International Journal of Applied Management Research,
Vol. 1(No. 1), April, 2013, Page: 133-151

Hurdle Technology: Its Potential Application in Food Industry

Mittal Kathiriya, JB Prajapati, Subrota Hati
Department of Dairy Microbiology, SMC College of Dairy Science, Anand Agricultural University, Anand-132001 (Gujarat), India
&
B.K. Mishra
Department of RDAP, North- Eastern Hill University, Tura Campus, Tura-794002, Meghalaya, India

Abstract

Consumers are very much aware of nutritive values, quality and safety of the processed foods available in the market. Hurdle technology is one of the food processing technology through which foods can be processed safely as well as nutritive values and quality will be maintained throughout the processing of the foods. Hurdle technology also provides different types protection layers against various food borne organisms present in foods and make the foods safe for consumption. Recently various non-thermal technologies like pulsed field technology, HHP, plasma technology combined with compounds of hurdles such as NaCl, organic acids, bacteriocins etc. are added to make these technology more strengthful against the food borne microorganisms.

Keywards:

Consumers, hurdle technology, consumption, plasma technology, microorganisms

Introduction:

Hurdle technology also called combined processes, combined methods, combination preservation, combination techniques or barrier technology. In other words we can elaborate this technique as- combined use of several preservation methods, possibly physical and chemical or a combination of different preservatives is an age-old practice. It has been commonly applied by the food industry to ensure food safety and stability. In smoked products, for example combination treatment includes heat, reduced moisture content and antimicrobial chemicals deposited from the smoke onto the surface of the food. Some smoked foods may also be dipped or soaked in brine or rubbed with salt before smoking, to impregnate the flesh with salt and thus add a further preservative mechanism. In recent years, the concept is now ready to be introduced for use with a much wider range of food products, including fruits and vegetables, bakery products, dairy products, fish and so on. The concept of combining several factors (Hurdle Technology) has been developed by Leistner. From an understanding of the hurdle effect, hurdle technology has been derived, which has the goal not just to understand why a certain food is safe and stable, but to improve the microbial quality of the food by an optimization and intelligent modification of the hurdles present. It employs the intelligent combination of different hurdles or preservation techniques to achieve multi-target, mild but reliable preservation effects. Hurdle technology is used in industrialized as well as in developing countries for the gentle but effective preservation of foods. Previously hurdle technology, i.e. a combination of preservation methods was used empirically without much knowledge of the governing principles. Since about 20 years the intelligent application of hurdle technology became more prevalent, because the principles of major preservative factors for foods (e.g. temperature, pH, a_w, Eh, competitive flora) and their interactions became better known. Recently, the influence of food preservation methods on the physiology and behaviour of microorganisms in foods, i.e. their homeostasis, metabolic exhaustion, stress reactions, are taken into account and the novel concept of multi-target food preservation emerged. Hurdle Technology also called: combined processes, combined methods, combination preservation, combination techniques, and barrier technology. Hurdle technology is a method of ensuring that pathogens in food products can be eliminated or controlled. This means the food products will be safe for consumption and their shelf life will be extended. Hurdle technology usually works by combining more than one approach. These approaches can be thought of as "hurdles" the pathogen has to overcome if it is to remain active in the food. The right combination of hurdles can ensure all pathogens are eliminated or rendered harmless in the final product. Hurdle technology has been defined by Leistner as an intelligent combination of hurdles which secures the microbial safety and stability as well as the organoleptic and nutritional quality and the economic viability of food products. The organoleptic quality of the food refers to sensory properties, i.e. its look, taste, smell and texture. Examples of hurdles in a food system are high temperature during processing, low temperature during storage, increasing the acidity, lowering the water activity or redox potential or the presence of preservatives. According to the type of pathogens and how risky they are, the intensity of the hurdles can be adjusted individually to meet consumer preferences in an economical way, without compromising the safety of the product. An effective combination of different hurdles can work in synergy and thereby provide a good antibacterial effect even though the "height" (level) of each individual hurdle may be small. A poor combination of hurdles may have an antagonistic effect means the effect of the combination is less than if any of the individual hurdles had been used alone. Hurdle technology used in the correct way is therefore a good tool for achieving safe and tasty products of high quality. Understanding how various hurdles work together and what effect they have on bacteria and other micro-organisms is important for ensuring safe food. About 60 different hurdles have been identified in food preservation. Apart from the most important and commonly used hurdles such as temperature, pH and water activity, there are many others of potential value. Other hurdles include: ultrahigh pressure, mano-thermo-sonication, photodynamic inactivation, modified atmosphere packaging of both non-respiring and respiring products, edible coatings, ethanol, maillard reaction products and bacteriocins.Some hurdles (e.g., Maillard reaction products) will influence the safety and the quality of foods, because they have antimicrobial properties and at the same time improve the flavour of the products. The same hurdle could have a positive or a negative effect on foods, depending on its intensity. For instance, chilling to an unsuitable low temperature is detrimental to some foods of plant origin ('chilling injury'), whereas moderate chilling will be beneficial for their shelf life. Another example is the pH of fermented sausage which should be low enough to inhibit pathogenic bacteria, but not so low as to impair taste. If the intensity of a particular hurdle in a food is too small it should be strengthened, if it is detrimental to the food quality it should be lowered. By this adjustment, hurdles in foods can be kept in the optimal range, considering safety as well as quality, and thus the total quality of a food (Leistner, 1994a). For each stable and safe food a certain set of hurdles is inherent, which differs in quality and intensity depending on the particular product, but in any case the hurdles must keep the `normal' population of microorganisms in this food under control. The microorganisms present (`at the start') in a food should not be able to overcome (`leap over') the hurdles present during the storage of a product; otherwise the food will spoil or even cause food poisoning.

