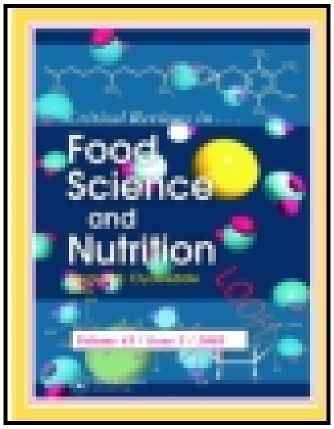

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Non-thermal Physical Technologies to Decontaminate and Extend the Shelf-life of Fruits and Vegetables: Trends Aiming at Quality and Safety

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Non-thermal physical technologies to decontaminate and extend the shelf-life of fruits and vegetables: Trends aiming at quality and safety

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Abstract

Minimally processed fruits and vegetables are one of the major growing sectors in food industry. This growing demand for healthy and convenient foods with fresh-like properties is accompanied by concerns surrounding efficacy of the available sanitizing methods to appropriately deal with food-borne diseases. In fact, chemical sanitizers do not provide an efficient microbial reduction, besides being perceived negatively by the consumers, dangerous for human health and harmful to the environment, and the conventional thermal treatments may negatively affect physical, nutritional or bioactive properties of these perishable foods. For this reasons, the industry is investigating alternative non-thermal physical technologies, namely innovative packaging systems, ionizing and ultraviolet radiation, pulsed light, high-power ultrasound, cold plasma, high hydrostatic pressure, and dense phase carbon dioxide, as well as possible combinations between them or with other preservation factors (hurdles). This review discusses the potential of these novel or emerging technologies for decontamination and shelf-life extension of fresh and minimally processed fruits and vegetables. Advantages, limitations and challenges related to its use in this sector are also highlighted.

Keywords:

food preservation, physical methods, emerging technologies, fruits and vegetables, quality and safety, food shelf-life

1. Introduction

1.1. The emergence of food preservation technologies

As a response to consumers' demand for fresh, healthy and minimally processed foods, conjoint with an altered lifestyle characterized by less time for planning and preparing convenient meals, a wide variety of minimally processed fruits and vegetables has been developed, being one of the major growing sector in food industry (Patrignani et al, 2015). In fact, consumers are more concerned about the nutritional and sensory properties of the food they eat, as well its safety (Ramos et al., 2013). However, the growing preference for minimally processed fruits and vegetables with fresh-like properties is accompanied by concerns surrounding efficacy of the available sanitizing methods to appropriately deal with food-borne diseases. These products are susceptible to microbial proliferation due to their high water and nutrients content and the loss of their natural resistance, having been repeatedly sources of foodborne illnesses. Additionally, the processing steeps of peeling, cutting or slicing favour the microbial growth due to the release of nutritive substances (Patrignani et al., 2015). Bacteria such as *Escherichia coli* O157: H7, *Salmonella* spp. and *Listeria* spp. are the pathogens most frequently linked to fruits and vegetables produce-related outbreaks, being a public health concern (Abadias et al., 2011; Drissner and Zuercher, 2014).

Fresh fruits and vegetables cannot be conveniently decontaminated using conventional thermal treatments without affecting their physical, nutritional or bioactive properties that may reduce the content or bioavailability of bioactive ingredients (Rawson et al., 2011). Besides, washing and chemical sanitizing treatments do not provide an efficient microbial reduction to afford safety consumers (Rowan et al., 2015) and are perceived negatively by the consumers, dangerous for human health and harmful to the environment (*e.g.*, sodium hypochlorite generates environmental and health risks associated with the formation of carcinogenic halogenated compounds (Escalona et al., 2014). The emergence of more resistant microorganisms to conventional food preservation techniques also increases the need for developing new decontamination processes (Abadias et al., 2011). Therefore, to meet these demands, the industry is investigating alternative non-thermal physical technologies, as well as possible combinations between them or with other preservation factors (hurdles).

1.2. Post-harvest decay of fresh produce

Fruits and vegetables are important inputs of nutrients and health-promoting compounds, including vitamins, carotenoids, minerals, fiber, polyphenols, etc. (Pereira et al., 2011; Pinela et al., 2012). After harvest, they remain living tissues and continue to carry out metabolic processes such as respiration and transpiration. Thus, from the moment they are detached from its source of nutrients, they become entirely dependent on their own organic reserves; they will consume carbohydrates, lipids, and organic acids, as well as oxygen (O_2), producing simple molecules of carbon dioxide (CO_2) and water with release of energy (Dey and Harborne, 1997). As a consequence, unwanted visual symptoms appear on the product, namely weight loss/dehydration, formation of wrinkles, and changes on colour and texture (Hodges and Toivonen, 2008).

The quality parameters of minimally processed fruits and vegetables can be affected by both internal and external factors. The internal factors represent metabolic processes and include morphological, physiological, and biochemical defence mechanisms, stress-induced senescence programs, and genotype. The external factors represent environmental situations which inhibit or exacerbate the manifestation of the internal ones; examples include processing treatments (washing, decontamination, peeling, cutting, slicing, wedging, etc.) and storage conditions (packaging system, temperature, humidity, light exposure, etc.) (Hodges and Toivonen, 2008; Tiwari and Cummins, 2013). Additionally, fresh-cut fruits and vegetables deteriorate faster than intact ones, because wounding plant cells and tissues increases the ethylene (C_2H_4) production and exacerbates water loss. The elevated C_2H_4 production stimulates respiration and consequently accelerates deterioration and senescence, and promotes ripening (Damodaran et al., 2007). Moreover, some stressful abiotic conditions may cause the *de novo* synthesis of antioxidant compounds (polyphenols, tocopherols, etc.) to fight against the reactive species that are produced under such unfavourable conditions, being a plant strategy to withstand to those situations (Munné-Bosch, 2005; Yusuf et al., 2010; Pérez-Gregorio et al., 2011). Post-harvest changes in fresh produce cannot be stopped, but they can be slowed within certain limits. Therefore, the reduced shelf-life of minimally processed fruits and vegetables requires a search for appropriated (effective and safe for health and environment) techniques to counteract the metabolic processes leading to rapid senescence, so that they can be of high quality for longer times, in order to be profitable for the enterprises dealing with them; as well to promote its decontamination and safety.

2. Non-thermal physical technologies

Different packaging systems, ionizing radiation, ultraviolet (UV) radiation, pulsed light (PL), high-power ultrasound (US), cold plasma (CP), high hydrostatic pressure (HHP), and dense phase carbon dioxide (DP-CO_2), are novel or emerging technologies that have already found application in the food industry or related sectors (**Figure 1**). An overview on the basic principles of these technologies inherent in the decontamination and shelf-life extension of fresh fruits and vegetables is presented below, as well as the main advantages, limitations and drawbacks concerning their impact on microorganisms, and quality parameters of these perishable foods. Some challenges and future needs are also identified in this paper. **Tables 1** and **2** summarize the critical parameters/variables of control, the inactivation mechanism/effect, advantages, limitations and drawbacks, and challenges inherent to the revised preservation technologies.

2.1. Packaging systems

Food packaging continues to evolve in response to the advancement of material science and technology, as well as the changing consumers' demand. In today's industrialized world, packaging not only is essential to enable effective distribution and preservation of food, but also to facilitate their end-use convenience and communication at the consumer levels (Galić et al., 2011; Mihindukulasuriya and Lim, 2014). Furthermore, the development of eco-friendly food packaging materials with improved mechanical and barrier properties has been an active area of research, in which the nanotechnology plays a fundamental role (Mihindukulasuriya and Lim, 2014; Hannon et al., 2015).

2.1.1. Vacuum packaging

Today is possible to find in the market several vacuum-packaged minimally processed fruits and vegetables. Vacuum-packaging involves removing the air from the package prior to sealing. This simple procedure reduces the O_2 level in the package and limits the growth of aerobic microorganisms, as well as the occurrence of oxidation reactions, which spoil the food (Deepa et al., 2013).

2.1.2. Modified atmosphere packaging

The use of modified atmosphere packaging (MAP) has greatly expanded during the last years and is now used around the world to extend the shelf-life of a wide range of foods, including minimally processed and ready-to-eat fruits and vegetables. After harvest, fresh produce continue respiration and transpiration processes and, since they are detached from its nutritional source, they become entirely dependent on their own reserves. MAP has the potential to delay these biological processes and the consequent rate of substrates depletion (Sandhya, 2010).

