


Edited by
Charis M. Galanakis

# Food Quality and Shelf Life

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# Emerging cold pasteurization technologies to improve shelf life and ensure food quality

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## Chapter Outline

**1 Introduction 55**
**2 Emerging technologies 57**
2.1 High pressure treatment 57
2.2 Ultrasound treatment 68
2.3 Dense phase carbon dioxide 72
2.4 CP application 79
2.5 Radiation methods for food decontamination 82
2.6 Other methods 94
**3 Hurdle approach and combinations of methods 96**
**4 Future trends and perspectives 97**
**5 Conclusions 99**
**References 99**
**Further reading 123**

## 1 Introduction

How to collect enough food in order to ensure survival? Why does food spoil? How to preserve food longer? These are among the most important questions ancient humans had to solve along with development. The deeper the knowledge mankind

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gained with time, the better these issues were addressed. Nowadays, most of the problems related to microbial contamination, food preservation, and foodborne illnesses are solved to a large extent, or a reasonable compromise has been made. However, all of the "traditional" methods for ensuring food safety suffer from some disadvantages. Thermal treatment (sterilization, pasteurization, etc.), although the modern technologies rely on short exposure to elevated temperatures, leads to some loss of valuable nutrients, chemical and physico-chemical changes, and could initiate Maillard reaction (Young et al., 2018). The decrease of water activity is another method of control and extending shelf life. Either performed by water content reduction or by addition of compounds binding water strongly, the process significantly changes final products, and does not ensure freshness and texture compared to untreated foodstuffs.

Freezing and cold storage are among the most popular methods for microbial development delay. The usual cold storage temperature is around 4°C. However, some nonproteolytic strains of *Clostridium botulinum* could even germinate at 2–3°C (Selby, 2017), and *Listeria monocytogenes* and *Yerstnia enterocoltttca* could grow below 1°C (Gould, 2000). This is particularly important when an improper temperature control (as the domestic one very often is) is applied. Freezing below −18°C ensures prevention of microbial growth but care should be taken to avoid large ice crystal formation, which could damage the cell walls and lead to valuable nutrients loss (Chemat et al., 2017). Chemical preservatives and antimicrobial agents usage is one of the earliest and easiest ways to control microbial growth. Nevertheless, it is a controversial issue, does not always completely fulfil the aim, and lately, consumers' opinion tends towards utilization of fewer chemicals. For example, application of chlorine-based treatment could lead to formation of chlorinated derivatives with possible carcinogenic activities (Rico et al., 2007). Poultry treatment with chlorine has been banned in the EU since 1997 due to uncertainties of successful disinfection of species of *Salmonella* and *Campylobacter* depending on how heavily the birds were initially soiled. A recent study questioned the complete eradication of *L. monocytogenes* and *Salmonella enterica* if they were induced by chlorine treatment to enter a viable-but-nonculturable state (Highmore et al., 2018). Synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, tertiary butylhydroquinone, etc., and higher amounts of salt and sugar in the diet are often blamed for provoking various socially significant diseases.

Contemporary world trends, however, are oriented towards higher and higher consumption of ready-to-eat and minimally processed foods. Consumers expect foodstuffs to exhibit a combination of the convenience of semiprepared products and, at the same time, the quality of freshly picked fruit, vegetables, or freshly prepared meat, milk, or other type of meal. Besides, concerns of the public health risks, the possibility of bioterrorism threats has to be taken into account. All these expectations and attitudes raise an additional question: how to ensure longer shelf life of food while keeping it fresh, minimally processed, and without food quality deterioration? In order to adequately answer this question, new "emerging" technologies and their combined use have entered into practice and technology.

## 2 Emerging technologies

All of the new technologies rely heavily on physical treatments of the products while trying to keep the temperature as low as possible. For this reason, the emerging technologies are also often referred to as "cold pasteurization." Some of the methods employ electromagnetic fields, such as pulsed light (PL), pulsed electric field (PEF), high voltage arc discharge (HVAD), cold plasma (CP), or microwaving (MW). Others use a combination of high-energy electromagnetic waves and particles (ionizing radiation, IR), sound waves (ultrasound treatment, US), high pressure (high pressure technologies, which could vary depending on the effect achieved and techniques employed), pressure and carbon dioxide (dense phase carbon dioxide treatment, DPCD), and mechanical waves (shockwave treatment). In general, the effects of some of the physical processes were well known from the past but, due to the lack of proper equipment at that time, these technologies only recently became practically applicable.

The main driving forces of the introduction and commercialization of the new technologies, according to Jermann et al. (2015), could be summarized and ordered by significance as follows:

- enhanced quality and/or added value of the food products;
- safety issues concerns;
- extending shelf life of food systems;
- convenience;
- lowering the price, increase in competitiveness, running costs decrease;
- regulations and legislation;
- better waste management and eco-friendly procedures;
- globalization trends;
- financial issues;
- research support; and
- installation quality and operability.

### *2.1 High pressure treatment*

The application of high pressure on food systems has been known since the end of the 19th century. The first attempts were made with raw milk with dubious success. The major limitation was lack of proper equipment. At the end of the 20th century, advancement of the technologies allowed construction of machinery able to achieve high enough pressures. Nowadays, several major approaches for utilization of high pressure in the food industry could be distinguished: high pressure processing (HPP, high hydrostatic pressure processing); high pressure homogenization (HPH); microfluidization (MF); and high-pressure jet (HPJ) processing. In general, microfluidization is performed in the 20–280 MPa range, HPH in the range 100–400 MPa, HPJ uses pressures up to 600 MPa, and HPP, in the range 400–1000 MPa (Tobin et al., 2015; Tran et al., 2018) (Fig. 1).

The European Commission, after several major producers submitted, according to Regulation (EC) 258/97, applications for placing on the market food products treated by high pressure, decided in May 2001 that "the use of high-pressure pasteurization

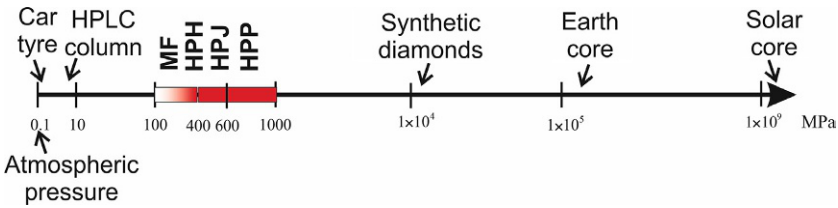



**Fig. 1** Comparative scheme of the high pressure technologies and the pressures utilized. *MF*, microfluidization; *HPH*, high pressure homogenization; *HPJ*, high pressure jet processing; *HPP*, high pressure processing.

in the production of fruit preparations is not likely to have an effect on public health so that a decision can be taken without consultation of the Scientific Committee for Food" (EU Commission decision, 2001). Similarly, the FDA ruled that HPP of fruit juices does not necessarily require specific FDA approval (US Food and Drug Administration, 2004), but the processors have to include in their plan the process time and pressure as critical limits. The Canadian authorities (Canadian Food Inspection Agency, 2016), based on a position from December 2016 of Health Canada (Health Canada, 2016), also stated that the results and proofs of safety issues with HPP treated food systems are already numerous and the novel status should be removed. However, the differences in the structure, microbial load, and texture for each product have to be carefully examined and taken into account.

The primary application of high pressure in the food industry is for nonthermal pasteurization and enzymes inactivation (Tran et al., 2018). Among the methods employing high pressure, HPP is the most investigated approach for food preservation, while at the same time retaining unchanged the nutrients, biologically active and aroma substances, and appearance of the treated products. HPJ, HPH, and MF are suitable for fluid or semifluid type food systems and mostly applied for emulsification, and droplet size reduction, especially for milk and creamy texture products (Mohan et al., 2016), although the higher pressures in HPH and HPJ also result in substantial microorganism reduction. HPP has several important advantages: it could be applied for a variety of products—liquid, solid and semisolid, the temperature could be easily controlled, and the product could be "cold" pasteurized after proper packing. Among the main disadvantages is the batch processing. HPJ, HPH, and MF are exclusively applied on liquids and the advantage is that they could be performed as continuous processes. The main disadvantages are related to packing after the treatment and the temperature control.

### *2.1.1 Effect of high pressure on microorganisms*

Microorganism destruction at high pressure goes through various stages and depends on the type and age of microorganisms, the pressure applied, the pH, the food matrix, etc. Initially high pressure affects the transport functions of the membrane. As the pressure rises, the membrane begins to deform, lose its integrity, and ultimately, under the effect of the difference in external and internal pressure, it breaks down. Depending on the cell type and the processing conditions, the effect on the transport

systems is due to protein denaturation in the cell membrane, modification of some protein structures, disruption of the membrane bonds, and lipid bilayer destruction. The pressurizing stage induces firmer packing of bilayered membrane, and gel-like structures are formed, which then, by diminishing the applied pressure at the end of the cycle/process, leading to puncturing and permeability of the cells (Barba et al., 2017).

Since there are a number of types of cellular structures in nature, the effect of high pressure in cell inactivation occurs differently and under different boundary pressure values (Abe, 2007; Hsiao-Wen et al., 2014). It should be noted that there is a correlation between the pressure and the processing temperature that can be used to enhance the treatment effect (Hsiao-Wen et al., 2014; Mohamed et al., 2012; Wang et al., 2013). Another important feature is the use of alternating pressure, i.e., the pressure is increased and released periodically, and the result is an increase in the final effect (Hsiao-Wen et al., 2014).

Simultaneously with membrane damaging, high pressure disturbs the physiological functions of the cellular structures. Firstly, the pressure disturbs the transposition mechanisms of substrates and metabolic products, which directly affects the cellular metabolic pathways naturally occurring in microbial cells. There is also evidence that high pressure treatment disturbs the synthesis of adenosine triphosphate. The proton flow in the cells changes and the intracellular pH, which is responsible for the physiological functions of the cellular structures, is affected (Hsiao-Wen et al., 2014; Tholozan et al., 2000). The enzyme functions are also impaired (Moreirinha et al., 2016).

High pressure changes the tertiary and quaternary structure of the cell proteins. Covalent bonds in proteins are not disturbed but breakdown of hydrogen bonds occurs (Hsiao-Wen et al., 2014). These variations are observed above 200 MPa and with pressure increase, the degree of change in the protein structure increases. Different authors, examining the influence of pressure on protein structure, state that at pressure values of up to 300 MPa changes in protein structures are reversible, but above this limit the changes become irreversible. Therefore, in order to substantially disturb the physiological functions of the cell, the pressure must be above 300 MPa (Hsiao-Wen et al., 2014; Yaldagard et al., 2008).

HPP affects the genetic material of the cell by disturbing DNA replication and transcription and also causes changes in the proteins of the nuclear cell structure. Stress factors such as high pressure may increase the resistance of some Gram-positive bacteria due to changes in their DNA structure. It is interesting to note that if the cells are processed at lower pressures they become resistant to shock conditions such as temperature and pressure due to genetic mutations (Hsiao-Wen et al., 2014; Wemekamp-Kamphuis et al., 2004). In addition, some bacteria could exhibit a recovery after HPP and survive in the food product (Muñoz-Cuevas et al., 2013).

Gram-positive bacteria, in general, showed better survivability compared to Gram-negative bacteria. Gram-positive bacteria have a thick cell wall composed of several layers of peptidoglycan (mucopeptide responsible for the thick solid structure) and two types of teihoic acids. Some species also have a layer on the cell surface called surface layer protein.The wall of Gram-positive bacteria contains several grid layers, while that of Gram-negative bacteria contains only one layer (Denkova et al., 2018;

Hsiao-Wen et al., 2014). Cells showed higher resistance in the presence of more unsaturated acids in the membrane because of greater membrane fluidity. Certain specific protein groups in the cellular membrane also increase cell stability (Allen et al., 1999; Denkova et al., 2018; Hsiao-Wen et al., 2014; Wemekamp-Kamphuis et al., 2004).

The age of the cell also affects its stability. In general, cells that are in an exponential growth phase are more sensitive due to the ongoing fission processes and the incomplete cellular structure. Cells in the stationary growth phase have well-formed membrane structures and are more resistant. However, there are exceptions, mostly related to the presence of RNA polymerase proteins in the cell wall. The spore-forming ability of some bacteria in the stationary phase is also important. Some spores can withstand pressure of 1000 MPa at room temperature. Spores have a multilayered structure and this leads to increased resistance. The best inactivation is application of periodically changing pressure, which induces spore germination and, further, their destruction (Hsiao-Wen et al., 2014; Minh et al., 2010).

The pH of the medium is important for cellular resistance. Data suggests that, at neutral pH, bacteria have a higher resistance than at lower (Bayindirli et al., 2006; Rastogi et al., 2007). The same applies to spore-forming bacteria, such as some *Bacillus* species. But this is not a rule, and the choice of a combination of pH-temperature-pressure processing conditions should be optimized for each particular type of microorganism. Islam et al. (2006) found that relatively low pressures and pH 7 did not affect significantly the resistance of *Bacillus coagulans*, but the pH reduction of three units increased the pasteurization effect even at lower pressures. Other authors investigated the condition of *B. coagulans* spores in model conditions and tomato juice applying a constant pressure of 800 MPa and a variable temperature of 25–75°C, and found that bacterial spores are much more sensitive to the combination of high temperature and high pressure under acidic conditions (Hsiao-Wen et al., 2014;Vercammen, et al., 2012).

For products with pH below 4.5 (most juices) it was suggested that pressures of 580–600 MPa and duration of 4–6 min, in general, are sufficient (Augusto et al., 2018). However, it had to be taken into account that even at conditions as harsh as 1200 MPa, the molds and bacterial spores could survive and germinate later in a better environment (Datta and Deeth, 2011), and spores are also resistant to heat, irradiation, and some chemical treatment (Hsiao-Wen et al., 2014). Increasing the pressure to 1000–1200 MPa at low temperatures does not significantly affect the spores of the species of *Bacillus* and *Clostridium.* As the temperature increases, the spore resistance of the two genera decreases, thus reducing the pressure needed (Aouadhi et al., 2013; Hsiao-Wen et al., 2014; Shao and Ramaswamy, 2011). Typically, the inactivation of spores occurs by combining the effects of temperature and pressure, quite often combined with other methods, including the addition of chemicals (Heinz and Buckow, 2010; Hsiao-Wen et al., 2014).

Destruction of protozoa and parasites is achieved at relatively low pressures (100–400 MPa), whereas inhibition of fungi and yeast requires pressure of more than 400 MPa. Furthermore, fungal spores are more resistant to HPP than vegetative cells (Rendueles et al., 2011). Viruses have different sensitivity to high pressure (Rendueles et al., 2011).

An important element in high pressure processing is the type of food matrix. Data suggested that solid food matrices protect cells better than liquid media (Tassou et al., 2007). The presence of inhibitory components such as bacteriocins and antimicrobial agents reduces cellular resistance, especially if these agents affect the cellular membrane structure (López-Pedemonte et al., 2003).

In conclusion, the reduction of the vegetative forms of microorganisms by 4–5 log $N$ is obtained at pressures of about 400–600 MPa at room temperature. Study data by a number of authors can be summarized as follows:

- Prokaryotic cells are more resistant to high pressure than eukaryotic ones;
- Gram-positive bacteria have higher resistance than Gram-negative bacteria;
- Cells with a spherical shape, in comparison with rod-shaped bacteria, are more resistant; and
- Spores of spore-forming microorganisms are also more resistant than vegetative forms (Hsiao-Wen et al., 2014).