In developing countries the application of hurdle technology for foods that remain stable, safe and tasty if stored without refrigeration is of paramount importance and has made impressive strides, especially in Latin America with the development of novel minimally processed, high-moisture fruit products. However, much interest in intentional hurdle technology is also emerging for meat products in China as well as for dairy products in India. There is a general trend in developing countries to move gradually away from intermediate-moisture foods because they are often too salty or too sweet and have a less appealing texture and appearance than high-moisture foods and this goal could be achieved by the application of intelligent hurdle technology. The progress made in the use of advanced hurdle technology in Latin America, China, India and Africa has been reviewed by Leistner (1999b).

Principle:

When cells are exposed to multiple hurdles, the intensity of damage may be higher since some of the hurdles may act on the same cellular target. The repair of multiple damages may also require much higher energy costs, leading to energy exhaustion and cell death. Therefore, the probabilities for survival and proliferation for cells confronted with multiple hurdles are very low. In addition, the synergy between different multiple hurdles may allow the use of lower doses compared to their individual application. An important phenomenon that is crucial with regard to hurdle technology is the so-called homeostasis of microorganisms. Homeostasis is the constant tendency of microorganisms to maintain the stability and balance (uniformity) of their internal environment. Although the pH values in different foods may be quite variable, the microorganisms living in them expend considerable effort keeping their internal pH values within very narrow limits i.e. they will actively expel proton against the pressure of a passive proton influx. Another important homeostatic mechanism regulates the internal osmotic pressure (osmohomeostasis). The osmotic strength (which is inversely related to the water activity) of a food is a crucial physical property, which has a great effect on the ability of organisms to proliferate. Cells have to maintain a positive turgor (pressure) by keeping the osmolarity of the cytoplasm higher than that of the environment and they often achieve this using so-called osmo-protective compounds such as praline and betaine. Preservative factors functioning as hurdles can disturb one or more of the homeostasis mechanisms, thereby preventing microorganisms from multiplying and causing them to remain inactive or even die. Therefore, food preservation is achieved by disturbing the homeostasis of microorganisms. The best way to do this is to deliberately disturb several homeostasis mechanisms simultaneously. This means that any hurdles included in a food should affect the undesired. microorganisms in several different ways, for example by affecting the cell membrane, DNA, enzymes, pH, Eh and water activity homeostasis systems thus a combination of multiple hurdles (hurdle technology) could increase the effectiveness of food preservation.

The success of hurdle technology also depends on ensuring metabolic exhaustion. Metabolic exhaustion of microorganisms could cause 'autosterilization' of a food. Microorganisms in stable hurdle-technology foods use every possible repair mechanisms for their homeostasis to overcome the hostile environment, by doing this they completely use up their energy and die, if they become metabolically exhausted. This leads to an autosterilization of foods (Leistner, 1995b). For example, Unilever laboratories at Vlaardingen have confirmed metabolic exhaustion in water-in-oil emulsions (resembling margarine) inoculated with *Listeria innocua*. In these products *Listeria* vanished faster at ambient temperature (25°C) than under refrigeration (7°C), at pH 4.25, pH 4.3, pH 6.0, under anaerobic conditions more quickly than under aerobic conditions. From these experiment it has been concluded that metabolic exhaustion is accelerated if more hurdles are present and this might be caused by increasing energy demands to maintain internal homeostasis under stress conditions.

