Non-thermal techniques and the “hurdle” approach: how is food technology evolving?

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Abstract

Background: Food technology has played a crucial role since the beginning of human civilization. Throughout the centuries, the evolution of food processing has led to an increase of food safety and quality, enhancing the overall quality of human life. Lately, academic research and industries have gained awareness about the impact of conventional preservation technologies like heat sterilization and chemical preservatives on environment and economy, besides the detrimental effects on the organoleptic and nutritional quality of foods. This consciousness oriented the efforts towards more sustainable techniques, paving the way to a new “green era” of food technology.

Scope and approach: This work explores seven non-thermal technologies, describing their theoretical principles, mechanism of action, effect on microorganisms, advantages, and limitations. Besides, the concept of hurdle technology to overcome the criticisms related to single processing techniques is highlighted.

Key findings and conclusions: Non-thermal technologies have the potential to substitute conventional techniques for microbial inactivation, improving the safety and quality of food. The efficiency of each technique strongly relies on the process parameters (treatment intensity; exposure time), equipment (geometry; conformation), product (physical state;

composition; viscosity; geometry), and microorganism characteristics (strain; concentration; growth phase; resistance mechanisms). In this sense, the hurdle approach allows to overcome the limitations related to the single technologies, broadening their efficiency and application range, and minimising their impact on food quality. Further studies are recommended to better understand the mechanisms of mutual interaction among these techniques when combined together in specific conditions, in view of their scaling-up for commercial applications.

Keywords: Non-thermal techniques; food preservation; hurdle; food decontamination; green technology

1. Introduction

Food safety has always constituted a matter of public interest in both developing and developed countries. Indeed, foodborne diseases are among the major concerns affecting public health (Luksiene, 2021). Foodborne diseases are mainly caused by the growth, spread, and metabolic activity of microorganisms such as bacteria, yeasts, moulds, and protozoa in food. Besides, the spread of spoilage microorganisms can compromise the quality and thus the shelf life of the product, generating relevant economical damage. Most food products are highly exposed to the risk of cross-contamination during the several processing steps from harvest to consumption. Therefore, the control of unintentional microbial contamination is compulsory in the food processing industries to guarantee the safety and quality of the products (Sakudo, 2017).

In ancient times, food preservation techniques mainly consisted in the control of parameters such as water activity (a_w), pH, and environmental conditions. Later, plant sanitisation with chemical disinfectants, high-temperature treatments, and chemical preservatives gained wide recognition due to their significant efficiency in food decontamination (Kaavya et al., 2021). These technologies, and particularly thermal treatments, represent a fundamental pillar of the food processing industry. Over years and years, these methods have been

extensively applied to ensure proper decontamination of foods, so much that today they are regarded as the “conventional” strategies to guarantee food safety (Aaliya et al., 2021). Despite the obvious benefits, the use of conventional decontamination technologies based on thermal treatment hides not negligible criticisms. Firstly, high temperatures can hinder food quality by lowering its nutritional values and freshness, affecting the organoleptic properties, and generating chemical derivatives harmful for consumers’ health (Rifna, Singh, Chakraborty, & Dwivedi, 2019). Besides, these technologies are highly energy consuming. This energy mainly derives from the combustion of fossil fuels, and this generates a remarkable ecologic and economic impact on human society and the environment (Chakka, Sriraksha, & Ravishankar, 2021).

The drawbacks related to the conventional decontamination processes prompted the development and adoption of novel non-thermal technologies, having the potential to improve product quality and safety with reduced water and energy consumption (Fengying, Min, Kai, & Arun, 2020). These physical techniques are effective for microbial decontamination, non-toxic, environment-friendly, and residue-free. However, they could present setbacks related to investments in equipment, maintenance costs, and required skills, which can hinder their spread in food industry (Priyadarshini, Rajauria, O’Donnell, & Tiwari, 2019). Moreover, the diffusion of some of these techniques is limited by the low consumer acceptability and the resistance of regulatory authorities (Khan, Tango, Miskeen, Lee, & Oh, 2017).

This work aims to give a synthetic overview about the trend techniques which are leading the food industry towards green technology and sustainability criteria, reducing energy consumption, waste generation, and footprints on the environment. Seven novel non-thermal preservation technologies will be highlighted, describing their theoretical principles, mechanism of action, and effect on microorganisms. The advantages and limitations of each technology will be described in each paragraph (Table 1). Besides, the concept of hurdle

technology, namely the combination of two or more preservation technologies at mild levels to overcome the criticisms related to single processing techniques, will be explored. A specific focus will be dedicated to the use of these techniques within “hurdle technology” approaches, and practical examples will be provided (Table 2).

2. Hurdle technologies

In recent years, many novel non-thermal technologies have gradually emerged across the food sector as promising substitutes of conventional treatments such as pasteurization or sterilization. These technologies are usually effective, non-harmful, environment friendly, and residue-free. Nevertheless, the “one-technology-per-treatment” strategy could face specific criticisms. On the one hand, some decontamination techniques could be not enough to guarantee the safety of a product when applied alone. On the other hand, single processing technologies with high dosage or exposure time could negatively affect the organoleptic and nutritional attributes of food, reducing consumer acceptability (Khan et al., 2017). These evident drawbacks prompted the adoption of a novel strategy, consisting in the sequential (i.e., one technology after another) or simultaneous application of different techniques at mild levels to improve the safety, nutritional and sensory qualities of the products, using lower individual treatment intensities (Liao et al., 2020). This approach is commonly known as “hurdle technology”.

The hurdle concept can be defined as “the combination of existing and novel preservation techniques to establish a series of preservative factors (hurdles) that any microorganism present should not be able to overcome” (Oh, Khan, & Tango, 2019). The primary scope of hurdle technology is to obstruct the growth and stability of targeted microorganisms by subjecting them to alternate or concurrent physical, chemical, and environmental stresses. This task is achieved by three main mechanisms: hindrance of homeostasis condition; metabolic exhaustion; deprivation of the stress reaction mechanism (Pal et al., 2017). These mechanisms are strongly related to each other. Firstly, homeostasis represents the natural

tendency of the organisms to maintain an internal status of uniformity and stability (Tsironi, Houhoula, & Taoukis, 2020). The most feasible way to disturb the homeostasis of microorganisms is by applying continuous and multiple hurdles. When this happens, microbes try to restore their homeostasis, and the loss of energy aids to metabolic exhaustion. This process induces the so-called “auto-sterilization” phenomenon (Pal et al., 2017). Moreover, the concurrence of different stressors impedes the expression of genes related to the synthesis of stress shock proteins, commonly produced by some microorganisms to face stress conditions and starvation.

The aforementioned complex of antimicrobial mechanisms induced by the co-action of different hurdles (i.e., physical techniques such as thermal treatments, freezing, irradiation, pressure etc., and chemicals, such as synthetic or natural preservatives) may lead to either additive or even synergistic effects. In the first scenario, the combination of two or more technologies results in an antimicrobial effect which is simply the sum of the effects observed for the single techniques. In the second case, representing the most attractive advantage of the hurdle approach, the interaction among technologies leads to antimicrobial effects which significantly exceed the sum of the single techniques. A synergistic effect can be achieved if hurdles applied on food can hit, at the same time, different targets (e.g., DNA, cell membrane, enzyme systems, pH and a_w susceptibility etc.) within the microbial cells, and thus disturb the homeostasis of the microorganisms under several aspects. If so, the recovery of the homeostasis state, as well as the activation of stress shock proteins, becomes impossible. Therefore, the simultaneous or sequential application of different preservative factors at mild intensities, intelligently selected according to the product characteristics, could be far more effective than the single preservative factors at high intensity for the preservation of a specific food product, without affecting its properties (Leistner, 2007). In practical terms, previous studies showed that mutual synergy or not-synergy among technologies not only relies on their specific combination, but also on a

complex of other parameters which include the intrinsic resistance of each target microorganism, the treated products, and others (e.g., environmental temperature; set-up of the plant etc.). Hence, each case study strictly requires a case-by-case investigation (Aalyia et al., 2021).

Hurdle technology is currently employed in various food processing sectors with the chief purposes to ensure the microbial safety and to prolong the shelf life of food matrices. In the last years, this strategy has been extended to several products including meat and derivatives, fruits and vegetables, milk and dairies, and starch products. Among multiple combinations of technologies that have been tested to date, some promising examples of hurdle technology involving the application of the most relevant non-thermal techniques are depicted in the following sections.

3. Non-thermal techniques: general description and "hurdle" approach

3.1. Ozone

3.1.1. Principles

Ozone (O_3) is a triatomic allotrope of oxygen. Discovered by Schoembein in 1840 and regarded as one of the most effective and sustainable biocidal agents, ozone treatment is commonly employed as sanitising technology in a broad range of processes to ensure and enhance the safety and quality of many food products (Niveditha et al., 2021). Ozone, as a powerful oxidant agent, is highly effective against several types of microorganisms including viruses, algae, bacteria, and fungi, and ably counteracts the presence of mycotoxin contaminants within foods (Deng et al., 2020). As ozone can spontaneously decompose into oxygen, leaving no hazardous residues on foods, its usage as a decontaminating agent has been approved as safe by the US Food and Drug Administration (FDA) since 2001. Progressively, ozone application either in aqueous or gaseous forms has been promisingly extended to the stages of post-harvest, processing, and storage of foods, including fruits, vegetables, meat, and poultry at levels set by central agencies (Pandiselvam et al., 2020).

The production of ozone requires a generator, an oxygen source, dust filters, a contact unit, and a destruct unit (Pandiselvam et al., 2018). Ozone production involves two subsequent processes, namely the splitting, also known as photodissociation or photolysis, and further re-combination of oxygen molecules. When a certain amount of energy is provided to diatomic oxygen, which splits into free radicals, the resultant oxygen atoms undergo numerous collisions with each other, and with stable O_2. These collisions release a combination of O_2 and O with an excess of energy, whose dissipation leaves intact and stable ozone molecules (Niveditha et al., 2021). This reaction is called a "three-body collision" and can be triggered by means of two key approaches: UV-light radiation and corona discharge. In the corona discharge method, the most commonly applied for ozone generation, pure oxygen or a gas mixture containing oxygen (e.g., air) passes through the gap between two electrodes supplied with a high-energy electrical field, which enables diatomic oxygen to split into free radicals. The two electrodes are separated by a dielectric material like glass. Another technique widely applied to produce ozone is the dielectric barrier discharge (DBD) method, which gained popularity due to the reduced power consumption and higher conversion efficiency. This technology employs high-voltage alternating current between two electrodes separated by an insulating dielectric barrier. Moreover, ozone can also be generated directly in water by applying electrolytic ozone generators. In these conditions, an electrical discharge is used to split the water molecules into H_2 and O_2, which is furtherly converted into O and then O_3 (Kaavya et al., 2021).

3.1.2. Antimicrobial mechanism

Concerning the antimicrobial activity of ozone, this gas aids in microbial destruction by interacting with the cellular components, which are gradually led to oxidation. Firstly, ozone interacts with the polyunsaturated fatty acids (PUFAs) of the cell envelope, converting them into acid peroxides (Khan et al., 2017). This process causes the progressive disintegration of the cell membrane, resulting in the leakage of cell contents, and eventually cellular lysis.

Secondly, ozone oxidizes amino acids, peptides, proteins, and enzymes, mainly attacking the sulfhydryl groups in these molecules. Accordingly, as the gas enters the microbial cells, all essential components, proteins, RNA, DNA, and enzymes undergo complete oxidation and result in the destruction of cells (Brodowska et al., 2017). Besides, the ozone decay in water leads to the formation of free radicals (ROS), which are also responsible for the antimicrobial activity (Deng et al., 2019) (Fig. 1).

Ozone has been discovered to actively degrade mycotoxins. In this case, the chemical pathway of degradation relates to the chemical structure of mycotoxin (Temba et al., 2016).

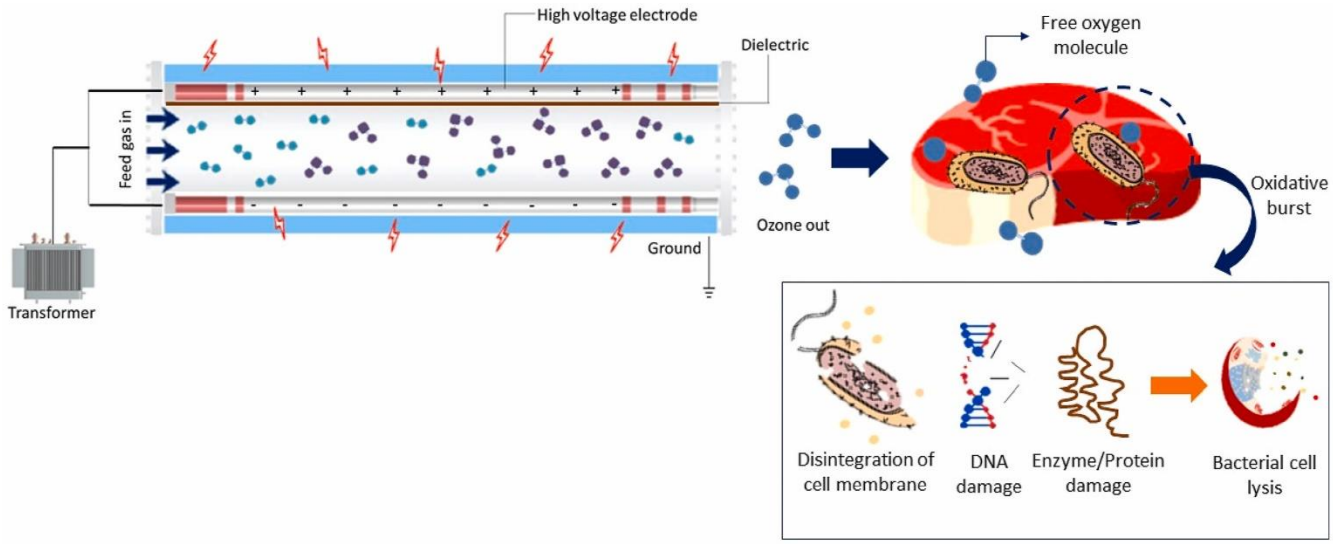


Fig. 1. Schematic representation of production and application of ozone on food products, with relative effect on bacteria (Kaavya et al., 2021).

3.1.3. Factors affecting processing efficacy

The key factors that affect the sterilizing efficacy of ozone are the exposure time, ozone concentration, treatment mode (i.e., aqueous or gaseous), inlet gas composition, and the intrinsic microbial sensitivity (Bahrami, Baboli, Schimmel, Jafari, & Williams, 2020; Rifna et al., 2019). A general rule, which was demonstrated in many studies, resides in the positive relation between the sterilizing activity of ozone and the exposure time/gas concentration (Brodowska, Śmigielski, Nowak, Czyżowska & Otlewska, 2015). Conversely, the influence of the treatment mode is less predictable, since different researches comparing the

effectiveness of aqueous and gaseous ozone for bacterial inactivation reported contrasting results. As an example, Pascual, Llorca, & Canut (2007) reported the higher efficiency of aqueous ozone with respect to the gaseous form. However, other studies showed that the decomposition rate of ozone in water is higher than in its gaseous form, inducing a shorter residual effect (Oner & Demirci 2016). Moreover, such variability of results is probably due to the diversity of products and the complex of conditions considered for each study. For this reason, ozone efficiency should be assessed concerning the target microorganisms, the product, and the processing conditions (Miller et al., 2013).

Many studies highlighted that spores are far more resilient to ozone than vegetative cells (Ramaswamy, Ahn, Balasubramaniam, Saona, & Yousef, 2019). As well, bacteria are more vulnerable than yeasts and fungi, while the greater or lower resistance of gram-positive and gram-negative bacteria is still debated (Deng et al., 2019).

Another crucial condition that determines the effectiveness of ozone treatment is the environment of application. The presence of organic material such as proteins and fat in the medium drastically decreases the effectiveness of ozone. As an example, Munhõs et al. (2019) showed a higher inactivation rate of *Pseudomonas aeruginosa* in ozone-treated skimmed milk with respect to whole milk. The same result was reported by Couto et al. (2016) for *Staphylococcus aureus* in the same products. This effect was due to the higher concentration of lipids in whole milk, which can directly react with ozone, affecting its efficiency. As well, specific food surfaces and structures could substantially change the antimicrobial efficacy of the treatment. Generally, smooth surfaces allow more precise contact between ozone and the microorganism, whereas fibrous and/or irregular structures may cause the possibility for pathogens to evade the action of ozone, as showed in previous studies for fish and vegetables (Luiz et al., 2017; Xu & Wu, 2014). Also, the stability of ozone increases with a lower pH of the medium, and decreases with the increase of temperature (Pandiselvam et al., 2020).