Increasing the effect of the treatment could be achieved by combining with other factors: changes in temperature, changes in pH, presence of chemical inhibitors, use of pulsating pressure, etc. (Rendueles et al., 2011). A nonexhaustive list of applications for inactivation of microorganism and potential structural, chemical, physicochemical, and biochemical consequences from the high pressure treatment of various food products is presented in Table 1.

### 2.1.2 *Effect of high pressure on low molecular substances, biopolymers and food structure*

The effective conditions for microbial reduction of applied preservation technology very often have negative effects on food structure. The emulsions are important biphasic systems in different food and cosmetic products. The emulsion stability determines the proper physical structure and appearance of the formulation and often could be disrupted by application of preservation methods. A recent study (Young et al., 2018) compared the stability of emulsion systems and biologically active substances after thermal treatment: short time-high temperature (90°C, 3 min), low temperature-long time (70°C, 30 min), and sterilization (121°C, 15 min), with HPP pasteurization (600 MPa, 5 min). The results implied that the disruption of the emulsion systems treated by HPP was the lowest and the recovery of retinol (used as a model of biologically active substance encapsulated in the micelles) was the highest. It should be noted, however, that the results depend on the type of matrix (e.g., the emulsifier used) and duration of the treatment, and the results could not be unambiguously interpreted. Other studies have found jellification of the food systems or food components treated by HPP (Ribeiro et al., 2018; Yang et al., 2016), which contributed to the better textural and functional properties of the food products. It has been found also that HPP leads to an improvement in the organoleptic profile of meat as some enzymes such as proteases are activated (Hsiao-Wen et al., 2014; Simonin, et al., 2012).

The volatile aroma compounds play a key role for the quality of food products. The effect of HPP (600 MPa, 6 min, 21°C) on volatile substances in Iberian dry-cured ham was studied (Martínez-Onandi et al., 2017b) and a significant change was found in the amount of the key odorants. In addition, an increase of substances with unpleasant

**Table 1** High pressure application for treatment of food systems

| Pressure/ technology | Product/medium | Microorganisms reduction | Optimum conditions | Co-processing; additives; storage | Structure related changes | Chemical changes | References |
|---|---|---|---|---|---|---|---|
| HPP | Fresh cheese | *Listeria monocytogenes* | 600 MPa, 10 min, (20°C) | Refrigeration, 4°C | Not investigated | Not investigated | Tomasula et al. (2014) |
| HPP | Whole milk<br>Skim milk | *Cronobacter sakazakii* | 400 MPa, 3 min | No | Not investigated | Not investigated | Jiao et al. (2016) |
| HPP | Korla pear (*Pyrus bretschneideri rehd*) juice | Total microorganisms count | 500 MPa, 10 min | Pretreatment by ultrafiltration | Clarity, color and degree of browning | Ascorbic acid, total polyphenols, aroma compounds | Zhao et al. (2016) |
| HPP | Mixed sweet pepper–orange juice | Total aerobic bacteria, molds and yeasts | 550 MPa, 5 min | Storage (4°C, 25 days) | Color, sensory analysis | Ascorbic acid, total polyphenols, antioxidant activity | Xu et al. (2015) |
| HPP | Strawberry (*Fragaria×ananassa Duch.*) puree and juice | Total aerobic bacteria, molds and yeasts | 500 MPa, 3 min | Storage (6°C, 49 days) | Color, pH, soluble matters, sensory analysis | Ascorbic acid, total, antocyanins | Aaby et al. (2018) |
| HPP | Carrot (*Daucus carota* L.) juice | Total aerobic bacteria, molds and yeasts | 550 MPa, 6 min | Storage (4°C, 20 days) | Color, pH, soluble matters, viscosity, clarity, sensory analysis | Total phenols, carotenoids, sugars, antioxidant activity | Zhang et al. (2016) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| HPP | Clear cucumber juice | Total aerobic bacteria, molds and yeasts | 500 MPa, 5 min | Storage (4°C, 20 days) | Color, soluble matters, clarity, pH, acidity, sensory analysis | Key odorants retained: (*Z*)-6-nonenal, (*E*,*Z*)-2,6-nonadienal, (*E*)-2-nonenal, and (*E*,*Z*)-3,6-nonadien-1-ol | Liu et al. (2016) |
| Thermally assisted HPP | Mango (*Mangifera indica* L.) pulp | Aerobic mesophiles count, yeast and mold, total coliforms, lactic acid bacteria, psychrotrophs | 600 MPa, 10 min (52°C) | No | Color, acidity, soluble matters | Ascorbic acid, total phenols and flavonoids, antioxidant activity | Kaushik et al. (2016) |
| Ultra HPH | Orange juice | *Alicyclobacillus acidoterrestris*, *Alicyclobacillus hesperidum* spores | 300 MPa | Inlet temperature 70°C | pH | Not investigated | Roig-Sagués et al. (2015) |
| Ultra HPH | Tiger nut (*Cyperus esculentus* L.) milk | Psychrotrophs, Aerobic mesophiles, Lactobacilli, Enterobacteriaceae, faecal coliforms, total mesophilic spores, molds and yeasts | 300 MPa, 40°C | Storage (4°C, 57 days) | Color (slightly changed), pH, sensory analysis | Not investigated | Codina-Torrella et al. (2018) |

*Continued*

**Table 1** Continued

| Pressure/ technology | Product/medium | Microorganisms reduction | Optimum conditions | Co-processing; additives; storage | Structure related changes | Chemical changes | References |
|---|---|---|---|---|---|---|---|
| HPP | Black tiger shrimp | Aerobic mesophiles, Psychrotrophs, *Escherichia coli*, *Staphylococcus aureus* | 300 MPa, 3–9 min; 400 MPa, 3 min | No | Color: loss in transparency; decreased redness, increased of yellow. Texture: higher hardness, springiness, cohesiveness, chewiness and gumminess | Trimethylamine nitrogen content: decreased in all conditions. Lipid oxidation: significant increase, but below the critical limits | Kaur et al. (2016) |
| HPP | Chilean jack mackerel (*Trachurus murphyi*) | Mesophilic and psychrophilic aerobic microorganisms; *Shewanella putrefaciens* ($H_2S$ producing bacteria) | 450 MPa, 3 min; 550 MPa 4 min (20°C) | No | Not investigated | Trimethylamine nitrogen content: decreased and remained for 26 days below 5 mg/100g pH increase slowly compared to control | Reyes et al. (2015) |
| HPP | Horse mackerel (*Trachurus trachurus*) | – | 150 MPa, 5 min | Frozen storage (1 and 3 months) | Texture and sensory analysis. Color change: loss in transparency | Water content decrease | Torres et al. (2014) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| HPP | Sea bass (*Dicentrarchus labrax*) fillets | Total microorganisms count | 600 MPa, 30 min | No | Appearance: cooked-like; whither and loss of translucency; WHC[a] decreased | Changes in the protein profiles | Teixeira et al. (2014) |
| HPP | Haddock (*Merlangius euxinus*) | Total mesophilic aerobic bacteria | 400 MPa, 5 min (15°C) | Refrigeration, 4°C, 15 days | Color changes: decreased *L* value (*L*a*b* system). Sensory analysis (odor): 15 days shelf life; control–7days | Increased TBA[b]; Decreased TMA-N[c], DMA-N[d] and TVB-N[e] values compared to control | Alpas and Akhan (2012) |
| HPP | Fresh farmed salmon (*Salmo salar*) and cod (*Gadus morhua*) Fresh wild mackerel (*Scomber scombrus*) | Total aerobic count; Hydrogen sulphide producing bacteria | 500 MPa, 2 min | Refrigeration, 0.5°C, 26 days | Not investigated | Increased levels of lipid oxidation (TBA[b] method). Decreased phosphatase activity | Rode and Hovda (2016) |
| HPP | Marinated herring (*Clupea harengus*) fillet | *Morganella psychrotolerans*; $H_2S$-producing bacteria; Total psychrophilic bacteria | 300 MPa, 10 min; 500 MPa, 5 or 10 min | 4% acetic acid, up to 90 days (for 500 MPa) | Not investigated | Histamine and cadaverine not observed for the storage period | Ucak et al. (2018) |
| HPH | Hen egg white lysozyme activity | Antimicrobial activity against *Escherichia coli*, *Enterococcus faecalis*, *Geobacillus stearothermophilus*, *Bacillus cereus* | 120 MPa, 20°C | Combined action of lysozyme and HPH | Not investigated | Not investigated | Tribst et al. (2017) |

*Continued*

**Table 1** Continued

| Pressure/ technology | Product/medium | Microorganisms reduction | Optimum conditions | Co-processing; additives; storage | Structure related changes | Chemical changes | References |
|---|---|---|---|---|---|---|---|
| HPP | Serrano ham | Aerobic mesophiles<br>Psychrotrophs<br>Lactic acid bacteria<br>*Micrococcaceae*<br>Moulds and yeasts | 600 MPa, 6 min (21°C) | No | Not investigated | Not investigated | Martínez-Onandi et al. (2017a) |
| HPP | Spanish chorizo sausage | *Listeria monocytogenes* | 550 MPa, 12.3 min | Maintaining $a_w$ at 0.86 | Not investigated | Not investigated | Rubio et al. (2018) |
| HPP | Saline solution; meat slurry | *Staphylococcus aureus* | 350 MPa, 6 min | Nisin (500 ppm) | Not investigated | Not investigated | Yao et al. (2015) |
| HPP | Ready-to-eat sliced dry-cured ham | *Listeria monocytogenes* | 600 MPa, 5 min | Nisin | Not investigated | Not investigated | Hereu et al. (2012) |
| HPP | Cooked ham mortadella | *Listeria monocytogenes* | 400 MPa, 5 min | No | Not investigated | Not investigated | Hereu et al. (2014) |
| HPP | Ground chicken | *Listeria monocytogenes* | 600 MPa, 3 min | NaCl, KCl, **$CaCl_2$** | Not investigated | Not investigated | Balamurugan et al. (2016) |
| HPP | Ground beef | *Escherichia coli* (Shiga toxin-producing) | 350 MPa, up to 40 min (4°C) | Observed some resistance | Not investigated | Not investigated | Sheen et al. (2015) |

[a] WHC: water holding capacity.
[b] TBA: thiobarbituric acid value (mg malondialdehyde/kg sample).
[c] TMA-N: trimethylamine-nitrogen.
[d] DMA-N: dimethylamine-nitrogen.
[e] TVB-N: total volatile basic nitrogen.

odor, such as nonanal, methanethiol, and dimethyl trisulfide, alter the sensorial profile of the product. Similar observations were made by Martínez-Onandi et al. (2016a,b) investigating Serrano dry-cured ham. The mechanism of formation of the volatiles in meat products containing higher fat levels is based on the lipolysis and further oxidative destruction of fatty acids. The increased formation of free radicals, especially at higher pressures (600–800 MPa), could also be related to the higher rates of formation and breakdown of hydroperoxides, and direct the reaction towards formation of less branched aldehydes (Fuentes et al., 2010). The upper limit of applied pressure that resulted in less radical formation, according to Bolumar et al. (2012), was 400 MPa (25°C) and 500 MPa (5°C). An additional advantage of HPP is inactivation of enzymes able to produce biogenic amines. Treatments at 350–400 MPa for 10–15 min significantly lowered the levels of cadaverine, putrescine, and tyramine in fermented meat products (Nuñez et al., 2016).

Although HPP was proven to ensure preservation with minimal heating, in certain cases a final product with the consumers' preferred textural characteristics and flavor cannot be achieved. Pingen et al. (2016) investigated ham production, trying to replace the traditional application of heating in the technological scheme by HPP (500 and 600 MPa). As a result, the authors failed to produce ham and the result might be described as a "ham-like" product with a softer texture and color diverging from the traditionally prepared product. Combined pretreatment with HPP and mild heating at 53°C gave better results, but in this case the advantage of shorter production time is lost.

HPP induces fewer changes compared to heat treatment but significant effects are observed and their influence on human health have to be carefully studied. In a recent study, Bogahawaththa et al. (2018) compared the impact of HPP and HTST on raw milk proteins. The results revealed that HTST has preserved to a large extent the natural conformations of proteins and this could suggest almost unchanged immunogenicity of milk. On the contrary, HPP at 400, 500, and 600 MPa (15 min, 30°C) led to denaturation of β-lactoglobulin and immunoglobulin G, and formation of protein clusters with participation of κ-casein. In addition, the authors observed alteration of immunogenicity of the treated milk (for pressures of 400 and 500 MPa) by measuring changes in the ratio of different types of T helper cells.

Today, it is roughly estimated that around 28% of vegetables and vegetable/fruit based products, 26% of meat products, 15% of seafood and fish, 14% of juices and beverages, and around 17% of other foods, are treated with high pressure (Liepa et al., 2016; Paterson, 2005). Fig. 2 presents an example of HPP treated meat products sold in Greece.

No doubt the application of high pressure technologies offers substantial advantages over traditional preservation methods, such as extending the shelf life, packaging before treatment, avoiding changes related to heating (even for a short time), etc. Nevertheless, limitations and shortcomings exist and often make the practical application of HPP difficult. Among the main disadvantages are the high initial investment, inability to utilize on all food systems, and structural and chemical changes, which need further research. It seems that the best way of applying HPP is in combination with other methods in order to ensure high quality longer shelf life foods and keeping processing at minimum levels.




**Fig. 2** Meat products treated by HPP and sold in the retail network of Greece.

## 2.2 Ultrasound treatment

Ultrasound (US) consists of sound waves with frequencies higher than the upper limit of human hearing—approximately 20 kHz. US techniques are simple, reliable, energy-saving, and cheap, and find application in many spheres of food processing. Based on the frequency, the applications of US in food processing and technology could be divided into low and high energy. Low power US, with frequencies higher than 100 kHz, at intensities below 1 W $cm^{-2}$, is among the most widely used nondestructive analytical methods for analysis and monitoring of foods, to ensure high quality during processing and storage. It is used for quality control of fresh vegetables and fruits, detection of honey adulteration, and assessment of the aggregation state, size, and type of protein, etc. High power ultrasound (HPUS) uses intensities higher than 1 W $cm^{-2}$ and frequencies between 20 and 500 kHz. These are disruptive and have effects on the physical, mechanical, or chemical/biochemical properties of microorganisms and organic matter. HPUS is recognized as an emerging technology with various application for controlling microstructure of foods, modifying textural characteristics of fat products, emulsification, defoaming; inactivation or acceleration of enzymatic activity, microbial inactivation, freezing, drying, and extraction of various food and bioactive components (Awad et al., 2012; Knorr et al., 2004).

### 2.2.1 Microbicidal effect of ultrasound

The antimicrobial effect of US on vegetative forms has been known since 1929, when Harvey and Loomis (1929) proposed the use of US for food processing and preservation. Since then, many studies have been performed to clarify the mechanisms behind the microbicidal effect of ultrasound. US-mediated inactivation of microorganisms occurs through physical, mechanical, and/or chemical mechanisms. Cavitation resulting from US treatment appears to be the main promoter of US-assisted microbial death. Cavitation can be classified into acoustic, hydrodynamic, optic, and particle, based on the mode of generation (Gogate et al., 2006). The chemical effects of US are due mainly to acoustic cavitation, which includes formation, growth, and implosive collapse of gas bubbles in liquids (Kentish and Ashokkumar, 2011). During bubble explosion, a considerable amount of energy is generated and resulting pressure causes mechanical weakening and destruction of cell walls (Maleki et al., 2013). In addition, hydroxyl radicals are generated from the dissociation of the water molecules

(Gogate and Pandit, 2011). Generated free radicals attack the bacterial cell wall and cause weakening and destruction (Kentish and Ashokkumar, 2011; Raso and Barbosa-Canovas, 2003). It has been proposed also that US could deagglomerate bacterial clusters or flocs (Joyce et al., 2003).

