH changes. Beck et al. (2015) compared UV-induced MS2 Phage inactivation and RNA damage by using phosphate buffered saline (PBS) as the buffer agent. On the other hand, Kim et al. (2019) demonstrated the synergistic effect of ohmic heating and UVC irradiation for inactivation of *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* in peptone water (PW). However, Jeon and Ha (2018) found an interesting phenomenon that in most UV treatment experiments, PW was significantly less effective at killing bacteria than PBS. Further research revealed that this is because the ultraviolet absorption mode of PW is

different from that of PBS. The results indicate that PW may not be used as a buffer solution in the study of ultraviolet sterilization, while PBS is an appropriate neutralizing buffer solution to ensure reliable data.

### *Types of PSs*

Several types of PSs, such as phenothiazine dyes (methylene blue (Klosowski et al., 2020), toluidine blue O (Moslemi et al., 2018)), phthalocyanine (Liang et al., 2021), porphyrin (Mala et al., 2021) have been proved to have significant inactivation effects. In recent years, consumers are more enthusiastic about natural products, therefor natural additives are very attractive to food manufacturers. Some natural PSs extracted from various plants and other sources have been proposed and characterized (Fig. 2) (Rodriguez-Amigo et al., 2015; Wang et al., 2021; Murakami et al., 2001; Buchovec et al., 2017; Li et al., 2021). A number of recent studies have tested the efficacy of PSs in food substrates, most of which have focused on fresh fruits, vegetables and meat. Table 1 summarized reported PSs that can be used in food products.

#### *Riboflavin*

Riboflavin, commonly known as vitamin $B_2$, has a special prospect because it is a water-soluble vitamin. Since water-soluble vitamins are regularly excreted from the body through urine, its allowable dose as food additive may be bigger. Riboflavin is now a colorant in the Chinese Food Additives Standard. Li et al. (2021) evaluated the ability of riboflavin to kill *Salmonella*, and the results showed that the irradiation dose of 9.36 J/cm$^2$ and 150 μM riboflavin inactivated more than 6 log of planktonic *Salmonella*. Moreover, under an irradiation dose of 93.6 J/cm$^2$ and 150 μM riboflavin, 2.1 log of *Salmonella* on tuna was inactivated effectively.

#### *Vitamin K*

Vitamin K (VK) is a photoactive chemical derived from natural products, which is essential for blood coagulation and bone health (Booth, 2012). VK exhibits high photoactivity under light irradiation, leading to the generation of ROS in various solvent systems, and $VK_3$ is the most effective photoactive compound that generates ROS in the ultraviolet–visible wavelength range among all five vitamin K compounds (Shearer & Newman, 2014). Even under sunlight, $VK_3$ still shows strong and long-lasting antibacterial function in the PBS buffer system, making it more convenient for daily use (Zhang, Si, & Sun, 2019). Zhang et al. (2020) fabricated VK-containing nanofibrous membranes (VNFMs) through blending hydrophobic polyacrylonitrile (PAN) and hydrophilic polyvinyl alcohol-*co*-ethylene polymers (PVA-*co*-PE) with three VK compounds. The research team used the suspension of T7 bacteriophage, a non-enveloped double-stranded DNA virus that infects bacteria, and discovered that PVA-*co*-PE/$VK_3$ VNFM achieved 6 log PFU after 30 min of sunlight. The reduction of phage showed its highly effective antiviral activity, and the author analyzed that the inactivation of T7 bacteriophage was attributed to the oxidation of viral nucleic acid and lipid membrane by ROS. Sheng et al. (2020) investigated the photosensitivity and antibacterial effects of $VK_3$ under UVA and simulated sunlight on *E. coli* O157:H7, *S. Enteritidis* PT30 and *L. monocytoge*nes on the surface of broth and lemon. The number of *L. monocytogenes* and *E. coli* O157:H7 inoculated on the surface of lemons immediately decreased more than 5 log after the treatment of $VK_3$ and simulated sunlight, and the number of *S. Enteritidis* PT30 decreased by about 4 log. After being stored in the dark at 4 °C for one day, the bacterial populations of all three strains were all reduced below the detection limit (<1 log). Moreover, as the storage time increased, the number of positive samples after enrichment further decreased, proving that $VK_3$ produced a continuous antibacterial effect in the surface of the lemon.

