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Symposium on 'Nutritional effects of new processing technologies'

New processing technologies: an overview

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Most food-preservation techniques act by slowing down or completely inhibiting the growth of micro-organisms. Few techniques act by inactivating them. While heat remains the technique most extensively used for inactivation, there has been increasing interest recently in the development of alternative approaches in response to the desires of consumers for products which are less organoleptically and nutritionally damaged during processing and less reliant on additives than previously. The new approaches, therefore, mostly involve technologies that offer full or partial alternatives to heat for the inactivation of bacteria, yeasts and moulds. They include the application to foods of high hydrostatic pressure, high-voltage electric discharges, high-intensity laser and non-coherent light pulses, 'manothermosonication' (the combination of mild heating with ultrasonication and slightly-raised pressure), and high-magnetic-field pulses. In addition, a number of naturally-occurring antimicrobials, including lysozyme and low-molecular-weight products of micro-organisms are finding increasing use. High pressure is being used commercially to non-thermally pasteurize a number of foods, while the other physical procedures are in various stages of development and commercial evaluation. Possible nutritional consequences have so far been given little attention compared with microbiological ones.

Food preservation: Food processing: Food safety: New food-processing technologies

Alternative food-preservation and processing technologies are being developed to a large extent in reaction to consumers' requirements for foods that are nutritionally healthier, more convenient in use (e.g. easier to store and prepare), fresher (e.g. chill-stored), more natural and therefore less heavily processed (e.g. mildly heated), less heavily preserved (e.g. less acid, salt, sugar) and less reliant on additive preservatives (e.g. sulfite, nitrite, benzoate, sorbate) than previously (Table 1). A potential consequence of these trends is a reduction in the intrinsic preservation of foods against microbial survival and growth, and therefore in food safety and keepability. Furthermore, many food-poisoning micro-organisms escape the attention of preservation processes altogether, reaching the consumer more or less directly from contaminated foods, most often foods of animal origin.

Most of the currently employed preservation techniques act by inhibiting the growth of micro-organisms (Table 2), slowing down or completely preventing their multiplication (e.g. by chilling, freezing, drying, curing, conserving, vacuum and modified-atmosphere packaging, acidifying, fermenting, adding preservatives). These techniques are being made less severe to meet the consumer trends mentioned earlier, mainly by their use in combinations ('hurdle technology'; Leistner, 1995), as well as by better-controlled methods of heating that deliver less damage to product quality, and by well-temperature-controlled cook–chill and '*sous-vide*'-like operations.

In contrast to the many micro-organism-inhibitory techniques, few of the currently-employed techniques act primarily by inactivating micro-organisms in foods (Table 2). By far the major inactivation technique remains heating. A major need is still to develop new and improved techniques for the elimination of spoilage and food-poisoning micro-organisms from the most-often contaminated foods. (The lapses of hygiene that will always occur in the home,

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Table 1. Changes in the requirements of consumers and food industry reactions

Consumer requirements
More convenience
Easier to prepare
Easier to store
Longer shelf-life
Higher quality
Improved flavour, texture, appearance
Fresher
More natural
Less use of additives
Nutritionally healthier
Minimally packaged
Safer
Industry reactions
Less-severe processing
Less-intensive heating
Minimal overheating
Less use of additives
Reduced use of chemical preservatives
Increased use of 'combination' or 'hurdle' technologies
More use of 'natural' preservation systems
Lower levels of salt, sugars and saturated fats
Reduced, environmentally-friendly packaging
New microbe-inactivation techniques to eliminate micro-organisms from the most-often contaminated foods and raw materials

Table 2. 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 O_2
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. sulfite, 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
Techniques that restrict access of micro-organisms to products
Packaging
Aseptic processing

in the food service establishment etc., would be of little consequence if the organisms of greatest concern were not present in the first place.)

It is encouraging, therefore, that most of the newer and 'emerging' technologies that are coming into use for food preservation act by inactivating micro-organisms (Table 3). Most successful of these technologies has been the use of high hydrostatic pressure to non-thermally pasteurize foods, and the incorporation of natural antimicrobials such as bacteriolytic enzymes and culture products into foods in order to kill specific bacteria. In addition, progress is being made in the evaluation of other physical techniques, in particular the application of high-voltage electric discharges ('electroporation'), 'manothermosonication' (the combination of mild heating with ultrasonication and slightly-raised pressure), high-intensity laser and non-coherent light pulses, high-magnetic-field pulses, and various steam, acid- and alkali-based surface treatments for the decontamination of carcasses.

Table 3. New and emerging technologies for food preservation

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
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

Treatment of various categories of foods with ionizing radiation is legal in over forty countries now (Patterson & Loaharanu, 2000), but remains little used, mainly due to the suspicions of consumers. Promotion for the eradication of *Escherichia coli* O157:H7 from meat, particularly in the USA, may encourage wider use, as may the recent recommendation by a joint WHO/International Atomic Energy Authority/FAO study group that there is no longer any toxicological, nutritional or microbiological reason to impose an upper dose limit for the irradiation of foods (World Health Organization, 1999).