3.1.4. Applications in the food sector

Due to its strong oxidizing power, ozone possesses a broad range of activities, which result in a countless number of prospective applications such as pathogens disinfection, reduction of mycotoxins (Rifna et al., 2019; Sert & Mercan 2021), starch modification (Pandiselvam et al., 2018), storage of fresh products (Feliziani, Romanazzi, & Smilanick, 2014), sanitation of processing equipment and packaging films (Almeida & Gibson, 2016; Bigi et al., 2021), and pesticide removal (Pandiselvam et al., 2020).

Disinfection of food products by aqueous and gaseous ozone treatment has been extensively studied and reviewed. Many studies tested the efficacy of this treatment for the decontamination of vegetables and fruits such as onions, lettuce, tomatoes, strawberries, and peaches (Chakka et al., 2021). Some works mainly focused on the capacity of ozone to inactivate specific foodborne pathogens such as *Salmonella enterica* (Kaavya et al., 2021) and *Escherichia coli* (Niveditha et al., 2021), while others took into account specific food categories such as milk, dairy products, and meat (Khanashyam et al., 2022). The in-depth interest and efforts that researchers and industries are currently focusing on this technology are symptomatic of its potential as a prospective substitute to conventional sanitizing techniques. In particular, the application of this technique has been recently extended to the meat and poultry sector (Gimènez, Graiver, Giannuzzi & Zaritzky, 2021; Kalchayanand, Worlie, & Wheeler, 2019), cream milk (Perna, Gambacorta, Simonetti, Grassi & Scopa, 2022), salmon (Qian, Zhang, Liu, Ertbjerg & Yang, 2022), soft cheese ripening (Tabla & Roa, 2022), and vegetables such as onions and grape tomatoes (Shelake et al., 2022; Wang, Fang & Guntler, 2022) with promising results. In particular, ozone treatment showed its potential as decontaminating process both as a short-term treatment (e.g., spray chill intervention before storage) and as a long-term treatment (e.g., low-dose gaseous ozone during refrigerated storage or ripening). As well, the efficacy of gaseous ozone was found to be lowered and enhanced in dry and humidified environments, respectively.

Along with the antimicrobial efficacy of ozone, a comprehensive overview about the impact of this gas on the intrinsic features of the product was also provided for the different food categories. Some of these outcomes will be highlighted in the following paragraph.

3.1.5. Advantages and limitations

Overall, the oxidising performances and the global sustainability of ozone make it a valuable potential alternative to conventional agents such as chlorine for food-related operations (e.g., cleaning and sanitisation) (Chakka et al., 2021). With about 50% higher oxidizing potential than chlorine, ozone efficiently inactivates fungi, viruses, bacteria, plus reducing the impact of mycotoxins and contaminants such as pesticides. This technology is suitable for both solid and liquid foods and can be applied both before (e.g., dipping/spraying of the product in aqueous ozone) and during storage (gaseous ozone). It requires shorter contact time for disinfection when compared to other techniques (Kaavya et al., 2021). An excessive amount of ozone rapidly auto-decomposes to O_2, leaving no chemical residues in food (Dilmaçünal & Kuleaşan, 2018). The treatment requires no thermal energy, and so it is suitable for heat-sensitive products and reduces the need for input energy. Not requiring storage or transportation, the treatment has relatively low running costs. Moreover, most materials applied in food industry are resistant to ozone at concentrations of 1 – 3 ppm. As an example, plastics such as PTFE (Teflon), PVDF (Kynar), and PVC (rigid and flexible) exhibited good resistance to corrosion during ozone exposure (Brodowska et al., 2017). Thus, it comprehensively constitutes an environmentally sustainable and commercially feasible technology.

Despite the relevant benefits, ozone at low concentrations or short exposures could be insufficient to remarkably affect the spread of microorganisms, mycotoxins, and spores. On the other hand, higher ozone concentration can lead to detrimental effects on sensory and nutritional attributes of food products, with notable differences related to the food category. Noticeable aroma loss and bleaching were observed for vegetables such as tomato and

carrot (Bridges, Rane, & Wu, 2018). Brodowska et al. (2015) reported that gaseous ozone treatment drastically decreased the concentration of essential oil and polyphenols in juniper berry. Sert & Mercan (2021) demonstrated that an excessive amount of ozone may cause the partial denaturation of proteins in milk and whey proteins, decreasing the overall firmness and consistency. The ozone treatment was found to induce a partial discolouration for both milk-based products and meat (Mohammadi et al.; 2017; Muhlisin et al., 2016). In the first case, this effect was attributed to the reduction of β-carotene content in milk, while in the second case the oxidation of myoglobin and oxymyoglobin was observed.

Several degradation products and/or by-products can be formed during ozone treatment, which could have toxicological properties (Temba et al., 2016). Moreover, the antimicrobial effects of this technology are limited to the surface of the product (for solid foods) (Pandiselvam, Kaavya et al., 2020).

From a technological point of view, ozone cannot be stored, and thus its generation needs to be on-site: this may increase the costs and difficulties of installation and maintenance (Pandiselvam et al., 2020). It also represents a toxic gas and thus the safety of operators and the rate of surfaces corrosion of equipment should be carefully considered (Oner & Demirci, 2016).

3.1.6. Ozone-based hurdle approach

Lately, the opportunity to combine ozone with other techniques to enhance the efficiency and to overcome the criticisms has gained increasing attention (Kaavya et al., 2021).

Different researches focused on the prospective synergistic or additive effect of UV-C light irradiation with ozone in aqueous and gaseous forms. This combination was tested for the disinfection of *E. coli* in flowing water (Hernàndez-Arias et al., 2019). The water was sequentially subjected to ozone and then to UV-C irradiation in cylindrical and rectangular reactors. Authors reported an inactivation efficiency of 99.32% for *E. coli*, with a treatment time of 180 s. Concerning the food products, Yang, Kalchayanand, Belk, & Wheeler (2019)

306 combined the effect of UV light with low-level oxidization to control the growth of pathogens
307 on fresh beef surface. In this case, the two technologies were applied not sequentially (as
308 described for the previous case), but simultaneously. This treatment (15-75 s), also known
309 as *photo-hydro ionization* process, was effective for the control of *Salmonella*, without
310 producing lipid oxidation or impairing the colour. Recently, Dogu-Baykut & Gunes (2022)
311 extended this combination of technologies for the decontamination of spices. Ozone and
312 UV-C treatments were applied alone, sequentially, or simultaneously to black pepper seed
313 to reduce the total aerobic mesophilic bacteria and *E. coli*. Interestingly, the combined
314 treatments did not show any additive or synergistic effects on the mesophilic population (with
315 ozone treatment slightly less effective than UV-C, and UV-C equally effective to combined
316 treatments), while they caused an additive effect on inactivation of *E. coli* in black pepper
317 seeds. The successive and simultaneous treatments caused 1.7- and 1.4-log reductions in
318 the *E. coli* population against almost 0.8-log reductions given by the single technologies,
319 respectively.
320 In all these studies, authors concluded that the direct impairment of proteins and enzymes
321 induced by UV-C photons was boosted by the oxidation of cell components caused by ROS
322 release from ozone breakdown. Besides, this ionization damaged the bacterial DNA/RNA,
323 hindering their replication and transcription process which subsequently inhibited the
324 multiplication of the cells.
325 The ozone/UV-C combination was furtherly integrated either with chemical treatments like
326 H_2O_2 for lemons decontamination (Hasani et al., 2019) or with physical technologies such
327 as infrared irradiation (IR) for the treatment of spices (El Darra et al., 2021;). In the first case,
328 the optimized oxidation process, obtained by spraying 1.88% v/v solution of H_2O_2 followed
329 by 2 g/h ozone exposure and 3.26 mW/cm^2 UV-C irradiation (applied simultaneously),
330 resulted in over 5 log-reductions for both *Salmonella* and *Listeria*, without altering the
331 sensory qualities of lemons. In the second case, onion flakes and black pepper were

inoculated with *E. coli* and exposed to each treatment alone and in combination with ozone sequentially followed by UV/IR, and UV/IR combined sequentially followed by ozone. Some differences between the two matrices were observed, since the efficacy of the treatments was generally higher for onion flakes that black pepper. Overall, the sequential treatments of ozone followed by simultaneous UV/IR gave improved results (higher than their additive effect) with respect to individual ones, with 99.99% of *E. coli* inactivation, and a shorter exposure duration for both ozone and UV/IR. Interestingly, the same authors had demonstrated in a previous study conducted on dried vegetables that IR/UV treatment followed by ozone was more effective than ozone followed by IR/UV (Watson, Kamble, Shanks, Khan & El Darra; 2020).

The biocidal efficacy of aqueous ozone was also found to surge when combined with physical treatments like high frequency ultrasounds for the sanitization of fresh vegetables (Mustapha et al., 2020). In particular, the authors found that the simultaneous application of multimode 20/40 kHz sonication and aqueous ozone washing (*sonozonation*) not only preserved the bioactive compounds and the physicochemical attributes of cherry tomatoes, but also caused a >3 log CFU/g reduction on their microbial population after 21 days of refrigerated storage, resulting in a synergistic effect of the two technologies. This effect could be due to the weakened cell walls as a result of cavitation and the enhanced penetration of ozone into bacterial cells after stimulating the mechanisms that aid the elimination of microbial cells from the surface of the produce.

García-Mateos et al. (2019) evaluated the combined effect of ozone and high-pressure processing (HPP) with respect to the individual effects of the two technologies over the microbial population of pitaya juice. Specifically, the juice was inoculated with either *Listeria innocua* or *Saccharomyces cerevisiae* and treated with ozone. Then, ozonated and not-ozonated juice samples were vacuum-packed in polyethylene bags and pressurized. Single HPP treatment (300 MPa/5 min) induced 6.89 log-reduction/mL and 0.89 log-reduction/mL

for *S. cerevisiae* and *L. innocua* populations, while single ozonation treatment (9.6 min) induced 0.47 log-reduction/mL and 2.51 log-reduction/mL for the same populations, respectively. However, exposure to ozone (7 min) followed by 316 MPa/5 min reduced the population of *L. innocua* of 5.1 log10 CFU/mL. Likewise, these combined treatments kept native microbiota of the juice at non-detectable level. These results confirmed the synergistic effect of ozone-HPP for the sanitization of fruit juices. Similar processing conditions (single technologies and in combination) were extended to solid food (i.e., catfish fillets) by Ling et al. (2022). In this case, the combined treatment was applied by soaking the fish fillets in 13.28 mg/L aqueous ozone (10 min), followed by packing in cooking bags and 200 MPa/10 min treatment. Again, the combination of aqueous ozone and ultra-high-pressure processing synergistically affected the microbial quality of the product, increasing its shelf life by at least 3 days compared to the control. Moreover, the combined processing seemed to reduce the lipid oxidation rate of the catfish during storage with respect to single treatments.

3.2. Atmospheric Cold Plasma (ACP)

3.2.1. Principles

Plasma treatment is an innovative biocidal technology which is becoming more and more popular in the food processing sector for decontaminating foods, plants, and packaging. Plasma represents the fourth state of matter after solid, liquid, and gas (Sakudo, 2017). It is mainly constituted by partially or fully ionized gases, involving the coexistence of positively and negatively charged ions, free radicals, excited molecules, UV photons, and other reactive species. It can be distinguished into quasi-equilibrium (thermal) plasma, produced at high pressure and temperature, and not in equilibrium (non-thermal or atmospheric cold) plasma based on its thermodynamic equilibrium (Pankaj, Wan, & Keener, 2018). Atmospheric cold plasma (ACP) is generated through different electric discharge methods such as dielectric barrier discharge (DBD), corona discharge (CD), radio frequency low discharge (RFLD), micro hollow cathode discharge (MHCD), plasma needle (PN) or

atmospheric pressure plasma jet (Deng et al., 2020). The gases currently subjected to ionization are air, nitrogen (N_2), oxygen (O_2) or specific mixtures of noble gases such as argon (Ar), helium (He) or neon (Ne). The ACP initiation leads to the formation of active species like OH•, O_3, H_2O_2, NO, NO_2, N_2O, and other reactive species (RS), widely known as reactive oxygen species (ROS) and nitrogen active species (RNS) (Deng et al., 2019). Depending on how the food is placed with respect to the generated plasma, 3 design systems including remote treatment, direct treatment, and electrode contact system can be identified. In particular, both direct and indirect methods can be used for any shape of food products (Kaavia et al., 2021).

3.2.2. Antimicrobial mechanism

ACP possesses an enhanced diffusion coefficient and a wide range of activity against pathogens and spoilage microorganisms, biofilms, and spores (Bahrami et al., 2020). ACP mechanisms for triggering microbial injury or death include: (i) ROS and RNS accumulation on the cell surface, which provokes the oxidation of amino acids and lipids and the disruption of the cell membrane (i.e., the "etching" effect of plasma) (Kaavia et al., 2021); (ii) UV photons intrinsic photo-desorption, which damage membranes and interior cellular components (Ansari et al., 2022); (iii) direct gene damage, which hinders the DNA replications and eventually leads to cell death (Misra & Jo, 2017). Other studies suggested that the intracellular accumulation of charged particles may induce apoptosis, electrostatic disruption, and electroporation (Ekezie et al., 2017).

3.2.3. Factors affecting processing efficacy

The factors influencing the antimicrobial efficacy of ACP may be categorized in 3 classes: processing, microbiological, and product/environmental factors (Feizollahi, Misra & Roopesh, 2020).

Processing factors include voltage, frequency, type and concentration of gas, treatment time, and exposure mode. Different studies reported that an increase in voltage, frequency,

and current supply led to significantly higher microbial inactivation rates. In some cases, this improved efficacy of the treatment was related to the higher concentration of ozone generated at high voltages. Nevertheless, the effect of power supply is still uncertain (Wang, Zhuang, et al. 2018). As well, the type of gas is critical, since the plasma chemistry is dependent on the properties of the gas medium (Deng et al., 2020).

Treatment time is another condition that can deeply influence the efficacy of ACP. Extended ACP treatments generate higher concentrations of reactive species, a reduction in pH, and an increased mortality of microorganisms, especially those residing in the inner layers of biofilms. Nevertheless, increased treatment times may result not relevant (Lis et al.;2018; Wang, Zhuang, et al., 2018).

The exposure mode is also crucial. Direct plasma exposure is generally considered more effective for microbial inactivation, due to the short half-life of reactive species which may decay to their normal state before reaching the sample in the cases of indirect or remote applications (Zhang et al. 2017). Instead, indirect modes can be useful to guarantee uniform three-dimensional exposure to all sides of large-sized food products.

A critical role is played by the target microorganisms. Bacterial type, strain, mode of existence (vegetative cells or spores), and concentration are the main microbiological factors related to the inactivation efficacy of ACP (Ekezie et al. 2017). Gram-positive bacteria are usually less sensitive than Gram-negative bacteria, owing to the outer features of their lipopolysaccharide membranes and peptidoglycan thickness (Lunov et al., 2016). Moreover, sporulated bacteria are more resistant than vegetative cells, due to the lower penetration capacity of reactive species (Feizollahi et al., 2020).

The ACP efficacy is strongly related to the product state, composition, and structural characteristics. For instance, solid and liquid food matrices interact differently with the reactive species of plasma, as most liquids can vaporize during treatment and partake in subsequent reactions. However, the plasma must be dipped inside the liquid to ensure

436 thorough contact (not necessary in solid products, for which the treatment is strictly
437 superficial). This makes the processing setup for liquid decontamination more complex
438 (Ansari et al., 2022). The composition, the surface topology, and the water content of the
439 product are other important factors. In particular, on rough surfaces, bacteria may attach as
440 multilayers, potentially hindering plasma diffusion. This concept was well explained by
441 Ziuzina et al. (2014), who demonstrated the better inactivation of bacteria on tomato surface
442 in comparison to strawberries. This was due to the smooth surface of the tomato, in contrast
443 with the presence of pores on strawberries.
444 Lastly, the water content, as well as the environmental humidity, are positively correlated
445 with the efficacy of plasma treatment. In both cases, the presence of water increases the
446 concentration of hydroxyl radicals, leading to higher oxidation rates.
447 *3.2.4. Applications in the food sector*
448 ACP represents a versatile technology, whose application has been investigated in several
449 sectors of the food industry, including the decontamination of foods, production plants, and
450 packaging.
451 ACP can be either applied directly on the food product (i.e., open-air treatment) or as in-
452 package decontamination technology (Fig. 2). In-package ACP treatment has two relevant
453 benefits. Firstly, the presence of packaging allows to avoid the loss of reactive species due
454 to diffusion to the open environment, although they have short lives. Besides, ACP can be
455 used as the final decontamination step after packaging, minimizing the chances of post-
456 packaging re-contamination (Feizollahi et al., 2020).
457 Amongst food products, in-package ACP was recently tested to ensure the safety and
458 extend the shelf life of vegetables such as romaine lettuce (Min et al., 2016) and fresh-cut
459 carrots (Kumar Mahnot, Siyu, Wan, Keener, & Misra, 2020) as a green alternative to chlorine
460 treatment for its disinfectant properties against *E. coli* O157:H7, *Salmonella*, and *Listeria*

monocytogenes. All these studies reported minimal changes in the pH, colour, texture, and nutritional contents of the products induced by ACP.

