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Methods for preservation and extension of shelf life

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Abstract

There is potential for spoilage of all foods at some rate or other following harvest, slaughter or manufacture and spoilage may occur at any of the stages between the acquisition of raw materials and the eventual consumption of a food product. These stages include processing, packaging, distribution, retail display, transport, storage and use by the consumer. They are under varying degrees of control that aim to deliver a satisfactory shelf life, to ensure that the finally-consumed product is of high quality and to ensure that it is safe. Spoilage may be caused by a wide range of reactions including some that are essentially physical, some that are chemical, some enzymic and some microbiological. The various forms of microbiological spoilage are preventable to a large degree by a wide range of preservation techniques, most of which act by preventing or inhibiting microbial growth (e.g., chilling, freezing, drying, curing, conserving, vacuum packing, modified atmosphere packing, acidifying, fermenting and adding preservatives). A smaller number of techniques act by inactivating microorganisms (e.g., pasteurization, sterilization and irradiation). Additional techniques restrict the access of microorganisms to products (e.g., aseptic processing and packaging). A major trend is that new and emerging preservation techniques which are coming into use or are under development include more that act by inactivation (e.g., ultrahigh pressure, electroporation, manothermosonication and addition of bacteriolytic enzymes). A further trend is towards the use of procedures that deliver products that are less heavily preserved, have higher quality, are more natural, freer from additives and nutritionally healthier. Less severe preservation procedures are therefore being developed that make use of preservative factors in combinations to deliver: (a) less damage to product quality (hurdle technologies); (b) new methods of heating that are better controlled and therefore deliver milder heat to products; (c) cook-chill combinations that deliver longer high quality shelf lives; (d) modified atmosphere packaging to retain quality longer; and (e) use of

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antimicrobial systems that are more natural. Many of the existing and emerging preservation techniques act by interfering with the homeostatic mechanisms that microorganisms have evolved in order to survive extreme environmental stresses.

Keywords: Food preservation; Shelf life

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1. Introduction

With few exceptions all foods lose quality and potential shelf life at some rate or other following harvest, slaughter or manufacture in a manner that is very dependent on food type, composition, formulation (for manufactured foods), packaging and storage conditions. Spoilage, or other changes that lead to loss of shelf life, may occur at any of the many stages between the acquisition of raw materials and the eventual consumption of a finished product.

The principal reactions that lead to spoilage, and that are consequently also the principal targets for effective preservation and control, are well known, and relatively few. They include some that are essentially physical, some that are chemical, some that are enzymic and some that are microbiological (Huis in't Veld, 1996).

While most preservation techniques therefore aim to control all the forms of spoilage that may occur, the overriding priority is always to minimize any growth of micro-organisms.

2. Preservation and the extension of shelf life

2.1. Basis of food preservation

Preservation is based firstly on the delay or prevention of microbial growth. It must therefore operate through those factors that most effectively influence the growth and survival of microorganisms. Such factors are not numerous. They include a number of essentially physical factors, some predominantly chemical ones and some microbial ones which depend on the nature of the microorganisms that are present. These factors have been categorized in a number of ways but the most widely quoted categorizations (ICMSF, 1980; Mossel, 1983; Huis in't Veld, 1996) separate the major factors into intrinsic factors, processing factors, extrinsic factors, and implicit factors. In addition, net effects take into account the fact that many of the other factors strongly influence the effects of each other, so the overall effects of combinations of factors may not be readily predictable, but may be derived from modern modeling studies (McMeekin et al., 1993; McClure et al., 1994) and may be of greater use than the perceived effects of the single factors would lead one to expect.