For most of the new, emerging and potential technologies, nutritional implications have so far received less attention than those relevant to processing for microbiological safety and preservation.

New and emerging technologies

High pressure

Vegetative cells of bacteria were first shown to be inactivated by pressures above about 100 MPa more than 100 years ago by Hite (1899), while bacterial spores were shown to be much more resistant, surviving pressures above 1200 MPa (Larson *et al*. 1918; Basset & Machebouf, 1932).

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Food preservation by pressure was demonstrated by extensions in the shelf-life of milk, fruits and vegetables (Hite *et al*. 1914), but difficulties in applying the technology delayed commercial use for food preservation until the 1980s (Mertens, 1995), when processes were developed for the non-thermal pressure pasteurization of a number of low-pH foods, in which survival of spores was not a problem because they were unable to outgrow under the acid conditions. Currently, pressure-processed foods include jams, fruit juices, jellies, acid sauces and fruit for inclusion in yoghurts (Selman, 1992). More recently, more attention has been given to materials science and other aspects of pressure effects on foods (Knorr, 1995), and further pressure-treated foodstuffs have been marketed, including chill-stored guacamole, dairy, fish and vacuum-packed sliced-meat products, some of which have higher pH values than those marketed earlier (Palaniappan, 1996; Grant *et al*. 2000).

The effects of pressure derive from the Le Chatelier principle, in which pressure favours any physical change or chemical reaction associated with a net volume decrease, and suppresses any change or reaction involving a volume increase. In biological systems the volume-decrease reactions that are most important include the denaturation of proteins, gelation, hydrophobic reactions, phase changes in lipids (and, therefore, in cell membranes) and increases in the ionization of dissociable molecules due to 'electrostriction' (Heremens, 1995). The changes that are brought about therefore differ from those brought about by heat, and may have nutritional consequences. For instance, pressure-denatured proteins differ in structure from heat-denatured ones (Heremens, 1995), with possible nutritional consequences. Small molecules are generally less affected than macromolecules, so that low-molecular-weight flavour and odour compounds etc. in foods tend to survive pressure treatment unchanged, with quality advantages in some types of products (Horie *et al*. 1991).

A structure as complex as a micro-organism will clearly have many potentially pressure-sensitive sites within it, e.g. enzymes, genetic material, macromolecular assemblies such as membranes, ribosomes etc. (Isaacs *et al*. 1995), and pressure has been shown to induce a variety of changes in vegetative bacterial cells that may contribute to their inactivation (Hoover *et al*. 1989). Kinetic studies have shown some examples of exponential inactivation of cells held at constant pressure (e.g. *E. coli*; Butz & Ludwig, 1986; Ludwig *et al*. 1992), but the majority of studies have reported 'tails' on survivor curves, i.e. a decreasing death rate with the time of treatment (Metrick *et al*. 1989; Earnshaw, 1995; Patterson *et al*. 1995*a*). It has been proposed that at higher temperatures (e.g. ≥40°C for *E. coli* at 250 MPa) inactivation is approximately first order, whereas at lower temperatures (e.g. 30°C for *E. coli*) it is more approximate to second order, and that temperature-dependent membrane-lipid changes may account for these differences (Eze, 1990).

More generally, over the range −20°C to +20°C pressure is more effective in inactivating vegetative micro-organisms at the lower temperatures than at the higher temperatures, e.g. for *Staphylococcus aureus, Salmonella bareilly* and *Vibrio parahaemolyticus* in buffers (Takahashi *et al*. 1991) and for *Citrobacter freundii* in beef (Carlez *et al*. 1992). Reduction in water activity resulting from the addition of a range of solutes led to substantial increases in pressure tolerance, e.g. of *Rhodotorula rubra* (Oxen & Knorr, 1993). Other factors that are not yet fully understood affect the pressure tolerance of micro-organisms in different foods. For instance, *Salmonella typhimurium* was inactivated about 10^6-fold in pork in 10 min at 300 MPa, but only 10^2-fold in chicken-based baby food at 350 MPa (Metrick *et al*. 1989). *Listeria monocytogenes* was more pressure tolerant in ultra-heat-treated milk than in phosphate-buffered saline (9 g NaCl/l; Styles *et al*. 1991). Strain-to-strain variability in sensitivity to pressure is greater than the variation to other inactivation techniques, such as heat. *List. monocytogenes* strains NCTC 11994, Scott A and an isolate from chicken were inactivated by <10-fold, approximately 10^2-fold and about 10^5-fold respectively by a similar 375 MPa pressurisation for 10 min in phosphate-buffered saline (Patterson *et al*. 1995*a*).

