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Current focus

Alternative food-preservation technologies: efficacy and mechanisms

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Abstract

High-pressure processing, ionizing radiation, pulsed electric field and ultraviolet radiation are emerging preservation technologies designed to produce safe food, while maintaining its nutritional and sensory qualities. A sigmoid inactivation pattern is observed in most kinetic studies. Damage to cell membranes, enzymes or DNA is the most commonly cited cause of death of microorganisms by alternative preservation technologies. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: High pressure; Injury; Irradiation; Kinetics; Non-thermal; Pulsed electric field; Resistance; Ultraviolet

1. Introduction

Consumers are increasingly aware of the health benefits and risks associated with consumption of food. To meet consumers' expectations, the food industry is devoting considerable resources and expertise to the production of wholesome and safe products. Production of safe food includes scrutinizing materials entering the food chain, suppressing microbial growth (e.g. storage at chilling temperature), and reducing or eliminating the microbial load by processing and preventing post-processing contamination. The presence of a processing unit operation aiming at microbial destruction is of primary importance to ascertain safety and stability of food. Heat treatments are traditionally applied to pasteurize or sterilize food, generally at the expense of its sensory and nutritional qualities. As consumers increasingly perceive fresh food as healthier than heat-treated food, the industry is now seeking alternative technologies to maintain most of the fresh attributes, safety and storage stability of food [1].

Satisfactory evaluation of a new preservation technology depends on reliable estimation of its efficacy against pathogenic and spoilage food-borne microorganisms. Research on alternative technologies was initially focused on process design, product characteristics and kinetics of microbial inactivation. The success of these new technologies, however, depends on progress in understanding microbial physiology and behavior of microbial cells during and after treatment. Consequently, this article reviews alternative preservation technologies with emphasis on (i) comparing their efficacy with conventional heat treatment, (ii) mechanisms of microbial inactivation, (iii) patterns of inactivation kinetics, (iv) microbial resistance mechanisms and (v) potential causes of underestimation of survivors during food processing by alternative technologies.

2. Food-preservation technologies

Thermal pasteurization and sterilization are predominantly used in the food industry for their efficacy and product safety record. Excessive heat treatment may, however, cause undesirable protein denaturation, non-enzymatic browning and loss of vitamins and volatile flavor compounds. Many US consumers, for example, consider the 'cooked' and caramelized flavors of sterilized milk as taste defects [2]. Advances in technology allowed optimization of thermal processing for maximum efficacy against microbial contaminants and minimum deterioration of food quality. High-temperature short-time (HTST) pasteurization and ultra-high temperature (UHT) sterilization, for example, minimize vitamin losses in milk in comparison with batch pasteurization and conventional commercial sterilization, respectively [3]. Products processed by modern thermal technologies, however, still lack the fresh flavor and texture.

Non-thermal alternative technologies have been investigated intensively in the past 30 years (Table 1) [4–9]. These technologies are named according to the main processing parameter leading to cell inactivation. Food treated with

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Table 1
Comparison of alternative food-preservation technologies

Process	Range of intensity	Solid food	Fluid food	Approval	Reference
Ionizing radiation	2-10 kGy	Raw poultry meat, raw red meat, seafood, spices	Liquid eggs	In 41 countries (http://ccr.ucdavis.edu/irr/inus2.shtml)	[4]
High pressure processing	100-1000 MPa	Ham, seafood	Fruit juice, guacamole, jam, salad dressing, milk	In Japan, north America, Europe	[5,6]
Pulsed electric field	5-55 kV/cm	–	Fruit juice, liquid eggs	Limited approval in the US (FDA, no objection letter from 07/07/1995)	[7]
Ultraviolet radiation	0.5-20 J/m^2	Meat surface, shell egg surface	Orange juice	Approval pending in the US	[8,9]

high-pressure processing (HPP) is exposed to a high hydrostatic pressure (up to 1000 MPa) for a few minutes [10]. Pulsed electric field (PEF) treatment is based on the delivery of pulses at high electric field intensity (5-55 kV/cm) for a few milliseconds [11]. Gamma radiations and electron beams generate doses of 2-10 kGy, and are commonly referred to as ionizing radiations [4]. Electron-beam technology is currently developed as a safer alternative to gamma radiation since radioactive isotopes are not used. Ultraviolet (UV) energy is a non-ionizing radiation with germicidal properties at wavelengths in the range of 200-280 nm [8,9].