The MAP technology consists in changing the air surrounding the food in the package to a desired composition in order to slow down natural deterioration processes, reduce microbial growth and retain all attributes that consumers consider as freshness markers (Niemira and Fan, 2014). In contact with food, the O_2 promotes several types of oxidative reactions and grow of spoilage microorganisms. Thus, for fresh fruits and vegetables, desired atmospheres consist usually in lowered levels of O_2 and heightened levels of CO_2 and/or nitrogen (N_2). Noble or inert gases (including argon (Ar), helium (He), neon (Ne), and xenon (Xe)) can also be used (Char et al., 2012; Silveira et al., 2014), but the literature on their application and benefits is still limited. CO_2 has significant implications in MAP; it is high soluble in water, which leads to the production of carbonic acid and consequent increased acidity, and may also cause package collapse due to the reduction of headspace volume (Sandhya, 2010). N_2 has a low solubility in water and other food constituents and does not support the growth of aerobic microorganisms

(Sandhya, 2010). Regarding Ar, it is biochemically active, probably due to its enhanced solubility in water, and appears to interfere with enzymatic oxygen receptor sites, thus reduces the metabolic activity of the food product (Char et al., 2012). This gas has also been reported to reduce microbial growth and to improve quality of fresh produce (Jamie and Saltveit, 2002). These gases can be injected singly or in combination to balance with the metabolic activity of the product.

Once after harvest fresh fruits and vegetables remain living tissues, their interaction with the package needs to be considered to design appropriated packaging conditions, *i.e.*, the gas and moisture permeability of the packaging film needs to be adapted to the product respiration in order to establish an equilibrium MAP for shelf-life extension. The storage temperature also needs to be considered since it has influence on plant metabolic processes; both ripening and C_2H_4 production rates are proportional to the temperature increase (Luengwilai et al., 2014). Additionally, sufficient light can promote photosynthesis in green vegetables, leading to the CO_2 consumption and O_2 production, as well as to weight loss and browning (Martínez-Sánchez et al., 2011).

Although there are several studies regarding MAP, it is still necessary to evaluate the influence of different packaging atmospheres and storage temperatures on quality parameters, microbial survival and shelf-life of selected fresh commodities, as well as possible combinations with other preservation treatments such as those described below.

2.1.3. Active packaging

Active packaging (AP) plays an additional role in maintaining the quality and safety of fresh foods compared with conventional packaging systems. AP involves an interaction between package and food product in order to provide favourable conditions for quality preservation and shelf-life extension. It is designed to control food deterioration processes by incorporating active ingredients that have been deliberately included in the package film or headspace, such as scavengers for O_2, CO_2 and C_2H_4, moisture absorbers, CO_2 emitters, antimicrobials, antioxidants, preservatives, and so on (Mehyar and Han, 2010; Mihindukulasuriya and Lim, 2014). Despite being necessary to design and optimize this technology for each specific food, it is expected to be ready for implementation by the food industry soon.

2.1.4. Intelligent packaging

An intelligent packaging (IP) system can interact with internal factors (food and headspace) and/or external environmental factors through innovative devices or sensors. As a result of this interaction, the sensor will generate a response that correlate with the state of the packaged food. The information generated not only is useful for communication with the consumers by informing them about the safety and quality of the products, but can also be utilized by the producers in their decision support systems to determine when and what actions are to be taken during the product distribution chain. Examples include indicators for integrity, freshness and time-temperature, sensors for O_2 and CO_2, radio frequency identification tags and security tags (Mehyar and Han, 2010; Mihindukulasuriya and Lim, 2014; Vanderroost et al., 2014). Despite all the potential of this technology, the use of IP for fresh fruits and vegetables preservation is still at an early development stage.

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2.1.5. Smart packaging

The concepts of smart and IP are often used interchangeably in the literature, but they are not the same. Smart packaging is an immature technology that could potentially be applied in the context of IP in the far future, being therefore a major challenge for the coming times. Smart packaging consists in the combination and integration of AP and IP concepts in one packaging system. This innovative combination offers the possibility to monitor changes in the food, package and/or environment, and to respond appropriately on these changes through a feedback mechanism. Sooner or later, smart packaging will undoubtedly result in a better control of food supply chains and in consequent improvements of their overall performance and security, but the development of new devices and technologies is still required (Vanderroost et al., 2014).

2.1.6. Edible coatings and films

The application of an edible coating (EC) on the food surface has become popular among the available techniques to preserve fresh or minimally processed fruits and vegetables. Although the concept is not new, recently considerable interest and advanced research activity has been observed in this field, which has been driven by an increasing consumer demand for safer, high quality, and convenient foods (Ciolacu and Nicolau, 2014). This technique is close to the AP technology, but EC do not act as a package by itself, they just reduce the barrier requirements of the package (Gómez-Estaca et al., 2014). Although many authors do not make distinction between EC and edible films (EF), the latter are used as covers, wraps or separation layers, while the EC are considered part of the final product and designed to protect or enhance its properties

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(Carocho et al., 2014). Actually, their mode of formation and application to foods is different. EC are applied and formed directly on the food, while EF are freestanding structures, formed and later applied as a wrapping on the food product (Olivas and Barbosa-Cánovas, 2009).

As with other MAP systems, EC can create a very low O_2 environment. Additionally, on fruits and vegetables, EC could provide replacement and/or fortification of natural layers to prevent moisture losses and to control exchange gases involved in respiration processes such as O_2, CO_2 and C_2H_4. Therefore, EC have the potential to improve food quality and shelf-life (Olivas and Barbosa-Cánovas, 2009; Andrade et al., 2012). These protective layers may also improve mechanical handling properties and structural integrity, and can be used as a vehicle for incorporating several active ingredients, including anti-browning, antioxidants, antimicrobials, colorants, flavours, fortifying nutrients, plant extracts, etc. (Carocho et al., 2014). These ingredients are more efficient into EC or EF than when directly added to foods, since they can be gradually and selectively released from the wrapping matrix to the food surface, keeping effective concentrations where and when they are needed; thus, smaller amounts of these ingredients will be required to achieve the desired effect or aspect (Olivas and Barbosa-Cánovas, 2009; Falguera et al., 2011). Therefore, EC can also enhance nutritional and sensory attributes.

The structural matrix of these edible layers can be developed from polysaccharides (starch, cellulose, alginate, carrageenan, chitosan and pectin), proteins (gelatine, casein, gluten, zein, keratin, albumin and soy protein), and lipids (waxes, acetylated monoglycerides, fatty alcohols and fatty acids) or blends between hydrocolloids (polysaccharides or proteins) and lipids, called composites (Han, 2013). Hydrocolloids can form cohesive molecular networks by strong interactions between molecules, imparting good barrier properties to gases and good mechanical properties. One of the most investigated compounds for EC is chitosan due to its antimicrobial properties. Regarding the application of EC, different methods such as panning, fluidized bed, dipping and spraying can be used (Andrade et al., 2012). The EC remains on the food during storage and will be disintegrated or dissolved during cooking or the mastication process. Thus, once the consumer acceptance of the coated product is influenced by the sensory and organoleptic properties of EC, its acceptability needs to be carefully evaluated (Andrade et al., 2012). A weakness of this technology relates to the difficulty in applying a regular thickness layer over the surface of the food, which can lead to irregular dispersion of the added active ingredients (Carocho et al., 2014). This can occur mainly in foods with irregular surfaces, which may present not covered areas that expose the food surface to unfavourable preservation conditions. In turn, EF are formed by casting and drying film-forming solutions on a levelled surface, drying a film-forming solution on a drum drier, or using traditional plastic processing techniques such as extrusion (Falguera et al., 2011).

Despite the benefits of EC, commercial applications on a broad range for different foods are still very limited, and research have focused mainly on searching for new coating materials. In order to overcome some limitations of this technology, a new generation of EC is under development by using nanotechnological processes. The main objectives are to improve mechanical and barrier properties through nanocomposite coatings, achieve a controlled release of active ingredients under specific conditions using nanoencapsulation techniques, and create multi-layered systems (nanolaminates) to coat highly hydrophilic foods (Andrade et al., 2012).

2.2. Ionizing radiation

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Food irradiation may be considered as the second big breakthrough after pasteurization. This novel physical treatment involves food exposure to ionizing radiations, such as gamma, electron-beam or X-ray, to eliminate microbial contamination, inhibit the germination of crops, and delay the ripening rate of fruits and vegetables, allowing to ensure safety and extend the shelf-life (ICGFI, 1999; Lacroix, 2014; Lung et al., 2015). It is considered as a safe and effective post-harvest treatment by several international authorities (WHO, 1999), and can be applied as an alternative to chemical fumigants. The irradiation technology can not only improve food safety but also reduce crop-related economic losses (Lung et al., 2015). In Europe as well as in Asia Pacific region it has been observed a positive trend in order to meet phytosanitary requirements in the international trade (Kuan et al., 2013). Nevertheless, the adoption of ionizing radiation for food applications has been a slow process due to some misunderstandings by the consumer who often chooses non-irradiated foods.

In food processing, the permitted sources of gamma-rays are mainly cobalt-60, a radioactive isotope produced from cobalt-59, and caesium-137, a spent fuel from nuclear reactors. Beta-rays, a stream of electrons, are another source; but, due to their low energy levels, they need to be accelerated to make them acquire the required energy to be used in food preservation (Lacroix, 2014). According to Clemmons et al. (2015), electron accelerators appear to be more successful compared to gamma-rays because the source can suspend the irradiation at any time; non-nuclear energy can accelerate the generation of radiation when required; little risk for occupational injuries; and applicability in high-flow and high-dose irradiation.