Otherwise, exponential-phase cells are more pressure sensitive than stationary-phase cells (Dring, 1976), and Gram-positive micro-organisms are generally more pressure tolerant than Gram-negative ones (Shigahisa *et al*. 1991). However, there are some important exceptions. For example, *E. coli* O157 H7 was found to be extremely pressure tolerant in some foods, e.g. exposure to 800 MPa in ultra-heat-treated milk only brought about a 10^2-fold reduction (Patterson *et al*. 1995*b*). Altogether, these various influences of, sometimes poorly-understood, environmental factors and strain-to-strain differences make it more difficult to predict accurately the effect of a particular pressure treatment than a particular heat treatment on micro-organisms in foods.

In contrast to vegetative cells, bacterial spores were shown in the earliest studies to be very pressure tolerant. Pressures up to 1200 MPa failed to inactivate spores of a number of species (Larson *et al*. 1918; Basset & Machebouf, 1932; Timson & Short, 1965). However, later it was shown that, surprisingly, under certain conditions inactivation of spores proceeded more rapidly and completely at lower pressures than at higher pressures (Clouston & Wills, 1969; Sale *et al*. 1970). An explanation for this difference was found when it was observed that inactivation of spores occurred in two stages (Clouston & Wills, 1969; Gould & Sale, 1970). First, pressure caused spores to germinate, then pressure inactivated the germinated forms. This finding led to the investigation of the combined use of pressure with raised temperature (Sale *et al*. 1970) and with low-dose irradiation (Wills, 1974) to achieve a higher level of spore inactivation. The overall pattern of inactivation showed a strong pressure–heat synergism. The effect has been confirmed for a wide range of spore types, although the effectiveness of the combination varies greatly in magnitude for different spores (Murrell & Wills, 1977; Kimugasa *et al*. 1992; Kowalski *et al*. 1992; Seyerderholm & Knorr, 1992; Hayakawa *et al*. 1994). The kinetics of pressure inactivation was approximately exponential for spores of *Bacillus pumilus* (Clouston & Wills, 1970), but for spores of *Bacillus coagulans, Bacillus subtilis* and *Clostridium sporogenes* concave-upward curves or long tails were reported (Sale *et al*. 1970).

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The fact that, although spores of some species are relatively sensitive to pressure (e.g. *Bacillus cereus*), those of other species, including some of special importance in foods such as *Bacillus stearothermophilus* and *Clostridium botulinum*, are very resistant (Knorr, 1995), has so far prevented the use of pressure to sterilize foods (Hoover, 1993). This situation may change with the development of presses that operate at higher temperature–pressure combinations, or the development of other effective combination techniques. For instance, the presence of bacteriocins such as nisin can amplify the effect of pressure against spores, for example, of *B. coagulans* (Roberts & Hoover, 1996).

Delivery of high pressures. The use of high pressure to inactivate micro-organisms involves the application of pressure isostatically (i.e. the pressure is equal throughout the material being processed; there are no gradients, as commonly occur during other processes, such as the application of heat). Pressure is applied either directly by forcing liquid into the treatment chamber or indirectly by forcing a piston into a liquid-filled vessel containing the material to be treated, usually in pre-packaged form. The sealed packages are sufficiently flexible to withstand the compression which occurs during pressurization. The pressure medium and the pack contents are compressed to about 80–90 % of their original volumes during pressurization in the 400–800 MPa pressure range but, of course, return to their original volumes when the pressure is released. There is a transient temperature rise during pressurization of about 11°C at 400 MPa and 23°C at 800 MPa (for water; Farr, 1990), which dissipates at a rate dependent on the volume of the treatment vessel and the conductivity of its materials of construction etc. The first commercial systems to be used for food processing operate as batch processes with treatment times commonly between about 0·5 and 5 min. The volumes of treatment vessels are between about 50 and 1000 litres (Barbosa-Canovas *et al*. 1995). Larger vessels are mostly limited to pressures lower than those capable of inactivating micro-organisms in foods. Fully-continuous processes are not yet used commercially, although cost-effective semi-continuous systems that can be used with pumpable liquid products have been developed (Barbosa-Canovas *et al*. 1995; Moreau, 1995).

High-voltage electric pulses

Effects on micro-organisms. While the application of electric fields to heat foods has become well established (Palaniappan, 1996), e.g. through electrical resistance or 'ohmic' heating (Fryer, 1995) and through microwave heating (Mullin, 1995), the use of electric pulses to bring about the essentially non-thermal inactivation of micro-organisms has only been explored and exploited more recently (Castro *et al*. 1993). The use of the technique at lower, non-lethal, voltage gradients has become established as the basis for 'electroporation', by which genetic material can be exchanged between protoplasted cells of micro-organisms, plants and animals (Neumann *et al*. 1989). A method was patented for inactivating micro-organisms with an electric field by Doevenspeck (1960). Later studies demonstrated the inactivation of bacteria and yeasts, and the lysis of protoplasts and erythrocytes (Sale & Hamilton, 1967, 1968). Even later studies concentrated on varying the electrical variables (Hulsheger & Niemann, 1980; Hulsheger *et al*. 1981, 1983), and on the effects of environmental variables (Mizuno & Hori, 1988; Jayaram *et al*. 1992).