The application of ACP has been recently extended to animal products such as avian eggs (Gavahian, Peng, & Chu, 2019b), and poultry meat, with drastic differences related to the shape of the treated portions (e.g., chicken thighs, breasts and skin) (Moutiq et al., 2020). In cured pork meat processing, direct DBD treatment has been demonstrated to be a prospective replacement for direct nitrite (antimicrobial agent) addition (Jung et al. 2017).

ACP technology was also applied to dairy and cereal products to reduce the impact of mycotoxin-producing fungi such as *Aspergillus*, *Alternaria*, and *Penicillium* (Gavahian & Cullen, 2019a; Gupta et al., 2020). In particular, a brand-new study conducted by Guo et al. (2023) evaluated and confirmed the possibility to apply ACP for the decontamination of molds and mycotoxins in rice grain.

ACP can be applied to food packaging for prevention of secondary pollution (Deng et al., 2020). Besides, the suitability of ACP for the decontamination of stainless-steel food-processing surfaces against *S.* Typhimurium and *L. monocytogenes* has been recently proved (Katsigiannis, Bayliss & Walsch, 2021; Katsigiannis et al., 2022).

The effect of ACP treatment on edible packaging materials was investigated by different authors, with considerable differences related to the packaging composition and the treatment parameters. As an example, Oh, Roh, & Min (2016) investigated the effect of ACP treatment on edible film made from defatted soybean meal (DSM) for covering smoked salmon. The results not only suggested the positive effect of the treatment on the microbiological quality of the product but also reduced rates of lipid oxidation.

Despite the sanitizing purpose of ACP, this treatment was found to enhance or worsen some specific features of the packaging material such as gas and water vapour barrier, microstructure, mechanical strength, and stretchability (Beikzadeh et al., 2020). The variety of effects is due to the specific pathway of interactions between the ionized gases and the

constituents of packaging like carbohydrates, lipids, proteins, phenolic compounds, and water.

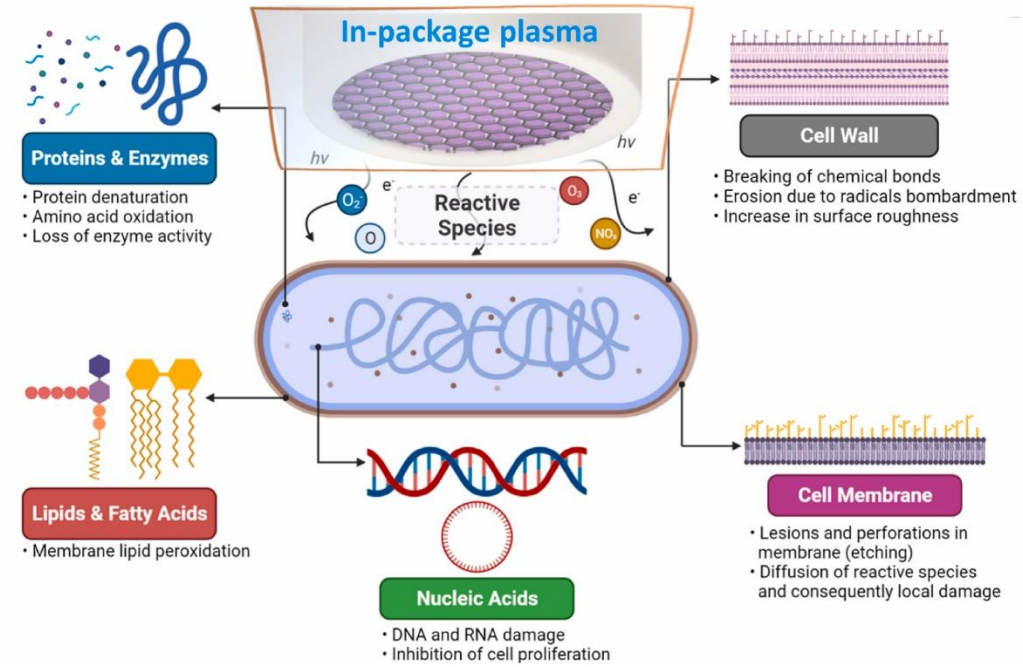


Fig. 2. Schematic representation of the effects of "In-package plasma" on bacteria (Zhou et al., 2022)

3.2.5. Advantages and limitations

ACP is an eco-friendly and flexible decontamination technology with low energy requirements, short treatment times, no utilisation of harmful solvents, and with the possibility to operate at low temperatures (Qiu, Zhang, Tang, Adhikari, & Cao, 2019). ACP can be produced in-situ and provides a range of gases according to treatment conditions and requirements, and therefore it is well suited and flexible with most of the already existing packaging and modified atmospheres. Cold plasma is also compatible with most food products (both liquids and solids) and packaging materials and does not cause a negative impact on the composition, organoleptic properties, and structural integrity of the food matrix (Chakka et al., 2021; Tappi, Tylewicz, & Dalla Rosa, 2020). Besides, the ionized gases are unstable and tend to recombine and react to form the original gas mixture. Thus, ACP was

503 demonstrated to release non-toxic substances and can be regarded as “clean label” (Misra
504 & Roopesh, 2019).
505 Despite the benefits, ACP presents some drawbacks. High-fat products were demonstrated
506 to be not suitable for the treatment due to the oxidation and derived rancidity of the lipids
507 (Liao et al., 2020). As well, ACP was found to increase the acidity, reduce the firmness, and
508 induce discolouration of some fruits and vegetables. Moreover, the presence of roughness
509 and irregularities on the food surface can hinder the biocidal efficacy of ACP. Clearly, the
510 interaction of the several radical species with multicomponent systems like food constitutes
511 a complex phenomenon, far from being widely understood (Denoya, Colletti, Vaudagna, &
512 Polenta, 2021).
513 *3.2.6. ACP-based hurdle approach*
514 ACP constitutes a sustainable and effective biocidal technique. However, the individual
515 application of ACP might be not sufficient to fully inactivate foodborne microorganisms. For
516 example, when subjected to ACP process, some microorganisms could enter into a sub-
517 lethal state known as the viable but non-culturable (VBNC) state (Chakka et al., 2021). Thus,
518 the combination of this technology with other hurdles (hybrid technology) becomes essential
519 to enhance the efficacy of each single treatment, also eliminating the microorganisms in
520 VBNC state (Chaplot, Yadav, Jeon, & Roopesh, 2019).
521 Since ACP represents a highly versatile technique, other hurdles could be introduced before,
522 after, or during the plasma treatment, allowing the use of plasma at lower intensity and
523 protecting the quality of the treated products.
524 The first form of hurdle technology based on ACP is known as chemical augmentation of
525 plasma. This method consists in the introduction of specific compounds such as water or
526 essential oils in the plasma generating chamber, which modify the plasma composition and
527 improve its biocidal performances. In particular, water-augmented plasma was shown to
528 possess a higher density of active molecular species such as H or H_2O_2 with respect to

normal plasma (Kaavya et al., 2021). As well, the presence of essential oils was proven to confer to the plasma an additive antimicrobial effect due to the high variety of compounds with intrinsic antimicrobial activity.

ACP was applied in combination with sanitizers such as H_2O_2 and peracetic acid (PAA), either in liquid or aerosolized forms (Chaplot et al., 2019; Liao et al., 2020). In particular, Chaplot et al. (2019) suggested that a sequence of ACP and peracetic acid treatments might result in a better antimicrobial effect due to enhanced penetration of reactive species. Generally speaking, ACP could produce a partial injury to the cells. This action could be integrated and finalized through the further action of mild chemical treatments.

Another hurdle approach consisting of ACP followed by post-treatment modified atmosphere storage (MAP) was assessed by Yadav et al. (2020). This combined process allowed to obtain over 6 log-reductions in cell counts of *L. monocytogenes* after 10 min ACP treatment, followed by 7 days of storage at 4°C.

Plasma processing was employed to improve the antibacterial properties of active packaging material based on silk fibroin nanofibers and incorporated with nano-capsules of thyme essential oil. The release of thyme oil by the nanofibers was relevantly boosted by ACP, with higher performances for the disinfection of *S.* Typhimurium in poultry meat wrapped using these treated nanofibers (Lin, Liao, & Cui, 2019).

Xiang et al. (2019) employed plasma-activated water along with mild heat treatment to reduce the population of *E. coli* O157:H7. The experiment revealed that the combined treatments affected the intracellular components and outer structure of bacteria by synergistic effect. As well, Lis et al. (2018) combined ACP with refrigeration temperature (8 °C) to inactivate *S.* Typhimurium in ham samples stored into N_2 flushed sealed packages.

Referring to the application of ACP in combination with other non-thermal techniques, Liao et al. (2018) investigated the combined effect of ultrasounds and ACP against *S. aureus* in saline solution. The two technologies were applied sequentially (both ultrasounds + ACP

555 and *viceversa*). The researchers found that the hurdle exposure resulted in over 6-log
556 reduction, which was significantly higher than the reduction of 2.55 and 0.55 logs recorded
557 for individual cold plasma and ultrasound treatments, respectively. During the process, the
558 plasma generated abundant ROS in the medium. These active species ((hydroxide (OH·),
559 nitric oxide (NO), hydrogen peroxide (H_2O_2)) effectively passed through the microbial cell
560 membrane thanks to the ultrasonic microjet effect, triggering oxidative damages in the
561 intracellular components. Therefore, cold plasma had a synergism with ultrasound on the
562 microbial inactivation. Another interesting application of this combination of techniques was
563 reported by Wang & Wu (2022) for ready-to-eat blueberry disinfection. In this case, in-
564 package ACP treatment was applied after chlorine or peracetic acid washing assisted by
565 ultrasounds. In this case, authors observed an additional effect of the combined techniques
566 with respect to the simple US-assisted washing for both the sanitizers.
567 Lastly, some researches focused on the application of ACP together with UV-C treatment.
568 Recently, this combination has been tested on black peppercorn (Bang, Kim, Lee & Min,
569 2020). The technologies were applied simultaneously. Interestingly, the bacterial
570 inactivation rate (3.4 log CFU/g) achieved by UV-CP treatment was higher than the sum (2.7
571 log CFU/g) of the inactivation rates of individual UV and CP treatments, which confirmed
572 their mutual synergy. Besides, UV-CP treatment did not alter the color of the product.

573 ***3.3. High-Pressure Processing (HPP)***

574 *3.3.1. Principles*

575 High-Pressure Processing (HPP), also known as pascalization, is a non-thermal
576 decontamination technology in which the food matrix (either packaged or non-packaged) is
577 placed in a pressure transmitting medium (PTM) medium (e.g., water), and subjected to
578 100-1000 MPa pressure at ambient temperature (do Rosàrio, Rodrigues, Bernardes, &
579 Conte-Junior, 2020). This technology has been defined as “cold pasteurisation” by FDA

since it has the potential to replace the conventional pasteurisation process due to its high biocidal efficacy.

HPP application obeys and takes advantage of three physical principles. The first principle, known as isostatic principle, relies on the instant and uniform transmittance of pressure within a medium. In other words, a product subjected to HPP is uniformly compressed independently from its geometry and size, since the transmission of pressure to the core is not mass and/or time dependent, and thus requires a minimum processing time (Neeto & Chen, 2012). The second principle, known as Le Chatelier's principle, defines that "when the pressure of a system in equilibrium varies, the system tries to re-establish its stability by adjusting the volume" (Rifna et al., 2019). The third principle, known as microscopic ordering theory, states that a pressure increase at constant temperature enhances the degree of molecular ordering of the sample subjected to the treatment (Chatterjee & Abraham, 2018). High pressure equipment usually comprises a pressure vessel and its closure, a PTM, pumps to generate pressure, and a temperature controller. The pressure vessel, mostly a forged monolithic cylindrical vessel, represents the heart of HPP equipment and can operate at different ranges of pressure depending on the internal diameter of the vessel. HPP treatment involves 5 steps: (i) filling of the chamber with PTM and product; (ii) increase of pressure; (iii) pressure holding; (iv) decompression; (v) removal of the product (Sehrawat, Kaur, Nema, Tewari, & Kumar, 2021).

High pressure is usually generated either by direct or indirect compression. In direct compression, PTM is directly pressurized into the pressure vessel through a piston. This method allows faster pressurization, but it is limited to laboratory research or pilot scale. Most of the industrial HPP systems use indirect pressurization method. In indirect compression, PTM is pushed into the vessel until the required pressure is achieved. Industrial HPP installations typically operate discontinuously and can attain pressures of up to 800 MPa. However, pressures exceeding 400 MPa are not normally used for solid foods

since they can lead to reversible or irreversible disruption of inter- and intramolecular bonds in the food matrix (Tappi et al., 2020).

Among the most interesting applications, HPP can be applied as a post-package process to reduce microbial spoilage in the final consumer package, eliminating the need for sterilisation before packaging. In this way, the product can be automatically handled, and avoids post-processing contamination (Roobab et al., 2022). The packaging material used in HPP should be able to withstand 15–20% compression of their original volume, and to immediately return to its volume after decompression. Common packaging materials used in HPP of foods are made of polyvinyl alcohol or ethylene–vinyl alcohol. In contrast, cans and glass bottles are not well suited as packaging material for HPP, since the compressive forces tend to fracture or deform them irreversibly (Sehrawat et al., 2021).

3.3.2. Antimicrobial mechanism

HPP exerts its biocidal effect against a wide range of bacterial, yeast, and mould vegetative cells by hindering the DNA synthesis, denaturing proteins and enzymes, and damaging the cell membrane (Deng et al., 2020) (Fig. 3). In particular, HPP can alter the cytoplasmic membrane structure via increasing the ordering degree of phospholipid molecules, which affects the cell membrane fluidity, and increases the thickness. Besides, HPP induces the detachment and inactivation of membrane proteins. Resulting in the modification of cell permeability and functionality, HPP can also promote cytoplasm acidification, oxidative stress, loss of the osmotic state, and organelle disruption (e.g., ribosomes) (do Rosàrio et al., 2020).

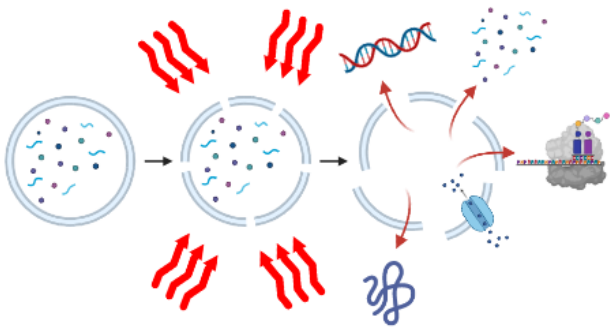


Fig. 3. Schematic representation of the effect of HPP over bacteria membrane.

3.3.3. Factors affecting processing efficacy

The antimicrobial efficacy of HPP depends on several parameters such as processing conditions (i.e., pressurization level, treatment time, compression/decompression rate, and temperature), physical state and composition of the matrix, and the intrinsic sensitivity of the microbial strains (Rifna et al., 2019).

The pressure level and the time of application represent the primary factors influencing the effectiveness of the method since both of these conditions are positively related with the inactivation rate of microorganisms (do Rosàrio et al., 2020). Besides, either very low or elevated temperatures enhance the susceptibility of microorganisms to pressure.

HPP can be applied in repeated pressure cycles, which improves the inactivation effect due to the rapid decompression of the matrix, leading to increased lesion and cell rupture. How fast the compression/decompression rate should remain object of debate. As an example, Syed et al. (2012) reported that slow compression/decompression rates increased the efficiency of high pressure for reducing spores. In contrast, faster compression/decompression rates were found to be more effective for the inactivation of vegetative bacterial cells (Noma et al., 2002). In this sense, pressure-mediated damages are difficult to predict since they are the result of interactions among the processing conditions and the other factors involved (e.g., the intrinsic sensitivity of the target microorganisms): thus, the optimisation of each process should be performed *case-by-case*.