### Factors affecting US microbicidal efficiency

Several factors affect the microbicidal efficiency of US treatment: type of microorganism and its vegetative form, degree of contamination, US frequency, heating, pressure, or other treatments. For example, US treatment is more efficient in inactivation of Gram-negative and rod-shaped bacteria, whereas spores, Gram-positive, and coccal bacteria are more resistant to US-mediated rupturing (Feng et al., 2008). Arroyo et al. (2011) revealed that US resistance of *Cronobacter sakazakii* in standard conditions was similar to that of other Gram-negative species and lower than that of Gram-positives. This effect is related to the higher amount of peptidoglucans in Gram-positive bacteria cell walls, which determines their higher strength and stability. Different strains differ in their resistance to US, as well. For example applying the same US parameters, *Escherichia coli* and *Saccharmyces cerevisiae* were reduced by more than 99% after ultrasonication, whereas *Lactobacillus acidphilus* was reduced by 72%–84% (Cameron et al., 2008). Usually a frequency of about 20 kHz is applied for microbial inactivation, but mortality rate is highly dependent on US frequency and wave amplitude (Anaya-Esparza et al., 2017). As already mentioned, cavitation occurs in water media and, therefore, moisture content of foods is crucial for microbicidal effectiveness. Therefore, US is more efficient in liquid foods in comparison to solid ones. Arroyo et al. (2011) demonstrated that decreasing water activity of the treatment medium resulted in a significant increase in the resistance of *C. sakazakii*. In general, HPUS is not very effective in killing bacteria in foods alone, and is often combined with other preservation methods, such as antibiotics, hypochlorite, mild heat, or pressure (Baumann et al., 2005, Furuta et al., 2004; Lee et al., 2003; Piyasena et al., 2003a; Villamiel and de Jong, 2000).

## 2.2.2 *Application of US for inactivation of food spoilage microflora and improving food quality*

### Application for liquid foods

The most widely US-treated liquid foods are fruit and vegetable juices. In general US treatment increases juices' microbial stability and retains their nutritional components better than conventional thermal treatments. Experimental results suggested that thermosonication leads to a significant reduction of the microbial count (1–9 logs) of different microorganisms such as *S. cerevisiae* (Bermúdez-Aguirre and Barbosa-Canovas, 2012; Char et al., 2010; Ferrario et al., 2015; Marx et al., 2011; Wordon et al., 2012), *E. coli* and *Salmonella enteritidis* (Char et al., 2010; Kiang et al., 2013; Muñoz et al., 2011, 2012; Sagong et al., 2011), *C. sakazakii* (Adekunte et al., 2010; Arroyo et al., 2011, 2012), *Alicyclobacillus acidophilus* and *Alicyclobacillus acidoterrestris* (Evelyn and Silva, 2016; Ferrario et al., 2015; Wang et al., 2010), and *Staphylococcus aureus* (Walkling-Ribeiro et al., 2009). For example, manothermosonication (4 bar,

43°C, 750 W, 20 kHz) significantly reduced microbial *E. coli* and *Pichia fermentans* counts inoculated in a fresh blend of apple and cranberry juice, compared to the untreated sample (Palgan et al., 2011). Thermosonication (24 kHz, 120 μm amplitude) at different temperatures (50°C, 54°C, and 58°C) was used to increase the shelf life of carrot juice. Samples sonicated at 58°C had the best quality, while microbial growth remained low at around 3-log for mesophiles, 4.5-log for yeasts and molds, and 2-log for enterobacteria after 20 days of storage. Furthermore, sonicated juice at 58°C retained more than 98% of its carotenoids and 100% of ascorbic acid.

Khandpur and Gogate (2015, 2016) investigated the effect of US (20 kHz, 100 W, 15 min) on the nutritional quality of different juices (orange, sweet lime, carrot, and spinach) in comparison to pasteurization (80°C, 10 min). US-processed juices retained most of the nutrients (polyphenols, vitamin C, carbohydrates, etc.) to a higher extent. Moreover, a scale up of the process with spinach juice confirmed that the technology could retain the required nutrient contents and microbial safety in refrigerated storage conditions for 10 weeks. The survival tests of *L. monocytogenes* in apple cider indicated the possibility of using a mild treatment condition in combination with ultrasound to achieve a 5-log reduction in pathogen populations (Baumann et al., 2005). Bermúdez-Aguirre and Barbosa-Cánovas (2008) used US to reduce *Listeria innocua* in milk and suggested that US-treatment efficiency depended on the fat content of the milk. Inactivation of *Listeria* cells occurred first in fat free milk, and rate of inactivation decreased with increasing fat content. No degradation of protein, a slight decrease in pH, and a minor increase of the milk's lactic acid content was observed.

### Application to solid foods

Fruit and vegetable are among the most widely US-treated foods. US was used by Aday et al. (2013), who investigated the effect of different ultrasound powers (30, 60, 90 W) and treatment times on quality of fresh strawberries. The authors revealed that power levels between 30 and 60 W improved quality of the fruits, resulting in higher oxygen concentration inside packages. The pH, total soluble solid content, and color (30 and 60 W treatments) were better than untreated control and 90 W treatment. Authors concluded that US power as high as 90 W is detrimental for the quality of strawberries. However, combining US with the chemical sanitizers (ozone and chlorine dioxide) was more beneficial for quality factors such as pH, total soluble solids, electrical conductivity, and texture, compared to the individual treatment or untreated fruit (Aday and Caner, 2014). In a similar study, freshly harvested strawberries were treated with 0, 25, 28, 40 or 59 kHz ultrasound at 20°C for 10 min. The results showed that 40 kHz US treatment significantly reduced decay incidence and numbers of microorganisms. US treatment also inhibited the decrease of firmness and maintained significant higher levels of total soluble solids, total titratable acidity, and vitamin C (Cao et al., 2010).

In a more recent study, strawberries were treated with ultrasound (33 kHz, 60 W) for different time periods (0, 10, 20, 30, 40, 60 min) and analyzed for the storage period of 15 days at 4°C. 20 and 30 min treatments were optimum for the firmness and color of the fruits. The pH, vitamin C, acidity, and total soluble solids were better retained between 30 and 40 min, while at 40 min treatment time, the bacterial count

decreased from 5.91 to 3.91 log CFU $g^{-1}$, while yeast and mold count decreased from 4.80 to 3.58 log CFU $g^{-1}$. 30–40 min US treated fruits were shelf stable for the entire storage period (15 days). The US treatment was found to be effective for color retention, texture loss, preserving sensorial quality, and microbial load reduction of whole tomatoes (Pinheiro et al., 2015). Vivek et al. (2016) used response surface methodology for optimization of US treatment combined with sodium hypochlorite solution of kiwifruit (*Actinidia deliciosa*). The optimal parameters obtained were US intensity (368 W $cm^{-2}$), temperature (25°C), treatment time (8 min), and concentration of the solvent (30 ppm). US combined with NaOCl was found to be the most effective treatment in inhibiting the microbial growth (bacteria, yeast, and mold) and preserving the quality of kiwifruits, suggesting that US treatment may provide an alternative for extending the shelf life of whole kiwifruit.

In a recent study, Amaral et al. (2017) demonstrated that sonication of potato strips (40 kHz, 5 min) coupled with an acid medium gives better results in comparison to blanching or coating with edible films, in respect to their quality and microbial inactivation. Thermosonication combined with slightly acidic electrolyzed water effectively prolonged the shelf life of fresh-cut kale during refrigeration storage (Mansur and Oh, 2015). Chen and Zhu (2011) investigated the effect of the simultaneous US treatment (100 W for 10 min) and aqueous chlorine dioxide (40 mg $L^{-1}$ $ClO_2$ for 10 min) on postharvest storage quality of plum (*Prunus salicina* L.). Combination of the two processes was effective in reducing the initial microflora and retaining sensory qualities of plum fruit, extending shelf life from 35 days (for controls) to 60 days after the treatment.

The first studies on the effect of US on the quality and shelf life of meat (dating back to the early 1990s) did not find any significant effect of US on microbial inactivation (Pohlman et al., 1996: Smith et al., 1991). More recently, Kordowska-Wiater and Stasiak (2011) investigated the removal of Gram-negative bacteria (*Salmonella anatum*, *E. coli, Proteus* sp., and *Pseudomonas fluorescens*) from the surface of chicken skin after treatment with US (40 kHz, 2.5 W $cm^{-2}$ for 3 or 6 min) in water and 1% lactic acid. Sonication in water alone or in lactic acid for 3 min resulted in a reduction of microorganisms on the skin surface by 1.0 CFU $cm^{-2}$; 6 min treatment reduced more than 1.0 CFU $cm^{-2}$ in the water samples, and 1.5 log CFU $cm^{-2}$ in the lactic acid samples. Hanieh et al. (2014) showed that steam treatment and US applied to chicken carcasses can significantly reduce the number of *Campylobacte*r on contaminated birds. The total viable count was reduced by approximately three logs by applying steam and US immediately after slaughter (Alarcon-Rojo et al., 2015).

More often, combined techniques are applied for microbial reduction of meats. US treatment inhibited the growth of psychrotrophic and lactic bacteria, reduced the lipid oxidation, and promoted only minor pH and texture modifications during storage, improving the pasteurization of hot dog sausages (Cichoski et al., 2015). Ferrentino and Spilimbergo (2016) revealed the feasibility and potential of combining US with DPCD for the microbial stabilization of cooked ham. The authors demonstrated that US with DPCD was more effective in comparison to DPCD alone, reducing the treatment time for inactivation of lactic acid bacteria, yeasts, and molds to undetectable levels. In another case, the same authors revealed that HPUS alone was not able to

induce any microbial inactivation of *L. monocytogenes*, while in combination with DPCD there was significant inactivation, depending on the parameters of the treatment (Spilimbergo et al., 2014). Morild et al. (2011) evaluated the inactivation of pathogens by the application of pressurized steam simultaneously with HPUS (30–40 kHz, 0.5–4.0 s) on the surface of pig skin and meat. Authors observed reduction of viable bacteria (*Salmonella typhimurium*, *Salmonella derby*, *Salmonella infantis*, *Yersinia enterocolitica*, and *E. coli*) with 1.1 log CFU $cm^{-2}$ after treatment for 1 s and 3.3 log CFU $cm^{-2}$ after treatment for 4 s. The reduction of microorganisms in pig skin was significantly greater than the reduction in meat.

Pedrós-Garrido et al. (2017) assessed the applicability of HPUS for surface decontamination of salmon (S. *salar*), mackerel (*S. scombrus*), cod (*G. morhua*), and hake (*M. merluccius*) fillets, and evaluated its impact on fish quality. US treatment reduced microbiological counts in oily fish species (salmon and mackerel) with 1.5 and 1.1 log CFU $g^{-1}$ for psychrophilic and mesophilic, respectively. In white fish species, the reduction was 0.5 log CFU $g^{-1}$. Lipid content did not change significantly by US treatment, whereas there was significant reduction of thiobarbituric acid reactive substance (TBARS) values.

HPUS is a cost-efficient and environmentally friendly technology to reduce microbial count and preserve nutritional components of various foods, thus extending their shelf life. However, its low efficiency in inactivation of bacterial spores impairs its application as a sterilization procedure. In combination with heat and/or pressure, HPUS can accelerate the rate of food sterilization. Among these combined techniques, HPUS under pressure at nonlethal and lethal temperatures appears to have great potential for industrial applications as they are more energy-efficient and effective in killing microorganisms. Furthermore, these techniques have shown several advantages compared to heat pasteurization, in terms of sensory and nutritional quality of treated foods.

## 2.3 Dense phase carbon dioxide

Fraser (1951) and Foster et al. (1962) studied the effect of DPCD on microorganisms and found that rapid decompression of $CO_2$ from high to atmospheric pressure resulted in the destruction of microbial cells. In 1969, the American company Swift & Co. received the first patent for food pasteurization by using $CO_2$ in "Super atmospheric" pressure. Various microorganisms had been shown to exhibit a different sensitivity to DPCD by 2002, when Spilimbergo et al. (2002) proposed the potential use of DPCD as an alternative nonthermal technique for pasteurization of foods.

### 2.3.1 Microbicidal effect of DPCD

Several mechanisms elucidate the microbicidal effect of DPCD: extracellular and intracellular pH decrease; inhibition of biological activities; cell rupture; modifications of cell walls; precipitation of proteins and ions; extraction of cellular substances; and disordering of the intracellular electrolyte balance (Erkmen, 2012). High pressure facilitates saturation of water with carbon dioxide, which in aqueous media transforms to $H^+$ and carbonate ions. The accumulation of $H^+$ decreases pH in both the media

(extracellular pH) and the microbial cells. The low extracellular pH does not have a significant direct microbicidal effect on the cells. Instead, it acts indirectly by increasing the permeability of membranes, which further facilitates the penetration of $CO_2$ in cells (Lin et al., 1994; Erkmen, 2012). Due to the high affinity of $CO_2$ to membrane phospholipids, $CO_2$ is accumulated fast in membranes, which modifies them and leads to an increased membrane fluidity and permeability (Hong and Pyun 2001; Damar and Balaban, 2006; Garcia-Gonzalez et al., 2007). Due to the high pressure, $CO_2$ accumulates in cells to a critical level that leads to a removal of important cellular constituents, compromised structure of membrane, and imbalance in biological systems (Erkmen, 2012).

Other studies indicate cytoplasmic leakage, rather than membrane modification, as a result of the elevated $CO_2$ in microbial cell (Hong and Pyun, 1999, 2001). Constant cytoplasmic pH is vital for microbial cells and, therefore, is maintained via different mechanisms. Several studies indicate that reduction of intracellular pH is the main microbicidal tool of DPCD (Dillow et al., 1999). Acidification of cytoplasm leads to denaturation of numerous enzymes, which actually is among the main reasons for the microbial inactivation (Erkmen and Bozoglu, 2008). For example, treatment with DPCD of *E. coli* leads to a lack of activity of its alkaline phosphatase and galactosidase (Zhang et al., 2006). Hong and Pyun (2001) revealed that DPCD inhibits the activity of 13 enzymes from *Lactobacillus plantarum*, while the activity of others remained unaltered. This indicates that DPCD-induced inactivation of enzymes is selective. The concentration of the dissolved bicarbonate is crucial for the inhibition of enzyme activity since it regulate the general anion-sensitive site on the enzymes (Damar and Balaban, 2006). Furthermore, it affects the carboxylation and decarboxylation reactions in microbial cells (Erkmen, 2012). Increased permeability and fluidity of cell membrane, as a result of DPCD treatment, could lead to extraction of cellular components. Depressurization could also facilitate the transfer of membrane and intracellular materials out of the cell. DPCD treatment triggers series of subsequent and/or parallel morphological or metabolic changes in microbial cells which could lead to either inactivation of the cells or rupturing (Hong and Pyun, 1999; Dillow et al., 1999; White et al., 2006).