Most stress reactions of microorganisms are active processes and this often involves the expenditure of energy, e.g. to transport protons across the cell membrane, to maintain high cytoplasmic concentrations of 'osmoregulatory' or 'compatible' solutes. Restriction of the availability of energy is then a sensible target to pursue. This probably forms the basis of many the successful, empirically derived, mild combination preservation procedures exemplified by hurdle technology. As an example, if a food can be preserved by lowering the pH, then it is sensible also to include a weak acid preservative which will amplify the effect of the protons or to allow a milder, higher pH to be employed. It is sensible if proton export is made more difficult by the additional requirement that cells be forced to regulate osmotic strength. Then, if the food can be enclosed in oxygen-free vacuum or modified atmosphere packaging, facultative anaerobes will be further energy-restricted at a time when the various stress and homeostatic reactions are demanding more energy if growth is to proceed (Gould, 1995). However, environmental stresses can provide varying results because some bacteria may become more resistant or even more virulent under stresses through stress reactions such as synthesis of protective stress shock proteins. If there is activation of genes for the synthesis of stress shock proteins, which help organisms to cope with stress situations, should be made more difficult if different stresses are received at the same time. Simultaneous exposure to different stresses will require energy consuming synthesis of several or at least much more protective stress shock proteins, which in turn may cause the microorganisms to become metabolically exhausted. Therefore, multi-target preservation of foods could be the key to avoiding synthesis of stress shock proteins, which otherwise could jeopardize the microbial stability and safety of hurdle technology foods (Leistner, 1995b).

List of hurdles:

Physical hurdles: Aseptic packaging, high temperatures (blanching, pasteurization, sterilization, evaporation, extrusion, baking, frying), microfiltration, low temperature (chilling, freezing), packaging films (including active packaging, edible coatings).

Physico-chemical hurdles: carbon dioxide, ethanol, lactic acid, lactoferrin, lactoperoxidase, low pH, low redox potential, low water activity, lysozyme, maillard reaction products, organic acids, oxygen, ozone, phenols, phosphates, salt, smoking, sodium-nitrite/nitrate, sodium or potassium sulphite, natural additives (spices and herbs), surface treatment agents.

Microbially derived hurdles: antibiotics, bacteriocin (nisin, pediocin etc.), competitive flora, protective cultures.

New emerging hurdles: Bactofugation, electromagnetic energy (microwave, radio frequency, pulsed magnetic fields, high voltage electric fields), modified atmosphere packaging, photodynamic inactivation, ultra-high pressures, ultrasonication, ultraviolet radiation, Ionic radiation, thermization

Microbial Hurdle Which Reduces Microbiological Load:

Bactofugation: This process includes the removal of microbial cells of high density from raw milk using high centrifugal forces.
Competitive microflora: The reduction of the number of undesirable microorganisms by lowering the pH. Consumption of nutrients and production of bacterial antimicrobial substances (nisin, other bacteriocins and hydrogen peroxide). Usually, this Hurdle is applied by choice of starter culture.
Microfiltration: This includes the removal of microbial cells, clumps and somatic cells by recirculation over a microfilter (0.22-0.44micrometer).

Thermization: This process includes the application of a heat treatment to milk of a lower intensity than pasteurization in order to reduce the number of microorganism. Thermized milk is alkaline phosphatase positive.

Ultrasonication: The application of high intensity ultrasound (18-500MHz) that cause cycles of compression and expansion as well as cavitation in microbial cells. Implosion of microscopic bubbles generates spots with very high pressures and temperatures able to destroy cells. 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 foods.

Elecromagnetic energy treatment: Electromagnetic energy results from high voltage electrical fields, which alternate their frequency millions of times per second (<108MHz). Examples are microwave energy (thermal effect), radio-frequency energy (non-thermal effects). The treatment destroys cells by establishing pores in the cell walls due to the build-up of electrical charges at the cell membrane.