Gamma-rays and electron-beams produce the so-called ionizing radiation constituted by electronically charged atoms or molecules. During the food exposure to the irradiation field, the quantity of energy absorbed by the food must be measured (in Gray or kiloGray (kGy)) to establish correct procedures for food preservation and quality control (Antonio et al., 2012). For this reason, dosimeters are placed within the food product to be irradiated to measure the distribution of the absorbed energy and to determine the maximum and the minimum dose absorbed by the food. The desired dose is achieved by the time of exposure and by the location of the food product relative to the source. The amount of energy absorbed by the food product also depends on its mass, bulk density and thickness (Lacroix, 2014). Additionally, gamma irradiation treatments can be divided into three levels based on dosage: low-dose treatment up to 1 kGy for insect disinfestation and parasite inactivation, delay in fruit maturity and prevention of germination; intermediate-dose treatment from 1 to 10 kGy to reduce non-spore forming pathogens and spoilage microorganisms and extend shelf-life of fresh commodities; and high doses above 10 kGy to reduce microorganisms to the point of sterility (ICGFI, 1999; Fan et al., 2012). Likewise, the gamma irradiation treatment provides an alternative way to eliminate pesticide residues from plant products (Wen et al., 2010).

The principal target of ionizing radiation is water that produces free radicals and other reactive species, able of breaking chemical bonds and modifies various molecules, leading to destruction or deactivation of bacterial components (Ramos et al., 2013). The inactivation of microorganisms by irradiation is primarily due to DNA damage, which destroys the reproductive capabilities and other functions of the cell (Rawson et al., 2011). Besides, changes in food quality parameters might vary depending on the basic raw food material, irradiation dose delivered, and type of radiation source employed (Lacroix, 2014). Depending upon the radiation dose, foods may be pasteurized to reduce or eliminate food-borne pathogens.

During irradiation, the dose, the food composition and the type of microorganism affect differently the efficacy of treatment (Moosekian et al., 2012). In general, the irradiation dose is positively proportional to the degree of killing microorganisms. When the food properties are adverse to microbial growth, their resistance to the radiation is reduced and can be killed by low irradiation doses. Regarding microorganisms, they show different tolerance levels towards specific doses of irradiation; normally, Gram-positive bacteria display stronger resistance than Gram-negative bacteria, while prokaryotic microorganisms are more resistant than eukaryotic microorganisms (Moosekian et al., 2012).

For fresh and minimally processed fruit and vegetables, low-doses (up to 1 kGy) are approved by the Food and Drug Administration (FDA) for shelf-life extension purposes. The treatment is applied for sprout inhibition, delay ripening, and to reduce bacterial, parasitic and protozoan pathogens (ICGFI, 1999; Ramos et al., 2013). If the dose is less than appropriate, the intended preservation effect may not be achieved, and if the dose is excessive, the food may be damaged and unacceptable for consumption. Additionally, for post-packaging irradiation, the package materials should be chemically stable to prevent polymer degradation and low molecular weight hydrocarbons and halogenated polymers formation which can migrate into foods (Galić et al., 2011). Moreover, all irradiated products need to be labelled with the RADURA-logo and a statement “treated by irradiation” or “treated with irradiation” either on the package or at the point of sale.

Currently more research is needed to evaluate the dose-response effects on different quality parameters of specific fresh produce, as well as to educate retailers and consumers about irradiation processing to advance commercial applications of this technology. The use of this technology as a hurled for packaged fresh produces also needs more research.

2.3. Ultraviolet radiation

Ultraviolet (UV) radiation can be used as a non-thermal treatment to decontaminate and reduce decay of fresh or minimally processed fruits and vegetables. UV radiation has been tested with UV-A (long waves, 400-315 nm), UV-B (medium waves, 315-280 nm) and most commonly with UV-C (short-waves, 280-100 nm) since it has more energy (US-FDA, 2002). The treatment can induce resistance mechanisms against pathogens or damage directly the bacterial DNA (Ramos et al., 2013). UV-C radiation also produces significant damage in the cytoplasmic membrane integrity and cellular enzyme activity (Gómez et al., 2011). However, the microbial inactivation is limited solely to the food surface, as UV-C has extremely low penetration into solids. Recent studies reported positive effects induced by this post-harvest treatment on physiological, microbiological and quality parameters of fresh fruits and vegetables. Martínez-Lüscher et al. (2014) demonstrated that the UV-B radiation has an overall positive impact on grape berry composition. Du et al. (2014) verified that the treatment can be used as an additional processing step on selected specialty crops to enhance their soluble phenolic content, and that the changes are species-dependent. Regarding medium waves, Syamaladevi et al. (2015) showed the UV-C radiation efficacy to reduce *Penicillium expansum* inoculated onto the surface of organic apples, cherries, strawberries and raspberries, and that the efficacy depends on the fruit surface morphology. The treatment also inhibits C_2H_4 production and delays the softening of tomato fruit during ripening (Bu et al., 2013; Severo et al., 2015). Additionally, an increase on the beneficial effect of tomatoes for human health was reported by Bravo et al. (2012) after UV-C irradiation, namely increased total phenolic levels and antioxidant activity. This novel technology also has high potential for surface decontamination of apricots (Yun et al., 2013) and fresh-cut melon (Manzocco et al., 2011).

2.4. Pulsed light

Pulsed light (PL), or high intensity light pulses, is a fast and environmentally friendly emerging technology to decontaminate food surfaces by inactivating microorganisms using pulses of an intense broad spectrum rich in UV-C light (Gómez-López et al., 2007). PL works with xenon lamps that can produce intense and short time pulses of broad spectrum "white light", from ultraviolet wavelengths of 200 nm to infrared wavelengths of 1000 nm, with peak emissions between 400-500 nm. The power is magnified many times by storing energy in a high power capacitor over relatively long times (fractions of a second) and releasing it over a short period of time (millionths or thousandths of a second) producing several high energy pulses per second. This phenomenon increases the instantaneous energy intensity that contributes to the inactivation of both spoilage and pathogenic microbial cells. In addition, it can limit the negative effects on food quality in terms of nutritional and organoleptic properties since there is no substantial increase in temperature during the treatment. This novel technique is proposed for the decontamination of solid and liquid foods, packages, medical devices, packaging and processing equipments for the food, medical and pharmaceutical industries (Gómez-López et al., 2005a; Rajkovic et al., 2010; Palgan et al., 2011; Ramos-Villarroel et al., 2012; Luksiene et al., 2012; Charles et al., 2013).

The inactivation efficacy of PL depends on the intensity (measured in J cm^{-2}) and numbers of pulses delivered. The UV-C part of the light spectrum (200-280 nm) emitted by the flash lamp is the most lethal and consequently the most important for microbial inactivation. As a result, cells are inactivated by photochemical damage of DNA, protein denaturation, agglutination of the cytoplasmatic content leading to a disruption of cell membranes, and other photothermal and photophysical effects (**Figure 2**) (Krishnamurthy et al., 2010; Ramos-Villarroel et al., 2012). Thus, significant and rapid microbial inactivation in short-time treatments, lack of residual compounds and great flexibility are some of the advantages of this technology. Additionally, recent evidence demonstrate that PL kills yeast through a multi-hit or mechanistic process that affects the cell membrane permeability along with DNA and macromolecule stability and functionality depending on the dose applied (Rowan et al., 2015). Nevertheless, the food composition affects the effectiveness of this technology. The presence of proteins and oil decreases the decontamination effect, because they absorb the effective wavelengths. On the other hand, foods rich in carbohydrates such as fruit and vegetables seem to be more suitable for PL treatments (Gómez-López et al., 2005b; Elmnasser et al., 2007). However, few studies have focused on the effects of PL on physiological and quality parameters of fruit and vegetables.

The inactivation of microorganisms naturally present or inoculated on fruits and vegetables surfaces by PL has been achieved and demonstrated in the last years. Izquier and Gómez-López (2011) investigated the dose-effect relationship between PL fluence (J cm^{-2}) and inactivation of naturally occurring microorganisms present on fresh-cut iceberg lettuce, white cabbage and carrot surfaces. They verified that a single low energy pulse (0.72 J cm^{-2}) was enough to achieve one log reduction, with an ultrafast treatment time of 0.5 ms, and a satisfactory inactivation level for shelf-life extension. Other study carried out by Charles et al. (2013) demonstrates that PL is an effective strategy to preserve the firmness, the colour and the carotenoid content of fresh-cut mangoes, compared to the control where quality decreased. They also reported an increased polyphenol oxidase (PPO) activity after 3 days, maintained phenylalanine ammonia lyase activity and unaffected phenolic and total ascorbic acid contents. On the other hand, Aguiló-Aguayo et al. (2013) achieved a surface reduction of natural and inoculated (*Saccharomyces cerevisiae*) microorganisms in fresh tomatoes without losses of nutritional quality, as vitamin C was unaffected and the carotenoids concentration was actually slightly increased. However, PL severely reduced the acceptability product quality by causing an important weight loss and visible wrinkles after three days. Likewise, a negative effect on surface colour and texture in fresh-cut watermelon, was reported by Ramos-Villarroel et al. (2012) after exposure to 30 light pulses (12 J cm^{-2}). The authors also found that inoculated *E. coli* (Gram-negative) is more sensitive to PL than *Listeria innocua* (Gram-positive) probably due to the cell wall composition of these bacteria. The cell walls of Gram-positive bacteria are more rigid and thicker than those of Gram-negative bacteria, which may give an extra protection against the PL treatment. Nevertheless, the inactivation of microorganisms present on food surfaces does not necessarily result in shelf-life extension. Gómez-López et al. (2005b) failed to prolong the shelf-life of minimally processed white cabbage and iceberg lettuce in spite of the reduction in the initial microbial load.