While the effects of high-voltage fields on micro-organisms are not understood at the molecular level, the gross effects and the mechanisms that cause them are well established. They result from the permeabilization of the cell membrane (Hamilton & Sale, 1967) that results when the voltage gradient is high enough to overcome its intrinsic resistance. Breakdown occurs when the potential difference across the membrane exceeds about 1 V (Chernomordik *et al*. 1987; Glaser *et al*. 1988). Massive leakage of cell contents occurs and the cell dies (Tsong, 1991).

Pulsed field inactivation has been reported for *E. coli, Sal. typhimurium, Salmonella dublin, Streptococcus thermophilus, Lactobacillus brevis, Pseudomonas fragi, Klebsiella pneumoniae, Staph. aureus, List. monocytogenes, Saccharomyces cerevisiae* and *Candida albicans* (Barbosa-Canovas *et al*. 1995). In contrast to vegetative organisms, bacterial spores (Sale & Hamilton, 1967) and yeast ascospores (Mertens & Knorr, 1992) are resistant, even at very high voltage gradients, i.e. >30 kV/cm.

A number of intrinsic and extrinsic factors influence the effectiveness of the electrical treatments. Inactivation increased greatly with rising temperature, e.g. for *E. coli* (Qin *et al*. 1994) and for *Lact. brevis* (Jayaram *et al*. 1992). Low ionic strength favours inactivation. A reduction in the KCl concentration in skimmed milk from 0·17 to 0·03 M resulted in a fall in the surviving proportion of *E. coli* following a 55 kV/cm twenty pulse treatment, from about 0·3 to about 0·002. Reduction in pH increased inactivation, e.g. it was doubled for *E. coli* by reducing the pH of skimmed milk from 6·8 to 5·7. Log-phase cells were more sensitive than stationary-phase cells.

Application of electric-pulse teatments to a number of liquid foods have indicated that useful 'cold pasteurization' inactivation of vegetative bacteria and yeasts can be achieved. For example, treatment of apple juice at temperatures below 30°C with less than ten pulses in a continuous-treatment chamber brought about $>10^6$-fold reduction in numbers of *Sacch. cerevisiae* at a voltage gradient of 35 kV/cm; 22 kV/cm caused about 10^2-fold inactivation (Qin *et al*. 1995). Studies of inactivation rates under different conditions have generally indicated kinetics that, on the basis of log survivor *v.* treatment time or log survivor *v.* number of pulses, show long tails. Approximately straight lines are seen for log survivors *v.* log treatment time or log survivors *v.* log number of pulses (Zhang *et al*. 1995). Some potentially-useful synergies have been described. For example, electroporated cells of *E. coli, List. monocytogenes* and *Sal. typhimurium* became much more sensitive than untreated cells to the bacteriocins nisin and pediocin (Kalchayanand *et al*. 1994).

Delivery of electric pulses. Electric fields may be delivered as oscillating, bipolar, exponentially decaying or square wave pulses. Bipolar pulses were more lethal than monopolar pulses because, it was presumed, rapid reversal in the direction of movement of charged molecules caused greater damage to cell membranes. Bipolar pulses generate less electrolysis in the material being treated, which may be

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advantageous for organoleptic reasons, and possibly also for toxicological and nutritional reasons, and they are energy efficient (Qin *et al*. 1994). It is generally most economic to raise the field strength as high as possible while reducing the duration of the pulses, without reducing pulse energy (Grahl *et al*. 1992). On the other hand, the use of very high field strengths demands more complex and expensive engineering (Zhang *et al*. 1994). As a result of these competing requirements, modern pulse-field devices employ field strengths from about 20 kV/cm up to about 70 kV/cm, with pulse durations between 1 μs and about 5 μs. Repetition rates are typically between 1 s and up to about 30 s at the higher voltages in order to minimize rises in temperature.

Treatment chambers may operate batchwise or continuously. The earliest versions were not fully enclosed and so were limited to voltage gradients of about 25 kV/cm because this level is the approximate breakdown voltage of air (Sale & Hamilton, 1967; Dunn & Pearlman, 1987). Enclosure and design improvements led to devices that could deliver 30–40 kV/cm (Grahl *et al*. 1992; Zhang *et al*. 1994). These devices were useful for laboratory studies to optimize design variables for efficient killing of micro-organisms. Continuous operation is essential for cost-effective commercial applications able to treat liquids and liquids containing particulates, and a number of such systems, mostly designed around coaxial cylindrical electrodes, have been developed (Boulart, 1983; Hoffman & Evans, 1986; Dunn & Pearlman, 1987; Sato & Kawata, 1991; Bushnell *et al* 1993; Qin *et al* 1995; Sitzmann, 1995).