The type of microorganism strongly influences the effectiveness of HPP treatment. In general, Gram-positive bacteria are more resistant to HPP than Gram-negatives, thanks to their tightly adherent layer of peptidoglycan. Besides, psychrotrophic bacteria are usually more sensitive to HPP than mesophilic bacteria since their cell membrane is richer in unsaturated fatty acids (USFA). The higher presence of UFSA increases the membrane permeability at low temperatures due to the smaller melting point of these fatty acids. HPP

increases the melting point of triacylglycerols reducing the ability of psychrotrophic bacteria to grow under refrigeration temperature, which makes them more sensitive to this technology (do Rosàrio et al., 2020). Bacteria were reported to be more resistant to high pressures than yeasts. In fact, molds and yeasts can be easily inactivated at 25°C using 300–400 MPa for a few minutes. The shape of the target bacteria is also important. Rod-shape bacteria (*E. coli*) were found to be more resistant than slender rod shape bacteria (*P. aeruginosa*), whereas maximum resistance was exhibited by cocci (*S. aureus*) (Sehrawat et al., 2021). Lastly, bacterial spores are far more resistant to pressure than vegetative cells. In particular, they can survive to pressures higher than 1000 MPa, unless HPP is carried out in combination with high temperatures (Chakka et al., 2021). For the inactivation of spores, a two-step treatment was found to be more effective than a simple one-step treatment. In the first step, HPP treatment was performed at low pressure to trigger the germination process in spores and the solvation of spore's component. In the second step, high-pressure treatment was then applied to inactivate the spores (Yaldagard et al., 2008).

Another variable that strongly influences the efficacy of HPP treatment is the food matrix. Generally, HPP results more successful in culture media and liquid foods than solid ones, principally due to the more homogeneous distribution of the pressure throughout the product. The composition of the product and its pH also interfere with the efficiency of the process. Proteins, carbohydrates, lipids, and metals ions can provide a shielding effect to the microorganisms, increasing their baro-tolerance (Dogan & Erkmen, 2004). As well, a deviation from neutral pH towards low pH increases susceptibility to microbial inactivation. Lastly, low water activity provides a shielding effect to microorganisms suspended in food, and thus HPP-induced inactivation is usually hindered by an increase in the saturation level of the suspension, regardless the kind of solute (e.g., sodium chloride; sucrose). In fact, the addition of solutes causes the shrinkage and the thickening of the cellular membrane, resulting in better survival of the microorganisms (Sehrawat et al., 2021).

3.3.4. Applications in the food sector

HPP has been tested as sanitising treatment for both solid and liquid foods. This process was shown to be particularly efficient when applied to foods stored at low temperatures and/or with high acid content (Aaliya et al., 2021). For instance, researchers focused their efforts on testing this technology on milk (Stratakos et al., 2019), cheese, fruit juices, coconut water (Raghubeer et al., 2020), soups, and smoothies (Ates, Rode, Skipnes, & Lekang, 2016) against the proliferation of various pathogenic bacteria such *E. coli*, *S. enterica*, and *L. monocytogenes*. All these studies showed that the application of 300-600 MPa for 1-5 min resulted in over 5-log reduction in the pathogenic populations, thus increasing the shelf life of the treated products. Thus, the suitability of HPP as a sanitizing tool to guarantee the safety and prolong the shelf life of liquid foods was well stated.

In recent years, HPP application on muscle-containing products like meat, fish, and oysters has been widely investigated (Ramaswamy et al., 2019). In particular, recent studies covered the impact of HPP for the reduction of *Salmonella* spp. (Chai & Sheen, 2021), *L. monocytogenes* (Cava, Higuero, & Ladero, 2021), Staphylococci, Enterobacteriaceae, lactic acid bacteria (LAB), yeasts, and moulds (Borges et al., 2020) in several meat products. Despite the relevant benefits observed, some studies observed that the HPP treatment could reduce the pathogen counts with over 5 log-reduction only when applied at pressures of ~400 MPa. As an example, this effect was observed by Roobab et al. (2021) for flexible vacuum-packed meat products. Unfortunately, high pressures not only promote microbial inactivation, but also physicochemical changes in texture, color, pH, as well as sensory alterations due to the alteration/denaturation of muscle proteins. In another study, assessed on chorizo sausage (Rubio, Possas, Rincon, Garcìa-Gìmeno, & Martìnez, 2018), even pressures higher than 500 MPa or longer holding times could not cause sufficient bacterial reduction.

Among the newest studies in this field, Lee e al. (2022) recently tested HPP as a prospective substitute of the conventional frozen shucking method for the preparation of marinated clams. In this case, authors reported that HPP conditions of ≥300 MPa for 3 min significantly impacted the microbiological quality and extended the shelf life of the product during cold storage, without affecting its overall attributes.

3.3.5. Advantages and limitations

HPP showed different advantages including enhanced nutrient retention and/or reduced loss of nutrients, minimal heat impact, shorter processing time, and unaltered color and organoleptic properties of foods with respect to other sanitising techniques (Qiu et al., 2019). Nevertheless, HPP resulted ineffective on low acid, shelf stable products since the microbial spores can survive even at a pressure of 1200 MPa when maintained at environmental temperature. This drawback can be overcome by combining this technology with other hurdles such as mild heat treatment, refrigeration or ultrasonication (Aaliya et al., 2021). Besides, the application of the principle of Le Chatelier's results in a reduction of the volume, which may affect the structure of larger molecules like proteins. This process can either produce positive effects, leading the inactivation of microorganisms and enzymes, or negative effects, causing undesirable changes in sensory and physicochemical characteristics of food (do Rosàrio et al., 2020). Moreover, the initial and maintenance costs of this system represent another significant drawback of this technology (Tappi et al., 2020).

3.3.6. HPP-based hurdle approach

The optimization of HPP biocidal efficacy by applying a hurdle approach has been extensively investigated, and different combinations of technologies were tested aiming for synergistic effects.

HPP was successfully combined with subsequent low-temperature storage on cantaloupe puree (400 MPa + 10 days at 15°C) (Mukhopadhyay et al., 2016), and on gilthead seabream and seabass fillets (600 MPa, 5 min + 0-2°C storage) (Tsironi et al., 2019). Both studies

731 revealed two relevant benefits of the combined treatment: (i) the possibility to reduce the
732 HPP treatment intensity with equal final results; (ii) a drastic extension of the shelf life with
733 respect to simply refrigerated products. As well, HPP was combined with mild heat treatment
734 (600 MPa, 70°C) for inactivating *Bacillus cereus* spores in beef slurry (Evelyn & Silva,
735 2016a), which resulted in a synergistic effect of the two treatments.
736 Recent studies showed the enhanced potential of HPP paired with virulent bacteriophages
737 and bacteriocins for the elimination of bacterial pathogens in animal products such as milk
738 (Komora et al., 2020) and fermented meat sausage (Komora et al., 2021) for the reduction
739 of *L. monocytogenes.* In both cases, the authors showed that the combined treatments
740 synergistically reduced the pathogenic population.
741 HPP was applied in combination with MAP to enhance the safety and extend the shelf life
742 of desalted cod (Rode & Rotabakk, 2021) and pre-cooked chicken breast slices (Dang,
743 Rode, & Skipnes, 2021). In these cases, the products were stored for different periods on
744 time in modified atmosphere (high concentration of CO_2), followed by HPP treatment.
745 MAP/HPP showed a synergistic antimicrobial activity for both the products.
746 Taking into account other non-thermal techniques, HPP has been combined mild heat
747 treatment and ultrasounds (i.e., thermosonication) to reduce the amount of *Alicyclobacillus*
748 *acidoterrestris* spores in commercial orange juice (Evelyn & Silva, 2016b). HPP (200-600
749 MPa, 15 min) was used as a pre-treatment to thermosonication (20.2 W/mL, 78°C, 60 min),
750 and their combined effect was compared with those of the single techniques. With 2.3–4.4
751 log-reduction achieved, HPP-assisted thermosonication was found to be drastically more
752 effective than the single treatments (synergistic effect). A similar combination of
753 technologies was then employed by Song et al. (2021) to inactivate bacteria and yeasts in
754 cold brew tea. In-package HPP treatment was performed on the product (sealed in PET
755 bottles), followed by treatment in an ultrasonic bath. The authors pointed out that the

synergistic effect of HPP and ultrasounds eliminated the total viable count, thus maintaining the concentration of tea polyphenols and the antioxidant capacity.

A large and exhaustive review about the application hurdle technology based on HPP for the preservation of raw and processed meat has been recently provided by Roobab et al. (2022).

3.4. Pulsed electric field (PEF)

3.4.1. Principles

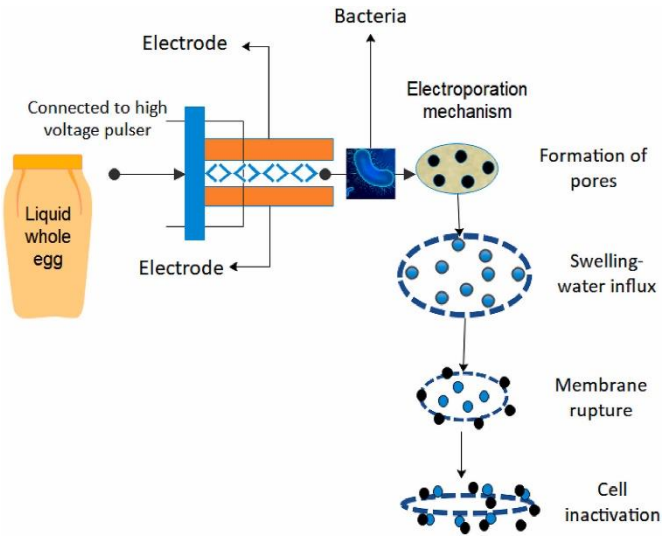
Pulsed electric field (PEF) represents a relatively novel and green technology, whose promising application in the food sector for various purposes has been extensively investigated in the last few years. During PEF treatment, the food product is placed between two electrodes and subjected to high-voltage electrical pulses (20-80 kV/cm) for short and repeated periods of time (1-100 μs) (Ziuzina, Los, & Bourke, 2018). Currently, PEF process is broadly employed to inhibit the spread of microorganisms within foods, to enhance the extraction efficacy of bioactive compounds from natural sources, and to enhance the performances of drying, dehydration, and freezing processes (Kaavya et al., 2021).

A PEF system comprises a treatment chamber, a high-voltage power source, a pulse generator, energy storage devices such as capacitors or inductors, a cooling system for temperature-rise balance, power switches, and a fluid handling system (when required) (Zhang et al., 2023). Basically, the generator converts alternate current (AC) to direct current (DC) using an energy storage device such as a capacitor. The fast pulses are generated by specifically designed components that act as a switch (Arshad et al., 2021). The treatment chamber consists of two electrodes, held in place by an insulating substance, which forms a food material enclosure. According to the specific design of the chamber, the configuration of the electrodes can be defined as coaxial, colinear, and co-field. Besides, the treatment chambers can be distinguished into continuous and static treatment chambers. The first ones are commonly used for the continuous processing of liquid, semisolid or pumpable

782 foods. On contrary, static treatment chambers are more suitable for solid foods (Zhang et
783 al., 2023).

784 *3.4.2. Antimicrobial mechanism*

785 PEF is able to induce cellular loss of integrity and death. The chief effects of this process
786 are known as electroporation (or electropermeabilisation) and dielectric breakdown
787 (Bahrami et al., 2020). When strong electrical pulses are applied to the cell cytoplasmic
788 membrane, the transmembrane potential is altered, which induces an enlargement of the
789 size of the existing pores and the formation of new ones. Repeated electrical shocks above
790 the critical transmembrane potential, which depends on the target microbial strain and the
791 processing conditions, progressively lead to the transition from reversible (i.e., recovery of
792 the integrity of the membrane after the treatment) to irreversible electroporation (Fig. 4). This
793 phenomenon causes the leakage of intracellular compounds towards the external
794 environment, and then the cellular rupture (Slavov et al., 2019).



795

796 Fig. 4. Schematic representation of an example of PEF technology to liquid whole egg and
797 relative effects on bacteria (Kaavya et al., 2021).

798 *3.4.3. Factors affecting processing efficacy*

799 The biocidal efficacy of PEF relies on multiple factors. Firstly, the performances of the
800 process are strictly related to the design of the plant and the treatment conditions (Khan et

al., 2017). In particular, PEF decontamination efficacy relies on the electric field intensity, time-per-pulse, number of pulses, and the treatment duration, which is a function of the previous two parameters (Ramaswamy et al., 2019). As an example, both electric field intensity and treatment time were found to be positively related with the decontamination rate of pathogenic bacteria such as *L. innocua* due to the shift from reversible to irreversible electroporation (Pyatkovskyy, Shynkaryk et al., 2018). Unfortunately, excessive treatment intensity or duration may lead to undesired changes in food quality (some of these effects will be highlighted in the following paragraphs). Hence, the right selection of these parameters allows to maximise the expected effects of this technology, thus maintaining the nutritional and sensory quality of the product.

The physical and dielectric properties of the target microbial cells play another crucial role. Gram-positive bacteria are generally more resistant than Gram-negative bacteria, due to the presence of a thick peptide glycan layer on the cell membrane (Slavov et al., 2019). According to Bahrami et al. (2020), *L. monocytogenes* is one of the most resistant microorganisms against PEF treatment, and thus the ability of PEF treatments to kill *L. monocytogenes* may be followed as an indicator for their decontaminating efficacy. The size and the shape of microorganisms strongly influence the PEF efficacy, since they greatly affect the membrane potential. As an example, the electric field required to destroy rod-shape cells was demonstrated to be five times stronger than the intensity required for a spherical shaped cells with the same characteristics. Moreover, vegetative forms of microorganisms are usually more unstable than spores (Slavov et al., 2019).

Lastly, PEF treatment efficacy drastically change depending on chemical factors such as pH, ionic strength, and conductivity of the medium of application. As a result, type, composition, and all the other properties of foods (e.g., shape; dimension etc.) should be carefully take into account for the correct set-up of the processing conditions (Bahrami et al., 2020). Firstly, this technology is highly suitable for processing liquid or semi-solid food

products, while its application on solid foods (e.g., powders) is very limited due to their low electric conductivity (Chakka et al., 2021; Priyandarshini et al., 2018). Besides, solid foods are rich in protective substances (i.e., proteins; fats), and have heterogeneous electrical resistivity, which could result in heterogeneous effects in the different areas of the product (Asik-Cambaz et al., 2022). For this reason, PEF treatment of solid products is mainly applied for other technological purposes such as extraction of bioactive compounds, inactivation of enzymes, freezing, and drying (Zhang et al., 2023). Another factor that strongly affects the sensitivity of the target microorganisms is the pH of the medium. In particular, an inverse relation between the pH of the treated product and the reduction rate of *L. monocytogenes* was observed for PEF-treated fruit juices (i.e., watermelon, orange, and apple).

3.4.4. Applications in the food sector

The enhanced lethality of PEF technology against a broad range of microbes such as molds, yeasts, and bacterial vegetative cells makes it a prospective substitute to thermal pasteurization for several high-moisture food matrices including milk, fruit juices, yogurts, and liquid egg (Bermùdez-Aguirre, 2018).

PEF treatment was found to achieve a promising decontamination result for milk, since it was able to reduce the *E. coli* population of 6-8.3 log, according to the experimental conditions (Sharma, Oey, Bremer, & Everett, 2014; Walter, Knight, Ng, & Buckow, 2016). Specifically, Sharma et al. (2014) pre-heated the milk to 55 °C for 24 s before the treatment, which substantially improved the log reduction. Recently, Ji, Sun, Sui, Qi & Mao (2022) investigated the feasibility of inactivating microorganisms in milk by low intensity electric fields. Results showed that the microbial population of milk, comprising pathogenic (e.g., *Acinetobacter* spp.) and spoilage bacteria (e.g., *Pseudomonas* spp.), could be reduced by over 5 log CFU/mL with a starting intensity of 5 V/cm-9 V/cm (50–55°C; 0.3 A–0.6 A; 5 min)

Besides, the process did not affect the fresh-like characteristics and the physical stability of the product.

Similarly, PEF treatments have been widely tested and provided acceptable microbial reduction (> 5 log) for a variety of fruit juices such as apple, pineapple, and orange (Dziadek et al., 2019; Indriani et al., 2019; Timmermans et al., 2019). All these studies proved that PEF treatment was able to improve the microbial quality of the products with minimal detrimental effects on their nutritional, physical, and sensory properties. In particular, PEF processing did not result in the loss of vitamins B1, B2, C, D, and E, and, in some cases, had a beneficial effect on the bioavailability of active compounds such as carotenoids and polyphenols (Slavov et al., 2019). Recently, Tanino et al. (2022) suggested the possibility to apply in-line continuous PEF treatment for sake pasteurization. The pilot tests were performed using NaCl solution as a treating medium, and the effect of temperature and flow rate on the treatment efficacy was assessed. PEF was able to fully inactivate sake yeasts and *Lactobacillus homohiocii* below 40°C when the flow rate was maintained low.