The effectiveness of DPCD for destroying microbial cells, and hence the effectiveness of pasteurization, depends primarily on the sensitivity of microorganisms to DPCD. In turn, microbial sensitivity depends on many genetic and/or environmental factors such as type of microorganisms, their vegetative form, initial microbial number, culture conditions and growth phase, presence of injured microorganisms, etc. Technological parameters such as water content, physical and chemical properties, $CO_2$ physical state and concentration, temperature, pressure, agitation, pressurization and depressurization rate, pressure cycling, and treatment time are even more important (Erkmen. 2012).

## Genetic and environmental factors affecting DPCD efficiency

The cell walls of Gram-positive bacteria are made up of a thick layer of peptidoglucans, which determines their strength and stability. In the cell walls of Gram-negative bacteria, the layer of peptidoglucans is thinner, making their cell walls more susceptible

to impacts and more permeable to DPCD (Erkmen, 2012). Different studies investigated the effect of DPCD on *E. coli* in different nutrient media, applying various pressures (10–31 MPa), temperatures (34–40°C), and contact times from 10 to 120 min (Chen et al., 2017; Erkmen, 2001; Kobayashi et al., 2009; Spilimbergo et al., 2003a,b; Wu et al., 2007) and reduction in the number of viable cells by 2.5–9 logs was observed. Similar results were reported for *L. monocytogenes* (21.7 MPa, 45°C for 60 min) in milk (Lin et al., 1994), *Listeria dunnifii* (20.5 MPa, 34°C for 36 min) and *Proteus vulgaris* (20.5 MPa, 34°C for 36 min) (Dillow et al., 1999), *Pseudomonas aeruginosa* (7.4 MPa, 38°C for 2.5 min and 20.5 MPa, 34°C for 36 min) (Dillow et al., 1999; Spilimbergo et al., 2002), *Serratia mercescens* (7.4 MPa, 38°C for 10 min) (Spilimbergo et al., 2003a), and *Salmonella* spp. (Dillow et al. 1999).

The effect of DPCD on Gram-positive bacteria (*Enterococcus faecium*, *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus innocua*, *Lactobacillus dextranicum*, *L. plantarum*, *S. aureus*, etc.) showed either higher or at least equal effect in comparison to Gram-negative microorganisms (Dillow et al., 1999; Hong and Pyun, 2001; Spilimbergo et al., 2002, 2003a,b; Wu et al., 2007). DPCD has been successfully used to inactivate vegetative forms of molds and yeasts (Erkmen, 2012; Ferrentino et al., 2017; Gunes et al., 2005; Isenschmid et al., 1995; Parton et al., 2007). All these studies revealed that DPCD is a promising processing alternative for pasteurization of foods at moderate temperature and pressure. Moreover, the inactivation effect was strongly dependent on the technological parameters such as pressure, temperature, and contact time. Isenschmid et al. (1995) revealed that DPCD treatment led to a removal of vital components from the cell membrane, rather than to rupture of the cell. The initial number of microorganisms also affects the effectiveness of DPCD, as a higher number would require a longer exposure to DPCD (Erkmen, 2012). In general, bacteria are more susceptible to DPCD at lower temperatures, which is very important in regards to the practical application of this technology for food processing. The same is valid also for the pH of the media, since DPCD is more effective at pH levels different from the optimal acidity levels for a given microorganism. Additionally, young cells are more sensitive to DPCD in comparison to mature cells. For example, Hong and Pyun (1999) revealed that *L. plantarum* cells in stationary phase are more resistant to DPCD than the culture in logarithmic phase.

Besides microorganisms, DPCD could be used as an alternative to the common fumigations techniques since it is very effective in eradicating eggs, larvae, or beetles after a short exposure (10–20 min). Even a pressure of 2.5 MPa killed completely the most common insects in rice, including their eggs (Capilla et al., 2003).

## Technological factors affecting DPCD efficiency

Probably, pressure and temperature are the most important parameters for the effectiveness of DPCD. Moreover, pressure and temperature determine the state of $CO_2$ and, hence, influence greatly its microbicidal activity (Illera et al., 2019). In supercritical conditions, the liquid-like state of $CO_2$ determines its increased density and solubility. On the other hand, the gas-like state is related to a decreased viscosity and elevated diffusivity, which allows quicker diffusion and effective penetration in

cells (Garcia-Gonzalez et al., 2007). Therefore, supercritical $CO_2$ is a more effective antimicrobial agent than pressurized liquid or gaseous $CO_2$. In general, the higher the pressure, the stronger is the effectiveness of DPCD. On one hand, pressure could act directly on cells but most probably its effect on $CO_2$ state and therefore its penetrating ability is more important for the microbicidal significance of this factor. However, the solubility of $CO_2$ in liquids and food matrices is limited and Spilimbergo et al. (2002) indicated that an increase of $CO_2$ pressure over 10 MPa does not influence significantly its solubility in water, which is the most common liquid in foods.

Temperature has a profound effect on microbial cells by destructing biological systems, which is used in many conventional technologies for sterilization, based on elevated temperatures. However, due to the milder temperatures (30–50°C) during DPCD treatment, the effect of this factor is related again mainly with its influence on $CO_2$ state. Additionally, elevation in temperature stimulates the diffusion of $CO_2$ into cells. To summarize, the microbicidal effect of DPCD could be increased by elevating pressure, temperature, and saturation of system with $CO_2$. However, technological optimum should be sought for every food matrix, since higher pressures and temperatures are related to more resistant equipment and higher production costs. Agitation of the system could also enhance the effectiveness of DPCD treatment, since it enhances the transfer of pressurized $CO_2$ and its solubilization rate, resulting in easier penetration of $CO_2$ through cell membranes (Oulé et al., 2006; Tsuji et al., 2005). The effectiveness of DPCD could be increased by the rate of pressurization and depressurization. Pressurized $CO_2$ rapidly expands through cells, leading to a mechanical rupturing of cell structures. The effectiveness of an explosive depressurization was demonstrated by Nakamura et al. (1994) with yeast cells. Conversely, Enomoto et al. (1997) revealed that even slower depressurization inactivates *S. cerevisia* cells similarly to the explosive depressurization of $CO_2$. Similar results were obtained by Kumagai et al. (1997), indicating that depressurization rate is not so essential in cell destruction.

The effect of pressure cycling (i.e., applying pressure repeatedly in cycles) on the effectiveness of DPCD processing is contradictory. Physical and chemical properties of food matrix strongly affect the effectiveness of DPCD treatment. pH is very important, since higher acidity could enhance the effect of DPCD (Damar and Balaban, 2006). Water content, viscosity, protein, and fat content are other factors affecting the treatment (Hong and Pyun, 1999). For example, under the same conditions *L. monocytogenes* in eggs is eliminated for twice the time in comparison to broth, and *E. faecalis* are eliminated for 24 h in whole milk, 16 h in skimmed milk, 3 h in orange juice, and 4 h in carrot juice (Erkmen, 2012). High moisture content is essential for an effective inactivation of microorganisms, since $CO_2$ must dissolve in an aqueous solution at a sufficient concentration, and dissociate into corresponding ions to reduce intracellular pH. Microorganisms in foods with low water content can show low or no inactivation behavior with DPCD treatment (Nakamura et al., 1994). From a technological point of view, batch systems require a longer time for microbial inactivation than continuous systems (Spilimbergo et al., 2003b). In general, inactivation increases with elongated exposure to DPCD, but the efficiency of the treatment depends on all the abovementioned factors.

### 2.3.2 Application of DPCD for pasteurization of foods

As already mentioned DPCD is used mainly for nonthermal treatment of liquid foods due to their high moisture content, but recently it is used more often for pasteurization of solid foods. DPCD is used not only for inactivation of food microflora but also for inactivation of some enzymes that improve food sensory properties and flavor stability.

#### Application for the treatment of liquid foods

There are many studies on the use of DPCD for reduction of microbes and enzyme activities in liquid foods and its effect on their nutritional components, quality parameters, and sensory properties (Balaban and Duong, 2014; Damar and Balaban, 2006; Garcia-Gonzalez et al., 2007). Fruit juices, and particularly orange and apple juices, are among the most investigated food matrices. Niu et al. (2010a) treated orange juice at 40 MPa and 55°C for 10–60 min and compared with thermally treated (90°C for 60 s) juice. Authors found that cloud and stability of the juice were improved due to the DPCD treatment. Similar findings were made by Kincal et al. (2005) in a continuous-flow system. Gui et al. (2006) reported significant reduction of the browning degree of cloudy apple juice after DPCD treatment. Gasperi et al. (2009) did not find significant differences in the sensory properties after DPCD treatment of apple juice. Similarly, Amaral et al. (2018a,b) did not find differences among physicochemical parameters of DPCD-treated whey-grape juice and untreated control. Niu et al. (2010b) reported that polyphenol oxidase in cloudy apple juice was completely inactivated by DPCD and pectin methylesterase activity was significantly reduced when compared to mildly heated control. More recently, Manzocco et al. (2017) reported that DPCD treatment (12 MPa, 35°C, 10 min) led to 80% inhibition of polyphenoloxidase activity and higher microbial stability of freshly squeezed apple juice in comparison to a control, while keeping a sensory fresh-likelihood higher than thermally pasteurized. Ferrentino et al. (2009a) revealed that DPCD is equally efficient in microbial inactivation of apple juice as a conventional thermal treatment (85°C, 60 min).

In another study, *A. acidoterrestris* spores in apple juice were completely inactivated by DPCD and, again, no influence on physical and sensory properties of the juice were observed (Bae et al., 2009). Lim et al. (2006) achieved maximal microbial reduction in mandarin juice at 35°C, 41.1 MPa, for 9 min at 7% $CO_2$. Moreover, cloud was enhanced by 38% and color parameters of juice improved. Five log reduction for yeasts and molds and total aerobic microorganisms occurred at 34.5 MPa and 7 min of treatment of fresh-squeezed grapefruit juice (Ferrentino et al., 2009b). Pineapple juice was completely free of microbiota after DPCD treatment (10 MPa, 31.5°C) at different contact times (3.06–4.6 min) and differences in quality attributes were minimal (Martínez et al., 2018). During storage, microorganisms were not able to recover and the vitamin C decrease could be limited to 8.2% after 4 weeks.

The treatment of watermelon juice at 34.4 MPa, 40°C, 10% $CO_2$ for 5 min led to reduction of the native aerobic microflora of the juice by 6 logs, which allowed subsequent refrigeration storage of the product for 8 weeks without spoilage (Lecky and Balaban, 2004). In a comparative study, Damar et al. (2009) revealed that there was no difference in the sensory properties of continuous-flow DPCD-processed (34.5 MPa,

25°C, 13% $CO_2$, 6 min) coconut water in comparison to that of fresh, untreated samples, whereas a heat-processed (74°C, 15 s) sample was liked less. DPCD can effectively inactivate yeasts (by 6 logs) in grape juice (Gunes et al., 2005). As the $CO_2$-juice ratio, temperature, and pressure increased, the inactivation rate increased, without detectable flavor degradation. In another study, Del Pozo-Insfran et al. (2006) showed that thermal pasteurization decreased anthocyanins (by 16%), polyphenols (by 26%), and antioxidant capacity (by 10%) of muscadine grape juice, whereas DPCD-treated grape juice retained fresh-like levels of these. As revealed by Zhou et al. (2009b), total soluble solids and carotenoid content of DPCD-treated carrot juice were stable. In a further study with peach juice, the same authors observed that DPCD significantly altered product particle size distribution. They observed decrease of protein and polyphenols, while pectin and calcium levels remained unchanged. The DPCD treatment of melon juice led to significant inactivation of microorganisms and enzymes (polyphenol oxidase, peroxidase and lipoxygenase), while retaining the flavor (Chen et al., 2010).

Besides fruit juices, DPCD was used for pasteurization of beer in a continuous system (27.6 MPa, 21°C, 5% $CO_2$, 5 min). DPCD-treated beer showed unaltered sensory properties in comparison to fresh beer after 1 month of storage, whereas those of heat-treated beer (74°C, 30 s) were significantly reduced. Haze in DPCD-treated beer was reduced, as well foam capacity and foam stability (Dagan and Balaban, 2006).

### Application for treatment of solid foods

There are several difficulties in the treatment of solid foods with DPCD: the continuous processing is difficult; the penetration of $CO_2$ in most of them is slow; and there are concerns about cellular damage leading to texture and/or surface changes, affecting food quality. DPCD was used for treatment of different solid foods, such as fruit and vegetables, meat, seafood, herbs and spices, etc. Continuous $CO_2$ flow was used by Valverde et al. (2010) to inactivate *S. cerevisiae* in fresh-cut pears. Using DPCD, at 55°C, the authors achieved inactivation of yeast by 5 logs. pH and °Brix of pears were not affected by DPCD treatment and peroxidase activity was partially reduced. However, some texture loss and darkening was observed. Less effective inactivation of *E. coli* and total aerobic bacterial count was achieved by Mazzoni et al. (2001) treating alfalfa seeds with DPCD (27.6 MPa, 50°C for 60 min). Jung et al. (2009) investigated the effect of DPCD with different intensity on the contamination of alfalfa seeds with *E. coli*, *Listeria* and *Salmonella*. DPCD treatment for 15 min (20 MPa, 45°C) reduced the pathogen levels by 7 logs, but impaired their germination capability. Seeds were able to germinate if treated for 10 min at 15 MPa and 35°C, but the reduction of *E. coli* was about 3.5 logs. DPCD (30 MPa, 65°C for 40 min) did not have any microbicidal effect on dry cocoa powder. However, after addition of 5%–10% water, microbes were fully inactivated (Calvo et al., 2007).

Similarly, Dehghani et al. (2008) achieved complete inactivation of bacteria and fungi on ginseng root, moisturized with water/ethanol/$H_2O_2$, after applying DPCD (10 MPa, 60°C, 6 h). However, when using dry ginseng, treatment was not effective and inactivation of microorganisms did not reach the required regulatory levels. Zhong et al. (2008) reported 5 logs reduction of *E. coli* on fresh spinach leaves treated with

DPCD (7.5–10 MPa, 40°C) for 40 min, which was accompanied by undesirable discoloration of the leaves and changes in texture. Calvo and Torres (2010) observed that water content and temperature were the influential parameters for microbial reduction of paprika powder. 35% water content and 6 MPa led to a 1.5 logs microbial reduction, while 30 MPa resulted in 5 logs reductions and retention of paprika color. Ferrentino et al. (2012) treated fresh-cut coconut pieces with DPCD (45°C, 12 MPa, 15 min) resulting in 4 logs CFU/g reduction of mesophilic microorganisms, lactic acid bacteria, total coliforms, yeasts, and molds. However, some microstructure irregularities occurred after the DPCD treatment. Beside microorganism inactivation, Zhou et al. (2009a) revealed that pectin methylesterase activity in carrots is inactivated by HPCD, whereas Manzocco et al. (2016) reported polyphenoloxydase inactivation in mushrooms.