The effectiveness of the PL treatment is limited by its low degree of penetration (~2 µm) or because of a shadow effect. Indeed, microorganisms may penetrate via crevices or irregularities present on the product surface or through the epidermis of the fruits or vegetables (Gómez-Lópes et al., 2005a,b; Lagunas-Solar et al., 2006; Huang and Chen, 2014). For this reason, Luksiene et al. (2012) evaluated the possibility to decontaminate fruit and vegetables with different irregular surfaces by PL, including plums, tomatoes, cauliflowers, sweet peppers and strawberries, from food pathogens and mesophiles. They verified that the surface irregularities just slightly, but not significantly, reduce the treatment efficiency, once significant decontamination effect was observed compared to control. Additionally, no effects on nutritional quality (vitamin C, total phenolics and antioxidant capacity), colour and texture have been observed in the processed samples. Therefore, an ideal food product surface would be smooth, clear and without roughness, pores and grooves which can “shadow” the microbial cells from the light, causing less complete light diffusion and thus reducing process effectiveness. For the same reason, food products to be treated should be clean and free of contaminating particulates (Lagunas-Solar et al., 2006; Cacace and Palmieri, 2014). Likewise, the PL decontamination efficacy decreases at high contamination levels. Thus, once at high population densities microorganisms overlap each others, only the upper layers will become inactivated, which shadow the remaining from the light (Gómez-López et al., 2005a and 2007).

In order to overcome these limitations is possible to add devices to the PL system to create multidirectional light pulses, or generate the random movement/rotation of the food product to provide uniform PL exposure of the entire surface and any protected microorganism can be attained. To achieve this, a novel setup using a water-assisted PL system was developed, in which the food samples are immersed in agitated water and can randomly move and rotate, allowing more uniform PL exposure of all the food samples surfaces. This wet PL treatment was already applied successfully to decontaminate green onions (Xu et al., 2013), blueberries (Huang and Chen, 2014), strawberries and raspberries (Huang and Chen, 2015) showing better results than the dry PL system, and being a promising chemical-free alternative to other sanitization treatments. Additionally, whenever possible or if appropriate, the food product could be cut into thin slices or pieces so that light can penetrate almost through the entire product.

Another study suggests that a brief post-harvest PL treatment stimulates coloration and anthocyanin accumulation in figs, and seems to be a feasible means of compensating for insufficient sunlight stimulation of colour development in figs and possibly other fruits as well (Rodov et al., 2012). However, current literature is scarce, therefore more research is needed to evaluate the effectiveness and suitability of this technology, namely to understand the effect of spectral range on specific pathogens and the impact on water soluble vitamins that are known to be sensitive to UV light, and then optimize its use.

2.5. High-power ultrasound

Ultrasound (US) is a green processing technology very promising in the food industry because of its potential to inactivate microorganisms present in fruit and vegetable surfaces (Chemat et al., 2011). The US treatment is simple, relatively cheap and energy saving (Awad et al., 2012); it also offers advantages in terms of cost, productivity and selectivity, with better processing time, improved quality, and reduced physical risks (Chemat et al., 2011). The US treatment is based on energy generated by sound waves with frequency beyond the limit of human hearing (Awad et al., 2012). Regarding the US system, it consists of an electrical power generator (source of energy), a transducer (to convert electrical energy at US frequencies) and a coupler or emitter (to emit the US waves from the transducer into the medium) (Chemat et al., 2011). The US band can be divided into low-power (high frequencies) and high-power (low frequencies) US (Kentish and Ashokkumar, 2011). The first one includes frequencies higher than 1 MHz and has non-destructive effects in the food properties through which they passes. On the other hand, the low frequencies, between 18 kHz and 100 kHz, induce mechanical, physical and chemical changes, such as physical disruption, which supports the inactivation mechanism on food surfaces (Awad et al., 2012).

In food preservation, high-power US is used to inactivate microorganisms. The antimicrobial effects are attributed to intracellular acoustic cavitation (**Figure 3**), phenomenon characterized by the formation, growth and implosion of small bubbles generated by the US waves. These bubbles pass through the biological structure and promote a series of compression and rarefaction (expansion) cycles leading to the production of localized energy (hot spots). Two cavitation phenomena may occur, the stable cavitation and the transient cavitation. The stable cavitation requires thousands of cycles of oscillating US waves to originate tiny and non-linear bubbles forming large bubble clouds whose collapse does not occur. In the transient cavitation (**Figure 3**), the bubbles are increased in size within a few oscillatory cycles and collapse quickly, releasing energy that contributes for the generation of reactive species endowed with bactericidal effects (Bilek and Turantaş, 2013; São José et al., 2014). These hot spots originated by the bubbles implosion events, characterized by presenting high temperature (up to 5500 °C) and pressure (up to 1000 MPa), occur in very short time periods (μs) and lead to the release of reactive species (*e.g.*, hydroxyl radicals) as a result of the dissociation of vapour trapped in the bubbles. Thus, apart from the physical effect responsible for the microbial cell death, the originated reactive species also contributes to the microbial inactivation (Gao et al., 2014; São

José et al., 2014). Furthermore, the released energy allows reaching food surfaces that are difficult to access using other sanitizing methods (Gao et al., 2014).

In microbial cells, the cavitation phenomenon can lead to the pore formation, cell membrane disruption and selectivity loss, and consequent cell breakage (**Figure 3**). Besides, the originated reactive species can demerge the DNA (Bermúdez-Aguirre et al., 2011; São José et al., 2014). Nevertheless, the type, shape and size of the microorganisms may influence the treatment efficacy. In general, Gram-positive bacteria are more resistant than the Gram-negative ones, because of its thicker cell walls and probably due to a more tightly adherent peptidoglycan layer in Gram-positive cells. Additionally, cocci are more resistant than rod-shaped bacteria, and larger cells are more sensitive than smaller ones. Moreover, spores appear to be more resistant than vegetative forms while enzymes are reported to be inactivated by ultrasound due to a depolymerisation effect (Chemat et al., 2011). The pH also affects the decontamination efficiency, being the inactivation rate increased at lower pH (São José et al., 2014). Additionally, since the frequency affects the cavitation phenomenon and larger bubbles are produced at lower frequencies, these are more effective in high viscous foods (Bermúdez-Aguirre et al., 2011; São José et al., 2014).

Recent studies demonstrated that the amplitude of ultrasonic waves, exposure time, treatment temperature, and volume and composition of the food can affect differently the fresh fruits and vegetables properties. Aday et al. (2013) evaluated the effects of different ultrasound powers (30, 60 and 90 W) and treatment times (5 and 10 min) on quality parameters of strawberry. Significant differences between treatment times were not found. Power levels between 30 and 60 W resulted in improved strawberry quality (colour, texture, pH and total soluble solids (TSS)), being useful for shelf-life extension. On the other hand, 90 W resulted in detrimental effects on the evaluated parameters. Nevertheless, decay incidence analysis confirmed that all treatment conditions were effective to reduce mould growth. Cao et al. (2010a) also investigated the effects of the ultrasound treatment on strawberry decay and physiological quality. Fresh fruits were treated with 0, 25, 28, 40 or 59 kHz frequency (operating at a power of 350 W), at 20 ºC for 10 min, and then stored at 5 ºC for 8 days. It was found that 40 kHz significantly reduced decay incidence and microbial population, and inhibited the decrease of firmness and maintained significant higher levels of TSS, total titratable acidity and vitamin C. It was also found that treatments with 25 and 28 kHz had no significant effect on decay and quality deterioration of this fruit. Other study demonstrated that 250 W and 9.8 min were the optimal conditions to treat strawberries, highlighting its suitability for quality maintenance and shelf-life extension (Cao et al., 2010b).

The decontamination effectiveness is not always severe enough when using the US treatment alone. Thus, combining US with other preservation factors may be advantageous due to the hurdle effect (Chemat et al., 2011). If combined with pressure, heat, both pressure and heat, or UV irradiation, the treatment is called manosonication, thermosonication, manothermosonication, or photosonication, respectively. However, a very few data regarding these combinations to preserve fresh or minimally processed fruits or vegetables are available. Cruz et al. (2008) determined the degradation kinetics of vitamin C in watercress (*Nasturtium officinale*) by thermosonication. The treatment was found to be a better blanching process, since it inactivates watercress peroxidase at less severe blanching conditions and consequently retained vitamin C content at higher levels. Additionally, the heat blanching required 70 s to produce the

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same degree of peroxidase inactivation (90% reduction), that was about 14-fold the processing time of the thermosonication treatment (5 s). This huge difference leads to a higher retention of vitamin C in the thermosonication treatment (about 94%) as compared to heat blanching, which reduced the content to 29%.