Other new and emerging physical technologies

High-intensity light pulses. High-intensity laser and non-coherent light has long been known to inactivate micro-organisms (Mertens & Knorr, 1992), although it is often unclear to what extent the lethal effects derive from the u.v. component of the radiation and, sometimes, local transient heating. The delivery of light to packaging materials, food surfaces and to transparent liquid products, in short pulses of high intensity has been shown to be capable of inactivating vegetative and spore forms of micro-organisms in these environments (Dunn *et al*. 1988) and in the medical area, particularly dentistry (Powell & Wisenart, 1991; Cobb *et al*. 1992; Rooney *et al*. 1994).

Commercially-practicable machines for treating foods and other materials have been patented (Dunn *et al*. 1988). These machines use broad-spectrum light with pulse durations from 10^{-6} to 10 s and with energy densities from about 0·1 to about 50 J/cm^2. Different spectral distributions and energies are selected for different applications. For example, u.v.-rich light in which about 30 % of the energy is at wavelengths shorter than 300 nm is recommended for treatment of packaging materials, water or other transparent fluids. In contrast, for food surfaces, when high intensities of u.v. may accelerate lipid oxidation or cause colour loss etc., the shorter wavelengths are filtered out and the killing effects are largely thermal. The advantage of delivering heat in this manner is that a large amount of thermal energy is transferred to a very thin layer of product surface very quickly, while the temperature rise within the bulk of the product can be very small (Dunn *et al*. 1988). Overall, therefore, these intense light treatments are effective predominantly because they deliver conventional micro-organism-inactivating treatments, u.v. irradiation or heat, but in an unconventional and sometimes advantageous manner (Mertens & Knorr, 1992).

High-intensity-magnetic-field pulses. Exposure to oscillating magnetic fields has been reported to have a variety of effects on biological systems ranging from selective inactivation of malignant cells (Costa & Hofmann, 1987) to the inactivation of bacteria on packaging materials and in foods (Hofmann, 1985). Treatment times are very short, typically from 25 μs to a few milliseconds and field strengths very high, typically from 2 T to about 100 T at frequencies between about 5 and 500 kHz. It has been suggested that the mechanism of action could involve alteration of ion fluxes across cell membranes, but this is not really known (Pothakamury *et al*. 1993). Efficacies of the treatments did not exceed about 10^2-fold reductions in numbers of vegetative micro-organisms inoculated into milk (*Strep. thermophilus*), orange juice (*Saccharomyces* spp.), bread rolls (mould spores) and no inactivation of bacterial spores has been reported (Hofmann, 1985), so the practical potential for the technique, as it has been developed so far, appears to be limited (Barbosa-Canovas *et al*. 1995; Mertens & Knorr, 1992).

Manothermosonication. The use of ultrasound to inactivate micro-organisms was first reported over 70 years ago (Harvey & Loomis, 1929). The mechanism of action derives from the rapidly-alternating compression and decompression zones propagating into the material being treated, and the cavitation that these zones cause. Cavitation involves the formation and collapse of small bubbles, generating shock waves with associated very high temperatures and pressures that can be sufficiently intense to catalyse chemical reactions and disrupt animal, plant and microbial cells (Scherba *et al*. 1991). Generally, large cells are more susceptible than small ones. Rod-shaped bacteria are more sensitive than cocci (Alliger, 1975) and Gram-positive bacteria more sensitive than Gram-negative bacteria (Ahmed & Russell, 1975), while spores are so resistant as to be essentially non-disruptable (Sanz *et al*. 1985).

A potentially useful synergy of ultrasound with heat was reported for the inactivation of bacterial spores (*B. cereus* and *Bacillus licheniformis*; Burgos *et al*. 1972), thermoduric streptococci (Ordonez *et al*. 1984), *Staph. aureus* and other vegetative micro-organisms (Ordonez *et al*. 1987), and for the inactivation of enzymes (Lopez *et al*. 1994). However, as the temperature was raised, the potentiating effect of ultrasound became less and less, and (for spores) disappeared at approximately the boiling point of water (Garcia *et al*. 1989). The important discovery that led to the development of manothermosonication was that this disappearance of the synergism could be prevented if the pressure was raised slightly (e.g. by only the order of tens of megapascals; Sala *et al*. 1995). The combination procedure generally has the effect of reducing the apparent heat resistance of micro-organisms by about 5–20°C, depending on the temperature, the organism and its z-value (change in temperature over which the rate of inactivation, or D-value, changes by a factor of 10). While the process has not yet been commercialized, it has been shown to operate in liquid foods (e.g.

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milk; Sala *et al*. 1995), offering the possibility of new sterilization or pasteurization processes for this and other liquid products with reduced levels of thermal damage.