2.2. Major food preservation technologies

The major food preservation techniques that are employed are therefore all based on a relatively limited set of factors, so that their range is necessarily limited also. They are summarized in Table 1 in such a way as to highlight the fact that most of the techniques act primarily by slowing down or, in some cases, completely inhibiting microbial growth. The newer techniques, reacting to consumers' needs, include more natural approaches, (e.g., modified atmosphere packaging, use of protective cultures, use of bacteriocins and other culture products and enzymes). In contrast to the inhibitory techniques, few of the most widely used techniques act primarily by inactivating the target microorganisms, indeed the only technique used substantially for this purpose is still heating. However, it is interesting that most of the newer or emerging techniques do act by direct inactivation, e.g., (a) irradiation; (b) the application of high hydrostatic pressure; (c) high voltage electric discharge (electroporation); (d) ultrasonication combined with increased temperature and slightly raised pressure (manothermosonication); and (e) addition of bacteriolytic enzymes (lysozyme).

Table 1
Existing and emerging antimicrobial techniques employed to preserve foods and to achieve desired shelf lives

Objective	Preservation factor	Method of achievement
Reduction or inhibition of growth	Low temperature	Chill and frozen storage
	Low water activity	Drying, curing and conserving
	Restriction of nutrient availability	Compartmentalization in water-in-oil emulsions
	Lowered oxygen	Vacuum and nitrogen packaging
	Raised carbon dioxide	Modified atmosphere packaging
	Acidification	Addition of acids; fermentation
	Alcoholic fementation	Brewing; vinification; fortification
	Use of preservatives	Addition of preservatives: inorganic (sulphite, nitrite); organic (propionate, sorbate, benzoate, parabens); antibiotic (nisin, natamycin)
Inactivation of microorganisms	Heating	Pasteurization and sterilization
	Irradiating	Ionizing irradiation
	Pressurizing	Application of high hydrostatic pressure
	Electroporating	High voltage electric discharge
	Manothermosonication	Heating with ultrasonication at slightly raised pressure
	Cell lysis	Addition of bacteriolytic enzymes (lysozyme)

Updated from Gould (1989).

Table 2
Antimicrobial food preservatives

Preservative	Examples of foods in which used
Weak organic acid and ester preservatives	
Propionate	Bread, cakes, cheese, grain
Sorbate	Cheeses, syrups, cakes, dressings
Benzoate	Pickles, soft drinks, dressings
Benzoate esters (parabens)	Marinaded fish products
Organic acid acidulants	
Lactic, citric, malic, acetic etc.	Low pH sauces, mayonnaises, dressings, salads, drinks, fruit juices and concentrates
Inorganic acid preservatives	
Sulphite	Fruit pieces, dried fruit, wine, meat sausages
Nitrite	Cured meat products
Mineral acid acidulants	
Phosphoric, hydrochloric	Drinks
Antibiotics	
Nisin	Cheese, canned foods
Natamycin (pimaricin)	Soft fruit
Smoke	Meat and fish

Adapted from Russell and Gould (1991).

The major preservation techniques currently employed to prevent or delay spoilage are reduction in temperature, reduction in pH, reduction in water activity and the application of heat. However, these and other techniques are increasingly used together in combination preservation or hurdle technologies (Leistner, 1995), and it is widely expected that these approaches will find increasing application in the future. Whilst the development of many of the most-used combination techniques was empirical, the basis of their efficacy has been worked out in some instances, and is already leading to a more rational approach to preservation, as indicated below.