#### *Chlorophyll*

Chlorophyll (Chl) and its sodium salt are photosensitizers. Copper chloride sodium salt has been allowed to be added in food according to the Chinese Food Additives Standard, and is usually used as a coloring agent. Since chlorophyll is not water-soluble, its water-soluble derivative, sodium chlorophyll, is commonly used. Buchovec et al. (2017) inactivated *Salmonella* based on the photosensitive activity of Chl and discovered the number of bacterial cells significantly reduced. Altering the incubation time of the bacteria in Chl, it turned out that neither the number of cell-binding PS nor the level of cell inactivation was changed, suggesting that Chl may bind to the cells from the outside, therefore it does not need longer time to accumulate inside the cells. Luksiene et al. (2010) inactivated food borne pathogen *Bacillus cereus* by Na-Chl-based photosensitization both in *vitro* and after adhesion to the surface of packaging material. The results suggested that *B. cereus* are reduced by 7 log in *vitro* at the lowest concentration of Na-Chl ($7.5 \times 10^{-8}$ mol/L) with the irradiation of 405 nm for 2 min, and that attached to the surface of

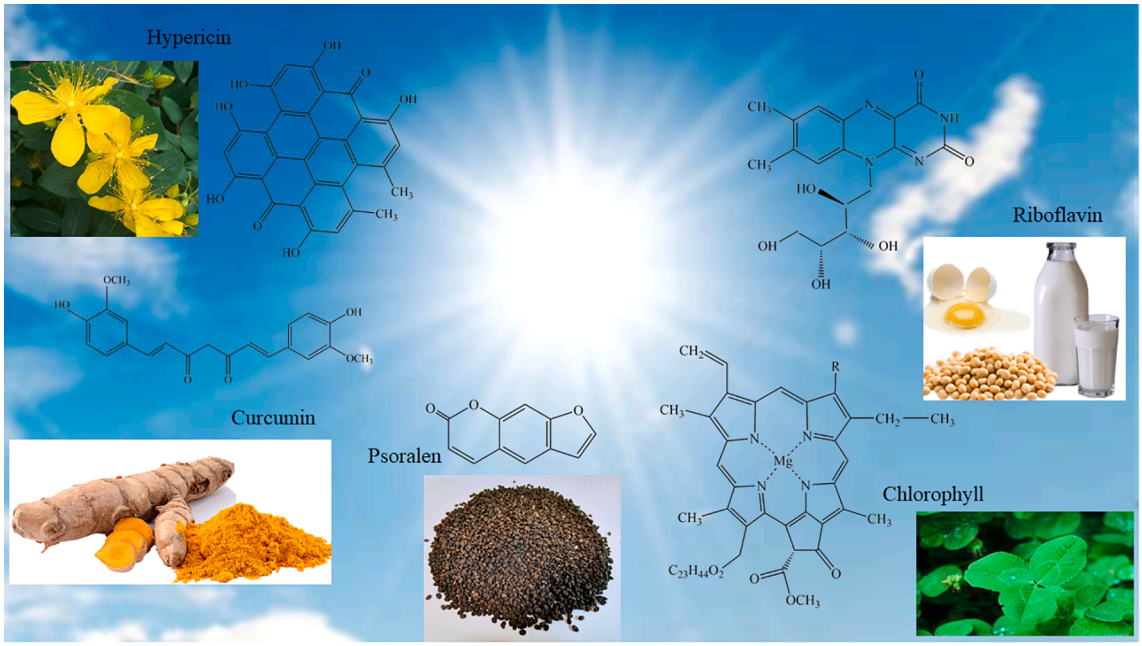


**Fig. 2.** Naturally available PSs extracted from various plants and foods.

**Table 1**
Reported PSs applied in food products.

| Exogenous PS | Wavelength (nm) | Microorganisms | Substrate |
|---|---|---|---|
| Sodium Chlorophyll | 400 nm | *L. monocytogenes* | Strawberries (Luksiene and Paskeviciute, 2011) |
| Chlorophyll | 405 nm | *S. typhimurium* | Strawberries (Buchovec et al., 2016) |
| Curcumin | 435 nm | *E. coli* O157:H7 | Cucumbers (Glueck et al., 2017) |
| | 470 nm | Total aerobic bacterial | Oysters (Liu et al., 2016) |
| | 435 nm | *S. aureus* | Chicken leg meat (Tortik, Spaeth, & Plaetzer, 2014) |
| | 410 nm | *E. coli* | Fresh-cut pineapple (Zou et al., 2021) |
| | 460 nm | Total viable counts | Fresh *Tremella Fuciformis* (Lin et al., 2021) |
| Erythrosine B | 200–1500 nm | *E. coli* O157:H7 *and L. monocytogenes* | Tomato juice (Jae-Won, 2020) |
| Riboflavin | 405 nm | Tulane virus | Blueberries (Kingsley et al., 2018) |
| | 405 nm | *L. monocytogenes* | Smoked salmon (Josewin et al., 2018) |