Natural antimicrobial systems

A wide range of natural antimicrobial systems have evolved in animals, plants and micro-organisms (Table 4). A number of them are already employed for food preservation, while many more have been investigated for use in foods. (Some of the new physical processes are being promoted as 'natural'.)

Animal-derived antimicrobials

The range of antimicrobial systems and substances that operate in animals is extensive (Table 4). It includes the immune system in higher animals and a variety of bacteriolytic and other enzymes that are components of antimicrobial systems, as well as non-enzymic proteins with antimicrobial activity. A steadily-expanding range of small antimicrobial peptides has been discovered recently. These peptides are usually membrane-active and lethal for a wide range of micro-organisms.

Of the animal-derived antimicrobials, most commercial use has been made of hen egg-white lysozyme. Lysozyme is present in many body fluids, and at levels up to about 3·5 % dry weight in egg white, so that it is readily available, relatively inexpensive and natural. It lyses many, although not all, Gram-positive bacteria in their vegetative forms, but is inactive against Gram-negative ones because their outer membranes prevent access to the underlying peptidoglycan that is the enzyme's substrate. The major successful use of lysozyme commercially has been to lyse cells of *Clostridium tyrobutyricum* as they outgrow from germinated spores (Carminati *et al*. 1985), thus preventing gas formation and spoilage by 'blowing' in certain types of cheeses (Wasserfall *et al*. 1976; Carini & Lodi, 1982). It has been estimated that more than 100 t lysozyme are used annually for this purpose (Scott *et al*. 1987). The antimicrobial spectrum of lysozyme can be broadened, e.g. by pretreating the target microbial cells in a number of ways. An example of this procedure is pretreatment with some chelating agents such as EDTA which will sensitize some Gram-negative bacteria to the action of lysozyme (Samuelson *et al*. 1985), but this process has not yet been exploited commercially. Conjugation of lysozyme with dextran increased its activity against Gram-positive and Gram-negative bacteria, particularly if the temperature was raised during treatment (Nakamura *et al*. 1990). Lysozyme and the bacteriocin nisin (see p. 470) act synergistically under certain conditions to inhibit the growth of, and to inactivate cells of *List. monocytogenes* (Monticello, 1989). Freeze–thaw treatments sensitize *E. coli* to lysozyme (Ray *et al*. 1984). Gram-negative bacteria, including salmonellae, become lysozyme-sensitive following an osmotic downshift

Table 4. Major natural antimicrobial systems and potential for synergy

Origin	Example
Animals: Constitutive	Phagosomes: Myeloperoxidase
	Serum: Transferrins
	Milk: Lactoperoxidase
	Lactoferrin
	Eggs: Lysozyme
	Ovotransferrin
	Avidin
Inducible	Immune system: Antibodies
	Complement
	Frogs: Magainins
	Insects: Attacins
	Cecropins
Plants: Constitutive	Herbs, spices and other plants: Eugenol (cloves; *Eugenia aromatica*)
	Allicin (garlic; *Allium sativum*)
	Allyl isothyocyanate (mustard; *Brassica nigra*)
Inducible	Injured or infected plants: low-MW phytoalexins
	high-MW polyphenolics
Micro-organisms	Lactic acid bacteria: Nisin, pediocin
	Other bacteriocins
	Other micro-organisms: Other antibiotics (pimaricin, subtilin)
	Bacteriophages
	Yeast 'killer toxins'
	Organic acids and other low-MW metabolites
Potential synergists	Low pH: Organic acids
	Low A_w: Specific solutes
	Chelators
	Low O_2: Raised CO_2
	Mild heat: Low temperature
	Raised pressure

A_w, water activity; MW, molecular weight.

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(e.g. achieved by sudden dilution of a salt solution). This procedure has been proposed recently as the basis for a dip- or spray-decontamination treatment for poultry and other animal carcasses (Chatzlopou *et al*. 1993).

All these treatments and adjuncts that act synergistically or raise the activity of lysozyme so as to improve its efficacy against vegetative bacteria have potential value in food preservation and safety but, to the author's knowledge, have not yet been employed commercially. Bacterial spores may be sensitized to lysozyme, which then causes them to germinate and so become heat sensitive, but only by severe treatments with reagents that break protein disulfide bonds (Gould & Hitchins, 1963), and which are unfortunately incompatible with foodstuffs.