2.3. *Low pH and weak acid synergy.*

Amongst the most extensively used combination treatments are those in which an antimicrobial acid is employed and its effectiveness is enhanced by lowering the pH. Indeed, the majority of the useful food preservatives fall into this category. There remain few wide-spectrum food preservatives that are effective at pH values near neutrality (see Table 2). The acids include the inorganic preservatives sulphite and nitrite and the weak organic acids. The organic acids generally increase in effectiveness in the order acetic, propionic, sorbic, benzoic, and this reflects their increasing lipophilicity. This gives a clue to one element of their modes of action, because it is the lipid solubility of mainly their undissociated forms that enables them to cross the microbial membrane and gain access to the cytoplasm within the cell (Booth, 1985; Booth and Kroll, 1989). The second element important in their modes of action is the dissociation constant of the acid, because it is the undissociated form that is most lipophilic and therefore most readily permeates the membrane, and it is the pH value and the dissociation constant together that determine the proportion of the acid that is in this form. The p*K* values of the common weak organic acid preservatives range from 4.2 (benzoic) to 4.87 (propionic), so that at pH values much above these, activity is greatly reduced. At the pH value of most foods microorganisms maintain an internal pH higher than that of their surroundings. On entering the cytoplasm, the undissociated acids therefore tend to dissociate, delivering hydrogen ions, along with the particular anion. The additional hydrogen ions may be exported to maintain a high internal pH, but this is energy-demanding, so cell growth is restricted. If the energy demand is overcome, the pH of the cytoplasm eventually falls to a level that is too low for growth to continue. In addition, careful uptake studies have shown that the preservative actions of some of the acids, and of sorbic and benzoic acids in particular, result as well from effects additional to the membrane gradient-neutralising ones mentioned above (Eklund, 1985), and from gradient dissipation effects that may be indirect.

Overall, therefore, an obvious practical conclusion is that, wherever possible, spoilage is best prevented by simultaneous reduction of pH and the presence of weak acid preservative and, since there is then an increased energy demand on the cell, by anything that restricts the efficient generation of ATP. For instance, for facultative anaerobes, removal of oxygen, e.g., by vacuum or modified atmosphere packaging, is a sensible further adjunct to apply wherever possible.

2.4. *Carbon dioxide in modified atmosphere packaging.*

Another weak acid that is widely used as a food preservative in some countries is carbon dioxide (Farber, 1991). When added to a food pack, it dissolves and equilibrates within the food to form bicarbonate anion and other chemical species according to the partial pressure of the gas, its relative volume and the pH and buffering capacity of the food. The efficacy of carbon dioxide results from a number of unrelated phenomena. The elimination of oxygen that is often achieved in modified atmosphere packs alters the spoilage flora generally by preventing the growth of strict aerobes and slowing the growth of facultative anaerobes through restriction of the amount of energy they can obtain from substrates utilised. However, carbon dioxide has a strong specific antimicrobial action itself that is very microorganism specific. For example, many oxidative Gram-negative bacteria such as species of *Pseudomonas* are sensitive to concentrations as low as 5%, while many lactic acid bacteria and yeasts are capable of growth in the presence of 100% and even in carbon dioxide under pressure. Carbon dioxide-enriched packaging of foods such as fresh meats therefore generally results in a shift of the spoilage flora from a rapidly growing Gram-negative one to a slower growing Gram-positive association of strains (Davies, 1995). In modified atmosphere-packaged fresh fish, in contrast, the Gram-negative *Photobacterium phosphoreum* is very carbon dioxide-tolerant and may therefore be selected as the dominant spoilage organism (Dalgaard et al., 1993).

Good temperature control is vital for effective use of modified atmosphere packaging because the antimicrobial efficacy of carbon dioxide is greatly enhanced as the temperature is reduced. While the reasons for the synergism with low chill temperature are not fully understood, they probably include the increase in solubility of carbon dioxide that accompanies reduction in temperature.

In addition to the antimicrobial effects of modified atmosphere packaging, there are often important additional effects on food colour, with these effects together contributing to the useful extension of organoleptically-acceptable shelf life. For instance, in the preservation of chill-stored fresh meat, carbon dioxide is usually employed at a concentration of 20–30% with oxygen making up the remainder. While the raised level of carbon dioxide delays growth of the Gram-negative spoilage flora, the raised level of oxygen ensures that the haem pigments remain in the bright red oxymyoglobin form that keeps the meat looking attractive to the purchaser, and with an approximate doubling of overall chill shelf life (Parry, 1993).