Application of DPCD to meat products is less effective in microorganism reduction in comparison to plant foods. Erkmen (2000) revealed that DPCD at 6 MPa, 45°C for 150 min inactivates 5 and 1 log microorganisms in minced and skinned beef, respectively. Applying 10 MPa for 15 min resulted in 1 log reduction of *E. coli* in beef trimmings (Meurehg, 2006). Choi et al. (2008, 2009a,b) investigated the inactivation of *E. coli*, *Listeria* and *Salmonella* in fresh, treated, and marinated pork and observed 1.9–2.5 log reduction of *Listeria*. In a more recent study, Ferrentino et al. (2013) totally inactivated *Listeria* in dry cured ham pieces at 50°C, 12 MPa, 15 min. DPCD treatment slightly affected the sensory attributes of the product. Ji et al. (2012) found that 15 MPa, for 26 min at 55°C reduced the microbial load of shrimps by 3.5 logs. Meujo et al. (2010) succeeded in reducing total aerobic plate counts by 3 logs and *Vibrio* by 6 logs, in the digestive system of oysters by exposing them to 17.2 MPa, 60°C for 60 min. In a more recent study, myosin and shrimp surimi from *Litopenaeus vannamei* were treated with DPCD at 5–25 MPa and 40–60°C for 20 min. DPCD had a greater effect on secondary structure of myosyn than heating, increased the hardness, springiness, and weight loss, meanwhile decreasing the redness of sausages.

De Matos et al. (2013) used pressure cycles and different depressurization rates to treat oysters with DPCD to reduce *Vibrio parahaemolyticus*. The authors observed that after 30 min, 75% of the initial count was inactivated and 100% inactivation was observed after 3 h. Investigating the inactivation of several bacteria on solid food matrices through supercritical $CO_2$, Galvanin et al. (2014) developed mathematical models to represent the DPCD pasteurization process at different experimental conditions. A more recent study investigated DPCD applicability to a solid-liquid product consisting of apples in syrup. The optimal process conditions (12 MPa, 55°C, 15 min) led to inactivation to undetectable levels of mesophilic microorganisms, total coliforms, yeasts, and molds. Polyphenol oxidase was totally inactivated, while pH, color, and total acidity were slightly affected. The shelf life study of DPCD treated products revealed their microbial, enzymatic, and quality stability.

DPCD is a nonthermal pasteurization technology, which is mainly applied to thermosensitive foods. $CO_2$ under pressure (either in supercritical, liquid, or gaseous state) is employed to destroy microbial cells and/or inactivate enzyme systems. There are different technological solutions that rely on a batch, semicontinuous, or continuous process. These solutions provide contact of the processed feedstock with $CO_2$, with a

pressure usually less than 50 MPa for a certain time period. The process temperature is low, generally from 30°C to 50°C. Therefore, DPCD largely retains the quality and nutrients in the food matrix. It is mainly used for liquid foods, but recently the accumulated knowledge and experience in the field is directed to DPCD application to many solid foods, as well. There is also great interest in combining DPCD with other nonthermal food processing technologies. Unfortunately, DPCD equipment is available mostly at a laboratory scale (Ferrentino and Balaban, 2012). With its potential for safe food without loss of nutrients or quality, and scientific background proving its efficiency, it is not surprising that DPCD treatment is listed among the emerging technologies for food processing with the most commercial importance in the next 5–10 years (Jermann et al., 2015).

## 2.4 CP application

CP processing is a new perspective and alternative method of sterilization that does not require high temperature. The nonthermal nature of the method allows treatment of thermosensitive materials (plastic foils) and heat-labile food systems (fresh fruits and vegetables). The substances formed during processing (atomic oxygen, ozone, ions) have a reactive character, and a short life span (Thirumdas et al., 2015; Yanakiev, 2017). CP is a type of nonthermal plasma, based on generation of ionized gas composed of positively and negatively charged particles (ions), chemical reactants such as atomic oxygen and ozone, UV rays, and accelerated electrons. Two types of CP are used in the food industry—CP generated under vacuum and CP generated at atmospheric pressure. The first type is generated in a hermetically sealed chamber filled with gas (Ar, N, $N_2O$, $N_2$, $O_2$) or a gas mixture in vacuum (0.01–0.02 MPa), via a high frequency electric field source, or source of microwave energy. Atmospheric CP is generated at atmospheric pressure through an electric field, generating a high potential difference between the two electrodes placed in air or other gas environment (Coutinho et al., 2018; Yanakiev, 2017).

Depending on the electric discharge and the generator design, CP may be classified as plasma generated via corona discharge, generated via a dielectric barrier discharge (DBD), and generated via an atmospheric pressure plasma jet (APPJ). According to the source of energy, CP is AC, Pulsed DC, and radio frequency (RF). Based on the composition of the feed gas, CP can be classified as atmospheric plasma or named after the gas used for generation: $Ar_2$, $He_2$, $N_2$, etc. (Pankaj et al., 2014b; Pervez et al., 2014; Shakila et al., 2012; Yanakiev, 2017). The most commonly used sources of CP are corona discharge, plasma jet, and DBD (Yanakiev, 2017).

The antimicrobial effect of CP is due to generation of reactive oxygen species (ROS) and reactive nitrogen species, such as: $H_2O_2$, $O_3$, $O_2^{\bullet -}$, $HO_2^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$, $^1O_2$, $^{\bullet}OH$, $NO^{\bullet}$, $^{\bullet}NO_2$, $ONOO^{\bullet}$, ROONO, etc. formed during discharge (Arjunan et al., 2015; Gaens and Bogaerts, 2013; Lu et al., 2016; Misra and Jo, 2017). ROS interact with various cellular structures. First of all, ROS are responsible for changing the DNA structure and bind to different proteins and enzymes in the microbial cell. ROS cause peroxidation of the lipids in the cell membrane, thus changing its biological functions. At the same time, the cells are subjected to intense tension from the electric

field leading to their destruction. ROS can attack the peptidoglycan in the membrane of the Gram-positive bacteria, which alters its functionality. In Gram-negative bacteria, ROS interacts chemically with lipopolysaccharides in the cell membrane. At the same time, the tension that the cells experienced leads to leakage of the membrane and loss of cell integrity. The cell growth inhibition during CP treatment depends on a number of factors. For example, it has been found that some fungal spores are not affected by the ozone and NO formed during discharge, but predominantly by atomic oxygen (Hashizume et al., 2013, 2014; Iseki et al., 2011; Ishikawa and Hori, 2014; Misra and Jo, 2017; Misra et al., 2016; Yusupov et al., 2013).

Of great importance for growth inhibition are the UV rays generated during discharge. These rays are largely adsorbed by the cell, resulting in a change in the DNA structure and chemical reactions in the cytoplasm. UV rays generation strongly depends on the method of obtaining (Misra and Jo, 2017; Schneider et al., 2011, 2012). The influence of CP on different groups of microorganisms largely depends on the generation source and the experimental conditions (Misra and Jo, 2017; Coutinho et al., 2018).

Recently, a comparison of different CP approaches in the treatment of fresh and cooked meat against the major groups of food pathogens was performed (Misra and Jo, 2017). Depending on the CP source, the gas phase present reduces microbial contamination by 0.5–3 $\log N$ (relative to the total bacterial abundance (TBA) of the samples tested) (Ulbin-Figlewicz et al., 2015a,b). The processing time of samples in order to kill the main psychotropic microorganisms is about 10 min. CP also has a strong impact on the two major pathogenic microorganisms in meat—*E. coli* and *L. monocytogenes*. Depending on the plasma source and the gas phase (He, $N_2$, $O_2$, $NO_2$), the reduction in the number of viable *L. monocytogenes* cells is more than 3 $\log N$. The trend with *E. coli* is similar. The structure of the food matrix is important in CP processing of meat. In general, it has been found that treatment in surface layers was more efficient (there was a reduction in the number of viable pathogen cells by more than 3 $\log N$), whereas in the inner layers, the reduction is up to 1 $\log N$. The processing time varies from 2 to 10 min (Misra and Jo, 2017).

Depending on the plasma generation, there are also differences in cell growth inhibition. Korachi et al. (2009) showed that corona-generated plasma is a suitable method for inhibiting the growth of *E. coli*, *S. aureus*, *P. aeruginosa*, *Streptococcus mutans*, *Candida albicans*, and *Aspergillus niger*, but the method did not show satisfactory results in the processing of *Bacillus subtilis*, most likely due to its inherent spore formation.

It has been found that plasma jet processing (20 min, 400–600 V/20–35 mA) significantly reduces the number of viable *S. aureus*. The pH medium was also important for the inactivation of all cells, for example, it can lead to 100% inhibition in combination with the CP. This is likely to be due to $HOO^{\bullet}$, which initiates oxidation of fatty acids in the membrane (Liu, et al., 2010). It was found that the treatment with CP generated by plasma jet resulted in the inactivation of *L. monocytogenes*, *E. coli,* and *Salmonella* sp. in nuts, fruits and vegetables by up to 3 $\log N$ or up to 90%, depending on the initial contamination quantity (Lee et al., 2011; Niemira, 2012; Wang et al., 2012).

Jayasena et al. (2015) investigated the effect of CP (generated by DBD) treatment in meat packaging. They determined its inactivation ability against several microorganisms (*L. monocytogenes, E. coli* O157: H7, and *S. typhimurium*) and the influence of plasma on the physicochemical properties of the product. A 10 min treatment reduced the number of viable cells of the three microorganisms tested by 2–3 log *N*. Miao and Yun (2011) investigated the bactericidal efficacy of CP generated by DBD in the treatment of samples infected with *E. coli* ATCC8099 at a different cell titer. Ziuzina et al. (2014) explored the possibilities for inactivation of *E. coli*, *Salmonella* sp., and *L. monocytogenes* on cherry tomatoes and strawberries by CP. Their experiments were conducted under different conditions, but it was found that processing up to 120 s provided a reduction in the number of viable cells from 3 to 7 log *N*. It has been found that *Salmonella* sp. and *L. monocytogenes* were more sensitive than coliforms. Patil et al. (2014a,b) applied direct treatment with DBD-CP when sterilizing the surfaces of packaged products. The achieved result was a reduction of *Bacillus atrophaeus* spores by 6 log *N* after treatment with 70 kV/50 Hz CP for 60 s.

Yanakiev (2017) examined the possibilities of applying CP treatment to surfaces coming into contact with food products. Antimicrobial activity of CP was found to be higher at low water activity on the treated surface. It has been demonstrated that the application of a longer treatment duration led to an increase in the effect and a higher level of reduction of the viable microorganisms was observed. The *Salmonella abony* and *S. aureus* had different resistance against the antimicrobial action of CP. The differences in the resistance of the nonspore-producing bacterial cells, *S. abony* and *S. aureus*, may be due to the different cell wall structure of the two microorganisms, being representatives of Gram-negative and Gram-positive bacteria, respectively. Duration of 4.8 s, a discharge voltage of 10 kV, and a discharge frequency of 550 Hz was the most energy efficient for a reduction by 1 log *N* of living cells number in both microorganisms. The highest reduction of the tested microorganisms was achieved (power required $141.75 \times 10^{-3}$ W h) for *S. aureus* (3.47 log *N* reduction) and for *S. abony*: 3.35 log *N*.

Besides decontamination, atmospheric pressure CP has shown promising potential for a number of innovative applications including surface hydrophobicity enhancement in biscuits (Misra et al., 2014), modification of dough rheology and mixing properties (Misra et al., 2015), modulation of whey protein functionality (Segat et al., 2015), technological performance of rice flour (Pal et al., 2016), enhancement of food grain (seed) germination (Jiafeng et al., 2014), and enhancement of mass transfer (Kodama et al., 2014).

The influence of CP on enzyme functionality in food systems was also assessed. Bernard et al. (2006) observed the degradation of immobilized lysozyme under the action of the afterglow of plasma in nitrogen-oxygen mixture, operating in a flowing mode. They proposed a model including destruction of functional sites under the influence of reactive species. Kylián et al. (2008) demonstrated etching of protein deposits (including lysozyme) on a range of substrates (e.g., silicon wafers, polystyrene, glass) using a low pressure plasma source. Surowsky et al. (2013) reported inactivation of

peroxidase and polyphenol oxidase in a model system using a CP jet with various gas mixtures. The changes in tryptophan emission fluorescence measurements brought by the duration of plasma exposure further corroborated the structural modifications in proteins. The inactivation of pectin methyl esterase and peroxidase in fresh cut fruit systems has also been studied by Tappi et al. (2014, 2016). CP was reported to inactivate a range of enzymes such as lysozyme (Takai et al., 2012), polyphenol oxidase, and peroxidase (Pankaj et al., 2014a; Surowsky et al., 2013).

CP technology is an emerging, green process offering many potential applications for food packaging. While it was originally developed to increase the surface energy of polymers, enhancing adhesion and printability, it has recently emerged as a powerful tool for surface decontamination of both foodstuffs and food packaging materials. New trends aim to develop in-package decontamination, offering nonthermal treatment of foods postpackaging. Food packaging materials retain very low barrier properties after plasma treatment and transparency is not affected even after 5 minute treatment (Pankaj et al., 2014a). Key limitations of CP are the relatively early state of technology development, the variety and complexity of the necessary equipment, and the largely unexplored impacts of CP treatment on the sensory and nutritional qualities of treated foods. Also, the antimicrobial modes of action for various CP systems vary depending on the type of CP generated. Optimization and scale up to commercial treatment levels require a more complete understanding of these chemical processes. Nevertheless, this area of technology is promising and is the subject of active research to enhance efficacy (Niemira, 2012).

## 2.5 Radiation methods for food decontamination

Radiation energy refers to energy sources that pass from one place to another through the air or other space. It can consist of particles or electromagnetic waves that are characterized by their length and have a different impact on microorganisms. The lower the wavelength is, the more energy is accumulated in them, and the stronger their effect on the organisms is. The basis of their action is different physical or chemical changes occurring in cells, microorganisms, and the environment (Fig. 3). Changes can only be the result of the absorption of the rays, that is, their effective penetration is important for effective irradiation.

Electromagnetic waves with a wavelength of 800–1000 nm in the infrared spectrum are used as an energy source by photosynthetic bacteria. Visible light with a wavelength of 380–760 nm is also used as an energy source by photosynthetic bacteria and higher plants. Electromagnetic waves with a wavelength of 200–380 nm are ultraviolet rays. Both infrared and ultraviolet rays are invisible to the human eye (Murgov and Denkova, 2012).

If the treatment includes the use of a source of radiation, for example, UV irradiation or pulsed light, FDA approval of the means of treatment for the control of microorganisms is required. Both UV radiation and pulsed light have been approved by FDA for the control of microorganisms, and the regulations specifying the conditions under which they may be safely used are at 21 CFR 179.39 (UV radiation) and 21 CFR 179.41 (pulsed light).

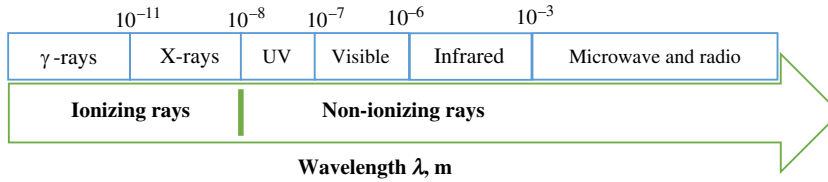



**Fig. 3** Radiation treatment methods in the electromagnetic spectrum according to the wavelength.

### 2.5.1 UV treatment

Ultraviolet rays are the most active rays of the solar spectrum, which also determines their bactericidal effect. They are characterized by high energy, with the greatest activity being at wavelengths of 250–260 nm (253.7 nm). The effect of the treatment depends on the intensity ($I$, in $\mu W\,cm^{-2}$), the treatment duration ($\tau$, in s), and the UV permeability of the medium in which they propagate (UVT, in %) (Smith, 2016):

$$\text{UV} - \text{dose} = \frac{I}{\text{UVT}}, \text{W s cm}^{-2}$$

The effective action of UV rays on microorganisms depends on the irradiation dose (amount of energy absorbed), the substrate properties, the pH, the degree of contamination by microorganisms, and the temperature. Low irradiation doses stimulate the individual functions of microorganisms, including hereditary ones. This is used, in practice, for obtaining antibiotics, enzymes, amino acids, and other biologically active substances.