Lactoperoxidase is an enzyme that is widely distributed, e.g. in colostrum, milk, saliva and other body fluids. It catalyses the oxidation of thiocyanate by H_2O_2, to form hypothiocyanite, which is cidal or static to a wide range of vegetative bacteria (Reiter & Harnulv, 1984). It has been effectively exploited to improve the keeping quality of milk; for example, in countries in which distribution infrastructure and refrigeration are poorly developed (Bjorck *et al*. 1979; Harnulv & Kandusamy, 1982), and in animal feeds to minimize enteric infections in young farm animals (Reiter *et al*. 1981). While generally ineffective against yeasts, moulds and bacterial spores, the lactoperoxidase system has been shown to be bactericidal or bacteriostatic for a wide range of spoilage and food-poisoning micro-organisms, including *Listeria*, *Staphylococcus*, *Campylobacter*, *Salmonella* and *B. cereus* (Ekstrand, 1994) and some yeasts (Pruitt & Reiter, 1985). Toothpastes are on sale that contain amyloglucosidase and glucose oxidase enzymes which, following the breakdown of food starches to maltodextrins by salivary amylase, generate H_2O_2 (Thomas *et al*. 1983). This process activates salivary lactoperoxidase and is claimed to promote, naturally, dental health.

The Fe-binding glycoproteins from eggs (ovotransferrin or conalbumin), milk (lactoferrin) and blood (serum transferrins), bind Fe^{3+} so tightly that, in low-Fe media like serum and egg-white, they prevent microbial growth (Tranter & Board, 1982). The requirement for a low-Fe environment limits their use in foods. They may, therefore, find more effective use in combination with other factors or procedures that limit the availability of Fe. Tranter (1994) suggested that, like EDTA, ovotransferrin may act to enhance the bactericidal activity of lysozyme. Transferrins have been shown to increase the activity of lysozyme against *List. monocytogenes* (Johnson, 1989). Lactoferricin, a polypeptide that can be derived from lactoferrin by acid hydrolysis (Saito *et al*. 1991) or by limited proteolysis (Tomita *et al*. 1991), interestingly acts on microbial cells by means that do not depend on Fe binding. A potential advantage of lactoferricin in food preservation is its relative heat resistance. It has been shown to be effective in extending the shelf-life of raw milk and in reducing the microbial contamination of raw vegetables when employed as a 1 % (w/v) dip (Kobayashi *et al*. 1990).

Small (usually between twenty and about forty amino acid residues) membrane-active peptides are common in animals. They include, for example, defensins in mammals, attacins and cecropins in insects and magainins in frogs (Board, 1995). There is considerable interest in the possibilities of their commercial application, particularly in the pharmaceutical industry and for topical application in medicine, although not yet in foods.

Plant-derived antimicrobials

Wilkins & Board (1989) reported that more than 1340 plants are known to be potential sources of antimicrobial compounds. These compounds include many low-molecular-weight substances (phytoalexins; Table 4), among which phenolic compounds predominate (e.g. caffeic, cinnamic, ferulic and gallic acids, oleuropein, thymol, eugenol). About sixty compounds are mentioned by Nychas (1995). Beuchat (1994) listed about sixty plants that are commonly used as herbs or spices and also are known to contain low-molecular-weight antimicrobial substances with activity against a wide range of bacteria, yeasts and moulds. Plant-derived phenolic compounds were particularly effective as antifungal agents (Lattanzio *et al*. 1994). Antimicrobial organic acids present in plants, of course, include many that are already employed as food preservatives or acidulants (e.g. benzoic, sorbic, acetic, citric etc.). Many herbs and spices contain essential oils that are antimicrobial. Deans & Ritchie (1987) list over eighty plants that contain high levels of antimicrobials with potential food use (e.g. sage (*Salvia officinalis*), rosemary (*Rosemarinus officinalis*), clove (*Eugenia aromatica*), coriander (*Coriandrum sativum*), garlic (*Allium sativum*), onion (*Allium cepa*)). So far, mainly the organic acids have been employed for food preservation, whilst the phenolic compounds, many of which are related chemically to the additives butylated hydroxyanisol and butylated hydroxytoluene that are employed as antioxidants, have not been exploited, although they do show antimicrobial activity as well (Kabara, 1991).

While many studies have been reported using laboratory media, too few have been undertaken using foods (Shelef, 1983). Furthermore, the levels necessary to inhibit micro-organisms in foods have sometimes been found to be much higher than those determined using cultures (Farbood *et al*. 1976). Other disadvantages associated with the use of many plant-derived antimicrobials in foods (e.g. the strong flavour of some phenolics and essential oils) may be ameliorated by using them in combination with other permitted antimicrobials, so reducing the concentrations necessary for efficacy (Nychas, 1995).

Micro-organism-derived antimicrobials

The tetraene antimycotic natamycin (pimaricin), produced by *Streptomyces natalensis*, is used to prevent mould growth in some foods, e.g. on the surfaces of some cheeses and dry sausages (Kliss, 1960), and is being considered for wider use in some countries, e.g. within the EU (EEC, 1989). However, the use of microbially-derived antimicrobials for food preservation is dominated by the peptide 'lantibiotic' bacteriocin nisin, produced by certain strains of *Lactococcus lactis*, and applied mainly in cheese and cheese products, and in canned vegetables (Delves-Broughton, 1990; Fowler & Gasson, 1991). Other applications proposed have been for

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meat, fish, milk and milk products, and alcoholic beverages (Delves-Broughton & Gasson, 1994).