Some exceptions to the aforementioned statement (i.e., PEF is not suitable for solid foods decontamination) have been recently reported (Rathod et al., 2022). Fresh seafood was the main subject of these studies. In particular, Shiekh & Benjakul (2020a) demonstrated that PEF treatment (15 kV/cm, 600 pulses) was able to inhibit the growth of total viable and psychrophilic bacterial counts, as well as *Pseudomonas* spp., H_2S-producing bacteria, and Enterobacteriaceae. Unfortunately, PEF slightly increased the rate of lipid and protein oxidation, which could be controlled through the addition of natural antioxidants. Overall, the effect of PEF processing on the sensory profile of the shrimps was negligible. Anggo & Suharto (2020) suggested the possibility of using PEF to control the total viable (4.6 log reduction) and the psychrophilic bacterial counts (3.7 log reduction), with increasing antimicrobial efficacy observed upon the increase of voltage from 30 to 90 kV. Wang, Wang, Xu &, Sun (2022) investigated the effect of extremely low frequency pulsed electric field (500

878 kV/m, pulse width of 6.5 s) on the safety, quality, and shelf life of tilapia fillets. PEF treatment
879 not only prolonged the shelf life from a microbiological point of view but also inhibited some
880 factors which commonly lead to quality deterioration during storage (i.e., lipid oxidation,
881 protein denaturation etc.). The higher suitability of fresh fish for PEF treatment with respect
882 to other solid foods may rely on their high water content, along with the homogeneous
883 distribution of muscle (i.e., proteins) and fats in their structure.

884 *3.4.5. Advantages and limitations*

885 PEF processing allows to obtain high rates of decontamination with minimum increase of
886 the product temperature. Thus, a relevant advantage of PEF consists in the ability to retain
887 the utmost nutritional and organoleptic qualities of the food material with respect to
888 conventional pasteurisation (Chakka et al., 2021). Moreover, this technique has been
889 reported to decrease the environmental impact of pasteurization, supporting food and
890 nutrition safety with minimal usage of energy and water, and without evident toxicity on
891 human health (Arshad et al. 2021).

892 Despite the relevant benefits of this green technology, its industrial implementation in the
893 different sectors of food industry is still difficult. One of the main obstructions is related to
894 the high costs of set-up and maintenance of the processing plants, still not comprehensively
895 justified through up-to-date literature enabling an efficient cost analysis of the process.

896 The biocidal efficacy of PEF strongly depends on the electrical conductivity of the product,
897 which mainly relies on its moisture content. Thus, solid and semi-solid foods are less suited
898 than liquid foods for PEF treatment (Rifna et al., 2019). Besides, Bermúdez-Aguirre (2018)
899 suggested that liquid products containing bubbles may not be suitable for PEF technology,
900 since arcing or electrical shutdown may occur when the bubbles come in contact with the
901 electric field.

902 PEF was found to significantly increase the lipid oxidation of some products with the increase
903 of current intensity, causing undesirable changes in the sensory quality (Shiekh & Benjakul,

2020a). Moreover, in some cases, electrodes may undergo corrosion and release metal particles in the treated material, affecting its overall safety (Aghajanzadeh & Ziaiifar, 2021). Hence, the selection of a proper set-up (e.g., electrode material compatible with the food properties etc.) and a constant maintenance of the machineries is strictly necessary to achieve the expected results, and to avoid undesired effects.

Lastly, PEF treatment must be necessarily accomplished before food packaging, which may represent an obstacle for the widespread commercialization of this technology.

3.4.6. PEF-based hurdle approach

Recently, PEF was applied in combination with one or more decontamination methodologies at low intensity to overcome the drawbacks related to this technology, depending on product composition and the specific microorganisms to be inactivated. These combinations allowed to achieve the required lethality with lower electrical input and a less intense electrical field. Different researches showed the benefits related to the application of PEF under mild heated conditions to decontaminate liquid foods such as fruit juice, egg, and milk. Particularly, this combination of hurdles was found to be very effective on pathogenic bacteria such as *Salmonella*. Where PEF alone could induce 0.5 log reduction on *Salmonella* population, the combination of PEF with mild heat treatment led to a synergistic effect, inducing over 3 log reduction in cells. The lethality of this treatment was furtherly enhanced by the addition of essential oils including citral, carvacrol, and limonene, yielding a maximum of 4 log reduction on liquid whole egg (Espina, Monfort, Alvarèz, García-Gonzalo, & Pagàn, 2014).

Recently, the sequential application of PEF pre-treatment followed by soaking in Chamuang leaf extract was tested by Shiekh & Benjakul (2020b) to prolong the shelf life of Pacific white shrimps. Despite the relevant impact of the microbial quality of the product, this combination of hurdles allowed to reduce the PEF treatment intensity, which reduced the impact on sensorial properties below detectable limits.

PEF processing has been applied in combination with other non-thermal techniques such as HPP and ultrasounds (US), with variable results related to the treated matrices and the target microorganisms. Among the first studies conducted in this field, Huang, Mittal & Griffiths (2006) analysed the effect of the sequential application of PEF-HPP, HPP-PEF, PEF-US, and US-PEF on liquid whole egg quality. In this study, while PEF alone was not able to significantly inhibit the growth of *Salmonella enteritidis*, an increase of processing temperature to 50° C reduced the bacterial population by 0.90 log CFU/mL. When used with US, 2.30 and 2.25 log reductions were observed for PEF-US and US-PEF, respectively. Similar results were observed for PEF-HPP and HPP-PEF combinations, giving 2.5-3.0 log reduction. Interestingly, all the combinations of technologies showed an additive effect, and no synergy was observed.

Pèrez-Won et al. (2021) investigated the effect of PEF (1-2 kV/cm; 20 µs/pulse; 15 s) alone or sequentially followed by MAP (CO_2 70%) and/or in-package HPP (150 MPa; 5 min) on the microbiological shelf life and physicochemical properties of coho salmon during refrigerated storage (25 days). The preserving techniques were applied either in pre-rigor or in post-rigor. In both cases, the combination of PEF with the other two hurdles was found to significantly increase the microbiological shelf life of the product (from ~6 days to ~11 days) with respect to simple PEF treatment. These techniques also had a relevant impact on reducing lipase and collagenase activity.

As well, ultrasound-PEF combination has been tested for the decontamination of liquid foods, with promising results comparable to conventional pasteurization treatments. As an example, the impact of single and combined PEF and US on the inactivation of different microorganisms in oil-in-water emulsions was investigated (Gomez-Gomez, de la Fuente, Gallego, Garcia-Perez, & Benedito, 2021). The processing conditions were set according to the target microorganisms. The technologies were applied sequentially, both for PEF-US and US-PEF combinations. The highest inactivation rates achieved with PEF were 2.6, 1.2

and 0.1 log-reduction for *E. coli*, *Aspergillus niger*, and *Bacillus pumilus*, respectively. These results were obtained by applying the highest energy level (152.3–176.3 kJ/kg). US led to higher reduction yields (5.4, 4.3 and 0.3 log CFU/g for *E. coli*, *A. niger*, and *B. pumilus*, respectively) after the longest treatment time (3 min). PEF (152.3–176.3 kJ/kg) followed by US (3 min) was reported to be the most effective sequence, leading to a synergistic effect (over 6.6 and 1.0 log-reduction for *A. niger* and *B. pumilus*, respectively).

Shiek, Zhou & Benjakul (2022) tested the efficacy of PEF pre-treatment (15 kV/cm, 800 pulses, 697 kJ/kg) followed by soaking in diluted Chamuang leaf extract and ACP (10 min) in modified atmosphere (argon/air 80:20) to improve the microbial quality and prolong the shelf life of prawns. The combined treatments attained the highest inactivation rate against the total bacterial load and the spoilage bacterial count, showing a synergistic impact on the quality of the product. Besides, the sensorial and nutritional properties of the prawns were not affected during the treatments, and their overall shelf life was prolonged from 9 (untreated prawns) to 18 days.

Other studies, mainly applied on apple juice, reported that PEF inactivation of microbes was enhanced when combined with UV-C treatment (Alzamora, Lòpez-Malo, Guerrero, & Tapia, 2018).

3.5. Ultrasounds (US)

3.5.1. Principles

Ultrasound (US) is defined as a sound wave that exceeds the threshold of human hearing (20 kHz). Basically, US represents a type of vibrational energy produced by specific transducers able to convert electrical energy into acoustic energy (Chen, Zhang, Yang, 2020). According to the frequency range, US can be classified as: power (20-100 kHz), high-frequency (100 kHz-1 MHz), and diagnostic (1-500 MHz). Specifically, power US have experienced an increasing interest in the food processing sector as a green, non-thermal, and reliable technology, with a variety of prospective applications including sterilization,

decontamination, hydrolysis, crystallization, homogenization, drying, and extraction (Bahrami et al., 2020; Deng et al., 2020).

The mechanism of US action, and thus its usefulness for several applications, is mainly related to a specific phenomenon known as cavitation effect. As the US waves propagate within a liquid medium, they generate a periodic alternance of positive and negative pressure, which makes the liquid molecules expand and stretch. This process induces the formation of micro-sized bubbles that grow, oscillate and burst, releasing a large amount of energy that increases the local temperature up to 5000 K and the pressure to 50 MPa. This process is accompanied by strong micro-jetting, shock waves, shear forces, and free radical formation (Chen et al., 2020).

US equipment usually comprises a US generator system (which can generate either a single or multiple frequencies), one or more transducers (i.e., a metal-ceramic bodies which receive and transmit the acoustic signal to the medium), and a stainless-steel treating chamber. According to the configuration, the transducers can be either installed on the external walls of the treating chamber (i.e., US bath or tank) or in direct contact with the matrix (i.e., US probe).

3.5.2. Antimicrobial mechanism

Many researchers investigated the opportunity to employ the disruptive effects of US to kill microorganisms and to inactivate enzymes in the food products (Slavov et al., 2019). The acoustic cavitation (both stable and transient) was found to affect cellular vitality directly, by damaging the cell wall and membrane through physical processes, and indirectly by generating free radicals with strong oxidative properties that break or modify the DNA structure (Liao et al., 2018; Wu et al. 2015) (Fig. 5).

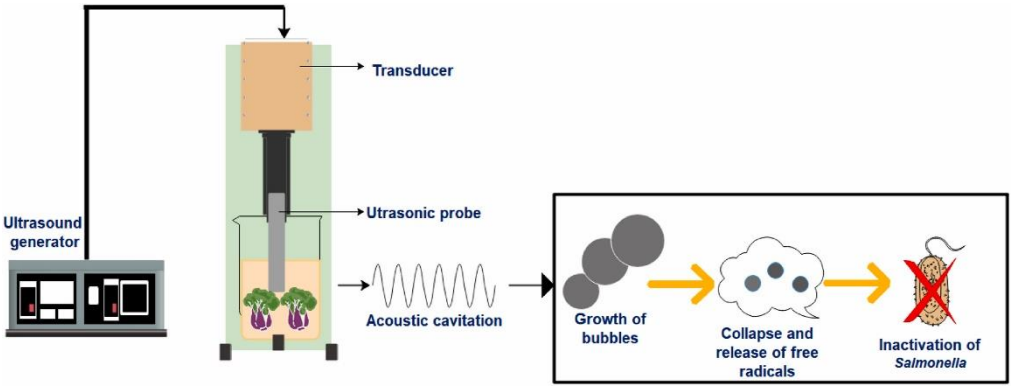


Fig. 5. Schematic representation of the effect of US on food contaminated by *Salmonella* (Kaavya et al., 2021).

3.5.3. Factors affecting processing efficacy

US biocidal efficacy firstly depends on processing parameters such as power intensity, frequency, exposure time, temperature, and geometry of the reactor (Bahrami et al., 2020). The correct evaluation of these factors allows to maximise the performances of this technology, obtaining valuable results against hazardous foodborne pathogens, without compromising the nutritional and sensory properties of the treated products.

Generally, increasing the power intensity and/or the exposure time enhances the antimicrobial performance of US. As an example, Huang, Wrenn, Tikekar, & Nitin (2018) observed that the inactivation yield of *E. coli* on lettuce was increased from 1 to 2.61 log CFU/cm^2 by prolonging the treatment from 1 to 10 min. A similar effect was observed by Afari, Hung, King, & Hu (2016) by increasing the power intensity from 130 to 210 W, which improved the reduction rate of *E. coli* on romaine lettuce from 1.92 to 2.24 log. Another critical factor which strongly influences the treatment efficacy is the uniformity of treatment. In fact, uneven sound pressure distribution in the medium may hinder the efficacy of the treatment, or even worse, damage the product. In this sense, Tangsopa & Thongsri (2019) recently found a strong correlation between the position of the emitting transducers (i.e., the installation set-up) and balanced/unbalanced distribution of sound pressure within a US cleaning tank.

The type of microorganisms, their shape, and their vital form (e.g., vegetative cells or spores) significantly influence the sterilization effect of US treatment. Gram-positive bacteria are generally more resistant to US than Gram-negative bacteria due to the presence of the tightly adherent peptidoglycan layer on the cell wall (Chemat et al., 2017; Huang et al., 2018). As well, rod-shaped bacteria are usually less resistant than cocci (Slavov et al., 2019). As described for the other non-thermal technologies, US cannot inactivate spores through the currently available equipment. Studies conducted on spores of *Neosartorya fischeri*, *Clostridium perfringens*, *Byssochlamys nivea*, and *Geobacillus stearothermophilus* confirmed the inadequacy of US treatment to kill these dormant forms (Bermudèz-Aguirre, 2018).

Lastly, the efficacy of the US treatment is strongly related with the physical state of the product. Although ultrasound technology has been well standardized and can fit in any kind of food production line (e.g., washing of vegetables etc.), sonication is more efficient in liquid foods with respect to solid ones (for which the effect is only superficial). Since cavitation occurs in water media, the moisture content of foods represents a key factor for microbicidal effectiveness (Slavov et al., 2019). This makes US treatment completely not suitable for the decontamination of food powders (Rifna et al., 2019), and confines its application to the pre-packaging steps of the production line. The composition of the product (e.g., the presence of fats) also plays a crucial role. As an example, Bermúdez-Aguirre & Barbosa-Cánovas (2008) showed that the US efficacy against *Listeria innocua* in milk decreased with an increase of the fat content (i.e., protective effect).

3.5.4. Applications in the food sector

So far, US technology has been tested as a sanitising tool on a variety of food products, either liquid or solid.

Among liquid foods, the US treatment of fruit and vegetable juices seems promising in view of its industrial application. Khandpur & Gogate (2015) reported that this technology (20 kHz,

100 W, 15 min) achieved results comparable to conventional pasteurisation (> 80°C, 10 min), but with a greater retainment of the nutritional characters (e.g., polyphenols, vitamin C, and carbohydrates), and lower energy expense. Campaniello, Bevilacqua, Speranza, Sinigaglia, & Corbo (2018) demonstrated that US treatment (130 W) could effectively reduce the population of *S. enterica* in rice beverage, prolonging its shelf life up to 13 days. Similar results were observed for liquid whole eggs (Techathuvanan & D'Souza, 2018).

Recently, researchers focused their attention on the potential of ultrasound for decontamination of fresh or ready-to-eat products (Bahrami et al., 2019). In particular, US treatment was found to reduce the population of *S. enterica* by 1.68–2.23 log CFU/cm^2 in fresh lettuce (Millan-Sango et al. 2016). Besides, a study conducted on freshly harvested strawberries showed that US-assisted washing (60 W; 33 kHz; 10-60 min) was able to decrease the bacterial (from 5.91 to 3.91 log CFU/g) and fungi counts (from 4.80 to 3.58 log CFU/g) of the fruits, prolonging their shelf life (15 days) and maintaining their overall quality (Slavov et al., 2019).

Besides the ability of US treatment to reduce the microbial load of food, many researchers have investigated the effects of US on the nutritional and sensorial quality of food. It was demonstrated that US may exert positive or negative effects on food quality depending on the applied processing conditions. For example, ultrasound was found to eventually improve or worsen the textural properties of meat, depending on the severity of treatment (Shi et al., 2020). Excessive exposures to US were shown to cause non enzymatic browning of food (Khanal, Anand, Muthukumarappan, & Huegli, 2014). However, a recent study highlighted that a proper treatment can significantly enhance the colour of blueberry wine, which may be due to the protection of anthocyanins by ultrasounds (Li, Zhang et al., 2020). As well, an appropriate US treatment can reduce the loss of volatile components in food and increase the availability of bioactive substances by affecting the cell membranes and making intracellular substances exude (De Souza Carvalho et al., 2020).