Low intensity irradiation: The submission of beams of photons/electrons to destroy viable microorganisms. 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 disinfection, to prevent sprouting of bulbs and tubers, and for antimicrobial pasteurization of foods in many 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 foods. 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.

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 to deliver 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 cells. 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 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 cells (Costa JL, Hoffman GA, 1987) to the inactivation of bacteria on packaging materials and in foods (Hoffman GA, 1985). Treatment times are very short, typically from 25 ms to a few milliseconds and field strengths are very high, typically from 2 Tessla to about 100 Tessla 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 reported (Hoffman GA, 1985), so the practical potential for the technique, as it has been developed so far, appears to be limited (Barbosa-Canovas GV, Weln-Chanes J., 1995).

Ultrahigh 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'. Vegetative forms of microorganisms are generally sensitive to pressures in the region of 400-600MPa (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'.

Microbiostatic Hurdles that limit and/or prevent growth by Biochemical means:

pH reduction: The creation of extra-cellular acid conditions that enables hydrogen ions to be imported into the cytoplasm of microorganisms, thus disturbing the homeostasis mechanism of the intracellular pH responsible for maintaining functionality of key cell components vital for continuing growth and viability. Low pH values are obtained by fermentation or addition of acids (inorganic or organic).

Carbon dioxide: The addition and/or formation of carbonic acid to obtain a multiple Hurdle effects, including the creation of anaerobic conditions by replacing oxygen, reducing pH, inhibiting certain intracellular enzymes (decarboxylation) and inhibiting the transport of water soluble nutrients across the membrane (by dehydrating the cellular membrane).

Use of Preservative: The addition of certain additives to enhance keeping quality and stability through direct or indirect antimicrobial and/or fungicidal activity. Most preservatives are rather specific and have effect only on certain microorganisms.

Modified Atmosphere: The establishing of gaseous environment (either low in oxygen and/or high in carbon dioxide or nitrogen) to limit growth of aerobic microorganisms by impairing biochemical pathways. Modified atmosphere packaging means that a modification of the gas atmosphere in the packaging is created.

Redox potential control: The redox potential (Eh) is a measure of the oxidizing or reducing potential of food systems that determines whether aerobic or anaerobic microorganisms are able to grow. Eh is influenced by removal of oxygen and/or addition of reducing substances (e.g. ascorbic acid, sucrose etc.).

Lactoferrins: The utilization of naturally present glycoprotein (highest concentration in colostrum) to prolong the lag phases of bacteria for 12-14 hours by binding iron in the presence of bicarbonates.

The lactoperoxidase system: The activation of lactoperoxidase/thiocynate/hydrogen peroxide system (indigenous system in milk) to inactivate several vital metabolic bacterial enzymes, consequently blocking their metabolism and ability to multiply.

Application in Food Industries:

Bacteriocins and heat treatments:

Bacteriocins can be used to reduce the intensity of heattreatments in foods without compromising microbial inactivation. Nisin and heat act synergistically against *L. plantarum*and *L. monocytogenes* (Mahadeo and Tatini, 1994; Ueckert *et al*., 1998), reducing the heat resistance of *L. monocytogenes* in milk (Maisnier-Patin *et al*., 1995). Nisin-resistant *L. monocytogenes* cells grown in the presence of nisin were more sensitive to heat at 55 °C than wild-type cells (Modi *et al*., 2000). The effectiveness of enterocin AS-48 was higher on *S.aureus* cells sub-lethally injured by heat due to the lower concentration of remaining viable cells and to the cell damage induced by the heat treatment (Ananou *et al*., 2004). Bacteriocins can also provide an additional protection during food storage against proliferation of endospores surviving heat treatments. Moreover, it has been demonstrated that the intensity of heat treatments against bacterial endospores can be lowered in combination with nisin as well as with enterocin AS-48 (Beard *et al*., 1999;Wandling *et al*., 1999; Grande *et al*., 2006), saving costs in the heat treatment and decreasing the impact of heat on the food. Sub-lethal heat has been shown to sensitize Gram-negative bacteria to several bacteriocins such as nisin or pediocin AcH (Kalchayanand *et al*., 1992; Boziaris *et al*., 1998), enterocin AS-48 (Abriouel *et al*., 1998; Ananou *et al*., 2005), or jenseniin G (Bakes *et al*., 2004), extending their spectrum of action. Highest sensitization was reported for combined treatments of bacteriocins, heat and a chelating agent (Abriouel *et al*., 1998; Ananou *et al*., 2005).

