

Preservation: past, present and future

Grahame W Gould

Formerly Unilever Research Laboratory, Colworth House, Bedford, UK

Foods deteriorate in quality due to a wide range of reactions including some that are physical, some that are chemical, some enzymic and some microbiological. The various forms of spoilage and food poisoning caused by micro-organisms are preventable to a large degree by a number of preservation techniques, most of which act by preventing or slowing microbial growth. These include freezing, chilling, drying, curing, conserving, vacuum packing, modified atmosphere packing, acidifying, fermenting, and adding preservatives. In contrast, a smaller number of techniques act by inactivating micro-organisms, predominantly heating (pasteurization and sterilization). Complementary techniques restrict access of micro-organisms to food products, e.g. aseptic processing and packaging. New and 'emerging' preservation techniques include more that act by inactivation. They include the application of ionizing radiation, high hydrostatic pressure, high voltage electric discharges, high intensity light, ultrasonication in combination with heat and slightly raised pressure ('manothermosonication'), and the addition to foods of bacteriolytic enzymes, bacteriocins, and other naturally-occurring antimicrobials. Major trends, reacting to consumers' needs, are towards the use of procedures that deliver food products that are less 'heavily' preserved, higher quality, more convenient, more 'natural', freer from additives, nutritionally healthier, and still with high assurance of microbiological safety.

With few exceptions, all foods deteriorate in quality following harvest, slaughter or manufacture, in a manner that is dependent on food type and composition, formulation (of manufactured foods) and storage conditions. The principal quality deterioration reactions, which are, therefore, the principal targets for preservation, are well known and relatively few (Table 1). They include some that are essentially microbiological, others that are chemical, enzymic or physical¹. When preservation fails, the consequences range from extreme hazard, e.g. if any toxinogenic micro-organisms are not controlled, to relatively trivial loss of quality such as loss of colour or flavour. The most serious forms of quality deterioration include those due to micro-organisms, following the survival and/or growth of infectious pathogenic bacteria or the growth of toxinogenic ones². The major food poisoning bacteria are listed in Table 2,

*Correspondence to:
Prof. Grahame W Gould,
17 Dove Road, Bedford
MK41 7AA, UK*

Table 1 Principal quality deterioration reactions of foods

Microbiological	Enzymic	Chemical	Physical
Growth or presence of toxinogenic micro-organisms	Hydrolytic reactions catalysed by lipases, proteases, etc	Oxidative rancidity	Mass transfer, movement of low MW compounds
Growth or presence of infective micro-organisms	Rancidity catalysed by lipoxygenases	Oxidative and reductive discolouration	Loss of crisp textures
Growth of spoilage micro-organisms	Enzymic browning	Non-enzymic browning	Loss of flavours
		Destruction of nutrients	Freeze-induced structural damage

Adapted from Gould¹

along with their abilities to grow at low, chill cabinet/refrigerator temperatures, and their resistance to heating, e.g. during cooking in the home or food service establishment, or during processing in the factory³.

Table 2 Major food poisoning bacteria and their temperature relationships

Minimum growth temperature	Heat resistance	
	Low ^a	High ^b
Low (0–5°C or so)		
<i>Listeria monocytogenes</i> (INF) ^c	<i>Clostridium botulinum</i> E and non-proteolytic B and F(TOX) ^d	
<i>Yersinia enterocolitica</i> (INF)		<i>Bacillus cereus</i> (INF and TOX) <i>Bacillus subtilis</i> (TOX) <i>Bacillus licheniformis</i> (TOX)
<i>Aeromonas hydrophila</i> (INF)		
Medium (5–10°C or so)		
<i>Salmonella</i> species (INF)		
<i>Vibrio parahaemolyticus</i> (INF)		
<i>Escherichia coli</i> enteropathogenic and verocytotoxigenic strains (INF)		
<i>Staphylococcus aureus</i> (TOX)		
Medium (10–15°C or so)		<i>Clostridium botulinum</i> A and proteolytic B (TOX) <i>Clostridium perfringens</i> (INF)
High (over 30°C)		
<i>Campylobacter jejuni</i> and <i>coli</i> (INF)		

^aIn excess of a 10⁶-fold inactivation of vegetative micro-organisms by pasteurization, e.g. at a temperature of about 70°C for 2 min

^bIn excess of a 10⁶-fold inactivation of spores at temperatures ranging from about 90°C for most heat-sensitive types to about 120°C for 10 min for the most heat-tolerant types

^cINF – organisms that may contaminate foods, and may multiply in them, and which cause food poisoning by infection

^dTOX – organisms that may contaminate foods and multiply in them to form toxins that then cause food poisoning by intoxication.