2.5. *Low water activity and adjuncts*

Reduction of water activity to values between about 0.65 and 0.86 –0.90 in intermediate moisture foods (IMF), depending on the pH value, prevents the growth of the majority of spoilage bacteria (Leistner and Rodel, 1976). However, many osmotolerant yeasts and moulds are able to multiply at these low water activities, so that IMF are not inherently stable by means of their low water activities alone. Addition of antimycotics such as sorbic or benzoic acids, however, can control the osmotolerant fraction of the potential spoilage flora, so that such combinations can ensure long ambient shelf life, as in many semimoist petfood products. These combination systems are less widely used for the preservation of human foods, however, because of the organoleptic penalties resulting from the extensive drying or the high levels of solutes that are needed to sufficiently reduce the water activity. It is for this reason that combinations of reduced water activity with other adjuncts, including mild heating, have been successfully developed, since higher water activities, and therefore lower concentrations of solutes, may be used.

2.6. *Heating with reduced water activity*

Mild heating may inactivate spore forms of microorganisms in foods and injure survivors at temperatures well below those necessary for sterilization. There are therefore a number of potentially useful synergies of heat with other stresses that may be imposed on microorganisms in foods, and one such stress is lowered water activity.

At pH values above 4.5 a botulinum cook would normally be required to ensure the safety of long ambient shelf life products, unless some other inhibitory factor was known to be operating. In particular in some cured meat products, such an additional factor is low water activity, sometimes with a reduction in pH value and the addition of nitrite as well. These combination preservation systems allow the production of a wide variety of so-called SSPs (Shelf Stable Products) that have long, safe ambient stability, and yet require only pasteurization heat treatments (Leistner, 1985, 1995). Many other alternative water activity-pH-mild heat process combinations have been reported but remain little used.

2.7. Heating with reduced pH value

The synergy of low pH with mild heating is the basis of the thermal processing of high acid foods, in which the pH, below 4.5, is low enough to prevent growth from spores of *Clostridium botulinum*. However, other mild heat low pH combination treatments with potential for the preservation of long ambient shelf life foods have been described, though little used. For example, it has been shown that mild heating of spores at low pH values decreases their resistance to subsequent heating, even if the subsequent heating is at a higher pH value (Alderton and Snell, 1963). This occurred with all the types of spores tested, including *C. botulinum*. It was proposed as a potentially useful procedure for reducing heating requirements for the sterilization of long ambient shelf life foods, but difficulties in designing suitable large scale processes were such that the acid-sensitization procedure has not been used commercially. The mechanism of the effect is partially understood. During the acid heating, hydrogen ions replace some of the spore-bound calcium and other cations to form the so-called H-form spores, and these have reduced tolerance to heat. The procedure is so efficient that virtually all of the calcium, which makes up about 2% of spore dry weight, can be removed from the spores of some species (e.g., *Bacillus megaterium*, Marquis et al., 1994) by this means. When the pH is raised, reequilibration occurs as hydrogen ions in the spore are replaced again by cations from the food, but this process is sufficiently slow that heating can be performed while the spores are still relatively heat-sensitive.

With the advent of modern aseptic processing and packaging techniques for pumpable liquid foods, it may be that a re-examination of the potential for 'acid sensitization' is opportune.