Bacteriocins and modified atmosphere packaging:

Modified atmosphere packaging (MAP) is frequently used in the food industry to prolong the shelf life of perishable food products. MAP may be defined as "the enclosure of food products in gas-barrier materials, in which the gaseous environment has been changed" (Young *et al*., 1988). Prolongation of the shelf-life of food by MAP is based on retardation of intrinsic food changes and inhibition of spoilage microbiota. In a modified atmosphere, the dissolved CO_2 will determine growth inhibition of microorganisms (Devlieghere *et al*., 1998). Gram-negative bacteria are generally more sensitive to CO_2, while lactic acid bacteria are much more resistant (Farber, 1991; Church, 1994). Since Gram-negative bacteria are usually not sensitive to bacteriocins, MAP and bacteriocins are therefore two complementary hurdles of advantage to food spoilage. Fang and Lin (1994a,b) found that growth of *L. monocytogenes* was completely inhibited on pork immersed in 10 IU/ml nisin and packed in 80% CO_2/20% air during 30 days of storageat 4°C. It has been reported that nisin and CO_2 atmosphere acted synergistically on the cytoplasmic membrane of *L. monocytogenes* by enhancing membrane permeabilization (Nilsson *et al*., 2000).

Bacteriocins and pulsed electric fields:

Pulsed electric field (PEF) technology is a non-thermal process where microbial inactivation is achieved by application of high-voltage pulses between a set of electrodes (Vega-Mercado *et al*., 1997). The effects of PEF resemble bacterial electroporation, but the higher intensity of this treatment causes severe damage to the bacterial cell membrane. Although this technology can only be applied to pumpable food products, it has gained attraction in recent years as an individual treatment or in combination with other hurdles such as bacteriocins. Since most bacteriocins act on the bacterial cytoplasmic membrane, the combined application of bacteriocins and PEF is expected to elicit increased bactericidal effects. Moreover, bacteriocins could also provide an additional hurdle against survivors from PEF treatments, such as sub-lethally injured cells or bacterial endospores. PEF could also be applied to extend the antimicrobial spectrum of bacteriocins, since PEF disrupts the bacterial outer membrane allowing bacteriocin molecules to reach the bacterial cytoplasmic membrane target. The efficacy of the combined treatments of PEF and bactericiocins in food preservation depends on several factors related to the PEF treatment (such as field strength, number of pulses, waveform or pulse duration), the food microbial load, composition and physiological stage, the added bacteriocin and other environmental factors (Wouters *et al*., 2001; Bendicho *et al*., 2002; Heinz *et al*., 2002). All of these may have an influence on the numbers and types of bacteria surviving the combined treatment and most important, their proliferation during the shelf life period of the processed food. For this reason, particular applications of bacteriocins and PEF must be studied in detail for each type of food and target bacteria.

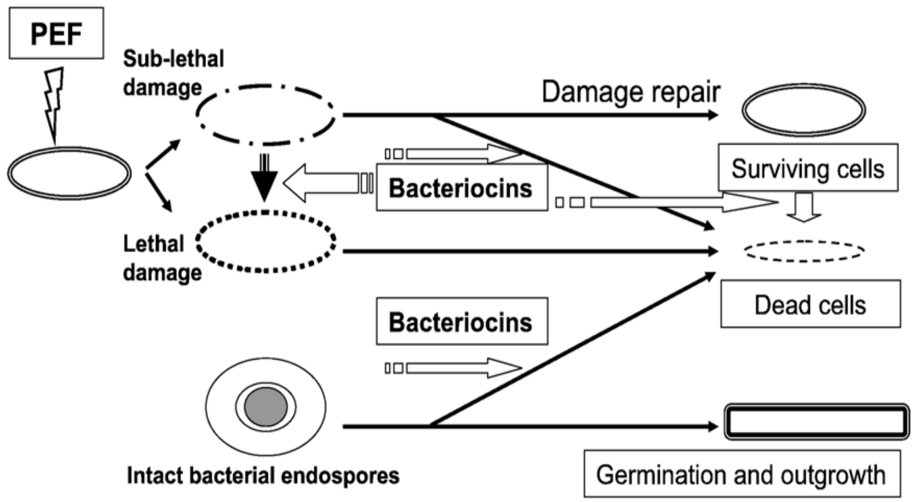

Figure: 2. Effects of Pulsed Electric Fields in Combination with Bacteriocins on Microbial Population