| Vitamin $K_3$ | 365 nm&D65 | *E. coli* O157:H7 and *L. monocytogenes and S.* Enteritidis | Lemon (Sheng et al., 2020) |

packaging material are reduced by 4.2 log.

*Curcumin*

Curcumin is widely used as a natural phenolic food coloring agent because of its low cost and non-toxicity. The light-absorption peak of curcumin is between 400 and 500 nm. Researchers discovered that the photodynamic effect of curcumin is effective in killing malignant tumors (Rao & Khanum, 2016), new research shows that low concentrations of curcumin can also kill a variety of pathogenic microorganisms in food. Al-Asmari et al. (2017) found that curcumin-mediated PDI significantly inhibited the growth of *Aspergillus niger*, *Aspergillus flavus*, *Penicillium griseofulvum*, *Penicillium chrysogenum*, *Fusarium oxysporum*, *Candida albicans* and *Zygosaccharomyces bailii* in aqueous buffer and on the agar plate. Zou et al. (2021) applied curcumin to fresh-cut pineapple for testing, and found a synergistic antibacterial effect occurred when combined with 410 nm light. After 10 min of light treatment (with curcumin of 100 μM), the growth of *E. coli* on the pineapple was inhibited during storage at 4 °C for 5 days, reducing 0.19–1.24 log. The author also analyzed the appearance and color of the treated pineapple and found that the browning of pineapple slices was well controlled. Besides, it turned out that the texture and sensory quality of pineapples were better in 10 min of light treatment than 20 min.

*Erythrosine B*

Erythrosine B (ERY), also known as cherry red, is a chemically synthesized functional food colorant with good antioxidant activity. Silva et al. (2018) irradiated ERY with green LED in its absorption range (450–550 nm), and found that it could inactivate bacteria in both planktonic and biofilm states, but the planktonic cells were more susceptible to PDI than the biofilm ones. The biofilms of *S. aureus* and *E. hirae* were both reduced to undetectable levels when 7.5 μM (295.83 J/cm$^2$) ERY were applied, while the planktonic ones were completely inactivated under 1000 nM (20.52 J/cm$^2$). Yassunaka et al. (2015) found that under 510 nm of LED lights, ERY-mediated PDI significantly reduced *Aeromonas hydrophila* by 4.3 log in 10 min (78 J/cm$^2$), and complete eradication was observed after 20 min and 30 min.