Alternative technologies are occasionally described as 'non-thermal'. Food is treated at ambient or refrigeration temperatures and heat generation during the process is not substantial. HPP and gamma radiation are more suitable than other alternative technologies for application in solid foods (Table 1). The lethal agent must penetrate these foods uniformly without degrading the food texture. The current design of PEF treatment chambers does not allow processing of solid foods. The shielding effect of solid particles restricts applications of UV radiation and electron beams to the treatment of food surfaces [9]. Some alternative technologies are inherently batch processes, while others are adaptable to continuous applications. HPP was designed primarily for processing of packed food, but new designs were developed recently for continuous processes. PEF is a rapid treatment that is well adapted to continuous processing. Maximal PEF treatment intensity is limited by equipment design and food's ability to withstand dielectric breakdown [12]. Maximal treatment intensity in HPP is also limited by equipment design, particularly when adiabatic heating is to be avoided. Irradiation is a batch process that is easily adapted to continuous applications. When irradiated, raw meats and seafood are commonly treated frozen or chilled [4].

3. Efficacy of alternative preservation methods, compared to heat

Most alternative preservation processes achieve the equivalent of pasteurization, but not sterilization [10,13,14]. For illustration purposes, results from different studies will be used to compare alternative preservation methods with a mild heat treatment (Tables 2 and 3) [13–19]. Substantial reductions in the population of *Escherichia coli* are possible using alternative technologies; these reductions are comparable to those achieved by heating at 63 °C for 16 s. HPP requires a comparatively long treatment time. This process may be combined with heat and applied intermittently for elimination of spores [13]. Treatment of food with PEF is a rapid process for inactivating vegetative cells such as *E. coli*. The current status of PEF technology does not enable it to be used to inactivate bacterial spores [14]. Gamma irradiation is effective against vegetative and sporulated bacteria [4].

Table 2
Inactivation of *E. coli* in milk by heat and alternative preservation technologies

Preservation process	Treatment conditions	Log count decreased	Refs.
Heat	63 °C, 16.2 s	5.9	[15]
γ-Irradiation	10 kGy	7.0	[16]
High-pressure processing	500 MPa, 5 min, 25 °C	5.9	[17]
Pulsed electric field	22.4 kV/cm, 330 μs	4.7	[14]

Table 3
Inactivation of *Bacillus* spp. spores by heat and alternative preservation technologies

Technology	Treatment conditions	Medium	Targeted spores	Log count decreased	Refs.
Heat	140 °C, 3 s	Milk	*Bacillus stearothermophilus*	3	[18]
γ-irradiation	12 kGy	Frozen yogurt	*Bacillus cereus*	3	[19]
High-pressure processing	2 × 5 min, 600 MPa, 70 °C	Soil medium	*Bacillus stearothermophilus*	3	[13]
Pulsed electric field	22.4 kV/cm, 250 μs	Milk	*Bacillus cereus*	0	[14]

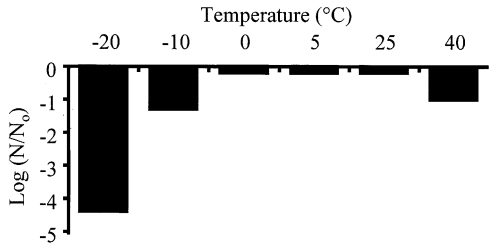


Fig. 1. Inactivation of *S. cerevisiae* after 10 min at 180 Mpa. Adapted from Hashizume et al. [21]. *N*, count after treatment; N_0, initial count.