Purine and pyrimidine bases of DNA and RNA absorb at their maximum UV rays at 260 nm while proteins absorb at 280 nm. The influence of UV rays on microorganisms is related to their effect on DNA and the most lethal effects of UV light are at a wavelength of 253.7 nm (Murgov and Denkova, 2012; Smith, 2016). They induce dimerization in adjacent pyrimidine bases in the DNA molecule. The dimers formed lead to changes in the genetic structure and to genetic mutations. There is evidence suggesting that UV rays induce mutations indirectly as well, by the formation of free radicals and peroxides having mutagenic properties (Bolton, 2001). Higher doses of UV rays cause microbial cell death. It should be noted that different microorganisms have different sensitivity to similar irradiation doses (Figs. 4–7).

Experimental data, as suggested by the figures, clearly indicate that bacteria that do not emit pigments into the environment are more sensitive to UV radiation. The carotenoid pigments contained in the cells have protective properties against UV rays (Fig. 4). Bacterial spores are significantly more resistant than vegetative cells. In order to destroy spores, more energy and longer exposure is needed (Fig. 5). The susceptibility of microorganisms to UV rays is as follows: Gram-positive bacteria < Gram-negative bacteria < yeasts < bacterial spores < mold spores (Figs. 6 and 7). Similar results were reported by Akshay (2013).

Microbial cell death can be due both to the direct effects of UV rays and to adverse changes in the substrate structure. UV rays inactivate enzymes, and being absorbed by important cellular substances (proteins, nucleic acids, etc.), cause damage to their

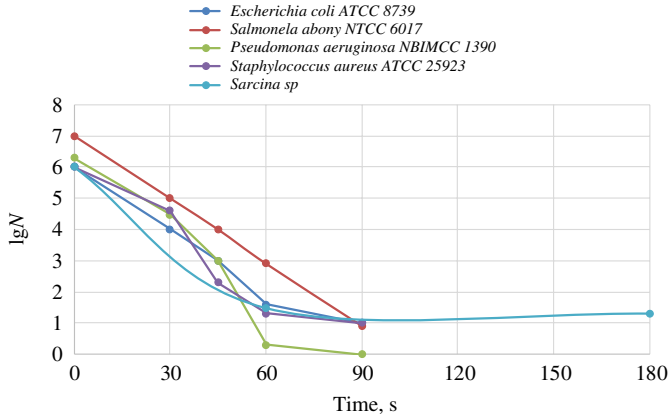



**Fig. 4** Death curves of various bacteria under the action of UV rays.

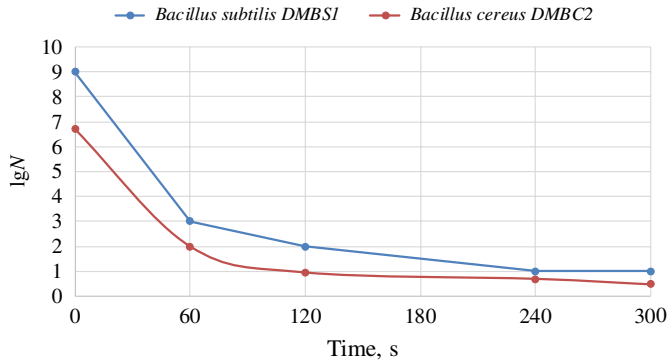



**Fig. 5** Destruction of spores of spore-forming bacteria under the action of UV rays.

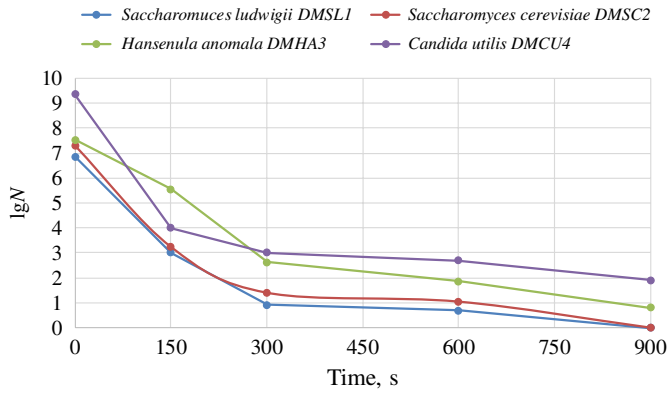



**Fig. 6** Death curves of yeast cells under the action of UV rays.

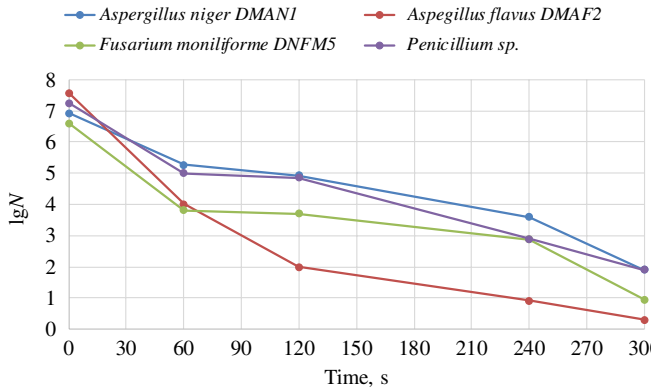



**Fig. 7** Destruction of fungal spores under the action of UV rays.

molecules. Substances ($H_2O_2$, $O_3$, etc.) that destroy microorganisms can also be formed in the irradiated environment. An often used artificial source of ultraviolet radiation is a low-pressure argon-mercury lamp (Bolton, 2001). UV rays are used to destroy microorganisms in the air and on the food surface (Fig. 8). Refrigeration chambers, healing, and production premises are disinfected by their application. Up to 100% of bacteria and fungi found in the air were destroyed after 3 hours (Table 2). UV rays are also applied in the packaging of foodstuffs, medical preparations, and for decontamination of trays, packaging materials, equipment, and utensils in public catering.

Food sterilization with UV rays is obstructed due to their low penetration ability (Sattar, 2011). Therefore, their effect is only on the surface or in a very thin layer. Positive results have been achieved in water disinfection by exposure to UV rays,



**Fig. 8** UV sterilization (90 W lamps) facilities at the Scientific laboratory of fermentation and lyophilization (Plovdiv, Bulgaria).

**Table 2** Influence of UV rays on microflora of air in production premises and equipment

| | | | | | | |
|---|---|---|---|---|---|---|
| Air in production premises (Protey-M Ltd, Bulgaria; Fermentation hall; 2 UV lamps 90 W); method of determination—Sedimentation method (Murgov and Denkova, 2012) | | | | | | |
| Time, min | 0 | 30 | 60 | 120 | 180 | 360 |
| TBA[a], CFU/$m^3$ | $2.2.10^2$ | $2.0.10^2$ | $1.76.10^2$ | $1.76.10^2$ | 44 | 0 |
| Freeze-drier equipment (Protey-M Ltd; 2 UV lamps 90 W); method of determination—Sedimentation method (Murgov and Denkova, 2012) | | | | | | |
| Time, min | 0 | 30 | 60 | 120 | 180 | 360 |
| TBA[a], CFU/$m^3$ | 44 | 0 | 0 | 0 | 0 | 0 |
| Air in production premises (Protey-M Ltd, Bulgaria; Packaging hall; 2 UV lamps 90 W); method of determination—Sedimentation method (Murgov and Denkova, 2012) | | | | | | |
| Time, min | 0 | 20 | 40 | 60 | 120 | 180 |
| TBA[a], CFU/$m^3$ | $1.76.10^2$ | 880 | 44 | 44 | 44 | 0 |

[a] Total bacteria abundance.

when viruses, bacteria, yeasts, and other pathogenic microorganisms were destroyed (Masschelein, 2002).

UV rays are successfully used in the processing of liquid-type foods with high transparency, such as sugar solutions and juices (Caminiti et al., 2011a; Choi and Nielsen, 2005; Fan, 2007; Franz et al., 2009; Koutchma et al., 2007). Application of UV rays for sterilization of fruit juices and wines (in a thin layer) allows their storage for longer periods than pasteurized ones.

Results have also been obtained in the treatment of solid food products in a thin layer, such as sand sugar, resulting in complete decontamination of *Bacillus stearothermophilus*, and thermal, ultrasonic, and ultraviolet inactivation of *Salmonella* in thin films of aqueous media and chocolate (Lee et al., 1989; Stermer et al., 1987).

For some foods (oil, milk) sterilization with UV rays leads to deterioration of taste and nutritional qualities of the food. When treating such products with UV rays, a decrease in the vitamin content (vitamins K, A, $B_{12}$, $B_2$, folic acid, and tocopherols) (Koutchma et al., 2009), deterioration in quality indicators such as appearance, color, flavor, and taste (Azzara, 1992; Borle et al., 2001; Frankel, 1991; Min and Boff, 2002), as well as physico-chemical changes in the composition of the food and the raw materials occurred (Kehoe et al., 2008; Wong and Kitts, 2001).

### 2.5.2 *Pulsed light treatment*

Among the existing nonthermal technologies, PL irradiation is one of the promising due to its ease of use, application to most types of microorganisms, nongeneration of chemical residues, and low cost of equipment compared with other sterilization methods

(Gómez-López et al., 2007; Ramos et al., 2013; Heinrich et al., 2015). The mechanism of generating intense PL can be described as follows: electric energy is stored in a high power capacitor and then discharged to a specially designed xenon lamp unit. The lamp converts the high voltage pulses to light pulses of the same duration and frequency as that of the received DC electric pulses. The high-voltage electric pulses (up to 70 kV $cm^{-1}$) applied to the lamp causes it to emit an intense PL focused on the treatment area between two electrodes, which typically lasts a few hundred microseconds (1 ms–0.1 s). This produces several high-energy flashes, typically pulsed at 1–20 flashes per second, and an energy density in the range of 0.01–50 J $cm^{-2}$ at the irradiated surface; each flash has intensity almost 20,000 times that of sunlight at sea level.

The spectrum of PL ranges from UV to near-NIR and at least 2/3 of the emitted electromagnetic radiation falls within the wavelength range from 170 to 2600 nm, with a peak emission between 400 and 500 nm (Gómez-López et al., 2005). The wavelength range of the PL spectrum includes the sun-light spectrum wavelengths and particularly about 25% UV wavelengths in the range 180–300 nm that do not normally reach the earth's surface, since they are filtered by the ozone layer (Gómez-López et al., 2005, 2007). When PL at wavelengths under 300 nm is blocked, the overall effectiveness of sterilization decreases dramatically. This indicates that the sterilization function of PL is primarily dependent on light in the UV range. Two main effects could be distinguished: photochemical and photothermal.

*Photochemical effect*: Although details of the mechanism by which PL inactivates microbial organisms is still being developed, it is generally believed that, basically, the germicidal effects of PL on microbial organisms are attributed mainly to DNA mutations induced from the absorption of UV energy of PL by proteins, DNA, and RNA, causing damage, which kills microorganisms (Cheigh et al., 2012; Muñoz et al., 2012; Turtoi and Nicolau, 2007; Wang et al., 2005).

According to Wuytack et al. (2003), a major reason for inactivation is structural changes in DNA, with minor reasons including membrane damage, and protein and other macromolecules modifications. In general, the UV light causes dimerization of the DNA thymine, resulting in mutations, damage to the genetic information, failure of replication, and gene transcription (Turtoi and Nicolau, 2007).

Another factor influencing the efficacy of PL treatment is the damage recovery of some microbes. According to Kao et al. (2005), there are generally two mechanisms through which microorganisms can recover from damage. The first is photo reactivation mechanism that occurs if products are exposed to harmless blue or near UV light during storage. The other is called dark repair, which does not need light to catalyze; however, it does not always lead to complete repair, which makes it less efficient than photo reactivation (Hijnen et al., 2006). It was also found that enzymatic repair of DNA does not occur after damaging by PL (Slieman and Nicholson, 2000).

*Photothermal effect*: When a PL lamp emits electromagnetic radiation, a portion of it lies in the visible and NIR range. When absorbed by the irradiated surfaces, these wavelengths may generate heat. According to Bang-Yuan et al. (2015), this heat may increase surface temperature of the objects up to 50–150°C. The generated heat is mainly concentrated on the surface of the objects, with a maximal penetration depth up to 10 mm (depends on the optical properties of the irradiated surface and moisture

content). The heat is evenly conducted to the center of the irradiated object so no considerable increase in the internal temperature can be expected. However, these temperature differences may generate thermal stresses that can eventually damage the cell wall. Thus, it can be suggested that the lethal effect of PL can also be due to photothermal effect. Wekhof (2000) hypothesized that the PL microbial lethal effect is achieved through a rupture/explosion of bacteria during their temporary overheating caused by absorption of NIR light with a fluence exceeding 0.5 J $cm^{-2}$, which was confirmed by SEM photographs of irradiated *A. niger* spores presenting severe deformation and rupture.

A possible coexistence of both photochemical and photothermal mechanisms could be expected also, along with other structural damages induced in cell walls, membranes, and internal structures (Artíguez et al., 2011; Elmnasser et al., 2007; Wang et al., 2005). Ohlsson and Bengtsson (2002) reported changes in ion flow, increased cell membrane permeability, and depolarization of cell membranes. The exact origin of these minor effects is not yet well understood, but experiments (Takeshita et al., 2003) confirmed that they were observed after treatment of yeast with pulsed UV light, but not observed after similar treatment with continuous UV light.

Parameters affecting the efficiency of PL on microbial inactivation can be grouped into two categories: process and target parameters. The process parameters include: fluence (light intensity), fluence rate, exposure (treatment) time, peak wavelength (spectral distribution), peak power, pulse frequency (pulse width and pulse duration), total number of pulses applied, and the distance between the lamp and sample. Fluence (or energy density) is the energy received by the sample per unit surface irradiated area during the PL treatment (measured in J $m^{-2}$). Fluence is believed to be the most important parameter when characterizing PL treatment (Gómez-López et al., 2007). There is an agreement in the published literature that effectiveness of microbial inactivation increases with the total fluence delivered to the object (Pataro et al., 2011). Cho et al. (2002) studied the inactivation effects by PL on lactic acid bacteria (*L. plantarum*, *L. brevis*, *Leuconostoc mesenteroides*, and *Pediococcus pentosaceus*) for 0–2500 s treatment at 15–25 kV and found an exponential relationship between the electric power and inactivation of microorganisms. After irradiating *A. niger* spores inoculated into a corn meal suspension, Jun et al. (2003) also reported that inactivation increases with both the electric power and treatment time.

The fluence rate can be defined as the received energy from the lamp by the sample per unit area per unit time (W $m^{-2}$). Exposure time is the time period in seconds during which treatment is given. Spectral distribution refers to the width in wavelength distribution of the PL. The lamp spectral distribution depends strongly on lamp characteristics and design, where the desired peak wavelength can be obtained by proper adjustment of the electrical pulse current by closed loop system equipped with photo-detectors. Alternatively, appropriate light filters can be used to block unwanted wavelengths in the PL before reaching the target or a laser with defined wavelength of emittance.