*Hypericin*

Hypericin (HYP) is one of the most effective photosensitizers (Pezzuoli et al., 2018). HYP is found in the plant *Hypericum perforatum* (commonly known as St. John's wort) (Luksiene & Brovko, 2013). Although it is not included in the Chinese Food Additives Standard, it is a very good candidate for food application because it has good photosensitivity and no toxic or genotoxic effects. Experimental results demonstrate that HYP exhibits strong absorption and high amount of singlet oxygen generation with high fluorescence yield in the range of 600–700 nm (Baptista & Wainwright, 2011; Kairyte et al., 2012). HYP is also known for its antidepressant (Eatemadnia et al., 2019), antiviral (Aygül & Şerbetçi, 2020) and anti-tumor (Agostinis et al., 2011) properties, it is also used as a flavoring agent in foods and alcoholic beverages abroad (de Araújo et al., 2020). The photosensitive properties of HYP, in particular, have been extensively studied for cancer therapy. However, HYP is highly hydrophobic, which makes it particularly difficult to be used in aqueous system, but copolymerized nanoparticles can be used to minimize the hydrophobicity of this compound (Singh et al., 2013). HYP-mediated (0.78 μM) polymer nanoparticles can reduce 4 log *S. aureus* after low-density orange LED (570–610 nm) irradiation for 30 min (Malacrida et al., 2020).

## Elimination of food-borne pathogens

Because of the advantage of having no specific cell target, PDI can effectively oxidize different biomolecules, thereby destroying a variety of cell types.

In general, gram-negative bacteria are significantly resistant to PDI, while gram-positive bacteria are more susceptible. Most of the gram-positive bacteria cells have a thick wall composed of peptidoglycan layer, which has relatively high porosity and permeability (Pereira et al., 2014). However, the cell wall of gram-negative bacteria is composed of a complex outer membrane of phospholipids, lipopolysaccharides, lipid teichoic acid and lipoproteins, which forms an impermeable barrier to antimicrobial agents (Nagata et al., 2012). Due to the complexity of the cell wall of gram-negative bacteria, it is necessary to use cationic PSs to promote decomposition through electrostatic interaction to maximize inactivation effects (Kashef & Hamblin, 2017). Besides, since most hydrophobic and amphiphilic PSs and lipids have similar polar and non-polar ratios, they usually locate on the cell membrane after enter the cells (Tsubone, Baptista, & Itri, 2019). Under the ideal process of PDI (Fig. 3), PSs attach to the bacterial cell wall to release ROS, further destroying the organelles (Ribeiro & Lourenço, 2021). Studies have found that the ROS produced by the photosensitive process can directly attack the functional or structural cellular components of bacteria.

Spores are of particular interest to the food industry because they can survive conventional sterilization processes such as pasteurization. Luksiene et al. (2009) evaluated the ability of the 5-aminolevulinic acid (5-ALA)-assisted photodynamic therapy to inactivate planktonic and sessile spores of *Bacillus cereus.* 400 nm LED with an irradiance of 20 mW/cm$^2$ and a dose of 18 J/cm$^2$ was applied to the strong planktonic population of *B. cereus*. Eventually, spores decreased approximately 2 log at an 5-ALA concentration of 3 mM, and 3.7 log at 7.5 mM 5-ALA concentration. Prado-Silva et al. (2021) tested the effect of new methylene blue (NMB) as a PS on the inactivation and sporulation of vegetative cells of *B. cereus*, and found that the use of NMB and red light together reduced both of the two forms of *B. cereus* (nutrition cells and spores).