Since the ultrasonic equipment has to be custom designed for each application, so far only a few treatments have reached industrial level (Knorr et al., 2011). Thus, future challenges are based on the optimization of adequate treatment conditions for different foods, which demands many research capacities and a close collaboration between researchers, equipment suppliers, and the food industry.

2.6. Cold plasma

Cold plasma (CP) is a new and green food preservation (decontamination) technology with the potential for application to a wide variety of foods. It is so recent that the terminology is still evolving and has only been applied at very small scales. Actually, different CP technologies have been developed for food processing, as well as technologies used to generate it (Niemira, 2012). The non-thermal plasma is formed by quasi-neutral ionized gases which are dissociated by an energy input. These ionized gases comprise particles such as photons, free electrons, positive and negative ions, atoms in their fundamental or excited states, and free radicals that, in combination, have the capacity to inactivate microorganisms on food surfaces (Niemira, 2012; Pankaj et al., 2014; Baier et al., 2015). The gas being ionized may be air, O_2 or N_2, or a mixture containing some proportion of noble gases (Ar, He or Ne). In turn, heat, electricity, laser light and radiation, among others, are used as energy sources (Niemira, 2012). Plasmas can be generated at atmospheric pressure and close to ambient temperature, thus, allowing non-thermal treatment conditions (Moreau et al., 2008; Ziuzina et al., 2014; Lacombe et al., 2015).

As a cloud of active particles, the recombination process of these species generates energy that is retained in the plasma for a period of time, and then is released as visible and UV light. The active particles in the plasma can react with the food substrate, releasing the stored energy into the target bacteria or viruses. Therefore, the microbial inactivation may occur by chemical interaction of radicals, reactive species, or charged particles with the cell membranes, by damage to membranes and internal cellular components by the UV radiation, or broken the DNA strands also by the UV light. However, the antimicrobial modes of action depend on the source and type of plasma generated, whose total energy varies with the gas used, density and temperature. Additionally, one mode of action may be more significant than another on a given commodity, and therefore the sanitizing efficiency can be improved by using plasmas with multiple antimicrobial mechanisms, taking advantage of synergistic effects (Niemira, 2012).

According to Niemira (2012), the CP systems can be classified in three categories based on where the food to be treated is placed with respect to the CP source and the nature of the CP chemistry, which delineates the half-life and reactivity of the charged active species within the plasma. The first category is the remote treatment system, where the food product is placed at some distance from the generation source, which is treated with plasma rich in secondary chemical species; the second is known as direct treatment system where the product is placed relatively close to the generation source and treated with active plasma; and in the third category, the electrode contact systems, the product is within the CP generation field.

Effective treatment time ranges from less than 3 s to 300 s, depending on physical (food shape) and chemical properties of foods and processing conditions. The decontamination efficacy also depends on the type of microorganism, inactivation medium, number and physiological state of cells, and operating gas mixture and flow (Bermúdez-Aguirre et al., 2013; Ziuzina et al., 2014). The early development stage of this technology also represents a limitation, as well as the diversity and complexity of the necessary equipment and the scarce information about physicochemical and functional changes that might occur in treated food product (Knorr et al., 2011; Bermúdez-Aguirre et al., 2013; Ramos et al., 2013).

Most studies on the application of CP in the food field are focused mainly on inactivation of microorganisms, often neglecting the effects on quality parameters. The antimicrobial activity of CP against Gram-negative and Gram-positive bacteria, yeast and fungi, as well as spores and biofilms that are generally very difficult to inactivate was shown in various studies (Montie et al., 2000; Laroussi and Lu, 2005; Vleugels et al. 2005; Brandenburg et al. 2007). Indeed, it is effective in reducing some human pathogens. Ziuzina et al. (2014) demonstrated that the atmospheric CP treatment for 10, 60 and 120 s resulted in reduction of *Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes* populations on packaged tomatoes to undetectable levels from initial populations of 3.1, 6.3, and 6.7 log_{10} CFU/sample, respectively. However, an extended treatment time (up to 300 s) was necessary to reduce bacterial populations attached on the more complex surface of strawberries. Baier et al. (2014) showed that keeping a distance of 17 mm to an Ar plasma-jet, corn salad leaves could be treated for up to 60 s, without exceeding the leaves surface temperature of 35.2 ºC. The authors also performed antibacterial tests on corn salad, cucumber, apple and tomato, achieving an inactivation of inoculated *E. coli* of at least 3 log cycles, after 60 s of treatment. However, the inherent inactivation mechanisms are not yet fully understood.

Once the emitted reactive species from plasma react with bacteria, they may also affect food properties. Nevertheless, the application of CP to improve shelf-life and safety of fresh or minimally processed fruits and vegetables is new and little is known about the effect on physicochemical or functional properties. Baier et al. (2015) studied the impact of CP on the external quality of apples, cucumbers, tomatoes and carrots, and its antimicrobial efficacy on indigenous and inoculated microorganisms. Significant changes were found on colour of tomatoes and carrots and on chlorophyll fluorescence parameters of cucumbers, whereas elasticity remained almost unaffected in all matrices. The treatment was suitable for apples, but for more susceptible produces such as carrots, some unwanted surface effects were induced. In turn, Lacombe et al. (2015) observed that CP can inactivate microorganisms on blueberries. However, after 60, 90 and 120 s, the treatment affected the firmness, reduced the anthocyanins content, and changed the surface colour, respectively. Contrariwise, other study demonstrated that the effects on strawberry colour and firmness were insignificant (Misra et al., 2014a). The suitability of CP has also been demonstrated for lettuce (Jahid et al., 2015) and cherry tomatoes (Misra et al., 2014b).

Today, this technology shows promises and is subject of active research to enhance efficacy. Nevertheless, optimization and scale up to commercial treatment levels require a more comprehensive understanding of the inherent chemical processes, namely in order to understand the mode of action of the treatment and the possible interactions with food constituents and properties. As the technology moves from the lab scale to industrial scale, the capital costs will be high but may be offset by advances in energy efficiency and overall engineering scale efficiencies (Niemira, 2012). Furthermore, with the technology evolution, it is also expected that the terminology and experimental methodologies will become more standardized.

2.7. High hydrostatic pressure

High hydrostatic pressure (HHP) processing is a relatively new and very promising non-thermal food processing technology that subjects liquid or solid foods, with or without packaging, to pressures between 50 and 1000 MPa. It is a good alternative to heat treatments to destroy food-borne pathogens and inactivate enzymes because, as a cold pasteurisation process, it does not lead to substantial modification of the nutritional, functional and organoleptic properties of foods, and enhance safety and shelf-life of perishable foods like fresh or minimally processed fruits and vegetables (Mújica-Paz et al., 2011; Castro and Saraiva, 2014; Huang et al., 2014).

In a HHP process, the food product to be treated is placed in a pressure vessel capable of sustaining the required pressure and submerged in a liquid (typically water), which acts as the pressure-transmitting medium (Knorr et al., 2011; Castro and Saraiva, 2014). The pressure is transmitted in a uniform (isostatic) and quasi-instantaneous manner throughout the food sample. Therefore, the time necessary for pressure processing is independent of food shape or size, in contrast to thermal treatments (Medina-Meza et al., 2013; Huang et al., 2014). Moreover, there is little variation in temperature with increasing pressure (the temperature increases approximately 3 ºC per 100 MPa, depending on the food composition). These particularities prevent the food from being deformed or heated, which would modify its organoleptic or nutritional properties (Considine et al., 2008).

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In general, low-molecular-weight compounds are only slightly affected by HHP because the break of covalent bonds does not occur at pressure below 2 GPa, due to its very low compressibility. Therefore, the primary structure of volatile compounds, pigments, vitamins, and other compounds connected with the sensory, nutritional, and health-promoting aspects of foods are less affected or rarely affected (Oey et al., 2008; Huang et al., 2014). This effect is important for fruits and vegetables once they are rich sources of bioactive ingredients (including vitamins) and pigments (Pereira et al., 2011; Pinela et al., 2012). However, the ionic bonds and hydrophobic interactions, responsible for maintaining the secondary and tertiary structure of proteins, are weakened/disrupted (in general above 200 MPa) and this event is associated with decreases in volume. The additional rupture of hydrogen bonds and electrostatic interactions can change the structure of large molecules and complex organized structures (Considine et al., 2008; Roeck et al., 2010; Rendueles et al., 2011). The global consequence for the food product is diverse, since the nutrient digestibility and bioavailability can be modified (Briones-Labarca et al., 2011; Linsberger-Martin et al., 2013), the activity of certain enzymes inhibited (Liu et al., 2013), or functional properties altered (Kim et al., 2014). For example, the structure of starch undergoes changes, such as gelling, similar to thermal-induced structure changes. Although, the physical structure of most high-moisture foods remains unchanged after exposure to HPP, since no shear forces are generated by pressure (Considine et al., 2008; Knorr et al., 2011; Rendueles et al., 2011).