Adapted from Russell and Gould¹⁵

Table 3 Changing consumer requirements and food industry reactions**Trends in consumer requirements***Improved convenience*

Higher quality	- in preparation, storage, shelf-life
Fresher	- in flavour, texture, appearance
More natural	- with fewer additives
Nutritionally healthier	
Minimally packaged	
Safer	

Food industry reactions

Milder processing	- minimal over-heating - less intensive heating - non-thermal alternatives to heat
Fewer additives	- less 'chemical' preservatives
Use of 'hurdle' technologies or 'combination preservation' systems	
Development and use of predictive models	- growth models, as a function of pH, a_w , temperature, preservatives - survival models, as above - thermal death models
Evaluation of natural antimicrobial systems as food preservatives	
Less use of salt, saturated fats, sugar; more low calorie foods	
Reduced, environmentally-friendly packaging	
Elimination of food poisoning micro-organisms	

Adapted from Gould⁴

Changes in the requirements of consumers in recent years have included a desire for foods which are more convenient, higher quality, fresher, more natural and nutritionally healthier than hitherto (Table 3). Food industry reactions to these changes have been to develop less severe or 'minimal' preservation and processing technologies (Table 3). However, minimal technologies tend to result in a reduction in the intrinsic preservation of foods, and may, therefore, also lead to a potential reduction in their microbiological stability and safety. Thus, an important challenge has been to ensure that new and improved technologies retain, or preferably improve on, the effectiveness of preservation and ensurance of safety that may otherwise be lost.

Major current preservation technologies

There is a limited range of techniques currently employed to preserve foods. These are commented on below, and listed in Table 4 in such a way as to emphasize the fact that most of them act by slowing down, or in some cases by completely inhibiting, microbial growth. Few act by

Table 4 Major existing technologies for food preservation*Techniques that slow or prevent the growth of micro-organisms*

Reduction in temperature	- chill storage, frozen storage
Reduction in water activity	- drying, curing with added salt, conserving with added sugar
Reduction in pH	- acidification (e.g. use of acetic, citric acids, etc), fermentation
Removal of oxygen	- vacuum or modified atmosphere packaging
Modified atmosphere packaging	- replacement of air with CO ₂ , O ₂ , N ₂ mixtures
Addition of preservatives	- inorganic (e.g. sulphite, nitrite) - organic (e.g. propionate, sorbate, benzoate, parabens) - bacteriocin (e.g. nisin) - antimycotic (e.g. natamycin)
Control of microstructure	- in water-in-oil emulsion foods

Techniques that inactivate micro-organisms

Heating	- pasteurization - sterilization
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Techniques that restrict access of micro-organisms to products

Packaging
Aseptic processing

Adapted from Gould¹

direct inactivation. A major trend is to apply these techniques in new combinations, in ways that minimize the extreme use of any one of them, and so improve food product quality. This has formed the basis of the successful 'hurdle technologies' of Leistner⁵ that have fostered the development of new routes to food preservation around the world. While traditional hurdle technologies were developed empirically, new logical developments are being made supported by the use of mathematical models⁶. These are generated using data derived from large multifactorial experiments, and allow confident computer-aided predictions to be made, e.g. of the effects of parameters such as pH, a_w , temperature, preservatives, gas phase, etc. on the growth, survival, and thermal death of specific micro-organisms in foods⁷.

Low temperature

As the temperature of a chilled food is reduced, the types of micro-organisms and their rates of growth are reduced also. Two particularly important temperatures are around 12°C, which represents the lower limit for growth of the strict anaerobes, *Clostridium perfringens* and the proteolytic strains of *Clostridium botulinum* (types A and some types of B), and 3°C, which is the lower limit for non-proteolytic strains of *C. botulinum* (types E and some types of B and F). A few years ago, this would have been the chill storage temperature below which no food poisoning micro-organisms would have been expected to multiply.