3.5.5. Advantages and limitations

Overall, US treatment constitutes a promising green technology with transversal applications for the food industry, supported by impressive academic results provided by several studies. This technology is rapid, low-cost, non-thermal, and non-destructive. If correctly conducted, it allows to achieve inactivation results comparable to conventional pasteurisation, thus maintaining a higher quality of the product in terms of nutritional, sensory, and aesthetical characteristics.

Despite the relevant benefits, some challenges still need to be solved before its commercial application. The first and most relevant challenge is the development of ultrasound equipment able to satisfy the needs of food industry for large-scale and continuous set-ups, which should be encountered by the willing of manufacturers to customise new designs according to the requirements (Azam et al., 2020). Secondly, further research is needed to explore more in depth the physical and chemical phenomena occurring on the food product during US treatment. Indeed, excessive US processing rates can hinder the quality of the product by causing off-flavours (e.g., lipid oxidation), affecting the physical parameters (e.g., color, texture), and inducing the decomposition of bioactive compounds (Bahrami et al., 2019). Lastly, ultrasonic technology alone was found to be not sufficient to inactivate a large number of bacterial species because of its lowered efficiency under certain circumstances (Mahmoud, Fagiry, Davidson, & Abdelbasset, 2022). This final drawback leads to the necessity to apply US technology in combination with other hurdles, which will be highlighted in the following paragraph.

3.5.6. US-based hurdle approach

The improved antimicrobial efficiency of US combined with other preservation techniques is well documented (Aalyia et al., 2021). In recent years, the combination of US with physical and chemical treatments has been extensively investigated.

Thermosonication, meaning the simultaneous combination of US and mild heat, was shown to induce macromolecule depolymerization on the structure of heat-shocked microbial cells, developing either a cumulative or synergistic effect (Guerrero, Ferrario, Schenk, & Carrillo, 2017). Thermosonication can be categorised as sublethal (<45-50 °C) or lethal (>45–50 °C) according to the temperature range (Mahmoud et al., 2022). In fact, most microorganisms (even spore-forming bacteria such as *S. aureus*) become highly sensitive to US at temperatures above 50 °C (Li et al., 2017). Jambrak et al. (2018) showed that US treatment (600 W; 20 kHz, 3–9 min) completely inactivated yeasts and moulds in fruit juices at 60 °C, while this effect was not observed at 20 °C and 40 °C. As well, Cichoski et al. (2015) tested ultrasound-assisted pasteurisation (200 W, 25 kHz,10.5 min, 74 °C) on hot dog sausages. The authors observed that US and heat treatments could synergistically inhibit the growth of lactic and psychrotrophic bacteria, without changing their chemical and physical properties. Overall, this technique is able to achieve microbial inactivation beyond the US FDA's 5-log cycle requirement, thus maintaining the sensory and nutritional quality of liquid products, making it a prospective option for improving food quality and extending the shelf life (Guerrero et al., 2017).

Mano-thermosonication represents an evolution of the previous treatment. Combining pressure, heat, and US, mano-thermosonication was found to inactivate most of the thermal-resistant enzymes, such as peroxidases and lipoxygenase, and to have a broader antimicrobial spectrum with respect to conventional thermosonication (i.e., efficacy against heat-resistant vegetative cells and spores). Moreover, it requires lower processing times than conventional US processing (Chatterjee & Abraham, 2018).

Sonication has been applied in combination with other non-thermal techniques to enhance their mutual effectiveness. As an example, the combination of UV irradiation with US gave rise to photo-sonication, which was successfully applied to decontaminate and prolong the shelf life of fruit juices (Khandpur & Gogate, 2016). In this study, US treatment (100 W, 20

kHz, 15 min) was applied simultaneously with UV-C light (254 nm, 8 W). The combined treatment induced over 5-log reduction on the tested microorganisms, thus maintaining the product quality. A similar combination was explored by Gabriel (2015) for the inactivation of *E coli* in apple and orange juices, but with the addition of mild heat treatment (45-60 °C). Also in this case, a synergistic effect between the technologies was observed.

Recently, Shi et al. (2022) suggested a new promising technique consisting of a US treatment (300 W, 40 kHz, 10 min) followed by in-package gamma irradiation to ensure the microbiological and physicochemical quality of fresh mushrooms. With an overall efficacy against all the tested microbial populations, the combination of US and ionizing irradiation had the best synergistic results, also considering the moderate impact of this technology on the nutritional parameters of the product.

Despite the combination with physical technologies, US was also tested in association with synthetic or natural compounds with antimicrobial activity, showing promising results against pathogenic and spoilage microorganisms (Afari et al., 2016). do Rosàrio et al. (2017) highlighted that the bactericidal impact of peracetic acid on pathogens like *S. enterica* was positively affected by US treatment, maintaining the sensory characteristics of strawberries. US, slightly acidic electrolyzed water (SAEW), and temperature (60 °C) were applied to guarantee the microbiological safety of bell pepper (Luo & Oh, 2016). Besides, the inactivation of *S. enterica* in green peppers was achieved by associating US with the addition of 1% lactic acid. Regarding to animal-based products, US treatment was paired with the addition of cinnamon essential oil to sanitise milk, and a strong biocidal effect was observed against both gram-positive (*L. monocytogenes*) and gram-negative (*S.* Typhimurium) bacteria (Mortazavi & Aliakbarlu, 2019).

A comprehensive review was recently released by Mahmoud et al. (2022), highlighting many different technologies which can be combined with US to ensure the microbiological preservation of food.

3.6. Ionizing irradiation

3.6.1. Principles

Ionizing irradiation is a physical process during which a matrix is exposed to the energy emitted by a source of ionizing radiation for a defined period of time. Irradiation treatment has been tested in many sectors, including the food industry, in which this technology is currently applied on different kind of matrices as a valuable alternative to conventional pasteurization for the microbial decontamination of foods (Deng et al., 2020). In view of that, in 1990 the FDA approved ionizing irradiation as an effective and safe biocidal technique for the treatment of fish, meat, eggs, poultry, vegetables, fruits, cereals, spices, and sprouts, either packed or in bulk (Ramaswamy et al., 2019).

The process of irradiation can be performed by applying three alternative types of radiation: gamma ray (γ-ray), electron beam (e-beam), or Roentgen ray (X-ray) (Bahrami et al., 2020). γ-rays are emitted during the decay of radioisotopes such as cobalt-60 (^{60}Co) or cesium-137 (^{137}Cs). The e-beams are generated by means of particle accelerators, and aimed at specific targets. X-rays are emitted within linear accelerators or Rhodotron-style accelerators when high energy electrons strike a metal plate (Aaliya et al., 2021). In particular, γ-rays possess an enhanced penetration ability through solid materials such as plastics, glass, and paper. This feature makes γ-rays the most applied ionizing energy source in food processing (i.e., post-packaging decontamination technology).

According to the applied dose of radiation, the industrial application of gamma irradiation can be distinguished in radurization (0.1-2.5 kGy), radicidation (3.0-10.0 kGy), and radappertization (> 10 kGy). In particular, radurization and radicization were shown to effectively inactivate pathogenic and spoilage microorganisms without causing alterations and damages of the food properties (do Rosàrio et al., 2020).

3.6.2. Antimicrobial mechanism

The main mechanism by which ionizing radiations induce inactivation and/or destruction of vegetative cells and spores is known as radiolysis. The exposed microorganisms undergo direct damage of the DNA, compromising their ability to replicate (Fig. 6). Additionally, radiolysis generates reactive molecules (hydroxyl radicals, hydrogen peroxide, atomic hydrogen) which corrupt the cellular metabolic pathways, leading to cellular oxidation and lysis (Ziuzina et al. 2018). This degradation process may involve a wide range of organic molecules, which makes this technology able to inhibit the germination of spores, disinfest insects and parasites, delay of ripening, and reduce the incidence of mycotoxins and mycotoxigenic fungi, besides the destruction of non-spore-forming bacteria (Bahrami et al., 2020).

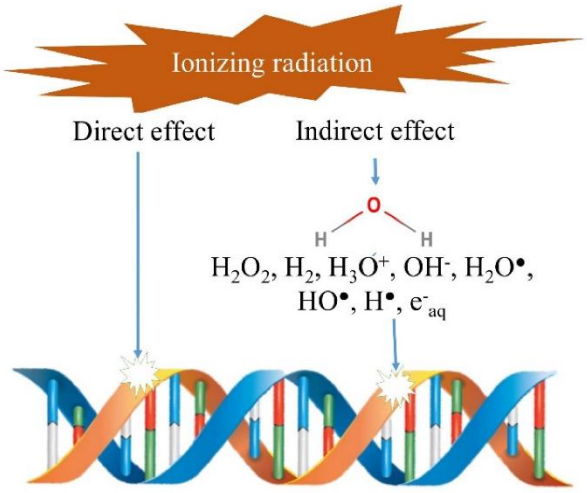


Fig. 6. Schematic representation of the effect of ionizing radiation over DNA (Munir et al., 2020)

3.6.3. Factors affecting processing efficacy

The biocidal efficacy of ionizing radiation relies on many factors, including radiation source and dosage (i.e., intensity x time treatment), intrinsic resistance and physiological state of the microbial strain, the food composition, and the environmental conditions.

In general, microbial inactivation rates tend to increase with an increase of irradiation dose level (do Rosàrio et al., 2020; Deng et al., 2020). As an example, when paprika was treated with γ-ray at doses of 1, 5, and 10 kGy, the aerobic mesophilic count was reduced by 1.76,

2.13, and 3.93 log CFU/g, respectively (Molnàr et al., 2018). Similarly, Song, Kim, & Kang (2019) observed that γ-ray irradiation at 0.5, 1.0, and 3.0 kGy reduced the *E. coli* population by 0.99, 1.50, and 4.32 log CFU/g, respectively. This effect is due to the fact that, at low doses of irradiation, specific reaction processes are activated in the microbial cell, triggering enzyme systems that may lead to cell restoration and hereditary mutations. At high doses, the cell metabolism is rapidly and significantly impaired, which leads to its destruction (Slavov et al., 2019).

In-depth investigations were conducted to describe the specific sensitivity of the main microbial classes to ionization, comparing them with each other. These studies highlighted that the prokaryotes are more resistant than eukaryotes, as well as Gram-positive bacteria resulted less susceptible than Gram-negatives (Deng et al., 2019). Song et al. (2014) demonstrated that *S.* Typhimurium resulted more resilient to ionization than *E. coli*, but their intrinsic sensitivity was significantly affected by the type of food matrix. Vegetative cells are more susceptible to radiation than spores (Slavov et al., 2019). Besides, other key-factors are represented by the size of the microorganism (generally, the smaller the target organism, the more resistant it is), their initial concentration (inversely related with the treatment efficacy), and the relative "age" of the cells (Ramaswamy et al., 2019).

The authors showed that the efficacy of the process also depends on the water content and the composition of treated foods. As shown in different studies, radiolysis is triggered by the action of ionizing irradiation on the liquid water of food. This process generates free radicals with enhanced oxidative effect, which contributes to microbial inactivation but can also trigger undesirable physicochemical changes of the product (when applied to high-fat products). When applied on frozen foods, the radiolysis process is partially hindered, thus reducing the antimicrobial efficacy of the process. Therefore, the quantity and state of water defines the maximum radiation dose that can be applied to reduce microbial load, avoiding detrimental effects on the product. This mechanism also explains the possibility to apply up

to 30 kGy irradiation on dry products like aromatic herbs or spices, which possess low water content. In fact, low moisture content generally improves the microbial tolerance against irradiation. Nevertheless, it also reduces the susceptibility of the products to oxidation (do Rosàrio et al., 2020).

The presence and concentration of lipids represent other critical factors defining the food tolerance to irradiation. In fact, polyunsaturated fatty acids may undergo oxidative reactions even at low irradiation doses (i.e., peroxidation-induced rancidity). For this reason, some products such as milk and other high-fat products are not suitable for irradiation (Priyadarshini et al., 2018).

3.6.4. Applications in the food sector

The inactivation of pathogenic and spoilage microorganism, and shelf-life extension are among the most common applications of ionizing irradiation in the food sector (Bahrami et al., 2020). The use of irradiation is well documented for spices and aromatic herbs, which represent a high-value commodity traded around the world. The quality of these products is usually threatened by the presence of sporulating (e.g., *Clostridium perfrigens; B. cereus*) and not-sporulating bacteria (e.g., *Salmonella* spp.), as well as molds. Another critical issue is related to the presence of mycotoxins. Among the first results obtained in this field, Sharma, Ghanekar, Padwal Desai, & Nadkarni (1984) reported that the use of γ-irradiation (7.5–10.0 kGy) was sufficient to decontaminate cardamom, pepper, and nutmeg with a starting microbial load of 2–7 log CFU/g. More recently, similar results were reported by Duncan et al. (2017) black peppercorn, cumin seed, and oregano. In this study, the authors also observed a minimal impact of the treatment on the sensorial properties of the products. The application of e-beam irradiation (2.5 kGy) resulted in 3.2 log reduction in the total number of aerobic bacteria of bay leaves (Gryczka, Migdał, & Bułka, 2018).

The efficacy of ionizing irradiation was also assessed on fresh vegetables. e-beam treatment was found to reduce the *Salmonella* Poona population on cucumber slices by 4.96 log CFU/

g (Joshi, Moreira, Omac, & Castell-Perez, 2018). As well, Mohácsi-Farkas, Nyirő-Fekete et al. (2014) reported that *L. monocytogenes* and *L. innocua* counts in carrots and fresh-cut tomatoes were reduced by almost 2 log CFU/g through γ-rays treatment (1 kGy), with no re-growth during the following storage period. In all this studies, the impact of the irradiation treatments on the nutritional properties of the products was negligible.

Similar results were reported for animal-derived products. As an example, high inactivation rates (6.65-7.56 logs, according to the specific serovar) in *L. monocytogenes* counts were reported for blue swimming crab lump meat when treated with 4-6 kGy irradiation (Suklim, Flick & Vichitphan, 2014). In the same study, the authors highlighted that, under lower irradiation doses (1-2 kGy), a partial recovery of injured *L. monocytogenes* was observed during the following storage period.

The impact of irradiation on packaging materials integrity is still subject of debate. Some reports argue that radiation may react with packaging polymer, and may produce low molecular harmful radiolytic hydrocarbons which could migrate into the product (Priyadarshini et al., 2018). Hence, future in-depth investigations will be necessary to furtherly investigate this phenomenon, and thus the suitability of the different packaging materials for this technology.

3.6.5. Advantages and limitations

Ionizing irradiation provides advantages of proven efficacy, being environmentally-friendly, and time effectiveness (Deng et al., 2019). The process takes less energy to inactivate microorganisms without increasing the temperature of food product. For this reason, it causes minimal modification in the color, flavour, nutrients level, taste, and other quality attributes of food (Priyadarshini et al., 2018). Unlike UV rays (which will be highlighted in the following chapter), ionizing rays possess a high penetration ability through solids such as glass, plastic, paper, and foods. Hence, irradiation can be applied on already packaged or

ready-to-eat products, allowing the reduction of microbial load and preventing the cross-contamination during the food processing (do Rosàrio et al., 2020).

Despite the evident advantages of ionizing radiations as non-thermal decontamination process, they include certain limitations. The first constraints are strictly technical, since the free radicals produced during irradiation (especially when high irradiation doses are required, such as spore inactivation) may cause the oxidation of nutrients such as lipids and vitamins, colour changes of the product, and formation of off-flavours in specific products. For example, irradiation was found to decrease the total carotenoid and tocopherol contents in paprika by 54.8–62.7% and 23.1–39.8%, respectively, and also the color and volatile aroma of the product were deeply affected (Molnàr et al. 2018). In addition, irradiation may induce the mutagenesis and selection of resistant pathogenic strains, while sub-inhibitory doses could stimulate the sporulation, growth and secondary metabolism of fungi. Lastly, the high penetrating ability of γ-rays makes them dangerous for people Therefore, this requires the use of shielded facilities and well-trained personnel for the proper handling of the irradiation equipment, which increase the capital costs of the technology (Slavov et al., 2019).

Another drawback is related to the lack of acceptance of consumers, related to health and safety (Denoya et al., 2021).

3.6.6. Ionizing irradiation-based hurdle approach

Low-dose irradiation along with other preservation treatments including freezing, addition of antimicrobial agents, and modified atmosphere packaging (MAP) aided to achieve satisfying results, comparable to those obtained by applying severe irradiation treatments alone (Khan et al., 2017). These synergistic combinations were mainly applied to treat fresh and processed meat, poultry, seafood, and vegetables.