The HHP effect on the microbial inactivation has been reported and reviewed (Patterson, 2005; Considine et al., 2008; Rivalain et al., 2010; Mota et al., 2013; Huang et al., 2014). In general, yeasts and fungi are more sensitive to pressure than vegetative bacteria, which can be inactivated

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at relatively low pressures (normally 200-400 MPa), at room temperature. Ascospores of heat-resistant moulds appear to be more pressure resistant, and many of them are not inactivated at pressures below 800 MPa, and conidia are much more pressure sensitive, with a sensitivity similar to yeasts. Nevertheless, bacterial spores are the most pressure-resistant life forms known; therefore, it is needed a combination of higher pressure and temperature once pressure treatment alone does not achieve a substantial inactivation of spores (Raso and Barbosa-Cánovas, 2003; Rivalain et al., 2010; Mor-Mur et al., 2014). Commonly, a pressure of 50 MPa can inhibit protein synthesis in microorganisms and reduce the number of ribosomes, 100 MPa can induce partial protein denaturation, and 200 MPa cause damage to the cell membrane and internal cell structure. Increasing the pressure above 300 MPa induces irreversible denaturation of enzymes and proteins, which causes rupturing of the cell membrane and the excretion of internal substances, resulting in bacterial death (Huang et al., 2014). However, the pressure resistance of the different bacterial species and strains is quite different. Generally, Gram-positive bacteria are more resistant to heat and pressure than Gram-negatives, probably due to the different chemical composition and structural properties of the cell envelope, and cocci are more resistant than rod-shaped bacteria (Russell, 2002; Patterson, 2005; Rivalain et al., 2010). Cells in the stationary phase of growth are also generally more resistant than those in the exponential phase. This is because microorganisms in the exponential phase undergo continuous cell division and synthesis, and the stress tolerance of cells in an adverse environment is lower (Considine et al., 2008; Huang et al., 2014). Additionally, the chemical composition of the food product can have a significant effect on the response of microorganisms to pressure, due to the ability to provide essential vitamins and amino acids to stressed cells. For example, proteins, carbohydrates and

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lipids can confer a protective effect against pressure. The low water activity of the system also protects microorganisms against the pressure effects, except those that are injured. It is also known that pH and pressure can act synergistically leading to an increased microbial inactivation (Patterson, 2005; Considine et al., 2008; Mor-Mur et al., 2014).

Regarding viruses, there is relatively little information in comparison to other microorganisms, but it is known that its pressure resistance varies greatly among their structural diversity and within related taxonomic groups or even strains. Enveloped viruses are usually more sensitive to pressure treatments than naked viruses. HPP can cause damage to the virus envelope preventing the virus particles binding to cells or even complete dissociation of virus particles, which may be either fully reversible or irreversible, depending on the pressure. Prions, associated with neurological disorders in animals and humans, are generally even more difficult to destroy than bacterial spores. The treatment requires very drastic conditions, with the application of pressure up to 1200 MPa for 10 min at a temperature up to 135 °C (Considine et al., 2008; Rivalain et al., 2010; Rendueles et al., 2011).

The effect of HPP on enzyme activity seems to be variable. In whole fruit and vegetables, enzymes are usually confined to compartments; but in fresh-cut fruits or vegetables this compartmentalization is destroyed and the enzymes released in the food product causing unwanted changes. Thus, it is expected that the pressure treatment changes the structural conformation of the enzyme/protein, which sometimes leads to a partial or complete inactivation or, in other cases, to its activation. In general, a relatively low pressure (~100-200 MPa) may activate some enzymes, while a high pressure (400-1000 MPa) may induce their inactivation. Indeed, in most vegetables, the pressure alone is insufficient to inactivate enzymes and hence needs to be combined with heat (Raso and Barbosa-Cánovas, 2003; Mújica-Paz et al., 2011; Mor-Mur et al., 2014).

Although this novel technology is being increasingly investigated, the main targets of research regarding plant-based foods are purees (González-Cebrino et al., 2013) and juices (Queirós et al., 2014), while few studies have been focused on whole or minimally processed fruits and vegetables. Apart from its use for preservation purposes, HHP might also influence the biosynthesis pathways and could lead to the formation of product variants with novel functional properties. In fact, HPP is used to develop tailor-made foods. Nevertheless, there is a lack of data about the behaviour of nutrients, allergens, and food-spoiling microorganisms under defined food matrices and treatment conditions, as well as the process conditions necessary to inactivate pressure-resistant bacterial spores.

2.8. Dense phase carbon dioxide

Recently, some attention has been paid to dense phase carbon dioxide (DP-CO_2), a collective term for liquid, high pressurized, or supercritical CO_2, as an alternative non-thermal treatment to preserve fresh fruits and vegetables among other foods (Calvo and Torres, 2010; Rawson et al., 2014; Zhou et al., 2015). It utilizes pressures in combination with CO_2 to kill microorganisms as a mean of food preservation. DP-CO_2 denotes phases of matter that remain fluid, yet are dense with respect to gaseous CO_2. CO_2 is inert, nontoxic, non-flammable, inexpensive, readily available in high purity, and leaves no residues, being generally recognized as safe. This gas is also a natural constituent of many foods. When operating at supercritical conditions (7.4 MPa and 31.1 ºC), it acquires very good mass-transfer properties (low viscosity and zero surface

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tension), providing effective penetration into solid matrices (Rawson et al., 2011). At the end of treatment, it can easily be removed from the solutes by mere expansion to ambient pressure (Choi et al., 2008; Calvo and Torres, 2010).

The microbial inactivation may occur by physical disruption due to a rapid depressurization and expansion of CO_2 within the cells. The CO_2 increases the membrane fluidity, due to an order loss of the lipid chains, resulting in increased permeability. The dissolution of CO_2 in the aqueous component of foods originates carbonic acid which lowers extracellular pH and consequentially inhibits many metabolic systems of the cell. However, microbial inactivation can also be achieved even when cells remain intact or exhibit only limited signs of deformation. In general, and as mentioned for some of the other reviewed technologies, Gram-positive bacteria are more difficult to inactivate than Gram-negatives, mainly due to the thickness and composition of the cell wall (Garcia-Gonzalez et al., 2007; Rawson et al., 2014).

One of the first food applications was the treatment of whole fruits to inhibit mould growth (Damar and Balaban, 2005). Recent published data demonstrated that this technology can effectively inactivate microorganisms and enzymes in minimally processed fruits (Valverde et al., 2010) and vegetables (Calvo and Torres, 2010; Bit et al, 2011), being considered as a promising and sustainable technology for preservation of a wide range of foods. Nevertheless, studies on the effects of this technology on food quality parameters are still limited. Thus, more research is necessary, especially on the elucidation of mechanisms underlying changes on food properties, especially on bioactive ingredients. Given the strong benefits of this technology, it is likely to be increasingly adopted in the food industry.

3. The hurdle concept

The hurdle concept provides a framework for combining (simultaneously or sequentially) more than one milder preservation factor in order to achieve an enhanced level of product safety and stability. These factors are hurdles, which microorganisms and pathogens need to overcome to survive in the food environment. Thus, they should be "high enough" so that the microorganisms cannot overcome them, and its selection must take into account the initial numbers and types of microorganisms. The correct combination of hurdles can ensure microbial safety, stability and quality of foods. Additionally, the hurdles can be physical, chemical or biological factors, and its intensity can be adjusted individually, depending on the objective, to meet consumer preferences in an economically sensible way, without compromising product safety. This concept fits with the actual consumer trend for minimally processed fruits and vegetables and has gained popularity regarding practical applications and research (Mukhopadhyay and Gorris, 2014). The combination of emerging technologies with conventional or novel preservation techniques has been explored in the last years; but a more deep understanding of the hurdle effect is crucial to obtain high quality and safety foods, and to support hurdles selection and their levels (Gómez et al., 2011).

4. Concluding remarks

The decontamination of fresh and minimally processed fruits and vegetables remains an important unsolved technological problem. Nevertheless, the reviewed non-thermal physical treatments are significantly useful for microbial decontamination and shelf-life extension of these perishable commodities and, due to its various advantages, can be used as an effective and

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safer alternative to chemical and heat treatments. Furthermore, the hurdle concept fits with the actual consumer trend for minimally processed foods and is expected to gain more popularity and practical applications due to its promising results. Besides, the future trends in food preservation cannot be considered independent of sustainability, eco-friendly, innovation, and advanced technologies, always intending to obtain safe and high quality foods.

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Table 1. Main advantages, limitations/drawbacks and challenges of the different packaging systems.