However, the number of other bacteriocins that have been discovered and that may have potential in food preservation continues to grow. They have been categorized into three classes: (1) small post-transcriptionally-modified lantibiotics, e.g. nisin; (2) small heat-stable unmodified peptides, e.g. pediocin PA-1; (3) larger heat-labile molecules, e.g. helveticin J. Hill (1995) lists more than twenty bacteriocins that have been well-characterized. Many bacteriocins are produced by strains of starter cultures (Ray & Daeschel, 1994). While these have not been applied as additive food preservatives in the same way as nisin, the potential for their use as additives, or by generation *in situ* in foods, e.g. from the addition of particular starter cultures, or in cultured-product food ingredients such as Microgard (Al-Zoreky *et al*. 1991), must be substantial.

Later on, the genetic modification of bacteriocins, that is the focus for much current research, may result in derivative molecules that have usefully modified antimicrobial activity spectra, or changes in other properties that increase their potential value in food preservation and safety, if regulatory hurdles can be overcome (Fowler & Gasson, 1991; Hill, 1995). Already genetic engineering has resulted in the production of nisins with changed amino acid sequences. For example, Kuipers *et al*. (1992) changed the serine residue at position 5 of prenisin Z to threonine, and the threonine was shown to be dehydrated to dehydroalanine during processing of the molecule. In this instance, the new nisin had lower antimicrobial activity than the wild-type molecule. Dodd *et al*. (1992) replaced the position 23 threonine of nisin A with a serine residue to produce a less-antimicrobially-active mature peptide. However, a nisin A variant in which the dehydroalanine was replaced by alanine at position 5 had near-normal antimicrobial activity (Delves-Broughton & Gasson, 1994).

Much recent research has concentrated on the potential for bacteriocins as antagonists for vegetative bacteria (e.g. *Listeria*). It should not be forgotten that nisin, the only widely used bacteriocin (its use is currently allowed in about fifty countries), is employed mainly as an anti-spore agent, e.g. finding substantial use to prevent the outgrowth from germinated spores of *C. tyrobutyricum* in processed cheese products (Delves-Broughton, 1990) and to prevent growth from spores of thermophiles such as *B. stearothermophilus* and *Clostridium thermosaccharolyticum* that may survive and grow in canned foods stored at high temperatures (Eyles & Richardson, 1988; Fowler & Gasson, 1991). However, nisin suffers from the disadvantage of being relatively ineffective against the proteolytic types of *C. botulinum* (Sommers & Taylor, 1987). Discovery of bacteriocins that are more effective against this organism, and which control the growth of a wider range of spoilage sporeformers as well, would have great potential value, because they may allow substantial reductions in the thermal processing of high-water-activity-low-acid foods, with potential advantages in energy saving and improved product quality.

Conclusions

The use of physical techniques to inactivate microorganisms without the the application of heat, or with the use of less heat than would be otherwise necessary, is attractive from the point of view of product quality, and the new and emerging techniques reviewed all aim to do this. Three facts limit their usefulness at the present time. First, bacterial spores remain the organisms most tolerant to all the techniques so that, with the possible exception of manothermosonication, sterilization (as opposed to pasteurization) is not yet possible. Second, the kinetics of inactivation that results from some of the techniques is different from that resulting from heating, so that a careful new approach, e.g. to product safety, will be needed if application of the techniques continues to be promoted. Third, with the exception of hydrostatic pressure, the efficacy of the other techniques is impaired by product structure, and may therefore be limited to liquid products or products containing small particulates, or (for light pulses) transparent products and surfaces etc. At the same time, combination techniques in which the new technologies are only one component of a total 'hurdle' preservation system (Leistner, 1995) have already been described, and if these systems are further developed and proven to be effective, the opportunities for use of the new techniques are very likely to grow in the future.

With respect to the exploitation of naturally-occurring antimicrobials, it is clear that, in nature, an enormous number of very effective antimicrobial systems exists. However, it is also clear that disappointingly few of them have been developed for positive use in foods. To some extent this situation may reflect industry's reluctance to embark on the substantial and expensive programmes of toxicological testing that can be necessary for the introduction of a new antimicrobial. To some extent it has probably been due to the absence of strong commercial and marketing incentives to develop their use, but this situation has changed in recent years in response to consumers' changing needs, as pointed out earlier. Most food technologists would predict that natural systems, particularly in additive or synergistic, hurdle, combinations with other factors and techniques that we can already make use of, as indicated in Table 4, will have an increasing role to play in the future (Banks *et al*. 1986); particularly if more studies are undertaken in realistic foodstuffs as well as in laboratory media. Whether important nutritional implications will arise from new combination uses of physical, natural and conventional techniques remains to be seen.

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