The greatest advantage of alternative processes, particularly HPP and irradiation, is their efficacy against microorganisms at ambient, chilling and freezing temperatures [4,7,20]. Holding food at mild temperatures favors rapid multiplication of pathogens, spore germination and toxin production. It is safer, therefore, to apply alternative preservation processes at chilling (< 5 °C) or hot (45-60 °C) temperatures than at ambient temperatures. Inactivation of *Saccharomyces cerevisiae* was not observed between 0 and 25 °C after 10 min at 180 MPa (Fig. 1), probably because cell metabolism was active and cell damage (e.g., membrane non-selective permeability) was repaired quickly [21]; higher pressures were required to kill this psychrotrophic microorganism at this temperature range. The greatest inactivation was accomplished when *S. cerevisiae* was treated at –10, –20 and 48 °C. Treating food with gamma irradiation and HPP at chilling or freezing temperature maintains heat-labile compounds, which contribute to the 'fresh attributes' of food. Temperatures ranging from 45 to 70 °C synergistically enhance the efficacy of HPP or PEF against resistant cells. For example, *Bacillus* spores are fairly resistant to pressures > 1200 MPa for several hours at 25 °C, but not to a 600 Mpa oscillatory pressurization at 50–70 °C for 30 min [13]. Heat treatment at 70 °C would be insufficient to kill these spores without pressure.

4. Mechanisms of microbial inactivation

Microorganisms are inactivated when they are exposed to factors that substantially alter their cellular structure or physiological functions. Structural damage includes DNA strand breakage, cell membrane rupture or mechanical damage to cell envelope. Cell functions are altered when key enzymes are inactivated or membrane selectivity is disabled. A preservation technology, e.g. heat, may cause cell death through multiple mechanisms. Limited information is available about the mechanisms of inactivation of microorganisms by alternative preservation technologies. To speed up the implementation of these technologies, research on mechanisms of cell injury and inactivation is urgently needed.

Microbial growth is retarded at pressures in the range of 20-180 MPa; these pressures also inhibit protein synthesis (Fig. 2) [5,22,23]. Loss of cell viability begins at approximately 180 MPa, and the rate of inactivation increases exponentially as the pressure increases. Lethal high-pressure treatments disrupt membrane integrity and denature proteins [23]. The irreversible denaturation of proteins above 300 MPa corresponds to the range of pressure necessary for the inactivation of most vegetative cells and bacteriophages, i.e. protein-coated viruses. Lipid-coated viruses such as the Sindbis virus can resist up to 700 MPa [6]. Membrane disruption is likely responsible for the changes in morphology observed in HPP-treated cells [5]. The formation of pores in spore coats during treatment at 50-300 MPa may indicate that HPP induces spore germination. No germination was observed at higher pressures, likely because the environmental conditions were potentially lethal to germinating spores [6].

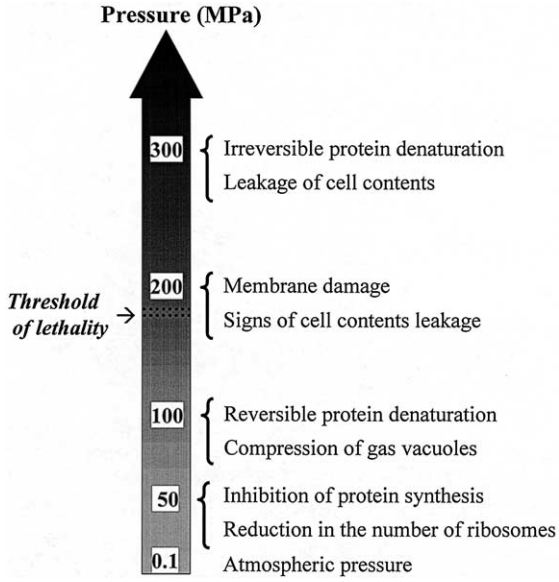


Fig. 2. Structural and functional changes in microorganisms at different pressures [5,22,23].