2.8. Mild heating and chill storage

There has been a recent expansion in the use of the combination of mild heating of vacuum-packaged foods with well-controlled chill storage, particularly for catering, but for retail also. These foods have been termed 'sous vide' products (Livingston, 1985) or REPFEDs (refrigerated processed foods of extended durability; Mossel et al., 1987; Notermans et al., 1990). The success of the procedure results primarily from the inactivation of the vegetative microbial flora by the mild heating, but also from the fact that the spores of psychrotrophic bacteria, which can grow at low chill temperatures are generally more heat-sensitive than those of mesophiles and thermophiles, which cannot grow at these temperatures. The mild heating therefore destroys the cold-growing fraction of the potential spoilage flora, whilst the minimal thermal damage and conditions of low oxygen tension ensure high product quality. Shelf lives at temperatures below about 3°C can then be very long, i.e., in excess of 3 weeks, with eventual spoilage resuling from the slow growth of psychrotrophic strains of *Bacillus* and *Clostridium*. In order to ensure safety, heat processes equivalent to about 90°C for 10 min are generally regarded as sufficient to ensure inactivation of spores of the coldest-growing pathogenic sporeformers (psychrotrophic strains of *Clostridium botulinum*; Notermans et al., 1990; Mossel and Struijk, 1991; Lund and Peck, 1994). For lower heat treatments, strict limitation of shelf life, efficient control of storage temperature below 3.3°C or some form of demonstrable intrinsic preservation of products is necessary (Anonymous, 1989; ACMSF, 1992).

2.9. Heating following irradiation

Ionizing irradiation sensitizes spores to subsequent heating, though the converse is much less effective (Gombas and Gomez, 1978). The magnitude of the effect varies with irradiation dose rate and is increased if the spores are additionally preincubated at low pH values, as described above. It has been suggested that the effect derives from radiation-induced decarboxylation of peptidoglycan in the spore cortex, which is known to play a role in maintainance of the low water content in the enclosed spore protoplast, but this is not proven. In support of this idea, it was shown that the heat sensitizing effect of irradiation was reversed when the treated spore were suspended in solutions of high osmolality before the spores were heated. It was proposed that this removed water from the partially-rehydrated spore protoplast by osmosis, so returning it to the original fully heat resistant state.

Should the irradiation of foods continue to expand, this synergism may by worth reexamination as a practical means for reducing thermal processing requirements and minimizing heat damage to the nutritional and organoleptic properties of some foods, while retaining a long, safe ambient shelf life.

2.10. High hydrostatic pressure and synergy with mild heating

With regard to the pressure sensitivity of microorganisms an important division is that between the vegetative and the spore forms of bacteria. While many environmental factors greatly affect pressure tolerance (in particular the water activity; Takahashi, 1992; Oxen and Knorr, 1993), the generality still holds that vegetative forms are inactivated by pressures in the region of 300 to 500 MPa but spores of some species resist pressures well in excess of 1000 MPa. For this reason, most current applications of high pressure technology for food preservation are for low pH foods, in which spores are not a problem because, even if they survive, they are unable to grow.

However, there is a strong synergism of pressure with heat such that the spores of some species can be inactivated even at pressures as low as one or two hundred MPa if the pressure is raised at the same time as the heat is applied. The basis for this synergy is partly explained by the fact the pressure in this intermediate region can directly cause spores to germinate (Clouston and Wills, 1969). Having germinated, they are then sensitive to heat, and also to pressure if it is high enough. While the required pressure-heat combinations are currently too extreme for wide commercial application for the sterilization of high water activity-high pH foods, the addition of other adjuncts (hurdles) may well introduce a new series of pressure combination procedures that are effective for long shelf life food preservation in the future. Presumably any such novel procedure would have to achieve at least a 12 log kill of spores of *C botulinum* (and other pathogenic spore formers ?), to match the level of safety that we have come to accept for thermally processed foods (Gould, 1995a).

2.11. *Manothermosonication*

While high intensity ultrasonic radiation has been known for many years to inactivate vegetative forms of bacteria, yeasts and moulds, by their physical destruction resulting from the effects of cavitation, spores are known to be much more resistant. However, application of ultrasonic energy with heat and with a slightly raised hydrostatic pressure together has been shown to be an effective combination that effectively reduces, by about 10°C or more, the temperature of heating necessary to achieve a particular kill of vegetative cells and of spores (Sala et al., 1995). While the detailed mechanism by which inactivation of the microorganisms occurs is not known, the physical basis is thought to include the effect of pressure on the vapour pressure of water. This increases the effectiveness of cavitation, and in particular reduces the fall off in cavitation efficiency that normally accompanies a rise in temperature.