The combined effects of γ-rays treatment and freezing on tiger prawn and fresh water prawn were evaluated. The results confirmed that low-dose irradiation (2.5-5 kGy) effectively

preserved the visual quality, mechanical attributes, and improved the microbial safety of the prawns during long-term storage (Mahto, Ghosh, Das, & Das, 2015). A synergetic inhibitory impact against *L. monocytogenes* was observed in ready-to-eat ham when γ-irradiation was applied in combination with nisin, oregano EO, and cinnamon EO. In this case, the total bacterial count was reduced under detectable levels, and the shelf life of the product was extended up to 28 days (Huq, Vu, Riedl, Bouchard, & Lacroix, 2015). More recently, Olanya, Niemira, Cassidy, Boyd, & Uknalis (2020) assessed the efficacy of a novel and promising sterilising protocol on fresh lettuce and carrots. This treatment consisted of *Bdellovibrio bacteriovorus* inoculation on the product, followed by ionization at mild levels (0.25-1.0 kGy). As a result, the combined treatment induced 0.9-2.6 and 0.4-3.9 log reductions on Salmonella and *E. coli* populations, resulting in a synergistic effect.

Another cutting-edge innovation in this field, namely the concurrent application of ionizing irradiation with active packaging to eliminate pathogens and assure food safety, has been recently reviewed by Fallha et al. (2023). Specifically, this brand-new combination of technologies was found to exert either synergistic or cumulative effects according to the treatment conditions, the intrinsic properties of the packaging solutions, and the characteristics of the treated products.

3.7. Ultraviolet irradiation (UV)

3.7.1. Principles

Ultraviolet (UV) light comprises the region of electromagnetic spectrum ranging between 100 and 400 nm. According to the wavelengths range, UV light can be classified as UV-V (100-200 nm), UV-C (200-280 nm), UV-B (280-315 nm), and UV-A (315-400 nm) (Sanchez-Maldonado, Lee, & Farber, 2018). UV light represents a non-ionizing electromagnetic radiation, widely employed as an effective alternative for thermal and chemical decontamination processes.

The conventional sources for emitting continuous UV light on laboratory and industrial scales are low-pressure mercury (LPM) lamps, medium-pressure mercury (MPM) lamps, low-pressure amalgam (LPA) lamps, and excimer lamps (do Rosàrio et al., 2020). Among these sources, LPM and MPM lamps, the most widespread ones for industrial applications, have different criticisms. In particular, these lamps are usually bulky and fragile, have a low life span, and require specific disposal due to their toxicity. Hence, novel UV emitting sources based on light emitting diode (LED) technology have recently gained attention for decontaminating processes (Josewin, Kim, & Yuk, 2018). LEDs are semiconductive diodes which produce light through electroluminescence principle. These diodes emit radiations with wavelengths at a narrow bandwidth, related to the nature of the employed semiconductive material. Recent researches were conducted in order to develop LEDs with radiating spectrum in the range 100-380 nm, defined as UV-LEDs (Hinds, O'Donnell, Akhter, & Tiwari, 2019). These LEDs can be used to produce either continuous or pulsed light, giving the opportunity to replace both conventional and xenon lamps for commercial applications.

3.7.2. Antimicrobial mechanism

The germicidal efficiency of UV light against prokaryotic and eukaryotic microorganisms is due to the ability of UV photons to induce a complex of chain reactions within the cell structure leading to DNA damage (Kaavya et al., 2021) (Fig 7). In particular, UV absorption promotes the formation of bonds between adjacent thymine bases, forming dimers. The thymine dimers block DNA replication and transcription, affecting the metabolic functions of the microorganism and causing its death. The maximum microbial inactivation rate is reached using light in the UV-C region, since the absorbance of DNA (around 254 nm) aligns with the emitted wavelength (Green et al., 2018).

Along with its own antimicrobial effect, UV light can be employed to trigger a non-thermal process known as photodynamic inactivation (PDI) mechanism (Corrêa et al., 2020). PDI relies on a series of simultaneous photochemical and photophysical interactions which

involve oxygen, light at specific wavelengths, and a non-toxic photosensitizer (Hamblin, 2016). Photosensitizers are light-sensitive molecules, which can be either naturally present (e.g., intercellular porphyrin) or supplied externally. When the cells are exposed to light at specific wavelengths, the photosensitizers absorb it and move to a higher energy state. While returning to their ground energy state, photosensitizers collide with the cytoplasmic molecules and release their energy. This process, conducted in presence of O_2, results in the formation of ROS such as peroxides, superoxide ion, and singlet oxygen. These compounds accomplish a cytotoxic effect by reacting with proteins, lipids, and nucleic acids (Du, Prasad, Ganzle, & Roopesh, 2020).

UV irradiation has also been considered as a potential effective tool to degrade the mycotoxins and inhibit their production by molds into low moisture foods like cereals and nuts. In particular, aflatoxin B_1 (AFB_1) was shown to absorb UV light in the range 220-370 nm, with a maximum of absorption at 362 nm. It was demonstrated that UV light affects the structure of the terminal furan ring and interacts with the active binding sites of AFB_1, hindering its toxicity (Hojnik, Cvelbar, Tavčar-Kalcher, Walsh, & Križaj, 2017).

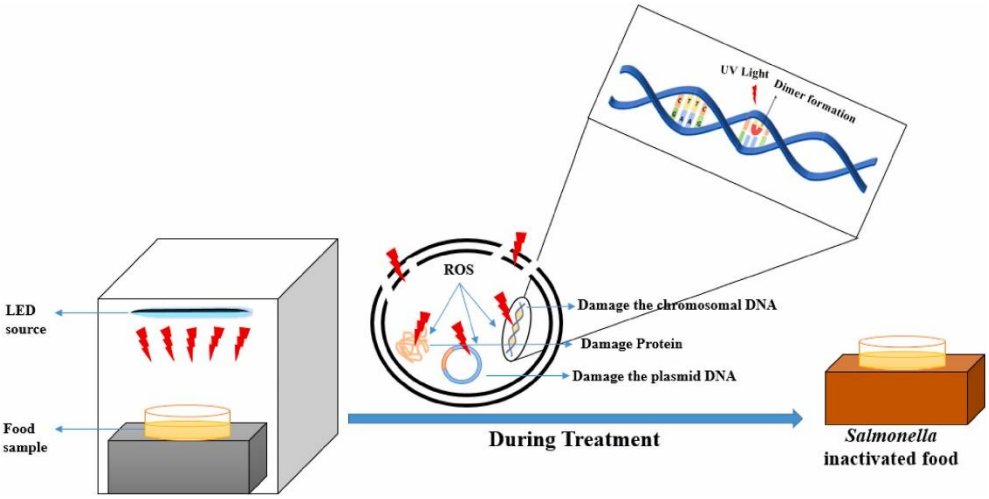


Fig. 7. Schematic representation of the effect of UV light on food contaminated by *Salmonella* (Kaavya et al., 2021).

3.7.3. Factors affecting processing efficacy

The disinfection efficacy of UV light depends on multiple parameters, including wavelength and intensity of emission, type of emitting source, O_2 concentration, exposure time, microbial strains and load, and properties of the product (e.g., composition, thickness, geometry, surface structure) (Lopez et al., 2018).

The inhibition rate of UV-C commonly increases with an increase of UV dose (i.e., the amount of UV energy applied to a specific surface over a given time). As an example, when tomatoes were exposed to UV-C light with a dose of 1.2, 2.4, and 4.8 kJ/m^2, *E. coli* O157:H7 was reduced by 2.70, 3.05, and 3.44 log CFU/fruit, respectively (Mukhopadhyay et al. 2014). As well, the population of *S.* Typhimurium in coconut water was reduced by 3.8, 5.2, and 6 log CFU/mL with UV-C treatment for 3.5, 7, and 10.5 min, respectively (Beristaìn-Bauza et al., 2018). Similar results were also observed by Dogu-Baykut & Gunes (2018) for the inactivation of total aerobic mesophilic bacteria in thyme. A greater lethal efficacy is achieved at wavelengths closer to the peak of DNA absorption (200-250 nm). For example, the *A. flavus* growth was inhibited by 92.53% and 87.37% when exposed to UV at wavelengths of 240 and 365 nm for 120 min (Hussein, Tuama, & Ali, 2015). Besides, the location of food with respect to the UV source is also important, since the action is mostly effective when the samples are positioned directly under the germicidal lamps.

Concerning the target microorganisms, the intrinsic microbial characteristics represent an important parameter for the process efficiency, as sensitivity to UV-C light varies considerably among different types of microorganisms, species, and strains. Gram-positive bacteria are usually more resistant to UV-C than Gram-negative bacteria due to the presence of a thick outer peptidoglycan layer, which hinders the penetration of UV photons within the cell. Yeasts possess large sized cells and lower pyrimidine content in the genetic material, which is associated to a higher resistance to UV compared to bacteria. For example, Ochoa-Velasco et al. (2018) observed reduction ranges of 7.54–7.79, 5.96–7.32, and 3.12–4.46 log CFU/mL for *L. rhamnosus*, *S.* Typhimurium, and *S. cerevisiae* in coconut

water when subjected to UV-C treatment for 10 min, respectively. Bacterial spores are more resistant to UV-C than vegetative cells, mainly due to the dehydrated state of the core, which obstructs the pyrimidine dimerization process. In addition, the physiological state of cells (i.e., their growth phase; growth conditions; stressors before the UV treatment; cell recovery after processing) and the initial inoculum concentration in food can also affect the antimicrobial efficacy of UV-C irradiation.

Lastly, the UV-rays antimicrobial performance strongly depends on the raw material to be treated, due to their low penetration ability (Slavov et al., 2019). The ability of UV light to penetrate solid foods relies on several factors, including thickness, shape, optical properties, density, color, roughness, and dirtiness. The effect of shape on the UV-C treatment efficacy was clearly described by Byun et al. (2020) for the inactivation of *Aspergillus flavus* and *Aspergillus parasiticus* in roasted coffee beans. Nevertheless, UV-C treatment is universally considered as a surface treatment for solid products (e.g., food; packaging; tools). Similarly, the efficacy of UV-C for the decontamination of liquid foods strongly relies on their color components, solids in suspension, and soluble compounds (i.e., turbidity), which may reduce the number of photons available to kill microorganisms by reflecting, absorbing, and scattering incidental light.

3.7.4. Applications in the food sector

The enhanced versatility of this technology prompted researchers and industries to extend its applications from water and water-like transparent fluids to liquid foods like tea, milk, coffee, vegetal juices, and sugar syrups (Koutchma, 2016).

More recently, UV irradiation efficacy was tested on different kind of solid food products including fresh and minimally processed fruits and vegetables (Bermùdez-Aguirre, 2018), meat (Corrêa et al., 2020). Both low moisture foods (Chen et al., 2019) and high moisture foods (Ochoa-Velasco et al., 2018) have been taken into account, with diverse impact of UV-C irradiation on the microbiological quality and physicochemical aspects of the products

according to the treatment conditions and the specific features of the products. Overall, all the studies stated the potential of UV light in inactivating pathogenic and spoilage microorganisms such as *S. aureus*, *E. coli*, *S.* Typhimurium, *L. monocytogenes*, *L. innocua*, *Pseudomonas* spp., and many others.

Some studies assessed the diverse efficacy observed between continuous and pulsed UV light treatment. As an example, Huang & Chen (2019) compared the activity of these technology for the inactivation of *Salmonella* population in fresh fruits and vegetables. Specifically, *Salmonella* count was reduced from 5.4 to 4.4 logs in tomatoes, 3.1 to 1.9 logs in lettuce, and 5.7 to 4.5 logs in blueberries. In this case, pulsed light and continuous light were found to perform similar effects on the microbial population of fresh products. Another interesting study conducted on wheat flour by Subedi et al. (2020) suggested that the impact of UV-C irradiation on the microbial population (i.e., *Salmonella*) strongly differ according to the wavelength of irradiation. In particular, the highest inactivation rate (3.67 log reduction) was observed after a UV-treatment 395 nm for 60 min, with significant differences with respect to the other tested wavelengths (i.e., 275, 365, and 455 nm). However, a significant change in the color of treated wheat flour was also observed in this study.

Concerning packaging, UV treatment may also be used in packaged products, but under a specific condition: the packaging material needs to be transparent to the applied wavelengths of irradiation. In this sense, a study about the impact of UV-C on various foodborne pathogens in packaged cheese surfaces suggested that transparent plastics such as polypropylene (PP) and polyethylene (PE) films in conjunction with UV-C irradiation could ensure the prevention of post-processing contamination (Ha, Back, Kim, & Kang, 2016).

3.7.5. Advantages and limitations

The application of continuous UV irradiation in the food sector represents a promising approach due to its broad spectrum of lethality, low cost, energy efficiency, easy setup and

maintenance, harmlessness, no residues generation, and minimal loss of sensory and nutritional quality of the food products (Deng et al., 2020).

Despite the advantages, UV treatment has specific hindering factors, mainly related to its limited penetration ability and the complex surface structure of some products, which creates the so-called “shade effect”. Pathogens and spoilage microorganisms can reside in wounds, crevices, and pores, being protected from UV light. Besides, light-sensitive nutritional compounds like unsaturated vitamins, fatty acid, and pigments can be altered by UV exposure, along with the organoleptic characters of food (Deng et al., 2020).

3.6.7. UV-based hurdle approach

In order to overcome the issues related to the single UV-C treatment (e.g., low microbial inactivation rates; adverse impact on product quality), different researchers suggested the opportunity to apply UV in combination with other technologies like mild heat treatment, cold plasma, and modified atmospheric packaging, causing a hurdle effect.

Concerning the first cited combination, Cheon, Shin, Park, Chung, & Kang (2015) investigated the efficacy of UV-C irradiation (20.4 kJ/m^2) in simultaneous combination with mild heating treatment (65 °C; 10 min) to inactivate *E. coli* and *S.* Typhimurium on powdered red pepper. When UV-C irradiation was applied alone, the *S.* Typhimurium and *E. coli* populations were reduced by 0.29 and 0.22 log CFU/g, respectively. Conversely, the addition of heat treatment decreased the surviving numbers of the pathogens by 3.06 and 2.88 log CFU/g, respectively. In this case, the combination of the two technologies exhibited a synergistic effect on the microbiological quality of red pepper, with minimum impact on the quality parameters. Similarly, UV-C irradiation and mild heat treatment exhibited a synergistic effect for the disinfection of pathogenic and spoilage bacteria including *S.* Typhimurium, *E. coli*, *Cronobacter sakazakii, L. innocua*, and *Bacillus* spp. (Fan, Huang, & Chen, 2017). Recently, Fenoglio, Ferrario, Schenk, & Guerrero (2020) suggested an alternative method to sanitise fruit juices under mild processing conditions. Clear pear juice,

turbid orange-tangerine juice, and orange-banana-mango-kiwi-strawberry juice were subjected to UV-C irradiation (390 mJ/cm^2) assisted by mild heating (50 °C) in a coiled tubing unit, and then stored at refrigeration condition (5 °C). Again, a synergistic bacterial inactivation was observed, attaining 5.5–6.7, 5.2–5.6, and 6.3–6.6 log reductions in pear, orange-tangerine, and orange-banana-mango-kiwi-strawberry juices, respectively.

UV-C irradiation was also tested in association with refrigeration and/or modified atmospheric packaging (MAP), showing a relevant increase of the product shelf life (e.g., fruits and fruit-derived products) and the effective reduction of bacterial population.

Kim, Tang, Bang, & Yuk, (2017) investigated the impact of LED UV treatment (405 ± 5 nm) on different serovars of *Salmonella* and the physicochemical properties of fresh-cut mango at different storage temperatures (4, 10 and 20 °C). The authors observed that, in all cases, the reduction rates were boosted at chilling temperatures (4 and 10 °C) with respect to environmental temperature (20 °C). As well, Choi, Park, Choi, Kim, & Chun (2015) demonstrated the synergistic effect of sequential UV-C irradiation (2 kJ/m^2), MAP (5.3% CO_2 + 5.5% O_2), and chilling temperatures for the inactivation of *S.* Typhimurium in cherry tomatoes. The study revealed that the count of *S.* Typhimurium in packaged cherry tomatoes, when subjected to UV-C irradiation, was significantly reduced during 9 days of storage at 4–8 °C.

Some researches were performed washing the products (mainly fruits or vegetables) with water or diluted antimicrobial substances followed by UV-C irradiation. Studies conducted on berries (Cao, Huang, & Chen, 2017) and tomatoes (Guo, Huang, & Chen, 2017) demonstrated that water-assisted UV-processing, which combined washing in turbulent water with UV light, had better biocidal performances than dry UV treatment, regardless of the inoculation method and the storage conditions. Moreover, the addition of antimicrobial substances during washing furtherly improved the efficacy of this hurdle approach (Leng et al., 2020).