Membrane structural or functional damage is generally accepted as the cause of cell death during exposure to high-voltage electric field. Zimmermann [24] suggested that PEF temporarily increases the trans-membrane potential of cells by accumulating compounds of opposite charges in the membrane surroundings. High trans-membrane potential exerts pressure on the cell membrane; this pressure decreases membrane thickness and ultimately causes pore formation. On the other hand, Tsong [25] introduced the poration theory to explain the mechanism of cell death by PEF. Electroporation in protein channels and lipid domains results in osmotic swelling of the cell and thus membrane weakening until the cell bursts. In both hypotheses, excessive pore formation causes irreversible loss of membrane functions. In addition, other researchers suggested different mechanisms for the biocidal action of electricity. Electric discharge in liquid media may generate small amounts of microbicidal agents such as chlorine, free radicals and H_2O_2 that alter the DNA and cytoplasmic activity during the treatment [26].

Ionizing and UV radiations damage microbial DNA and to a lesser extent denature proteins [8,27]. Potentially lethal DNA lesions are scattered randomly through the cell population during ionizing and UV radiations. Cells that are unable to repair their radiation-damaged DNA die. Sublethally injured cells are often subject to mutations. Ionizing radiations generate hydroxyl radicals from water, which remove hydrogen atoms from the sugar and the bases of the DNA strands. UV energy at 254 nm induces the formation of pyrimidine dimers; this distorts the DNA helix and blocks cell replication. In addition, UV radiation cross-links aromatic amino acids at their carbon–carbon double bonds. The resulting denaturation of proteins contributes to membrane depolarization and abnormal ionic flow [28]. Irradiation with long-wave UV (320-400 nm) causes the formation of hydroperoxide radicals in the membrane's unsaturated fatty acids, which induces changes in membrane permeability [9]. Exposure of shell eggs to UV light (254 nm) at 4350 μW/cm^2 for 15 min reduced the aerobic microbial population by 3 $\log_{10}$ units [8].

5. Kinetics of microbial inactivation

The most widely recognized theories explaining the death behavior of cell populations in response to exposure to lethal factors are the vitalistic and the mechanistic concepts [29]. The former considers permanent phenotypic variations in the degree of resistance of individual cells within a population. A symmetric, but not normal, distribution of the sensitivity of the population to the treatment is assumed. High treatment intensity is therefore needed to kill the most resistant cells. In contrast, the mechanistic concept considers that each cell of a population has the same degree of resistance, but only a fraction of the population is affected by the lethal agent at a given time. When microorganisms are treated with heat, the logarithm of cell population normally decreases linearly with the treatment time for constant treatment intensity. The resulting straight logarithmic plots support the mechanistic concept. The vitalistic concept describes a logarithmic inactivation only when process intensity is high compared to the variations in resistance among the cells within the population. Alternative technologies were also believed to inactivate microorganisms logarithmically, as found for *Staphylococcus aureus* pressure-inactivation in milk (Fig. 3). The decimal reduction time (*D*-value) corresponds to the treatment time required to reduce the microbial population by 90% at constant treatment intensity. The *D*-value is calculated from the following equation:

$$\mathrm{D-Value} = \frac{t_2 - t_1}{\mathrm{Log}\ X_2 - \mathrm{Log}\ X_1} \qquad (1)$$

with X_1 and X_2 corresponding to the viable counts after treatment times t_1 and t_2, respectively.

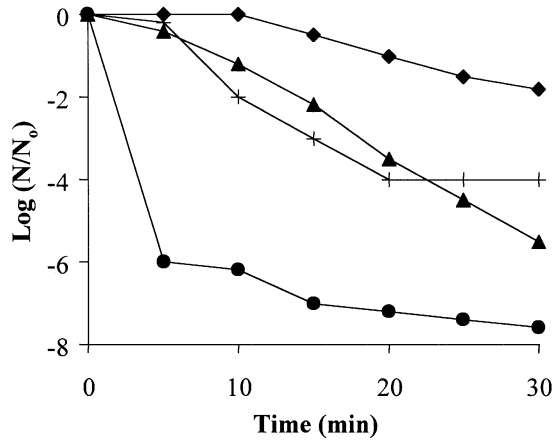


Fig. 3. Linear and biphasic patterns of microbial inactivation during HPP processing. Adapted from Patterson et al. [20]. Pressure treatments: –♦– 375 MPa, *L. monocytogenes* in milk; –|– 600 MPa, *S. aureus* in poultry meat; –▲– 600 MPa, *S. aureus* in milk;–•– 600 MPa, *E. coli* in 10 mM phosphate saline.