At the moment manothermosonication is laboratory or pilot scale, but may well have potential as an alternative pasteurization or sterilization method for liquid foods in the future. Its application for solid foods is less likely because of the difficulty of delivering sufficiently high ultrasonic intensities into solid substrates economically.

2.12. *High voltage electric discharge*

Substantial inactivation of vegetative cells of bacteria, yeasts and moulds, though less effectively bacterial spores, can be achieved by delivering pulsed electric discharges to liquid foods (Sitzmann, 1995). A major part of the mechanism of action is the puncturing of the cell membrane at the poles of the cell with respect to the voltage gradient (Sale and Hamilton, 1968). This damage occurs because, at the high voltage gradients used, a potential difference of about 1 V or more becomes applied across the cell membrane, which represents an enormous voltage gradient. The technique therefore acts by breaching one of the most important structures in the cell that is involved in many key homeostatic mechanisms, e.g., control of cytoplasmic pH, maintenance of chemiosmotic ion gradients and operation of osmoregulation mechanisms.

2.13. Naturally-occurring antimicrobial systems

In addition to the more traditional and emerging systems that are employed to achieve desired safe shelf lives, there is consumer pressure for still milder, more natural means for the preservation of foods. This has concentrated attention on the wide range of extremely effective naturally-occurring antimicrobial systems that are employed by animals, plants and microorgainsms, with the aim of exploiting some of them in foodstuffs (Davidson and Brannen, 1993; Dillon and Board, 1994).

Animal-derived systems include enzymes such as lysozyme, lactoperoxidase, other proteins such as lactoferrin or lactoferricin derived from it, ovotransferrin, serum transferrins, small peptides such as histatins and magainins and even the immune system. Plant-derived systems include phytoalexins, low molecular weight components from herbs and spices, phenolics such as oleuropein from olives, essential oils. Microorganism-derived ones include acids, hydrogen peroxide, diacetyl and other low molecular weight substances and bacteriocins such as nisin and pediocin and many others dicovered in the last few years (Abee et al., 1995, Table 3).

While many of these have been described and explored for food use, very few are actually exploited at this time. Only lysozyme, to prevent defects in some types of cheeses caused by *Clostridium tyrobutyricum* (Carminati et al., 1985) and nisin

Table 3
Naturally-occurring antimicrobial systems

Origin	Example
Animals—constitutive systems	Myeloperoxidase in phagosomes
	Transferrins in serum
	Lactoperoxidase, lactoferrin in milk
	Lysozyme, ovotransferrin, avidin in eggs
Animals—inducible systems	Antibodies, complement in immune system
	Attacins, cecropins in insects
	Magainins in frogs
Plants—constitutive systems	Eugenol in cloves, allicin in garlic, allyl isothiocynate in mustard, oleuropein in olives, etc.
Plants—inducible systems	Low MW phytoalexins and high MW polyphenolics in injured or infected plants
Microorganisms	Nisin, pediocin and other bacteriocins from lactic acid bacteria
	Other antibiotics (natamycin, subtilin) from other microorganisms
	Bacteriophages
	Yeast 'killer toxins'
	Organic acids and other low MW metabolites

Adapted from Gould (1996).

(Delves-Broughton and Gasson, 1994), to help to preserve some cheese products and to protect some canned foods from spoilage by thermophilic spore-formers, have found relatively wide use.

However, it is widely believed that the future potential for application of such systems is substantial, in particular as their efficacy is demonstrated in combinations with the other antimicrobial factors and procedures that can already be used to preserve foods (Dillon and Board, 1994).