Pulse frequency (known also as pulse duration or pulse repetition rate) has similar meaning from a physical point of view—the number of pulses per unit time or the time interval during energy delivery. For a fixed input current density, fluence is determined

by the pulse duration. High fluence (pulse power) can be achieved with a low current density if the pulse duration is shortened so that the generated light energy can be dissipated within a short time. The maximum frequency of a pulse light system that can be effectively achieved is usually limited by the design characteristics of the flash lamp. When higher pulse frequency must be achieved, two or more lamps arranged in sequence can be used.

The total fluence delivered on a given surface depends on the pulse power, as well as the total number of pulses delivered. From a practical point of view, the so-called Bunsen-Roscoe law states that the product of intensity of light multiplied by the time of exposure equals a constant. Therefore high energy density can be attained by using either high fluence rate with low time of exposure or low fluence rate with high time of exposure. However, in some experiments, especially where the pulse fluence is low, a threshold for number of pulses exists, below which no inactivation can be achieved.

The distance from the light source has an impact on the light intensity and, therefore, on the penetration depth. As the distance from light source and depth of the substrate increases, the absorption and scattering diminishes. The term optical penetration depth represents the distance over which light decreases in fluence rate to 37% of its initial value. The optical penetration varies with wavelength, with shorter wavelengths providing deeper penetration into the food than longer wavelengths (Abida et al., 2014). The target parameters include the optical properties of the objects, such as scattering and absorption, color, opacity, thickness, and surface topography of the irradiated object, viscosity, and flow conditions of the liquid samples, as well as moisture content (Pataro et al., 2011) The chemical composition and physical properties of the irradiated cells and the wavelength of the incident light determine to a great extend whether light will be reflected, scattered and absorbed or transmitted. In the case of biological tissues and photothermal effect, absorption and scattering are the most relevant types of light-substrate interaction (Abida et al., 2014).

Esbelin et al. (2013) found that the color of the fungal spores (*A. niger* vs. *Fusarium culmorum* spores) played a significant role in PL disinfection, i.e., darker *A. niger* spores are more resistant than *F. culmorum* spores. This was explained by the presence of protective dark pigments in the wall layers in *A. niger* spores that absorb more UV-C light than the pigment in *F. culmorum* spores. Turtoi and Nicolau (2007) also reported similar dependence and found that pigment extracts of *A. niger* absorb strongly UV in the range 240–480 nm.

Bang-Yuan et al. (2015) and Ringus and Moraru (2013) hypothesized that the physical properties of the treated surfaces, including surface topography and reflectivity, affect the efficacy of PL. For smooth surfaces, the incident light on the surface comes out at the same angle as the incident beam, with the same spectral distribution of energy. For rough surfaces, light travels through the outer layers of the material, where the incident light is partly absorbed, this phenomenon is called diffuse reflection. Surface roughness and crevices have also been suggested to shield microbial cells during treatment as they can shadow some microorganisms from the intensive light and may reduce the effectiveness of inactivation. Therefore, samples subjected to microbial inactivation with PL should have a smooth surface and must be placed in containers that are transparent and not reflective to at least the UV range of the spectrum.

Hillegas and Demirci (2003) indicated that the efficacy of PL is also closely related to the moisture content of the product. Higher moisture content typically increases UV and NIR absorbance by the material and, therefore, the lethal effect of the treatment. However, Fine and Gervais (2004) demonstrated that PL can also be successfully applied for food powders, such as wheat flour and black pepper, and suggested that PL has a potential to inactivate *S. cerevisiae* and *L. monocytogenes* cell population in powdered infant foods.

PL has been widely used for the decontamination of different food products: meat products, eggs, milk and dairy products, mushrooms, juices, baby foods, etc. Hierro et al. (2011) demonstrated inactivation of *L. monocytogenes* in ready-to-eat cooked processed meat products. Paskeviciute et al. (2011) reduced viability of *S. typhimurium* and *L. monocytogenes* inoculated on the surface of chicken, and organoleptic properties were not affected. It has been found that PL at a dose of 11.9 J $cm^{-2}$ effectively reduces the number of viable cells of *L. monocytogenes* and *S. typhimurium* in ready-to-eat cured meat products (Ganan et al., 2013). Lasagabaster et al. (2011) irradiated eggs and reported that 2.1 J $cm^{-2}$ caused death of *Salmonella* cells on the egg surface with slight increase in temperature. When fluence was increased to 10.5 J $cm^{-2}$ no penetration of *Salmonella* cells to the egg contents from the shell was observed.

Continuous flow PL was also used for the inactivation of *S. aureus* in milk and of brine during Mozzarella cheese production (Li and Farid, 2016). Matak et al. (2005) reported reduction of *L. monocytogenes* in goat milk. However, according to Krishnamurthy et al. (2007), the milk temperature increased during PL treatment in a continuous flow system, causing a fouling effect and undesired changes in milk. Fresh slices of mushrooms were exposed to PL treatment varying between 4.8 and 28 J $cm^{-2}$ and was found to increase in shelf life by 2–3 days in comparison to untreated samples (Oms-Oliu et al., 2010). However, the authors reported side effects of the treatment at higher fluence levels (12 and 28 J $cm^{-2}$), such as affected texture and decrease of phenolic compounds and vitamin C.

Pataro et al. (2011) inoculated apple and orange juice with Gram-positive (*Listeria innocua* 11288) and Gram-negative (*E. coli* DH5-α) bacteria and then irradiated with continuous flow PL system. The authors reported microbial load reductions in apple and orange juices for *E. coli* to be 4.00 and 2.90 log-cycle, respectively, and for *Listeria innocua* were 2.98 and 0.93 log-cycles, respectively, when fluence of 4 J $cm^{-2}$ was applied.

The sterilization effects of PL treatment on commercial infant foods (infant beverages, an infant meal and infant powdered milk) inoculated with *L. monocytogenes* were investigated at 15 kV, which level showed higher energy efficiency for the inactivation of *L. monocytogenes* (Choi et al., 2010). PL was also successfully used for packaging and surface decontamination (Rajkovic et al., 2010; Turtoi and Nicolau, 2007).

PL treatment delivers to the irradiated objects high energy density but without generating significant thermal effects, which can affect quality and nutrients (Abida et al., 2014; Ohlsson and Bengtsson, 2002). The bactericidal effect is based mainly on the photochemical and photothermal effects. In contrast to the continuous UV radiation where the effects of inactivation are reversible due to the presence of repairing enzymes, the absence of such enzymes in the damaged DNA molecules of cells irradiated

with PL makes it much more effective (Palmieri and Cacace, 2005). Moreover, PL has shown potential in reducing allergies associated with some foods, that is, peanuts and soy extracts (Yang et al., 2010, 2012).

The major challenge for PL is possible resistance of some strains (Caminiti et al., 2011a; Manzocco et al., 2011; Rajkovic et al., 2010; Uysal and Toklucu, 2011). Another limitation of PL is the depth of penetration of light. It is usually limited by the power and wavelength distribution of the PL and by the optical properties and chemical composition of the object and, in general, it hardly exceeds 10 mm. When very rough surfaces are irradiated, folds or fissures in the food may protect microbes from exposure to the PL (Brown, 2008). In general PL is useful mostly for liquids and solid foods surfaces.

### 2.5.3 Ionizing radiation (food irradiation)

At the end of the 19th century, two discoveries, of X-rays by Röntgen (1895) and natural radioactivity by Becquerel (1896), paved the way for the introduction of new physical effects of high energy particles on living organisms. The application of ionizing radiation for food treatments dates back to the beginning of the 20th century, when the first patents were issued. Commercially, the method was introduced in 1957 (Germany) for treatment of spices (Harder et al., 2016) and nowadays, more than 60 countries allow food irradiation, with more than 500,000 tons irradiated yearly (Eurofins, 2016). International organizations (the UN International Atomic Energy Agency, WHO, FAO) have approved foodstuffs that can undergo radiation treatment and the radiation doses. The most commonly irradiated food items in the United States are spices/herbs (around 78%), fruit and vegetables (14.6%), and meat and poultry (7.8%). In the EU, 46% of the irradiated products are frog legs, followed by dried spices and herbs (around 25%). In comparison with other methods for pasteurization, the labeling for irradiated foods is compulsory. It has to be strictly pointed out that the food product is treated by ionizing radiation and a specific symbol, called Radura, has to be on the packing. The food legislation in the EU is regulated by two directives, 1999/2/EC and 1999/3/EC, which determine the type of the food systems, the sources of radiation used, and the facilities (which also need prior registration and approval). In France, food irradiation is regulated by Decree 2001/1097 of 16 November 2001 and the Decree of 20 August 2002, and the following irradiated products may be marketed: frozen aromatic herbs, onions, garlic, shallots, pulses, dried fruit, flakes and germs of cereals, rice flour, meat and offal of poultry, frozen shrimps, shelled or headstocks, frozen frog legs, egg white, casein, and animal blood caseinate.

Ionizing rays are more powerful than nonionizing rays and include corpuscular (flow of accelerated elemental particles, α-rays, β-rays, high-speed electrons, and neutrons) and electromagnetic (γ-rays and short-wave X-rays). Characteristic for radioactive rays is their ability to induce ionization of atoms and molecules, which in turn leads to the destruction of molecular structures. They cause ionization of water and other substances. The mutation effect is indirectly due to ionization, which generates free radicals, hydroxyl radicals ($OH^{\bullet}$) being the most significant among them. Free radicals react with DNA, which is probably as sensitive to ionizing rays as other

macromolecules, but since each gene is present in only one copy in it, its inactivation has severe consequences for the microbial cell. The effectiveness of ionizing radiation on microorganisms depends on the dose of radiation absorbed and other factors. At low doses of radiation, some vital processes in the microbial cell are activated, triggering enzyme systems. They induce hereditary changes in the properties of the microorganisms leading to mutations. At high doses, metabolism is significantly impaired, pathological changes occur in the cell and they lead to its destruction. It should be noted that microbial cells are significantly more resistant to radioactive radiation than the cells of the higher organisms. The lethal dose for them is hundreds to thousands times higher than for animals and plants. Microorganisms most sensitive to radioactive irradiation are those with higher carbohydrate and proteolytic activity, containing more $Mn^{2+}$ and $Ca^{2+}$ in their cells, as well as those with high levels of protective metabolic processes. *E. coli*, *Proteus* sp., and *Pseudomonas* sp., which typically cause microbial spoilage of meat and fish foods, are sensitive to irradiation. Micrococci are characterized by remarkable resistance. *Micrococcus radiodurans* is particularly resistant to ionizing rays and is easily isolated from foods that are subjected to ionizing radiation (Murgov and Denkova, 2012).

Vegetative cells are more susceptible to radiation than spores of bacteria and fungi. The persistence of microorganisms is enhanced by reduced humidity (dry products). Some substances, mainly organic, containing sulfhydryl groups protect microorganisms against the detrimental action of ionizing rays.

Many enzyme systems, membrane structures, the nuclear apparatus, especially DNA, are sensitive to ionizing radiation, which affects cell proliferation. Different microorganisms are distinguished by their resistance to ionizing radiation (e.g., the radiation dose that reduces the number of live *E. coli* cells 10 times is within the range of 5–10 kGy, for spore-forming bacteria of the genera *Bacillus* and *Clostridium*; it ranges from 10 to 50 kGy for vegetative cells and from 5 to 10 kGy for sporulation forms; for yeast and fungal cells it is between 5 and 10 kGy, and for viruses, in the 5–10 kGy range. Depending on the radiation resistance, microorganisms can be divided into four groups (Murgov and Denkova, 2012):

- radio-sensitive—with lethal dose of up to 5 kGy;
- relatively radio-resistant—up to 10 kGy;
- meso-radio resistant—up to 15 kGy; and
- radio-resistant—up to and over 20 kGy.

The absorption dose is influenced by the age of the culture, the composition of the medium, the temperature, and the dose power (irradiation dose for a certain period of time) (Farkas and Mohácsi-Farkas, 2011; Smith and Pillai, 2004). The microbial cells have the ability to repair radiation damage. The nature of the repairing process is determined by the species specificity, physiological state, the absorption dose, and the γ-radiation power.

Ionizing rays, unlike UV rays, have high penetration through solids such as glass, plastic, paper, etc. (Silindir and Ozer, 2009). This penetrating ability makes them dangerous for people and they are, therefore, used in shielded facilities. This actually necessitates proper handling of the irradiation equipment, isolation of the radiation

source, and well-trained personnel. γ-Rays are the most frequently applied form of radiation as they have the greatest penetrating ability and upon irradiation they do not cause the appearance of so-called induced radiation in the product, but minor chemical changes in the composition of the food, as well as in their flavor might occur (Aquino, 2012).

In food processing, the radiation effect depends on the microflora composition, food matrix, absorbance dose (the time for the reduction of the number of viable cells in the population by 1 log $N$ at a given exposure), and power.

The International Atomic Energy Agency has proposed a specific terminology for the radiation treatment of foodstuffs (Arvanitoyannis et al., 2009; Farkas and Mohácsi-Farkas, 2011; Smith and Pillai, 2004) with doses sufficient to destroy human pathogenic microorganisms:

- radiation (6–10 kGy) applied to reduce the number of microorganisms causing microbial spoilage; and
- radarization (10–50 kGy) performed in industrial sterilization of food under conditions excluding re-contamination with microorganisms.

The main concerns of consumers still remain in the sphere of safety of the irradiated foods. Although the effect of the process on food quality and safety has been investigated over more than 100 years, there are no significant reports raising awareness of the ionizing radiation application. There were some issues reported related to possible negative effect of the irradiated foods but the studies seem not to have adequately addressed public concerns (Ehlermann, 2016). An experiment in India with malnourished children was performed with only 20 persons and few of them were given irradiated wheat (Bhaskaram and Sadasivan, 1975). The authors claimed that the patients receiving irradiated food developed polypoid and certain abnormal cells and the effect disappeared after stopping irradiated wheat feeding. However, the experiment was set up in a manner that could be difficult to repeat, the number of the patients was too small, and the initial severity of the children's health conditions were not taken into account.

Another isolated incident in Australia with irradiated cat food was again used as an occasion to raise issues about the safety of irradiated food (Ehlermann, 2016). This incident, however, was isolated and related to the sole importer of irradiated cat food. It is well know that irradiation generates free radicals, but in presence of water the radicals are neutralized very fast; some sensitive substances in food are partially destroyed, such as anthocyanins and vitamins. There were also concerns about generation of potential toxic substances in the irradiated foods (Ehlermann, 2016). It was found that in most cases the compounds generated during irradiation could also be found in thermally treated foods. The particular case with 2-alkylcyclobutanones (2-ACBs) led, in the beginning, to the opinion that these substances were generated only in the irradiated foods but later they were discovered in untreated products, altough in minimal amounts. Studies, however, till now did not find any evidence for potential harmful effects and, for this reason, The Scientific Committee on Food (SCF, today EFSA) of the EU had already concluded that the occurrence of 2-ACBs is of no concern (Statement of the Scientific Committee on Food on a report on 2-alkylcyclo-butanones)

(expressed on 3 July 2002); European Commission; https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out135_en.pdf (last visited 20 September 2018). Nevertheless, ionizing radiation has some clear disadvantages, such as high investments cost, precautions with the source of radiation, packaging safety and migration characteristics, etc., which to a large extent hamper the wider application of the method (Luksiene and Paskeviciute, 2011).