Different from bacteria, the fungal cell wall contains chitin, glucan, lipoprotein, and a medium permeability barrier. Under the same conditions, yeast cells are more resistant to PDI (Calzavara-Pinton et al., 2012), which may be related to the difference in the cell wall structure. Therefore, to kill yeast cells requires multiple damage to important organelles. Lyon et al. (2011) has confirmed that PDI is more effective against yeast by enhancing PS concentration and light intensity. De

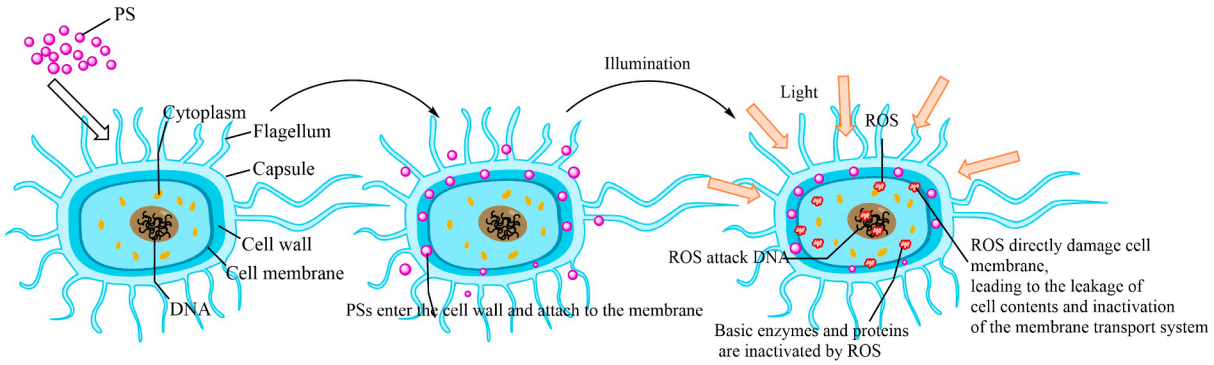


**Fig. 3.** Schematic diagram of PS entering and destroying a bacterial cell when exposed to light.

Menezes et al. (2014) tested a novel pentacyclic phenothiazinium photosensitizer S137 combined with 634 nm LED light against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* that usually infect tropical fruits. The authors found that after penetrating into fungal cells, PS was located in the cytoplasmic structure of conidia. In addition, PDI can cause additional damage to the fungal proteome, which may lead to changes in the isoelectric points of some proteins (Brancini et al., 2016).

The main threat caused by bacteria and viruses is food-borne diseases, but the risk of food spoilage caused by mold is greater. Unlike bacteria, molds require higher oxygen, thus they usually grow on the surface of solid substrates, hence PDI is particularly suitable here. Imada et al. (2014) investigated the effects of three wavelengths (405, 415 and 450 nm) on grey mold (*Botrytis cinerea*), and found that 405 nm illumination not only inhibited the growth of spore filaments of *Botrytis cinerea* on tomato leaves within 120 h of light, but also inhibited spore germination (<20%) within 18 h of light. In the study of Luksiene et al. (2005) about the inhibition of *Alternaria alternata*, *Fusarium avenaceum*, *Acremonium strictum* and *Rhizopus oryzae* under a broadband tungsten light (370–680 nm), they observed that a large number of PS accumulated in the conidia of the mold. However, photodynamically inactivated fungi are still mainly used in the medical industry, such as the use of amino functionalized mesoporus silica-rose bengal nanoconjugates to inhibit *Candida albicans* (Paramanantham et al., 2018).

Enveloped viruses are more easily inactivated than non-enveloped viruses, because lipids and proteins in the envelope are considered to be binding sites of PSs, and protein damage caused by ROS may be a potential mechanism for virus inactivation (Kashef, Huang, & Hamblin, 2017). However, some studies have shown that non-enveloped viruses can also be inactivated effectively by the phototoxicity of cationic PSs, with an inactivation efficiency similar to gram-negative bacteria (Lim et al., 2012). Foodborne viruses are difficult to become non-infectious because they have a relatively simple design made up of protein capsids and nucleic acids, and are often a problem with fresh products as they are usually raw and unwashed. Kingsley et al. (2018) evaluated the potential of 405 nm irradiation to interfere with Tulane virus in the surface of blueberry. It turned out that PSs or other chemical enhancers are required in order to effectively inactivate the virus by photoactivation on the surface of berries.