Advantages	Limitations/drawbacks	Challenges	References
Vacuum packaging			
Residual O_2 levels; Limits the growth of aerobic microorganisms and the occurrence of oxidation reactions; Economic and easy to apply.	Permeability of the packaging film.	-	Deepa et al. (2013)
Modified atmosphere packaging			
Lowered levels of O_2. Controlled respiration rate and deterioration processes. Controls the microbial growth. Provides high-quality foods. Improved shelf-	The headspace gas composition depends on the food product. The permeability of the packaging film needs to be adapted to the product respiration.	Determine suitable packaging atmospheres and films for different food commodities.	Jamie & Saltveit (2002); Sandhya (2010); Luengwilai et al. (2014); Niemira & Fan (2014)

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life.			
Active packaging			
Controlled respiration rate and deterioration processes. Controls the microbial growth. Improved food safety and shelf-life.	Possible migration of substances from the package.	Research on the migration behaviours of nanomaterials and their potential impacts on health/safety, as well as the environment. Optimization for specific food commodities. Labelling requirements.	Mehyar & Han (2010); Mihindukulasuriya & Lim (2014)
Intelligent packaging			
Capacity to monitor the integrity and safety of the packaged foods. Improved food safety and shelf-life.	Early stage of development. The increased cost per package.	Development of new devices and technologies is required. Labelling requirements.	Mihindukulasuriya & Lim (2014); Vanderroost et al. (2014)

Smart packaging			
Both of active and intelligent packaging. Better control of the food supply chains and improvements on overall performance and security.	Both of active and intelligent packaging.	Both of active and intelligent packaging,	Vanderroost et al. (2014)
Edible coatings			
Control of respiration processes. Reduced exposure to O_2 and moisture losses. Replacement and/or fortification of natural layers. Improve mechanical and handling properties and structural integrity. Vehicle for the incorporation of active	Difficulty in applying regular thickness layers. Sensory and organoleptic properties of the EC affect its consumer acceptance.	Achieve a controlled release of active ingredients under specific conditions using nanoencapsulation techniques. Creation of multilayered systems (nanolaminates) to coat highly hydrophilic foods.	Olivas & Barbosa-Cánovas (2009); Andrade et al. (2012); Carocho et al. (2014)

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ingredients.			

Table 2. Summary of critical parameters/variables, inactivation mechanism/effect, advantages, limitations and drawbacks, and challenges inherent to the revised technologies.

Parameters/variables	**Inactivation mechanism/cause/effect**	**Advantages**	**Limitations and drawbacks**	**Challenges**	**References**
Ionizing radiation					
Radiation source Dose (kGy) Dose rate (kGy h^{-1})	Formation of free radicals and other reactive species. DNA damage and loss of reproductive capability and other functions of the cell.	Availability of different systems (gamma-rays, electron beams, x-rays). Excellent penetration into foods. Insect	Efficacy depends on food composition and type of microorganism. Possibility of affecting quality parameters. High capital cost. Strict safety standards.	Consumer education and regulatory approval required.	Rawson et al. (2011); Fernandes et al. (2012); Moosekian et al. (2012); Lacroix (2014)

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		disinfestation and parasites inactivation. Delay ripening and senescence. Sprout inhibition. Post-packaging treatments. Suitable for sterilisation. Reliable and safe. Low energetic inputs.	Needs training to operate. Consumer acceptance.		

		Suitable for large-scale processing. Suitable for packaging materials.			
Ultraviolet radiation					
UV wavelength and source Treatment time Distance of product from the radiation source	DNA damage, membranes and enzyme activity induced by radiation absorption.	Delay ripening and senescence. Equipment of moderate to low cost and easy to use.	Pre-treatment can be necessary. Low degree of penetration (surface treatment). Occurrence of shadow effects.	Evaluate the effects on specific pathogens and the impact on water soluble vitamins.	Gómez et al. (2011); Ramos et al. (2013); Du et al. (2014); Severo et al. 2015 ; Syamaladevi et al. (2015)

		Little changes in quality at low doses. Stimulates the synthesis of health-promoting compounds. Suitable for food contact surfaces.	Process parameters difficult to standardize. The efficacy depends on food composition and microbial concentration.		
Pulsed light					
Intensity (J cm^{-2}) Number of pulses delivered Treatment time	Photochemical damage of DNA, protein denaturation, agglutination of the cytoplasmatic	Significant and rapid microbial inactivation in short-time	Low degree of penetration (surface treatment). Occurrence	Evaluate the effects on specific pathogens and the impact on	Elmnasser et al. (2007); Krishnamurthy et al. (2010);

	content and disruption of cell membranes	treatments. Lack of residual compounds. Great flexibility. Little or no changes on foods. Stimulates the synthesis of health-promoting compounds. Suitable for packaging materials. Medium	of shadow effects. Efficacy depends on food composition and physical properties, and microbial concentration; Possible occurrence of some appearance defects.	water soluble vitamins.	Izquier and Gómez-López (2011); Ramos-Villarroel et al. (2012); Rodov et al. (2012); Xu et al. (2013); Huang & Chen (2014 and 2015); Rowan et al. (2015)

		cost and low energetic input. Availability of water-assisted systems.			
High power ultrasound					
Power, amplitude and frequency Treatment time Temperature Pressure	Acoustic cavitation. Mechanical energy. Production of reactive species. DNA damage.	Enhanced penetration to inaccessible sites. Effective against vegetative cells, spores and enzymes.	Efficacy affected by food size and composition. Efficacy depends on type, shape and size of the microorganisms. Possible	Optimization of adequate treatment conditions for different foods, which demands a close collaboration between researchers,	Bermúdez-Aguirre et al. (2011); Knorr et al. (2011) ; Awad et al. (2012); Bilek & Turantaş (2013); Gao et al.

		Reduced processing time. Heat transfer increased.	physical changes on food. Possible food damage by reactive species. Problems related to scaling-up and lack of suitable industrial scale processing units.	equipment suppliers and the food industry.	(2014) ; São José et al. (2014)
Cold plasma					
Type of plasma generated Energy (which varies with the gas	Chemical interaction of radicals, reactive species or charged	High efficiency. No shadow	Efficacy depends on the type of microorganis	Understand the mode of action of the treatment	Niemira (2012); Bermúdez-Aguirre et

used, density and temperature) Treatment time	particles with the cell membranes, by damage to membranes and internal cellular components or broken the DNA strands	effects. In-package treatments.	m, inactivation medium, number and physiological state of the cells. Efficacy also affected by physical and chemical properties of foods, as well as operating gas mixture and flow. Technology in an early development stage.	and the possible interactions with food constituents and properties.	al. (2013); Ziuzina et al. (2014).
High hydrostatic					

pressure					
Pressure (MPa) Treatment time Adiabatic heating Compression and decompression rate	Membrane damage. Proteins denaturation. Leakage of cell content. Dissociation of ribosomes.	Independent of food shape or size. Uniformity of treatment throughout food. The primary structure of molecules remains intact. Kills vegetative bacteria. Colour, flavour	Foods should have ~40% free water for antimicrobial effect. Efficacy depends on type of microorganism and physiological state of cells, as well as the food composition. Spores not inactivated. Mixed effects on enzymes. Limited	Evaluation of the behaviour of nutrients, allergens, and microorganisms under specific foods and conditions, as well as the process conditions necessary to inactivate bacterial spores.	Russell (2002); Patterson (2005); Considine et al. (2008); Rivalain et al. (2010); Castro & Saraiva (2014); Huang et al. (2014)

		and nutrients are preserved. Reduced treatment times. Post-packaging treatments; Positive consumer appeal. Easy to use. Commercial systems available. Approved by regulatory	packaging options. Batch processing. High cost of equipment.		

		agencies.			
Dense phase carbon dioxide					
Pressure (MPa) Treatment time Temperature Compression and decompression rate	Physical disruption due to a rapid depressurization and expansion of CO_2 within the cells. CO_2 increases the membrane fluidity and permeability.	At supercritical conditions CO_2 has effective penetration capacity into solid matrices. CO_2 can easily be removed from the solutes by mere expansion	High capital costs are associated with this technology.	Elucidation of the mechanisms underlying changes on food properties, especially bioactive ingredients.	Choi et al. (2008); Calvo & Torres (2010); Rawson et al. (2011); Rawson et al. (2014)

		to ambient pressure. Relatively low operating costs.			