Survivor plots in alternative preservation technologies commonly exhibit a shoulder and/or a tail [20,30] (Fig. 3). The initial delay in inactivation of *Listeria monocytogenes* in milk and *S. aureus* in poultry meat during HPP for 5 and 10 min, respectively, are typical 'shoulders'. The intensity of the deleterious agent (e.g. heat) must exceed a threshold value before microbial inactivation happens, which would explain the shoulder. The constant numbers of *S. aureus* in poultry meat and *E. coli* in a buffer solution during extended HPP treatment correspond to the tailing effect (Fig. 3). The mechanistic concept considers the shouldering and the tailing effects as an artifact due to factors including sublethal injury, cell clumping, and heterogeneous treatment zones. The presence of low electric field regions during the PEF treatment of liquid food has not been clearly established [30]. On the other hand, presence of resistant subpopulations explains biphasic curves in the vitalistic concept. A highly resistant sub-population remains viable over a long period of treatment time, causing a substantial decrease in the efficacy of the process observed at low cell concentration. From a practical point of view, the intensity of the treatment should be high enough to ascertain 'a virtual absence' (i.e. undetectable levels) of targeted pathogens in ready-to-eat food with minimal impact on the product's quality and production costs.

Numerous models have been developed to fit sigmoid patterns of inactivation; these have been reviewed by Van Gerwen and Zwietering [31]. Inactivation by HPP is initiated during the pressure come-up time, i.e. the time needed to raise the pressure to the targeted level. The pressure come-up time depends on the equipment and the headspace in the package [32]. Lethality during come-up time should not be included in constructing survivors' plots. Kinetics in HPP, therefore, is based on inactivation while the product is at the targeted pressure [20].

Dose–response models are derived from kinetic data to predict the efficacy of the preservation treatment [31]. These

models depict the relationship between treatment intensity and a population resistance parameter. Treatment intensity corresponds to the temperature, pressure, electric field intensity and radiation dose in the case of thermal, high pressure, PEF and radiation treatments, respectively. The *D*-value is a commonly used population resistance parameter. Heat treatments sufficient to pasteurize or sterilize food are traditionally predicted from thermal death time plots. In linear models, the *z*-value is an estimate of the treatment intensity necessary to decrease tenfold the *D*-value:

$$z - \text{Value} = \frac{I_2 - I_1}{\text{Log } D_2 - \text{Log } D_1} \quad (2)$$

with *I* corresponding to the intensity of the preservation treatment.

Dose–response models describing the PEF treatments are based on sigmoid inactivation plots. The survival fraction during the PEF process (X_1/X_0) at a fixed electric field (*E*) and a treatment time can be predicted from Fermi's equation [33]:

$$\frac{X_1}{X_0} = \frac{1}{1 + e^{\left(\frac{E - E_c}{k'}\right)}} \quad (3)$$

where *k*' is the slope of the steepest segment of the survival plot, and E_c, the critical electric field value for 50% survival.

For industrial applications, however, both the treatment time (*t*) and the intensity of the electric field (*E*) should be considered. The Hulsheger empirical equation takes into account these two process parameters [33]:

$$\frac{X_1}{X_0} = \left(\frac{t}{t_c}\right)^{-\left(\frac{E - E_c}{k'}\right)} \quad (4)$$

with t_c, the time for inactivation threshold.