Protozoa is another group of single-celled eukaryotes that affects food safety. The complexity of their cellular structure and life cycle, including the active feeding phase and the dormant cyst phase, as well as their relatively large size, make them a challenging target. However, photodynamic antimicrobial chemotherapy has been shown to be effective in killing parasitic protozoa that cause infections such as malaria, leishmaniasis and other diseases (Baptista & Wainwright, 2011). However, the mechanism of photolysis of protozoan cells with different PSs is remain unclear, which will allow the selection of suitable PSs and the development of efficient methods to control these organisms.

## Application of PDI in food

PDI can slow down the growth of microorganisms, viruses and other organisms in food by releasing ROS, and even eliminate them. Although this PDI method has been widely used in the medical field, such as killing tumor cells and treating periodontal disease (Ramanauskaite et al., 2021), it is still less studied in the food field, therefore, it is of great significance to apply it in the food industry. In contrast to traditional pasteurization methods, PDI minimizes the changes in nutritional and sensory properties and meets the growing demand of consumers for microbiologically safe foods without chemical additives. PDI has the potential to be applied in a variety of food substrates with disparate traits, as illustrated in Fig. 4.

To date, most studies have involved PSs in food substrates by immersion or spraying. When choosing a PS for food-related applications, considerations should be given regarding its influences to food and food processing environment. PSs used should not impair the appearance, composition, nutrition or flavor of food. Moreover, the hydrophilicity of PS will affect its ability to reach target cells due to different compositions of food substrates (Pezzuoli, et al., 2018). Hydrophilic PSs are more effective on fruit surfaces than meat on account of the water-based surfaces of fruits. In this case, add PS into food packaging films or coatings may promote wider application of PSs in food.

### *Aquatic products*

PDI can be applied to disinfect microbiologically contaminated water before contacting with fish, so as to reduce the probability of fish disease. However, the treatment of fish diseases through PDI has not received sufficient scientific research. Wohllebe et al. (2009) put very low concentrations of chlorophyll acid into the water where the larvae lived, and it turned out the larvae were killed under solar irradiation. Afterwards, Wohllebe et al. (2012) tried chlorophyll-mediated PDI to inhibit *Ichthyophthirius mulftifiliis* (a protozoan parasite which causes the so-called white spot disease), which has become a promising, inexpensive and practical new treatment for controlling ectoparasites in fish.

Liu et al. (2016) analyzed the effects of curcumin-mediated PDI on the shelf life and quality of Pacific oyster stored at 51℃. After treatment, there was no significant change in texture, and the shelf life of oysters was extended from 8 days to 12 days. The treated oysters also showed the lowest degree of oxidation, as well as less decomposition of nutrients and lower level of spoilage microorganism metabolism at the end of the shelf life.

### *Fresh fruits and vegetables*

An adequate intake of fresh fruits and vegetables is an important part of a healthy diet. Consumption of raw vegetables has increased in recent years as people have begun to cultivate healthy eating habits. However, raw fruits and vegetables are actually a major vector of human disease