A synergistic effect was observed as the electric field intensity, number of pulses and nisin concentration increased both in liquid whole egg and in skim milk (Calderon-Miranda *et al*., 1999a, b). *L. innocua* treated by PEF-nisin in skimmed milk exhibited an increase in the cell wall roughness, cytoplasmic clumping, leakage of cellular material and rupture of the cell walls and cell membranes (Calderon-Miranda *et al*., 1999c). It has been reported recently that the efficiency of the combined treatment of nisin and PEF in liquid whey protein concentrate was strongly dependent on the sequence of application, since exposure to nisin after PEF produced a lower effect on *L. innocua* inactivation. This behaviour was mainly attributed to changes in the cell envelope and to modifications of the medium caused by PEF application (Gallo *et al*., 2007). The application of nisin clearly enhanced the lethal effect of PEF treatment on other Gram-positive bacteria such as *S.aureus* in skim milk (Sobrino-Lopez and Martin Belloso, 2006) and vegetative cells of *B. cereus* (Pol *et al*., 2000).

Several studies have shown that the efficacy of PEF against Gram-negative bacteria can be enhanced by nisin. Although nisin was totally inactivated by PEF treatment in simulated milk ultrafiltrate media, a 4-log cycle reduction of inoculated *E. coli* cells was accomplished with nisin (ca. 1,000IU/ml) and three pulses of 11.25 kV/cm or 500 IU/ml for five pulses of the same intensity (Terebiznik *et al*., 2000). Nisin-PEF inactivation of *E. coli* in simulated milk ultrafiltrate media was enhanced by water activity reduction. Decreasing water activity to 0.95 with NaCl and applying PEF at 5 kV/cm (a non-lethal intensity when no other hurdle is used) with the further addition of nisin (1200 IU/ml) resulted in a 5-log cycle reduction of the bacterial population (Terebiznik *et al.*, 2002). Nisin also increased PEF effectiveness against *Pseudomonas aeruginosa*. At high PEF intensities (i.e., 11 kV/cm), the inhibitory effect of nisin increased with the number of pulses applied (Santi et al.,2003). In conclusion, addition of nisin can improve the efficacy of PEF treatments against vegetative cells of foodborne pathogenic and spoilage Gram-positive and Gram-negative bacteria.

Bacteriocins and high hydrostatic pressure (HHP):

High hydrostatic pressure (HHP) is an innovative food processing and preservation method that causes injury and killing of microbial cells (Kalchayanand *et al.*, 1994; Farkas and Hoover, 2000; Patterson, 2000; Ray, 2002). During pressurization, the disruption of H-bonds, ionic bonds and hydrophobic interactions of the macromolecules adversely affects their structures and functions (Hoover, 1993). The sub-lethal damage is initiated by membrane phase transitions (Kato and Hayashi, 1999), affecting mainly ATP-generating and transport proteins.Cell death caused by HHP increases with pressure and so does the synergism with bacteriocins. Since most bacteriocins act on the bacterial cytoplasmic membrane it can be hypothesized that the observed synergy between bacteriocins and HHP results from cumulative damage to this structure. Several studies have described the combined effects of bacteriocins and HHP on bacteria. Nisin in combination with HHP showed strong synergistic effects against *L. plantarum*, *E. coli* and *L. monocytogenes* (ter Steeg *et al.*, 1999; Farkas *et al.*, 2003). Pediocin AcH also increased decimal reductions caused by HHP on food spoilage and pathogenic bacteria in peptone solution (Kalchayanand *et al.*, 1998b) and increased cell lysis in *L. mesenteroides* through cell wall degradation (Kalchayanand*et al.*, 2002). The synergistic activity of pediocin AcH in combination with nisin was greatly enhanced during pressurization (Kalchayanand *et al.*, 1998a,b, 2004a). A combination of nisin/pediocin AcH added to the plating medium was also effective on killing *Clostridium* spores induced to germinate by HHP treatment (Kalchayanand *et al.*, 2004b). Combining HHP and nisin (500 IU/ml) also increased inactivation of bacteria associated with milk (Black *et al.*, 2005). Similarly, the combination of lacticin 3147 and HHP increased the viability loss of *S. aureus* and *L. monocytogenes* in milk and in whey (Morgan *et al.*, 2000) and residual lacticin still showed inhibitory effect in the food, inactivating and preventing growth of sub-lethally injured cells. However HHP may have unwanted effects on milk components (Trujillo *et al.*, 2002), bacteriocin-HHP treatments could serve to decrease the intensity of HHP treatment without compromising microbial inactivation.