However, both *Listeria monocytogenes* and *Yersinia enterocolitica* can grow at temperatures below 1°C, so that indicated shelf-lives and sell-by dates can play an important role in ensuring safety, particularly when temperature control can not be assured, e.g. in the home⁸. Many types of spoilage micro-organisms may continue to grow at sub-zero temperatures, multiplying slowly at temperatures down to about -7°C. Badly stored frozen foods may, therefore, slowly spoil through the activities of micro-organisms, but not become dangerous if thawing has not occurred. At the temperature of properly stored frozen foods, nominally -18°C in many countries, microbial growth is completely prevented, although slow loss of quality may still occur through the activities of enzymes and through chemical reactions and physical changes (see Table 1).

Reduction in water activity

Water activity values (a_w) are widely used to predict the stability of foods with respect to the growth of micro-organisms and the chemical, enzymic and physical changes that lead to quality deterioration⁹. Values range from 1 (pure water) to zero (no water), equivalent to equilibrium relative humidities (ERH) on a scale from 100% to 0%. The water activity of foods is reduced by drying or by adding solutes such as salt, as in cured products, or sugars, as in preserves, or by combinations of these treatments. Small reductions, e.g. to about 0.97, are sufficient to prevent the growth of some important spoilage micro-organisms, e.g. *Pseudomonas* species that grow at high a_w s, and rapidly spoil foods such as fresh meat stored in air. Cured meats generally have a_w s sufficiently reduced to ensure longer *Pseudomonas*-free shelf-lives. Slow souring, caused by lactic acid bacteria occurs instead. If the a_w is lower still, below about 0.95, as in some salamis and dry-cured meat products, even these are inhibited, and slow spoilage by low a_w -tolerant micrococci takes over. These and similar relationships are widely used to explain and predict the storage stability and safety of foods. Of the food poisoning micro-organisms, *Staphylococcus aureus* is the most tolerant, with a low a_w limit for growth of about 0.86 in air, but only 0.91 anaerobically, so that it may grow and produce enterotoxin in relatively low a_w foods if other conditions are conducive, e.g. temperature and time of storage. At a_w values below 0.86, few bacteria, and no bacteria of public health concern, can grow, and food is spoiled by yeasts or moulds, some of which can multiply slowly at a_w s as low as 0.6. Below this a_w , no micro-organisms are able to grow. Shelf-stable dried foods are generally formulated around a_w 0.3, where lipid oxidation and other chemical changes are minimal.

An interesting extrapolation of a_w -control of microbial growth into the clinical area was made by Herszage and his colleagues in Buenos Aires¹⁰. He built on the ancient uses of honey and other highly soluble solutes by promoting the treatment of infected wounds with cane sugar. The sucrose was not highly absorbed into underlying tissues, but served to reduce the a_w within a wound, and apparently without interfering with macrophage activity, sufficiently to prevent the growth of pathogens, including *Staph. aureus*. Efficacy was demonstrated in a number of clinical studies¹¹, and the procedure was said to have potential value, e.g. where particularly antibiotic-resistant micro-organisms were involved, or in third world countries where sugar is much cheaper than antibiotics.

Vacuum and modified atmosphere packaging (MAP)

The effectiveness of vacuum and MAP derive firstly from the removal of oxygen, with the consequent inhibition of strictly oxidative micro-organisms. Fermentative organisms continue to multiply but they do so more slowly and, for some types of foods, they have less unpleasant consequences for food quality. Special attention is always given to the possibility of encouraging the growth of strictly anaerobic food poisoning micro-organisms, such as *C. botulinum*, so that for foods such as 'sous vide' products, which are vacuum packed and pasteurized rather than sterilized, minimal heat treatments and tight temperature control in distribution are recommended¹². Carbon dioxide is widely used in MAP foods because it has a specific antimicrobial activity, acting as a preservative that uniquely dissipates when the food pack is opened¹³. For example, much supermarket meat is packed in gas mixtures containing about 70% O₂ and 30% CO₂. The O₂ maintains the meat in the bright red oxymyoglobin colour that consumers prefer, while the CO₂ slows down the growth of Gram-negative spoilage bacteria so as to about double the useful shelf-life.