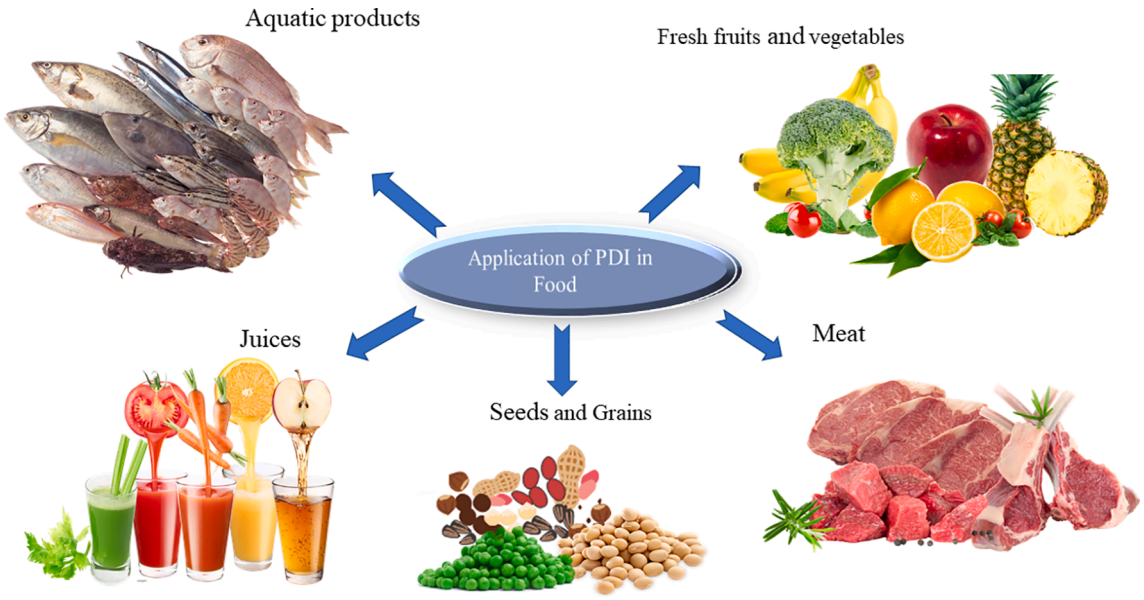


**Fig. 4.** Potential applications of PDI in different food substrates.

and the second leading cause of foodborne illness (Pui et al., 2011). Clearly, new processing technologies need to be developed to eliminate foodborne pathogens without compromising the sensory properties and nutritional value of the fresh products. Luksiene and Paskeviciute (2011) soaked strawberries in Na-Chl for 5 min and irradiated them at 400 nm for 20 min, it turned out that the naturally occurring yeasts and microorganisms were inhibited by 86% and 97% respectively. After PDI treatment, the shelf life of strawberries was extended by 2 days and the total antioxidant activity was increased by 19%. No effects on phenols, anthocyanins or surface color were detected. Yu et al. (2021) evaluated the curcumin based PDI applied to fresh-cut potato slices, with 30 μM curcumin solution and exposed to 420 nm LED, the *Escherichia coli* and *Staphylococcus aureus* reduced 2.43 log and 3.18 log respectively. Additionally, PDI decreased the activity of polyphenol oxidase and peroxidase, increased the activity of phenylalanine ammonialyase, thereby increasing the total antioxidant capacity.

*Vegetable and fruit juices*

Cho and Ha (2020) discovered that the cell counts of *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* decreased by 6.77, 2.74 and 6.43 log respectively after 15 min treatment of xenon light (200–1500 nm), with no sublethal damage found. The color, flavor, pH and lycopene content of treated tomato juice samples were evaluated, and there were no significant changes. However, studies have shown that photosensitive nutrients such as vitamin C can be severely degraded during UV treatment (Bhat, 2016). Usaga et al. (2017) found that ascorbic acid, benzoates and sorbates increased the UV absorption coefficient of juices and negatively affected their UV properties. The increase of ascorbic acid concentration reduced the inactivation rate of *E. coli*. UV irradiation also causes degradation of ascorbic acid, sulfur dioxide and potassium sorbate. Therefore, additives that may adversely affect or degrade UV efficiency should be added after UV treatment.