Microbial inactivation and Shelf Life comparison of 'Cold' Hurdle processing with pulsed electric fields and microfiltration and conventional thermal pasteurization in skim milk (Walkling-Ribeiro *et al.*, 2011)

The application of non-thermal processes pulsed electric fields (PEF) and cross-flow micro-filtration (CFMF) continuous to be studied with the purpose of controlling microorganisms in milk. Thermal pasteurization (TP) is the established food technology for commercial processing of milk. However, degradation of valuable nutrients in milk and its sensory characteristics occurs during TP due to substantial heat exposure. Pulsed electric fields (PEF) and microfiltration (MF) both represent emerging food processing technologies allowing gentle milk preservation at lower temperatures and shorter treatment times for similar or better, microbial inactivation and shelf stability when applied in a hurdle approach compared to TP. Incubated raw milk was used as an inoculums for the enrichment of skim milk with native microorganisms before PEF, MF, and TP processing. Inoculated milk was PEF-processed at electric field strengths between 16 and 42 kV/cm for treatment times from 612 to 2105 µs; accounting for energy densities between 407 and 815 kJ/L, while MF was applied with a transmembrane flux of 660 L/h m^2. Milk was TP-treated at 75 °C for 24s. Comparing PEF, MF and TP for the reduction of the native microbial load in milk led to a 4.6 log_{10}CFU/mL reduction in count for TP, which was similar to 3.7 log_{10}CFU/mL obtained by MF ($P \geq 0.05$), and more effective than the 2.5 log_{10}CFU/mL inactivation achieved by PEF inactivation (at 815 kJ/L ($P<0.05$)). Combined processing with MF followed by PEF (MF/PEF) produced a 4.1 (at 407 and 632 kJ/L), 4.4 (at 668 kJ/L) and 4.8 (at 815 kJ/L) log_{10}CFU/mL reduction in count of the milk microorganisms, which was comparable to that of TP($P \geq 0.05$). Reversed processing (PEF/MF) achieved comparable reductions of 4.9, 5.3 and 5.7 log_{10}CFU/mL (at 407, 632 and 668 kJ/L respectively ($P \geq 0.05$)) and a higher inactivation of 7.1 log_{10} (at 815 kJ/mL($P<0.05$)) in milk than for TP. Microbial shelf life of PEF/MF-treated (815 kJ/L) and TP-treated milk stored at 4°C was analyzed over 35 days for total aerobic; *Enterobacteria; Yeasts* and *Moulds*; *Lactobacilli*; psychrotroph; thermoduric psychrotroph, mesophilic and thermophilic and *Staphylococci* counts. For both PEF/MF and TP-treated milk an overall shelf stability of 7 days was observed based on total aerobic counts ($P \geq 0.05$). Milk hurdle processing with PEF/MF at its most effective treatment parameters produced greater microbial inactivation and overall similar shelf stability at lower processing temperatures compared to TP. With higher field strength, shorter treatment time, larger energy density and rising temperature the efficacy of PEF/MF increased contrary to MF/PEF. Thus, PEF/MF represents a potential alternative for 'cold'pasteurisation of milk with improved quality.

Effect of HHP on S*taphylococcus aureus* in milk (Tabla *et al.,* 2012)

The combined effect of bacteriophages and high hydrostatic pressure (HHP) on *Staphylococcus aureus* Sa9 was evaluated in pasteurized whole milk under a simulated cold chain break, which was simulated by incubation of milk at 25°C for 48h. Four-hundred MPa was found to be the most suitable pressure to be used in combination with these phages. Two different levels of *staphylococcal* initial contamination (1×10^4 and 1×10^6 CFU/mL) were tested. A synergistic effect between HHP and phages was observed in both cases. Compared to each single treatment, the combined treatment was able to reduce the initial *S. aureus* contamination below the detection limit (<10 CFU/mL). Bacteriophage performance in pressurized milk against *S. aureus* enabled milder hydrostatic pressure treatments, therefore phages can be regarded as a valuable hurdle on minimally processed food.