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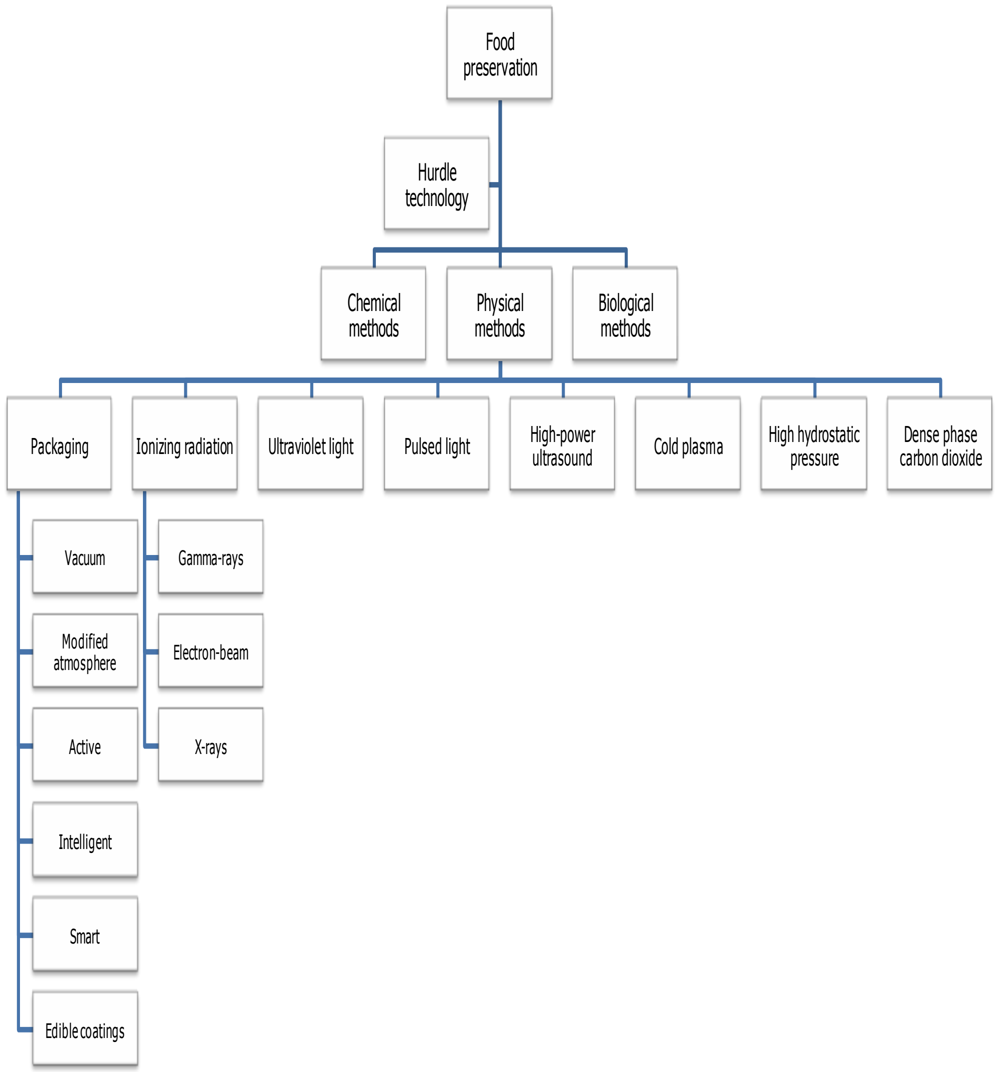

Figure 1. Non-thermal physical technologies to preserve fresh and minimally processed fruits and vegetables.

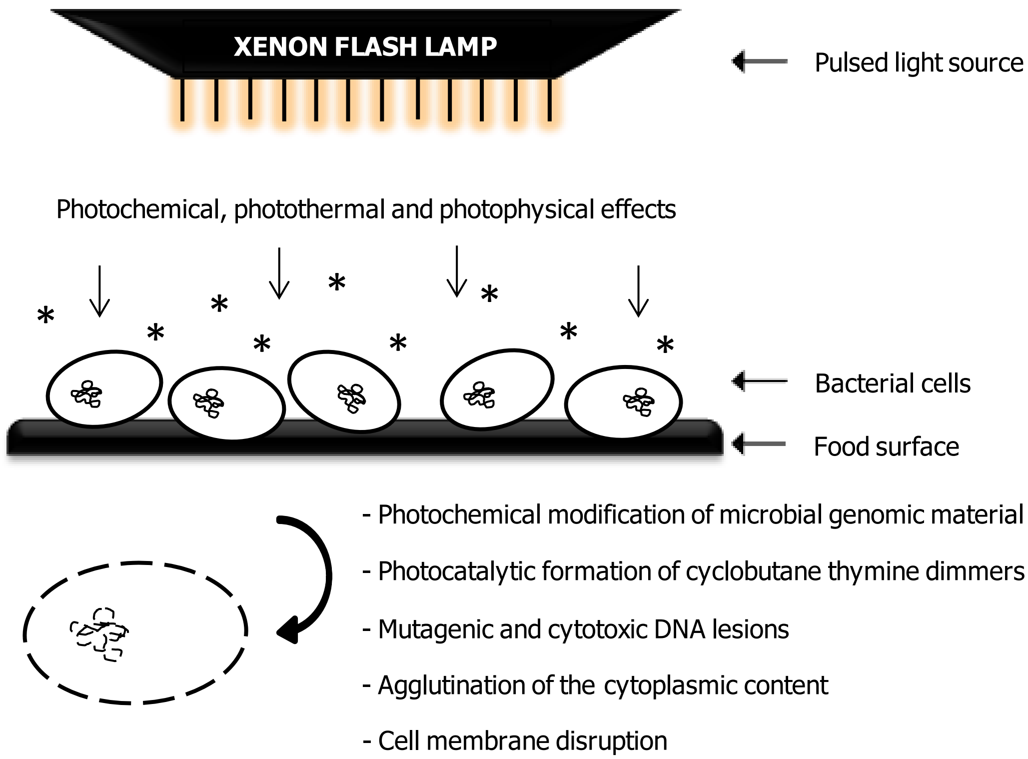

Figure 2. Mechanism of microbial inactivation by pulsed light.

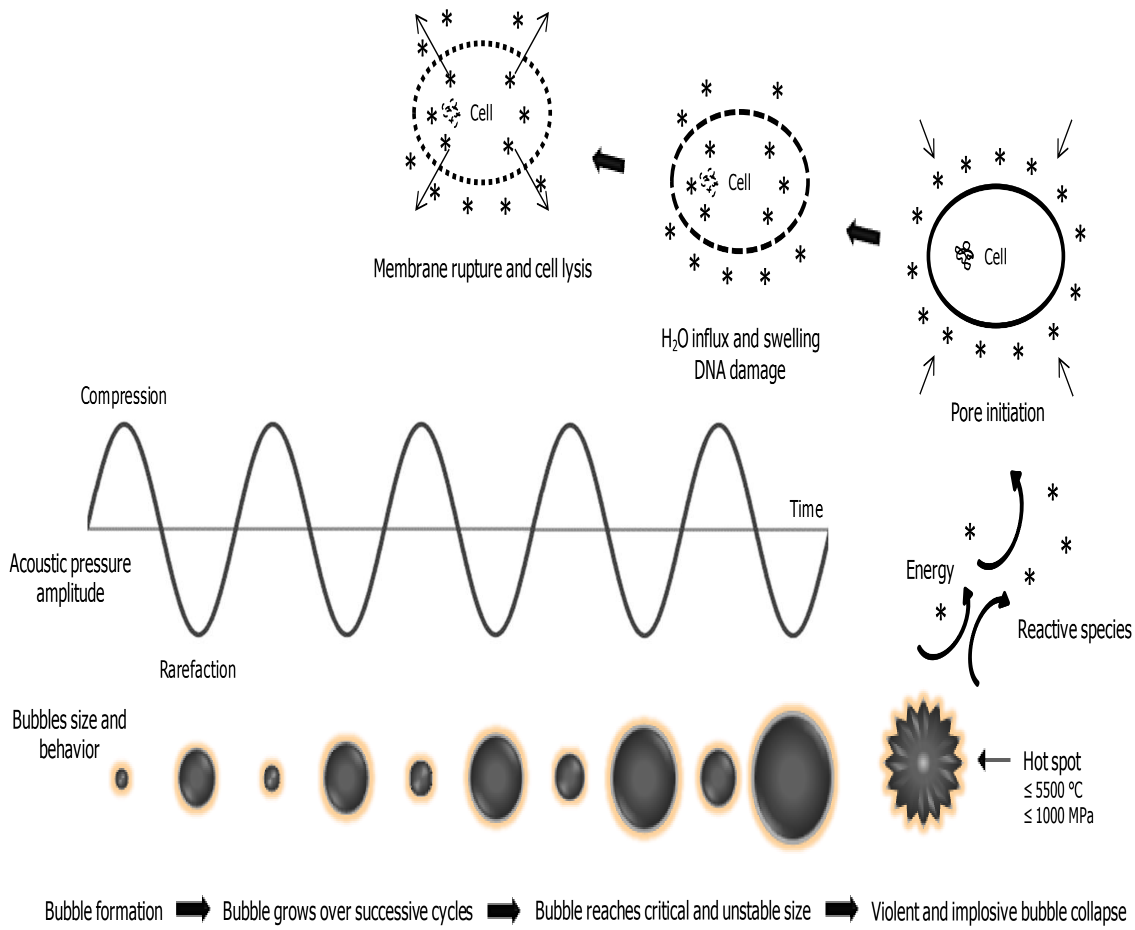

Figure 3. Mechanism of transient acoustic cavitation and microbial cells inactivation. Adapted from Leonelli and Mason (2010) and Chemat et al. (2011).

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