Hulsheger's model takes into account the minimum treatment time without loss of viability; therefore, the model describes well the inactivation plots with shoulders. Good fits have been found at high electric strength for both Fermi's and Hulsheger's models, probably because the shoulder is reduced at high treatment intensity [33]. Models of Fermi and Hulsheger should be used cautiously, as resistance of microorganisms to PEF increases with cell concentration, which could be due to a shadowing effect, cell clusters or the presence of low electric field regions [7]. To ascertain food safety, an adequate dose-response model must include a safety margin for cell heterogeneity and stress-adaptation mechanisms.

6. Microbial resistance to alternative processes

Bacterial spores are generally the most resistant to inimical processes, followed by Gram-positive and Gram-negative bacteria [13]. The higher resistance to HPP and PEF of Gram-positive, compared to Gram-negative, bacteria (Fig. 4) may be linked to the rigidity of the teichoic acids in the peptidoglycan layer of the Gram-positive cell wall. Bacteria of small size and coccoid in shape are generally more resistant to HPP than the large rod-shaped ones [34]. Reduced cell surface area in contact with the environment may limit cell leakage at a given treatment intensity, and thus minimize the effect of the treatment. Resistance to high pressure, however, was greater in *S. cerevisiae* than bacteria [34]. This finding indicates that other factors than the shape and size of the cell are involved in cell resistance to alternative preservation treatments. The nature of the cell membrane influences cell resistance to preservation processes [34]. High membrane fluidity increases the resistance to HPP and low temperatures. Membranes with relatively high fluidity are rich in unsaturated fatty acids. Pressure-resistant cells also have low-diphosphatidylglycerol content [35]. A specific porin has been associated with increase in pressure resistance [34]. The enhanced resistance of bacterial spores to physical stress, solvation and ionization is linked to the protective effect of the membranes and coat layers surrounding the core, the low-water activity in the spore core and the presence of dipicolinic acid [36].

Microorganisms are more likely stressed or injured than killed in food processed by alternative preservation technologies. Adaptation of microorganisms to stress during processing constitutes a potential hazard. Sub-lethal stress induces the expression of cell repair systems (Fig. 5). For instance, exposure of cells to UV induces enzymatic photorepair and expression of excision-repair genes that may restore DNA integrity. *E. coli* that survives UV exposure has an activated *htpR* gene, i.e. a transcriptional regulator involved in multiple stress resistance. The chaperones GroEL and DnaK, which are under the control of *htpR*, were isolated post-irradiation [37]. Strains surviving HPP have a similar type of enhanced gene-regulated resistance to both HPP and other stresses [38]. Pressure-induced proteins are

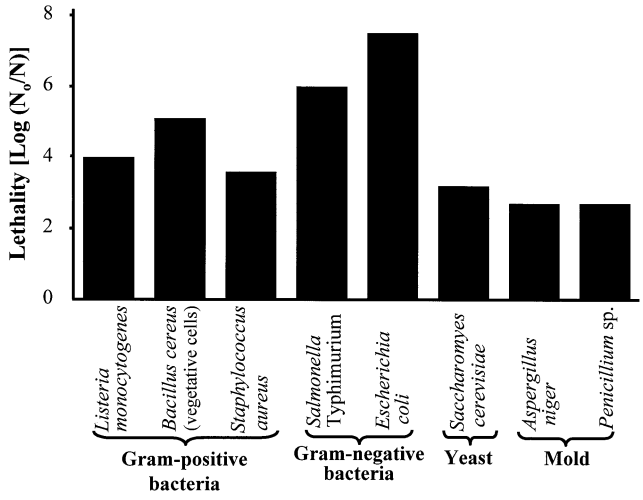


Fig. 4. Lethality of microorganisms after high-pressure processing at 300 MPa (5 °C) for 30 min. Adapted from Arroyo et al. [34]. *N*, count after treatment; N_0, initial count.