Acidification

Many yeasts and moulds are able to multiply at very low pH values, *i.e.* well below pH 2, so that they predominate in the flora of spoiling acidified foods. Few bacteria grow below about pH 3.5 or so. Those that do are adapted to acid environments, e.g. the lactic acid bacteria, and indeed are employed in numerous acid-generating food fermentations such as those for yoghurts, cheeses and salamis. A particularly important pH for food safety is pH 4.5, because it is the pH below which *C. botulinum* is unable to multiply. Consequently, in thermal processing,

Table 5 Most-used food preservatives

Preservatives	Examples of foods in which they are used
<i>Weak lipophilic organic acids and esters</i>	
Sorbate	Cheeses, syrups, cakes, dressings
Benzoate	Pickles, soft drinks, dressings
Benzoate esters (e.g. methyl, propyl)	Marinated fish products
Propionate	Bread, cakes, cheese, grain
<i>Organic acid acidulants</i>	
Acetic, lactic, citric, malic, etc	Acidulants for low pH sauces, mayonnaises, dressings, salads, drinks, fruit juices and concentrates
<i>Mineral acid acidulants</i>	
Phosphoric, hydrochloric	Acidulants, as above
<i>Inorganic anions</i>	
Sulphite (SO ₂ , metabisulphite)	Fruit pieces, dried fruits, wine, meat (British fresh sausages)
Nitrite	Cured meats
<i>Antibiotics</i>	
Nisin	Cheese, canned foods
Natamycin (pimaricin)	Soft fruit, dry-cured meats
<i>Smoke</i>	Meats and fish

Adapted from Russell and Gould¹⁵

it is not necessary to heat foods that are more acid than this to the same extent as higher pH 'low acid' foods. Below about pH 4.2, other food poisoning and spoilage bacteria are mostly controlled. However, recently the spore-forming bacterium *Alicyclobacillus acidoterrestris*, capable of growth at pH values as low as 2, has caused spoilage problems ('disinfectant taints') in some low pH foods.

Survival of micro-organisms at low pH may be important, even if they are unable to multiply. For example, *Escherichia coli* O157 has an acid tolerance that may have contributed to some food poisoning outbreaks in which the vehicle was a low pH food, e.g. American (non-alcoholic) apple cider. Furthermore, acid tolerance may aid passage of such organisms through the stomach. Food processors are aware that acid tolerance may be increased by prior exposure to mild acidification, or even by seemingly unrelated stresses, such as mild heating¹⁴.

Preservatives

Most of the preservatives that are used in foods are acids (Table 5), such as the weak lipophilic organic acids (sorbate, benzoate, propionate) or

the inorganic ones (sulphite, nitrite). All are more effective at low rather than at high pH¹⁵. Indeed, with the possible exceptions of the alkyl esters of *p*-hydroxybenzoate ('parabens'), there are no wide-spectrum antimicrobial food preservatives that are effective at near-neutral pH. There is a well-established rationale for the effectiveness of the weak acids and for their synergy with hydrogen ions, *i.e.* with low pH. This derives from the fact that in their unionized forms, which are favoured at low pH, they are able to readily equilibrate across the microbial cell membrane and access the cytoplasm of the cell. The pK value of the common weak 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 of most foods, micro-organisms maintain an internal pH higher than that of their surroundings. Consequently, on entering the cytoplasm, the undissociated acids tend to dissociate, delivering hydrogen ions along with the particular anion. The additional hydrogen ions may be exported by the micro-organisms, but this is energy-demanding, so cell growth is restricted. If the energy supply is overcome, then the pH of the cytoplasm eventually falls to a level that is too low for growth to continue. In addition, the accumulated anion may have specific antimicrobial effects¹⁶.

From the point of view of practical food preservation, it is, therefore, sensible to include a weak organic acid whenever possible, then to acidify the food product as much as is organoleptically acceptable to capitalize on the weak acid-low pH synergy, then to vacuum pack it if possible because this will restrict the amount of energy that is available for the extrusion of hydrogen ions, then to reduce the a_w as much as possible, because this will place additional energy requirements on the cell, and so on. In this way, many empirical preservation 'combination technologies' can be rationalized, and new, logically-based ones sought.