3. Conclusions

While the range of well-established, new and emerging techniques for food preservation and for achieving a satisfactory shelf life may seem to be diverse, there are two underlying themes that have emerged as fundamental to most of the newer methods that have been successfully applied. Firstly, from the practical standpoint, many of the techniques are milder, less damaging to product quality and sometimes more natural than present techniques, in reaction to the changing requirements of consumers. Secondly, from the scientific standpoint, it has become clear that most of the techniques are effective because they overcome the various homeostatic mechanisms that microorganisms have evolved in order to survive extreme environmental stresses (Table 4). There is a logic to many of the combination preservation procedures that are increasingly employed and researched because many of them act by amplifying this interference with homeostasis (Gould, 1995b).

Many of the homeostatic mechanisms are active and therefore require energy for their operation. A sufficiently low food pH interferes with the internal pH homeostasis of cells. Weak acid preservatives cross the cell membrane in their protonated forms and interfere with pH homeostasis. Microrganisms in lowered water activity foods osmoregulate in order to remain hydrated and to maintain membrane turgor. Like pH homeostasis, osmoregulation is energy-dependent, so that anything that reduces the availability of energy to the cell tends to amplify the antimicrobial activity of low water activity. Reduction of pH and water activity together is therefore a logical combination to employ whenever compatible with food quality. And any additional procedure that further reduces a microorganism's ability to generate energy (eg: vacuum or modified atmosphere packaging) will introduce another 'hurdle' that will help to ensure that he energy demands for homeostasis cannot be met.

While such preservation systems are effective because they interfere with homeostasis by imposing stresses on microbial cells, it must be remembered that the cells' responses to stress may result in an increased tolerance to that stress and even to other, apparently unrelated, stresses as well (Foster and Spector, 1995; Gutierrez et al., 1995; Hill et al., 1995). And in pathogens the stress response may include an increase in virulence (Mekalanos, 1992). Care must therefore be excercised in the development of milder preservation techniques in case situations arise in which microorganisms more easily, and less safely, overcome the applied stresses (Knochel and Gould, 1995). On the other hand, Archer (1996) has pointed out that milder

Table 4
Active and passive homeostatic mechanisms in microorganisms

Environmental stress	Example of homeostatic reaction
Active homeostasis	
Low nutrient levels	Nutrient scavenging, oligotrophy, generation of viable non-culturable forms
Low pH, presence of weak organic acids	Extrusion of hydrogen ions, maintenance of cytoplasmic pH and membrane pH gradient
Reduced water activity	Osmoregulation, avoidance of water loss, maintenance of membrane turgor
Low temperature—growth	Membrane lipid changes, cold shock response
High temperature—growth	Membrane lipid changes, heat shock response
High oxygen levels	Enzymic protection from oxygen-derived free radicals
Biocides and preservatives	Phenotypic adaptation and development of resistance
Ultraviolet radiation	Excision of thymine dimers and repair of DNA
Ionizing radiation	Repair of DNA single strand breaks
Passive homeostasis	
High temperature—survival	Low water content in the spore protoplast
High hydrostatic pressure—survival	Low spore protoplast water content ?
High voltage electric dicharge	Low conductivity of spore protoplast
Ultrasonication	Structural rigidity of cell eall
High levels of biocides	Impermeable outer layers of cells
Population homeostasis	
Competition	Formation of biofilms, aggregates with some degree of symbiosis

Adapted from Gould (1995b).

preservation procedures may, by delivering less extreme stresses to microorganisms, actually lead to a reduction in microbial stress responses in foods and therefore to an improvement in food safety.

Other homeostatic mechanisms are passive, having been built in to the microbial cell when it was formed. Low pH sensitizes bacterial spores to heat by overcoming a part of the spores passive homeostatic resistance mechanism. High hydrostatic pressure and manothermosonication somehow do likewise. Electroporation interferes with all those homeostatic mechanisms that depend on an intact semipermeable cytoplasmic membrane for their operation. Altogether, therefore, interference with homeostasis, particular by the logical use of combinations of techniques, remains an attractive focus for future work.

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