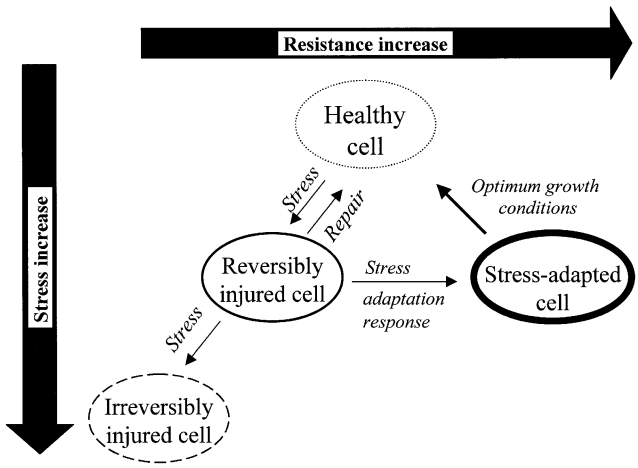


Fig. 5. Microbial stress, injury, adaptation and resistance to processing.

produced in a transitory manner, some of which are also induced by heat and cold shocks [39]. The production of high levels of shock proteins is typical of gene-controlled resistance to stress and may contribute to the bacterial stress hardening effect, i.e. the expression of cross-protection mechanisms against multiple types of environmental stresses [40]. Stress-adapted cells are particularly challenging to the food industry; they may survive processes combing several preservation factors (i.e. hurdle technology). In addition, repetitive exposure of bacterial contaminants to the preservation process can select for highly resistant mutants [22].

Most alternative preservation technologies affect DNA structure and expression. Reversible DNA supercoiling protects cells against osmotic pressure [6]. Slow replication under sub-optimal growth conditions increases the time allowed for cell repair activity and thus favors recovery of sub-lethally injured microorganisms [41]. The highest sensitivity of cell populations is when they are in mid-exponential phase of their growth cycle. This applies to all preservation treatments. Transcriptional regulators such as *rpoS* are not expressed at this stage of bacterial growth. The growth region of yeast cells during budding was found particularly sensitive to PEF [42]. The shift in gene expression and the higher sensitivity of the cell membrane during division therefore contribute to the susceptibility of actively growing cells.

7. Enhancing the efficacy

Adaptation of pathogens to environmental and processing stresses constitutes a serious challenge to the food industry [40]. Alternative preservation technologies should inactivate unusually resistant contaminants and prevent or minimize stress adaptation. Improved efficacy was reported with the addition of antimicrobial agents (Fig. 6) [43]. Nisin has a synergistic effect with PEF treatment, and an additive effect with HPP treatment [23,43]. Incorporation of a Nisin

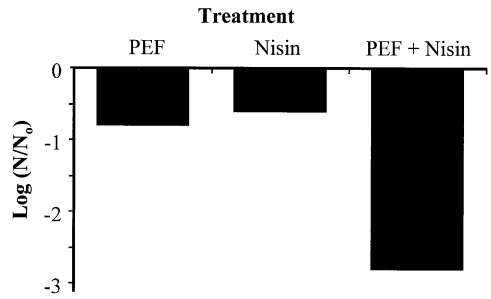


Fig. 6. Inactivation of *Bacillus cereus* with 0.06 µg/ml Nisin and PEF treatment at 16.7 kV/cm for 100 µs. Adapted from Pol et al. [43]. *N*, count after treatment; N_0, initial count.

molecule through the membrane may be facilitated during HPP and PEF treatments, which may cause the formation of higher numbers of permanent pores. Ozone contributes to the breakdown of cells during mild PEF treatments [30]. The addition of antimicrobial agents does not, however, eliminate the tailing effect exhibited by the resistant cell sub-population [30,43].