Heat

Pasteurization at times and temperatures sufficient to inactivate vegetative micro-organisms, and sterilization at times and temperatures sufficient to inactivate bacterial spores, remain the bases of large industries around the world¹⁷. With the slow acceptance of irradiation for food preservation in most countries, heat remains the only substantial means for inactivating micro-organisms in foods. However, most of the new and 'emerging' technologies that have been investigated and promoted in recent years act by inactivation, but without the need for substantial heating.

New and emerging food preservation technologies

Natural additives

A few natural additives are widely used (Table 6)^{18,19}. For instance, egg white lysozyme is employed at levels in excess of 100 tonnes per annum to prevent 'blowing', by lysing vegetative cells of *Clostridium tyrobutyricum* outgrowing from spores in some cheeses. Activation of the lactoperoxidase system has been shown to be useful to extend the shelf-life of bulk milk in those countries in which pasteurization soon after milking is not possible and refrigerated transport systems are poorly developed. The small post-transcriptionally modified peptide bacteriocin, nisin, is increasingly used to prevent spoilage of some cheeses and to prevent spoilage of some canned foods by thermophilic spore-forming bacteria such as *Bacillus stearothermophilus* and *Clostridium thermosaccharolyticum*. More than 40 other bacteriocins have been discovered and some are being evaluated for food use. Hundreds of herb, spice and other plant-derived compounds have been described and shown to have antimicrobial properties in laboratory studies²⁰. While some of them are effective in foods, their efficacy is often reduced because of binding of the compounds to food proteins, partition into fats, *etc.*

New physical procedures

It is likely that new physical procedures will provide the most effective alternatives to heat. Some of them are already in commercial use, while other are attracting substantial research and development support (Table 6)⁴.

High hydrostatic pressure

The application of high hydrostatic pressure is now well-established for the non-thermal inactivation of vegetative bacteria, yeasts and moulds in foods, by 'pressure pasteurization'²¹. Vegetative forms of micro-organisms are generally sensitive to pressures in the region of 400–600 MPa (Megapascals) or so (equivalent to 4000–6000 atmospheres), though with large differences in the sensitivities of different species and sometimes large strain-to-strain variations too. Foods so treated include jams, fruit juices, dressings, and avocado dip (guacamole). The advantage of the treatments is that, whereas pressure may greatly alter the state of macromolecules in foods, such as proteins and polysaccharides, it has little effect on small molecules, so that flavours and odours remain relatively unaltered and 'fresh-like'.

Table 6 New and emerging technologies for food preservation**Natural additives**

Animal-derived antimicrobials	– lysozyme – lactoperoxidase system – lactoferrin, lactoferricin
Plant-derived antimicrobials	– herb and spice extracts
Microbial products	– nisin – pediocin – other bacteriocins and culture products

Physical processes

Gamma and electron beam irradiation
High voltage electric gradient pulses ('electroporation')
High hydrostatic pressure
Combined ultrasonics, heat and pressure ('manothermosonication')
Laser and non-coherent light pulses
High magnetic field pulses

High pressure has so far been exploited mainly for the preservation of foods in which spores are not a problem, *e.g.* foods in which the pH is too low for spores to outgrow, or which are stored for limited times at chill temperatures. These limitations result from the fact that bacterial spores are far more tolerant to pressure than are vegetative cells. However, it has been found that pressure can be highly synergistic with mild heating for the inactivation of spores. This seems to occur because pressure, in some as yet unknown manner, actually triggers spores to germinate. Having germinated, they lose their resistance to pressure, and to heat, so that the two physical processes applied together inactivate many more spores than either alone. Further development along these lines, and the possibility of other synergies (*e.g.* pressure has been shown to be synergistic with nisin) may eventually allow it to be used as an alternative to heat-sterilization of foods, and possibly of some pharmaceuticals too. Pressure was first evaluated for vaccine production.

Ultrasonication

Ultrasonication at high enough intensities has long been known to inactivate vegetative bacteria and to reduce the heat resistance of spores; the effect is amplified by increasing the temperature. However, as the temperature is increased, the relative magnitude of the amplification becomes reduced. It is thought that this occurs because, as the vapour pressure rises, it has the effect of reducing the effectiveness of cavitation (the rapid formation and collapse of tiny bubbles), which is the main vehicle of killing. However, application of a slight overpressure (*i.e.* a few atmospheres) has been reported to overcome this fall in effectiveness, so that the amplification is maintained at higher temperatures. The

combination procedure ('manothermosonication'), therefore, has been claimed to have potential for reducing pasteurization and sterilization temperatures for pumpable liquid and semisolid foods²².