## 2.6 Other methods

Other emerging and promising methods were also proposed. Pulsed electric field processing (PEF) has a dual advantage: prolonging the shelf life of food with minimal changes in nutrient content and sensory quality. PEF technology involves food processing by placing food between electrodes and processing with high voltage electrical pulses of 20–80 $kV\,cm^{-1}$ (several microseconds). PEF has a lethal effect on the vegetative cells of bacteria, yeasts, and molds (Pan et al., 2017). A series of short high-voltage impulses destroy cytoplasmic membranes of cells in liquid media by expanding existing pores or forming new ones. The vegetative cell membranes become permeable to small molecules, permeability causes swelling, penetration of macromolecular components within the cell, and eventually, cell membrane rupture. The PEF affects the proteins and the phospholipid bilayer of the cell membrane, resulting in leakage of cellular contents (Jaeger et al., 2014). The other way to inactivate cells is the loss of transport functions due to the electric field. In the latter case, electroporation of the membrane and leakage of the cellular content is observed. The stability of the microorganisms in this case depends on the medium conditions: type of microorganisms, electric field, impulse size, medium permeability, pH, and temperature. In general, vegetative forms of microorganisms are more unstable, while bacterial spores are more resistant. Data shows that yeasts are more resistant than bacteria, which is quite natural, as they are eukaryotes. Due to the presence of a thicker peptide glycan layer, Gram-positive bacteria are more resistant than Gram-negative bacteria (Pal, 2017).

PEF processing can also be used to inactivate some groups of food enzymes, but in this case, the electric field should be more powerful than the one used for the inactivation of microorganisms. When treating dairy and meat products with a field of 21.5 $kV\,cm^{-1}$, a decrease in lipase and peroxidase activity in the range of 25%–65% in raw milk was observed. At the same time, there was no loss of alkaline phosphatase activity. PEF processing is extremely useful in treating thermally unstable products such as juices and soups. It has been shown that PEF processing does not result in losses of vitamins B1, B2, D, and E, whereas some losses are seen in vitamin C content (Pal, 2017). PEF was also found to have a beneficial effect on bioactive compounds, such as carotenoids (García-Parra et al., 2018; Vallverdú-Queralt et al., 2013), polyphenols (Vallverdú-Queralt et al., 2012), etc.

Shockwave treatment (hydrodynamic pressure processing) was initially used as a novel approach for meat tenderization (Bowker et al., 2011; Ha et al., 2017; Warner et al., 2017). The first attempts were made at the end of the 20th century by Solomon and coworkers (Solomon et al., 1997, 1998) using controlled explosions in sealed vessels. Due to difficulties in controlling the explosions, safety issues with explosives,

and packaging problems, nowadays electric discharge processes are used (Ha et al., 2017; Long and Ayers, 1999; Long and Waits, 2000). Although the main purpose of shockwave treatment is related to tenderization of meat and meat products, there is also observed clear bactericidal effect (Hazell et al., 2010; Leighs et al., 2014; Schiffer et al., 2017; Udagawa and Suzuki, 2013). Studies related to changes in the appearance of the treated meat suggested that no substantial change in the meat color in response to shockwave were observed (Ha et al., 2017). Considering the cooking loss of treated meat, there was a small, but significant increase compared with the control (untreated) samples. Data for the disruptive effect of the shockwaves on the collagen microfibril network were reported (Zuckerman et al., 2013). In generall, neverthelss, the technology offers some advantages for modification of physico-chemical and microbiological properties of meat products; however, the technology met difficulties with industrial commercialization, especially the variant using explosions (Warner et al., 2017).

Photosensitization (photodynamic treatment) is a method relying on the action of visible light on the microbial cells loaded with compound (photosensitizer—accumulating preferentially in the microbial cells), which can absorb the light and provoke photochemical reactions detrimental for the host organisms (Luksiene and Paskeviciute, 2011). Various photosensitizers were explored: porphyrin derivatives, phtalocyanins, phenothiazinium dyes, xanthylium dyes, fullerens, etc. The main requirements for the substances used are efficiency in formation of triplet excited state when light with specific wavelength is used, and preferential affinity to the microbial cells (Brovko, 2010). According to Luksiene and Zukauskas (2009), photodynamic treatment has the advantages that there is no bacterial resistance (or mutagenicity and antibiotic resistance influence on efficacy), no harmful effect on the surrounding observed, the cost is comparatively low, and it is a generally environmentally friendly procedure. Nevertheless, there are some points of concerns that should be mentioned: the necessity for presence of oxygen, addition of compounds (although natural and allowed for food purposes), which usually are dyes and could interfere or change the appearance of the surface, the penetration ability of the light, etc.

Ultrahigh-frequency electric field technology (UHF), relying on waves with short wavelengths of 10–50 m and ultra-short wavelengths (from 10 m to mm) also had a sterilization effect. This is due to the high frequency (HF) and ultrahigh frequency (UHF) current changes during the passage of waves with short and ultra-short wavelengths through the medium. In the electromagnetic field, the electrical energy is transformed into thermal energy. The nature of heating in the UHF field is different from conventional heating techniques and has a number of advantages: the object is heated rapidly and evenly throughout the whole volume/mass, and heating may be selective, as individual parts of a nonuniform object have different electrophysical properties and are heated to different degrees.

The death of microorganisms in the UHF field occurs as a result of the thermal effect, but the mechanism of action of the UHF field is still not fully understood. Some researchers believe that electromagnetic waves have a specific impact. It has been found that a low-intensity UHF field does not cause medium heating and affects some physiological and biochemical properties of microbial cells. Data on the death of certain bacteria and yeasts at temperatures of 35–40°C were reported (Youn et al., 2011;

Ziuzina et al., 2018). Due to its specific features, this treatment method is used for pasteurization and sterilization of foods, including canned fruits. Compared to ordinary steam treatment in autoclaves, the heating time with UHF energy to the same temperature is reduced, thus completely preserving the taste and nutritional properties of the product while the effect on its microflora is the same. It has been found that UHF energy in the processing of spices, whole meats and meat products has a detrimental effect on *Salmonella* sp., *Staphylococcus aureus*, and *Escherichia* sp. These microorganisms, as well as their toxins, are destroyed under different modes of treatment at power of 410–540 W (Awuah et al., 2005; Youn et al., 2011; Ziuzina et al., 2018). UHF electromagnetic food processing is widely used in the food and catering industry for cooking, drying, baking, thawing, etc. (Piyasena et al., 2003b).

## 3 Hurdle approach and combinations of methods

In most cases, a failure of single technologies to achieve proper microbial control with minimal and mild processing has led to the introduction of the idea of combining several approaches to ensure high-quality safe foods. This approach became known as "hurdle technology" (Leistner and Gorris, 1995). More than 50 hurdles were proposed; some are well-known from the past, some are recently introduced. Based on the type of influence on microorganisms and products, they can be categorized as physical (temperature, electromagnetic energy, pressure, ultrasound, packaging, etc.), physicochemical (water activity, pH, redox potential, etc.), chemical (nitrite, polyphenols, spices, oxygen, organic acids, etc.), and microbial (competitive microorganisms, bacteriocins, protective cultures, etc.) (Khan et al., 2017).

Ideally, the technological processes and steps necessary to obtain the desired product have to serve as treatments ensuring microbiological safety. Unfortunately, in most cases, additional steps (hurdles) have to be introduced, depending on the type of food system. The lower the additional hurdles the better, from a technological point of view, from a sensory point of view, for consumers, and for economical reasons, etc.

The inability of HPP alone to ensure complete eradication of bacterial spores gave ground for utilization of combinations of two or more methods. Concepts like mild pressure assisted thermal sterilization (Wimalaratne and Farid, 2008), relying on thermal expansion generated pressure, and pressure assisted thermal sterilization (Wilson et al., 2008), in which heating is applied before HPP treatment, has proven inactivation of microorganisms and spores (Li and Farid, 2016). Similarly, combined action of HPP and bioprotective microorganisms on cooked ham was investigated (Ramaroson et al., 2018) and it was found that, from 63 investigated *Lactobacillus* strains, three gave promising results to regrow after HPP and for suppression of *Bacillus* and *Clostridium* spore-forming bacteria. Combinations of HPP and US with mild heat treatments were also very often proposed for microorganism reduction (Evelyn et al., 2017). The data suggested that heat assisted HPP (600 MPa, 70–75°C) ensured better bacterial and mold spore inactivation than thermosonication and heat treatment. Similarly, García-Parra et al. (2018) concluded that combine action of PEF and HPT could ensure food safety of pumpkin puree.

Spilimbergo et al. (2003b) investigated the synergistic effect of PEF and DPCD on *E. coli*, *S. aureus*, and *Bacillus cereus* and found that it could be an alternative approach for treatment of thermally unstable substances. Various combinations were also tested and promising results were achieved: US followed by mild heating (Wordon et al., 2012); thermosonication and PEF for *S. aureus* inactivation (Walkling-Ribeiro et al., 2009); UV and PEF and PL plus PEF for *E. coli* and *P. fermentans* inactivation (Palgan et al., 2011); US and steam treatment of broilers (Musavian et al., 2014); DPCD and US of cooked ham and dry cured ham (Ferrentino and Spilimbergo, 2016; Spilimbergo et al., 2014), etc.

High intensity PL with PEF was used to inactivate *E. coli* in apple juice with no significant effect on the juice quality (Caminiti et al., 2011b). Caminiti et al. (2011a) applied UV light (5.3 J $cm^{-2}$), high intensity PL (3.3 J $cm^{-2}$), PEF (34 kV $cm^{-1}$, 18 Hz, 93 ms), and manothermosonication (5 bar, 43°C, 750 W, 20 kHz) to a blend of apple and cranberry juice, and found that the high intensity PL and PEF combination was the most effective.

Combining US with pressure at nonlethal (manosonication) and lethal (manothermosonication) temperatures appears to have great potential (Condón et al., 2005, 2010). Knorr et al. (2004) inactivated *E. coli* more easily when US was combined with heat and Álvarez et al. (2006) used heat and US under pressure for inactivation of *Salmonella senftenberg* 775 W. Other works have demonstrated the microbicidal efficiency of US with chemical antimicrobials against *Salmonella* and *E. coli* on alfalfa seed (Scouten and Beuchat, 2002), and for reduction of bacteria in contaminated water (Phull et al., 1997).

Promising results were obtained using combinations of physical and chemical treatments of food systems: US with ozone and $ClO_2$ (Aday and Caner, 2014), US with NaOCl (Vivek et al., 2016), thermosonication with acidic electrolyzed water (Mansur and Oh, 2015), US with $ClO_2$ (Chen and Zhu, 2011), and US combined with organic acids (Sagong et al., 2011), etc. Terpens present in essential oils of many herbs and spices have a pronounced antibacterial activity against different microorganisms. Utilization of both carvacrol and PEF was investigated (Ait-Ouazzou et al., 2013) and it was found that the combination was able to reduce 5 $\log_{10}$ of *E. coli* O157:H7 in mango juice. The study also revealed that 20–50 pulses (30 kV $cm^{-1}$ electric field strength and 1 Hz frequency of repetitions) were necessary for mango, apple, tomato, and orange juice preservation. The inclusion of carvacrol in food formulations is an excellent example of consumers' demand for utilization of a naturally occurring chemical preservative (Wang et al., 2016). Additionally, various plant extracts or wastes of the essential oil industry were also investigated as antimicrobial agents. However, very often the concentration of chemical natural constituents either isolated or as part of natural extracts, is not sufficient for effective microbial preservation. Besides, the higher amount of such natural extracts very often leads to negative alteration of the product taste and flavor (Vasileva et al., 2018).

## 4 Future trends and perspectives

A recent survey on the importance and application of the novel "cold" pasteurization technologies outlined the current status, development, major advancements, limitations, trends, and future perspectives for the next 10 years (Jermann et al., 2015).

Specialists from the industry, government, and academic institutions from North America and Europe (two separate surveys) were interviewed and the main purpose was to identify which of the emerging technologies (commercially exploited now or with potential for commercialization) will be the most utilized in the near future. With regard to the present situation the most utilized technologies seems to be HPP and microwave heating (as alternative to the traditional thermal treatments). Nowadays more than 300 HPP units are used globally and the HPP market is estimated to be more than $10 billion (Huang et al., 2017). The UV (in North America) and PEF (for Europe) pasteurization methods were outlined also as currently often used. The predictions for future implementations and applications of the technologies revealed HPP as the most utilized method for the next 5–10 years. For Europe PEF and microwave heating will be the second and the third mostly used technologies, while for North Americans the prognoses suggested microwave heating, thermal sterilization assisted by applying pressure and UV to be the next after HPP important techniques. It is interesting to note also that according to the opinions of the participants the HPP was the most researched and developed method for the past 10 years. More than 200 HPP installations are industrially available and intensively used in North America (Higgins, 2016).

With regard to potential for application in different food sectors, HPP was the most promising and potentially applicable technology for the meat, poultry, and seafood industries, and UV was considered to be the best alternative for treatment of liquid state food systems and the fresh produce industry. No doubt the major limitation of further spread of the new technologies is the initial investment needed, which actually reflects later on production costs and final products prices. Additionally, lack of funding is also a limitation for some of the food processors. The applications of the emerging technologies will remain mainly for shelf life extension and preservation but also trends toward obtaining new functional and value added foods with reduced salt content are observed (O'Flynn et al., 2014; Yang et al., 2015). Another survey (Lindell, 2018), conducted in October 2017 with participation of food producers and retailers, revealed growing interest on HPP application and the main reasons are shelf life increase and food waste reduction.

From another point of view it is very important to consider consumers' preferences and expectations. The conclusions of a survey organized in Bourg-en-Bresse, Rennes, and Strasbourg (Bourgade et al., 2015) on the technologies for amelioration of food quality and safety, reduction of food wastes, and consumers' awareness, showed that a novel technology is better accepted if it allows substantial reduction or elimination of some additives (especially preservatives), and if it is more understandable by the consumers and closer to the "natural." Similarly, consumers react negatively if a technology is too complex, introduces too many changes in the final product, and relies on materials which could lead to usage of too much packaging (from the point of view of waste reduction, and for minimization of possibility for chemical compounds migration into foodstuffs). Based on the above-mentioned, the consumers ranked as accepted technologies like HPP, microwaving, usage of biopreservatives, etc., while technologies like pulsed light and especially food irradiation were more "suspicious" to consumers.

Generally, the overall impression is that these new technologies for now are spread out mostly in the developed countries from where the consumers' demand for fresher, healthier, minimally processed products mainly comes. Underdeveloped, poorer countries, where the hygienic conditions are not at the highest levels often, could benefit from the emerging "cold" pasteurization technologies with development of research, improvement of the techniques, and mostly with decrease of the installation and final products costs.

## 5 Conclusions

Conventional techniques for thermal sterilization of food have certain disadvantages that may affect the quality of food, the organoleptic properties, and nutrients. The advantages and merits of the new emerging technologies, such as HPP, UV, US, PEF, PL treatment, CP application, ionizing radiation, etc., have led to a rapid spread in the production facilities. Nevertheless, with the continuous growth of utilization and commercialization of the emerging technologies and the new solutions come many more questions and challenges.

How to ensure longer shelf life of food products and in the same time keeping food fresh, minimally processed, and without food quality deterioration? Can the emerging technologies answer this important question? In general, the solutions offered were closer to yes, but yet with some restrictions and limitations. At this point no single technology could offer a perfect solution, and combinations, and applications based on case by case studies should be regarded as the best possible solution.

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## Further reading

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