8. Measurement of cell survival

An important issue in food safety is to ascertain that alternative preservation processes kill targeted microorganisms rather than merely cause sub-lethal injury or phenotype transition into a resistant dormant state. This minimizes the risk of cell resuscitation and expression of virulence during food storage or after food ingestion [44]. Injured *L. monocytogenes*, *Salmonella typhimurium* and *E. coli* O157:H7 were detected after HPP, when comparing viable counts on non-selective and selective media [45]. The heterogeneity of the cell population after processing is likely due to variations in levels of injury, metabolic activity and viability among the cells. Vital staining with fluorescent dyes showed the presence of viable sub-populations that do not form colonies and are therefore not detected on agar media [34]. It has not been demonstrated whether such viable but non-growing cells are the lineage of scarce injured cells. If this hypothesis were true, an extended lag phase would allow the restoration of the original membrane permeability and metabolic activity [46,47].

Several methods are available to directly assess the extent and nature of cell injury within a microbial population. These include culturing on selective agar media and cell staining using epifluorescence techniques. Propidium iodide was used to detect cells that lost selective permeability after HPP [34]. Surface hydrophobicity and ATPase activity may help identify the nature of the membrane damage. The combination of fluorescent dyes in flow cytometric analyses can provide information on different types of cell injury, simultaneously. For instance, ethidium bromide, bis-oxonol and propidium iodide can be used to quantify, in a single experiment, the populations that are reproductively viable, metabolically active and/or with

intact polarized membrane [48]. Staining with acridine orange differentiates viable from dead cells, based on their relative proportion of DNA and RNA [48]. The combination of propidium iodide and SYTO 9 in the *Live/Dead BacLight*™ system gives a similar indication [49]. These dyes are potentially useful in rapid detection of cell viability and injury during treatment with alternative processes.

9. Microorganisms for efficacy testing

Effective food-preservation processes eliminate hazardous pathogens and decrease the loads of spoilage microorganisms. The canning industry targets *Clostridium botulinum* and uses heat treatment sufficient to eliminate 12-log of spores (i.e. a '12-D process'). Safe pasteurized milk is 'virtually free' of *Mycobacterium* sp. Challenge and validation studies using food-borne pathogens should not be carried out in the food-processing facility. Surrogate biological indicators have been proposed as alternatives to pathogens in these studies [50]. Surrogate microorganisms should be slightly more resistant than the targeted pathogens, in order to conservatively estimate the level of pathogens remaining in the treated food. The rapid detection of low levels of pathogens or their corresponding non-pathogenic surrogates may be facilitated when the microorganisms are tagged with a selectable marker such as bioluminescence or resistance to antibiotics, provided these mutations are unlikely to be transmitted to other microorganisms in the factory [50]. The *Clostridium sporogenes* spore is the conventional indicator to predict the heat-inactivation of *C. botulinum* spores. *Listeria innocua* is a non-pathogenic strain that grows in environments similar to those suitable for *L. monocytogenes* [50] and this surrogate is suitable for studying efficacy of milk pasteurization. Surrogate organisms for alternative preservation processes are yet to be clearly defined.

10. Conclusion

HPP, PEF, ionizing radiations (gamma and electron beams) and UV light can inactivate food-borne microorganisms without substantially heating the food. These technologies are developed to produce safe food with high sensory and nutritional values. The choice of a technique for industrial application depends on food properties and process design. High pressure and irradiation are the most frequently used alternative technologies, partly because of suitability for solid and liquid food applications. UV radiation and electron beams are limited to surface decontamination applications. PEF allows the processing of liquid foods rapidly and in a continuous fashion. Antimicrobial agents such as Nisin synergistically enhance the PEF treatment. The combination of HPP or PEF with heat (45-70 ºC) increased their efficacy and reduced bacterial spore populations. Alternative preservation technologies, however, may not be suitable for food sterilization. Thermal sterilization has remained nevertheless the simplest and most effective method for spore inactivation.

Microbial lethality by HPP and PEF is mainly attributed to changes in the membrane structure and functionality. Nucleic acids are the primary target of ionizing radiations and UV light. Most kinetic studies on alternative technologies reveal a sigmoid inactivation pattern. The tailing effect and cell injury are some of the factors that should be elucidated for improving the efficacy of these new technologies. The role of cell structure, physiology, and gene regulation in microbial resistance to alternative preservation technologies should be investigated.

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