High voltage electric discharges

High voltage electric discharges ('electroporation') are most effective for the inactivation of vegetative bacteria, yeasts, and moulds, while spores are much more tolerant. The cell membrane is one of the most important structures controlling many of the vegetative cell's homeostatic mechanisms. It is not surprising, therefore, that electroporation, which breaches this structure, has such a lethal, and essentially non-thermal, effect on vegetative cells. Voltage gradients in the region of 20–60 kV/cm are used, delivered in a series of microsecond pulses, at pulse repetition rates sufficiently low to avoid too much heating. Foods such as milk and fruit juices can be pasteurized using this technique in flow-through continuous treatment cells²³. The reason for the resistance of spores is not known for certain, but probably results from the fact that the central cytoplasm of spores is thought to be relatively dehydrated. This would reduce its conductivity, and make difficult the development of a sufficiently high voltage gradient to breach the surrounding membrane.

High intensity light

High intensity laser and non-coherent light pulse generators have been developed for the decontamination of surfaces of foods and packaging materials, and possibly transparent foods also²⁴, as well as in dentistry²⁵. The killing effect results partially from the UV content for some applications and partially from intense but local heating for others. Additional non-UV and non-thermal effects have been claimed by some researchers.

High intensity magnetic field pulses

Exposure to high intensity oscillating magnetic fields has been reported to have a variety of effects on biological systems ranging from selective inactivation of malignant cells²⁶ to the inactivation of bacteria on packaging materials and in foods²⁷. Treatment times are very short, typically from 25 ms to a few milliseconds, and field strengths are very high, typically from 2 Tesla to about 100 Tesla at frequencies between about 5–500 kHz. Efficacies of treatments did not exceed about 100-fold reductions in numbers of vegetative micro-organisms inoculated into milk (*Streptococcus thermophilus*), orange juice (*Saccharomyces* spp.), bread rolls (mould spores) and no inactivation of bacterial spores has been reported²⁷, so the practical potential for the technique, as it has been developed so far, appears to be limited²⁸.

Irradiation

The use of ionizing radiation, including gamma radiation from isotopes such as ^{60}Co , and electrons and X-rays from machine sources, is legal for disinfestation, to prevent sprouting of bulbs and tubers, and for antimicrobial pasteurization of foods in nearly 40 countries. Doses allowed have generally been up to 10 kGy (kilogray). Recently, the World Health Organization recommended that there are no toxicological or other hazards associated with higher doses, so that there should be no upper dose limit imposed for the irradiation of foods²⁹. The technology is relatively simple to apply, with straightforward inactivation kinetics and geometry that makes dose control and processing requirements much easier than for many heat processes. The potential value to consumers, in the area of prevention of food poisoning through the elimination of pathogens such as *Salmonella* and *Campylobacter* from some foods of animal origin and some sea foods, is substantial. However, this is not widely recognized by consumers, so that slow acceptance by the public continues to restrict its introduction in most parts of the world.

Conclusions

While the most-employed preservation technologies have a long history of use, there is currently a real need for improved techniques, to meet the developing needs of consumers. Some improvements are being derived from the use of established techniques in new combinations or under improved control, and other improvements are being derived essentially from the development of new techniques. These are finding, at first, new and attractive, but niche, markets. It is expected that these will expand as experience in the new techniques is gained. If the resistance of bacterial spores to some of the new techniques could be overcome, and in a manner that was widely proven and accepted to be safe, then the potential markets could be immeasurably larger. A particular attraction of the newer techniques is that they act by inactivation rather than by inhibition. With regard to reducing the incidence of food poisoning disease, the introduction of effective inactivation techniques that lead to the elimination of the pathogens must be the ultimate target of primary food producers, processors, distributors, and retailers. Occasional lapses of hygiene will continue to occur in the food service establishment and in the home, but would be of no public health consequence if the organisms of concern did not enter these premises in the first place.

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