Recently, different studies investigated the impact of UV-C irradiation combined with chemical preservatives on the microbiological quality of poultry meat. For example, Possas et al. (2021) evaluated and modelled the combined effect of UV-C irradiation (0-15 J/cm^2) and caffein (0–20 mM/g) on *E. coli* counts on raw chicken breast fillets. *E. coli* inactivation rates were found to grow by increasing both UV-C doses and caffeine concentrations. Reductions over 5 log CFU/g were observed in caffeine-free samples at UV-C doses higher than 12 J/ cm^2, while the pre-treatment of samples with caffein (20 mM/g) resulted in undetectable levels of the tested pathogen after UV-C irradiation at doses higher than 6 J/cm^2. Adopting a similar approach, Byun et al. (2022) evaluated the efficacy of peroxyacetic acid (50–500 µg/mL) or lactic acid (0.5–2.0%) treatment followed by UV-C irradiation (5-10 min) against *S. enteritidis* biofilms on food contact surfaces (i.e., stainless steel, silicone rubber, and ultra-high molecular weight polyethylene) and chicken skin. The results showed that the combination of peroxyacetic acid with UV-C reduced the *Salmonella* population by 3.10–6.41 log CFU/cm^2, while lactic acid with UV-C induced a 3.35–6.41 log (CFU/cm^2) reduction on surfaces. *S. enteritidis* biofilms on chicken skin was reduced by around 2 log CFU/g through both the combined treatments, without affecting the color and texture of the product. Once again, the mutual synergy derived from the treatments' interaction was observed.

4. Future challenges

Non-thermal techniques and hurdle technology represent fascinating trend areas of research. Novel combinations of preservation technologies are constantly tested on laboratory or pilot scale to maximise their technological performances, ensuring the safety of food products without compromising their quality aspects. Despite that, perfecting the hurdle approach still involves notable challenges and requires efforts from the academic research and industries in view of their massive application on industrial scale (Aalyia et al., 2021).

- In-depth investigations highlighting the microbial mechanisms of stress reaction, adaptation and “cross-tolerance” (both generic and specific of the single strains) are strongly required to overcome the actual limitations of this approach;
- The combination of hurdles might have an additive, synergistic or an antagonistic effect compared to a single hurdle. Thus, it is extremely important to assess the uncertainty related to combining different preservation techniques;
- Monitoring and validation of microbial inactivation mechanisms and rates by each hurdle approach represents a central area of interest, to maximise the efficacy of the treatment and to avoid the generation of undesired chemical reactions;
- Implementation of tools such as predictive microbiology and data integration from genomics, metabolic pattern recognition, and protein expression could be used to predict the microbial activity in food and clarify the response of microbial cells, helping to set-up a suitable combination of treatments for each specific case study;
- Nowadays, most of the hurdle approaches are expensive and time-consuming because of its high set-up cost and the need for trained personnel. Cost-effectiveness of hurdle technologies should be taken into account in future studies;
- The lack of regulations regarding hurdle technology is a major limitation for establishing the real potentialities of this approach. However, due to the promising results obtained on research and pilot scale, official standards regulating the application of this novel approach are keenly expected in the next future.

5. Concluding remarks

The adoption of adequate control tools throughout the food production chain represents the key-factor to supply safe, high-quality foods to the final consumers. In this sense, non-thermal techniques and their synergistic combination represent a prospective way to counteract the spread of harmful microorganisms in the food matrices, thus minimizing the impact of each technology on the quality characters. In fact, since each technique exerts a

different antimicrobial mechanism on the microbial cell, the combination of different treatments at mild levels leads to multiple sublethal stresses on bacterial cells, which force the bacteria to disburse their energy for overcoming stressful conditions, resulting in metabolic exhaustion and death.

Overall, all the cited studies are fundamental, since the constant progress of research is the propulsion engine that will drive the change in food technology, paving the way for novel, more efficient, and more sustainable strategies for food decontamination. Nevertheless, it is good to remember that the true key to ensure and maintain the safety of food is the awareness and responsibility of each person that comes along, from the fields to the table.

Author contributions

Francesco Bigi: Conceptualisation, Investigation, Writing - Original draft preparation; **Enrico Maurizzi:** Conceptualisation, Reviewing, Tables preparation; **Andrea Quartieri:** Methodology, editing administration; **Riccardo De Leo:** Reviewing, editing; **Maria Gullo:** Reviewing; **Andrea Pulvirenti:** Resources, Supervision and Reviewing.

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Table 1

Advantages and limitations of the main non-thermal technologies for food decontamination

Technology	Advantages	Disadvantages	References
Ozone	No-thermal energy required; no harmful compounds; short contact period; low running cost	Corrosive; Degradation by-products; Oxidation	Chakka et al. (2021); Temba et al. (2016)
Atmospheric Cold Plasma	Low energy requirement; no solvents; clean-label; no harmful compounds	Not suitable for high-fat foods; Degradation of fruit quality	Liao et al., (2020); Misra & Roopesh (2019); Qiu et al. (2019)
High-Pressure Processing	Reduced nutrient loss, minimal heat impact, short time; unaltered organoleptic properties	Ineffective on low acid, shelf stable products; possible impact on food macromolecules; initial and maintenance costs of the plant	Aaliya et al. (2021); Qiu et al. (2019); do Rosàrio et al. (2020); Tappi et al. (2020)
Pulsed electric field	Minimum increase of product temperature; retainment of nutritional and organoleptic qualities	High costs of set-up and maintenance; dependence on the electrical conductivity of the product	Arshad et al. (2021); Rifna et al. (2019)
Ultrasounds	Effectiveness; non thermal energy; no solvents; no harmful compounds produced	Difficult development of ultrasound customised equipment; ambiguous effect on the product quality	Azam et al. (2020)
Ionizing irradiation	High antimicrobial efficacy; low effect on the product	Oxidation of nutrients; mutagenesis and selection of resistant pathogenic strains; large capital costs; lack of acceptance from customers	Denoya et al. (2021)
UV-C irradiation	Broad spectrum of lethality; low cost; energy efficiency; easy setup and maintenance; harmlessness; no residues generation; minimal loss of quality	Limited penetration ability; alteration of photo-sensitive nutritional compounds	Deng et al. (2020)

Table 2

Recent hurdle approaches based on non-thermal technologies for food decontamination – practical examples (2014-2022)

Technology	Combined with	Target microorganism	Application	Reference
Ozone (aqueous or gaseous)	**UV-C irradiation**	*E. coli*	Flour slurries	Jewsuwan & Thipayarat (2015)
		E. coli,	Flowing water	Hernàndez-Arias et al. (2019)
		E. coli *Salmonella*	Fresh beef	Yang, Kalchayanand, Belk, & Wheeler (2019)
		E. coli	Black pepper	Dogu-Baykut & Gunes (2022)
	UV-C irradiation and hydrogen peroxide	*Salmonella*, *L. monocytogenes*, *Aspergillus, Penicillium*	Lemons	Hasani et al. (2019)
	UV-C irradiation and infrared	Mesophilic bacteria	Dried vegetables	Watson, Kamble, Shanks, Khan & El Darra (2020)
		E. coli	Onion flakes, black pepper	El Darra, Xie, Kamble, Khan, & Watson (2021)
	Ultrasounds	Mesophilic bacteria Molds and yeasts	Cherry tomato	Mustapha et al. (2020)
		E. coli *Salmonella*	Lettuce	Sun, Wu, Zhang & Wang (2022)
	High-Pressure Processing	*S. cerevisiae* *L. innocua*	Pitaya juice	García-Mateos et al. (2019)
		Enterobacteriaceae *Pseudomonas*	Catfish fillets	Ling et al. (2022)
	Pasteurisation	Mesophilic bacteria	Milk	Varga & Szigeti, 2016
	Lyophilization	Mesophilic bacteria	Chicken breasts	Cantalejo, Zouaghi & Pérez-Arnedo (2016)
	Lactic acid	*E. coli* O157:H7	Fresh-cut lettuce	Wang et al. (2019)
	Electrolyzed water	*E. coli*	Goat meat	Degala, Scott, Nakkiran, Mahapatra, & Kannan (2016)
	MAP (carbon monoxide)		Beef	Lyu et al. (2016)
Atmospheric Cold Plasma	**Ultrasounds**	*S. aureus*	Saline solution	Liao et al. (2018)
		Pseudomonas *Enterobacteriaceae*	Silver Pomfret	Esua, Sung, Chen & Li (2022)
	Ultrasounds, peracetic acid/chlorine	*E. coli* *S.* Typhimurium	Blueberry	Wang &Wu (2022)
	UV-C	*L. innocua* *L. monocytogenes* *E. coli* *S. aureus* *S. enteritidis*	Smoked salmon	Colejo, Alvarez-Ordònez, Prieto, Gonzalèz- Raurich & Lopèz (2018)

		S. Typhimurium *Plesiomonas shigelloides* *Aeromonas hydrophila*		
		Bacillus tequilensis Mesophilic bacteria	Peppercorns	Bang, Kim, Lee & Min (2020)
	Ionizing irradiation	*E. coli*	Raw beef	Stratakos & Grant (2018)
	Hydrogen peroxide	*S.* Typhimurium, *L. innocua*	Grape tomatoes, apples, cantaloupe, romaine lettuce	Song & Fan (2020)
	Peracetic acid	*S.* Typhimurium	Raw poultry meat	Chaplot et al. (2019)
	Vitamin C			Liao et al. (2020)
	Refrigeration	*S.* Typhimurium	Ham	Lis et al. (2018)
	MAP (20% O_2, 40% N_2, 40% CO_2)	*L. monocytogenes*	Ready-to-eat ham	Yadav et al. (2020)
	MAP (65% O_2, 30% CO_2, 5% N_2), citric	*L. monocytogenes* *E. coli*	Tender-coconut water	Mahnot, Mahanta, Farkas et al. (2019)
High-Pressure Processing	**Ultrasounds**, thermal treatment	*Alicyclobacillus acidoterrestris* spores	Orange juice	Evelyn & Silva (2016b)
	Refrigeration, ascorbic acid	*S. enterica,* *L. monocytogenes*	Cantaloupe puree	Mukhopadhyay et al. (2016)
	Refrigeration		Seabass and seabream fillets	Tsironi et al. (2019)
	Freezing	*Salmonella*	Frozen chicken breast fillets	Cap et al. (2020)
	Mild heat	*Bacillus cereus* spores	Beef slurry	Evelyn & Silva (2016a)
	Pasteurization, germinant	Endospores	Cream cheese	Song et al. (2023)
	Virulent bacteriophages	*Shigella flexneri* *Vibrio cholerae*	Ground beef Sea-food	Ahmadi, Anany, Walkling-Ribeiro, & Griffiths (2015)
	Virulent bacteriophages, pediocin	*L. monocytogenes*	Milk	Komora et al. (2020)
		L. monocytogenes	Fermented meat sausage	Komora et al. (2021)
	MAP (100% CO_2)	Total bacterial count	Desalted cod	Rode & Rotabakk (2021)
		Total bacterial count *L. monocytogenes* *C. jejuni* LAB	Chicken breast slices	Dang, Rode, and Skipnes (2021)
	Edible packaging (sodium alginate films + probiotic bacteria)		Ham slices	Pavli et al. (2017)

	Edible packaging (chitosan films + rice bran extract/nisin)	*L. monocytogenes*	Sliced dry-cured Iberian ham	Martillanes et al. (2021)
Pulsed electric field	**High-Pressure Processing**	Mesophilic bacteria *Bacillus* spores *Enterobatteriaceae*	Pumpkin	García-Parra et al. (2018)
	High-Pressure Processing, MAP (70% CO_2)	Mesophilic bacteria	Coho salmon	Perez-Won et al. (2021)
	Ultrasounds	*E. coli* *A. niger* *B. pumilus*	Oil-in-water emulsions	Gomez-Gomez et al. (2021)
	Atmospheric cold plasma (Ar/Air 80:20) + soaking in Chamuang leaf extract	Total viable count Psychrophilic bacteria Enterobacteriaceae H_2S bacteria *Pseudomonas* *Clostridium perfrigens*	Prawns	Shiek et al. (2022)
	Mild heat, essential oils	*Salmonella,* *L. monocytogenes*	Liquid whole egg	Espina,et al. (2014)
	Nisin + Grapefruit extract + Cinnamaldehyde	*L. monocytogenes*	Pork loin	Zhao et al. (2020)
	Chamuang leaf extract MAP (CO_2)	Total viable count Psychrophicil bacteria Enterobacteriaceae H_2S bacteria *Pseudomonas*	Pacific white shrimps	Shiekh et al. (2020b)
Ultrasounds	**UV-C irradiation,** mild heat, natural extracts	*E. coli*	Apple and orange juice	Gabriel et al. (2015)
		Total viable count Yeasts	Fruit and vegetable juices	Khandpur & Gogate (2016)
	Ionizing irradiation	Total viable count Yeasts Molds *Pseudomonas* Enterobatteriaceae	Fresh mushrooms	Shi et al. (2022)
	Mild heat	Psychrophicil bacteria LAB	In-package sausages	Cichoski et al. (2015)
		Alyciclobacter acidoterrestris Yeasts Molds	Apple, cranberry, and blueberry juices and nectars	Jambrak et al. (2018)
	Peroxiacetic acid, sodium chloride	*Escherichia coli* *Bacillus cereus* *Penicillium expansum*	Dried figs	Gorguc et al. (2021)
	Thyme EO nano-emulsion	*E. coli*	Cherry tomato	He et al. (2021)
	Propyl gallate	*L. monocytogenes* *E. coli*	Apple juice	Nguyen, Tikekar & Nitin (2022)
	Nisin, oregano EO	*E. coli*	Raw cabbage	Takundwa et al. (2022)

Ionizing irradiation	Frozen storage	Mesophilic bacteria Molds Coliforms *Salmonella*	Fresh water prawn Tiger prawn	Mahto, Ghosh, Das, & Das (2015)
	Bdellovibrio bacteriovorus	*E. coli* *Salmonella*	Lettuce Carrot	Olanya, Niemira, Cassidy, Boyd, & Uknalis (2020)
	Edible coating (0.5% rosemary EO)	Enterobacteriaceae *S. aureus* *B. cereus* *Vibrio* spp. *Salmonella* spp.	Silver carp	Abdeldaiem, Mohammad & Ramadan (2018)
	Chitosan films (cumin EO nanoemulsion)	Mesophilic bacteria Psychrophilic bacteria Enterobacteriaceae LAB	Beef loin	Dini, Fallah, Bonyadian, Abbasvali & Soleimani (2020)
	Pectin coating (curcumin nanoparticles and ajowan EO nanoemulsion)	Mesophilic bacteria Psychrophilic bacteria Enterobacteriaceae LAB	Lamb loin	Fallha et al. (2022)
	Alginate or alginate/cellulose nanocapsules (EOs)	Mesophilic bacteria *E. coli* *L. monocytogenes* Fungi LAB	Dry fermented sausages	Ji, Allahdad, Sarmast, Salmieri & Lacroix (2022)
UV-C irradiation	Mild heat	*E. coli* *Salmonella*	Red pepper	Cheon, Shin, Park, Chung, & Kang (2015)
		Mesophilic bacteria	Fruit juices	Fenoglio, Ferrario, Schenk & Guerrero (2020)
	Refrigeration	*Salmonella*	Fresh-cut mango	Kim, Tang, Bang, & Yuk (2017)
	MAP, refrigeration	*S.* Typhimurium	Cherry tomato	Choi, Park, Choi, Kim, & Chun (2015)
	Lemongrass oil	*E. coli*	Goat meat	Degala, Mahapatra, Demirci & Kannan (2018)
	Cinnamaldehyde, refrigeration	*S.* Typhimurium	Coconut water	Beristaìn-Bauza et al. (2018)
	Chlorine, **ultrasounds**	*Salmonella*	Lettuce	Huang & Chen (2018)
	Peracetic acid, *Pseudomonas graminis* CPA-7	*S. enterica* *L. monocytogenes*	Lettuce Spinach leaves	Collazo et al. (2019)
	Vanillin and citral emulsions, mild heat	*Lactobacillus plantarum* *E. coli* *Saccharomyces cerevisiae*	Fruit juices	Ferrario et al. (2020)
	Blended sanitizers	*Salmonella*	Cherry tomato	Leng et al. (2020)
	Caffein	*E. coli*	Raw chicken breasts	Possas et al. (2021)
	Peroxyacetic acid, lactic acid	*S. enteritidis*	Chicken skin	Byun et al. (2022)

	Antagonistic yeast (*Wickerhamomyces anomalus*)	*Alternaria tenuissima*	Potato tubers	Leng et al. (2022)

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