*Meat*

Tortik et al. (2014) inoculated chicken legs with *S. aureus* and exposed them to 435 nm LED light in the presence of curcumin, resulting in an average reduction of 1.5 log of bacteria on the chicken surface. Kim and Yuk (2017) investigated the condition of three strains of *S. enteritidis* on cooked chicken after being illuminated by 405 nm LED at 4, 10 and 20 °C. About 1 log of bactericidal effect was observed at 4 ℃, and the bactericidal effect at 10 ℃ and 20 ℃ is 1 to 2 log. Guffey et al. (2016) reported that *E. coli* O157:H7 on hot dogs was completely inactivated after irradiation of 405 nm LED with an irradiance of 83.3 mW/cm$^2$. Josewin et al. (2018) found that the combination of 460 nm LEDs and riboflavin may minimize the risk of infection of smoked salmon with *L. monocytogenes* during storage.

*Seeds and grains*

Lukšienė et al. (2007) proved that 5-ALA is a growth stimulant for wheat seedlings and roots without compromising germination vigor and viability of seeds. It also induces an increase in chlorophyll content, increases the rate of photosynthesis and the activity of antioxidant enzymes, which can promote cell detoxification of ROS. Its water solubility, stable shelf life, non-bleaching properties and simplicity of production make it potential to be used as a food additive. Moreover, because it is colorless and tasteless, spraying 5-ALA on the food will not change the sensory characteristics, nor will it damage the food ingredients.

Purify small substrates by using optical techniques is particularly difficult. This is because the geometry of the seeds provide space for microbes to hide and prevent light from being evenly distributed on the surface of the seeds. Glueck et al. (2017) studied the sterilization efficiency of curcumin at 435 nm on rotating spherical substrates (ungerminated fenugreek seeds and mung beans). During the process, the substrates needed to be shaken constantly to rotate them to promote the uniformity of the light distribution, and the final bacteria concentration was reduced by 2 to 4 Log.

*Edible biopolymer coatings and films*

The combination of PSs and traditional packaging materials to prepare composite packaging films with bactericidal activity has received widespread attention. It is a completely safe and environmentally friendly method.

Coatings and films usually use edible biopolymers, which consist of polysaccharides, proteins, and lipids (Li et al., 2020). Polysaccharide-based biopolymers include starchy, cellulose derivatives, and chitosan, etc. Protein-based biopolymers include zein, gelatin, and whey proteins, etc. Lipid-based biopolymers include beeswax, ancient wax, stearic acid

and palmitic acid, etc. Riboflavin was mixed with chitosan to make packaging films (CS-RB) (Su et al., 2021). The composite films exhibited good UV resistance, and the 1% CS-RB composite film under LED light (6.84 J/cm$^2$) can led to a decrease of at least 8 log *V. parahaemolyticus* cells, while *L. monocytogenes* and *S. baltica* exhibited 2.5 log reduction. When applied 1% CS-RB composite film to salmon sample with 5 log of the initial bacterial population, it was found that after irradiation treatment of 4 h, the cells of *L. monocytogenes* and *S. baltica* on salmon were reduced about 3 log, and the cells of *V. parahaemolyticus* were completely inactivated. The results suggested that the application of PS in the film matrix can also retain its strong antibacterial activity.

## Conclusion

Green and healthy foods with no chemical preservative have been popular all over the world, and more people prefer natural and safe foods. Many natural PSs have good bacteriostasis effects, which just caters to this trend. For this reason, as a truly effective technology against food-borne bacteria, yeast and other microorganisms, PDI is worthy of wider application on the basis of current medical antibacterial functions. The various applications proposed in recent years and the research progresses in this field indicate that PDI is a very promising sterilization method. In addition, PDI can also combine with the hurdle technology to achieve a stronger antimicrobial effect.

It is promising that with the development of PDI, it will get more attention and good practical applications in the future. Some PSs are food additives and can be added into packaging materials to directly cover on food surface, thereby playing a bacteriostatic effect. However, considering the complexity of food matrix and its microbial ecology, as well as the shelf life and food quality after photosensitive treatment, the application of PDI in food matrix still needs further research.

## Declaration of Competing Interest

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

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