Cinnamon antimicrobial effect against *Salmonella typhimurium* cells treated by PEF in pasteurized skim milk beverage (Pina-Pérez *et al*., 2012)

This study shows a possible synergistic effect of hurdle technology using a combination of Pulsed Electric Fields technology and a natural ingredient, cinnamon, on inactivation of *Salmonella typhimurium* in skim milk (SM). Firstly, different cinnamon concentrations (1, 2.5 and 5% (w/v)) were added to inoculated SM (10^7CFU/mL) for quantitative study of the effect of the natural ingredient on microbial growth behaviour (bactericidal/bacteriostatic) at different temperatures (8, 25, 36°C). 5% (w/v) cinnamon supplementation of SM (SM-5%C) was bacteriostatic with regard to *S. typhimurium* growth according to μ_{max} [0.016 ((CFU/mL)/h) (8°C); 0.259 ((CFU/mL)/h) (25°C); 0.603 ((CFU/mL)/h) (37°C)] and λ [282.8 (h) (8°C); 15.38 (h) (25°C); 2.587 (h) (37°C)] values. Secondly, PEF treatments of 10, 20 and 30 kV/cm were applied, at treatment times ranging between 60 and 3000 μs. All cinnamon supplementation levels (1, 2.5 and 5% (w/v)) had a synergistic effect within [0.171–0.989] $\log_{10}$ cycles ($p<0.05$) due to non-thermal combination processing. The maximum synergistic effect was achieved by 10 kV/cm–3000 μs PEF treatment with 5% (w/v) cinnamon SM supplementation. The maximum inactivation level (1.97 $\log_{10}$ cycles) due to hurdle combination was achieved at 30 kV/cm–700 μs in SM-5% C.

Conclusion:

There is definitely a real need for improved food preservation techniques, to meet the nutritional quality and safe processing of foods. Some improvements are being derived from the use of established techniques in new combinations and other improvements are being derived essentially from the development of new preservation techniques in a combined manner. 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 and developments in this respect are also expected in the near future. A better understanding of the occurrence and interactions of different preservative factors (hurdles) in foods can form a powerful and logical basis for improvements in food preservation technologies. Although recently, there have been many studies that investigated the effect of combining hurdles in laboratory media and foods, the interactions of many hurdles are still not clear. This technology could be a better option for safe food processing along with nutritional qualities and further research is also needed to enhance its efficiency, safety or any changes in food products during its processing or addition of any substances to enhance the efficacy.

Hurdle Technology is a method of ensuring that pathogens in food products can be eliminated or controlled by combining more than one approach. These approaches can be thought of as "hurdles" the pathogen has to overcome if it is to remain active in the food. The right combination of hurdles can ensure all pathogens are eliminated or rendered harmless in the final product. This technology is an intelligent combination of hurdles which secures the microbial safety and stability as well as the organoleptic and nutritional quality and the economic viability of food products. The organoleptic quality of the food refers to their sensory property, which are its look, taste, smell and texture.

Examples of hurdles in a food system are high temperature during processing, low temperature during storage, increasing the acidity, lowering the water activity or redox potential, or the presence of bio-preservatives. According to the type of pathogens and how risky they are, the intensity of the hurdles this can be adjusted individually to meet consumer preferences in an economical way, without sacrificing the safety of the product.

References

Abriouel, H., Valdivia, E., Gálvez, A., Maqueda, M., 1998, Response of *Salmonella choleraesuis* LT2 spheroplasts and permeabilized cells to the bacteriocin AS-48, Applied and Environmental Microbiology 64, 4623–4626

Ananou, S., Gálvez, A., Martínez-Bueno, M., Maqueda, M., Valdivia, E., 2005, Synergistic effect of enterocin AS-48 in combination with outer membrane permeabilizing treatments against *Escherichia coli* O157:H7, Journal of Applied Microbiology 99, 1364–1372

Ananou, S., Valdivia, E., Martínez Bueno, M., Gálvez, A., Maqueda, M., 2004, Effect of combined physico-chemical preservatives on enterocin AS-48 activity against the enterotoxigenic *Staphylococcus aureus* CECT 976 strain, Journal of Applied Microbiology 97, 48–56

Antonio Galvez, Hikmate Abriouel, Rosario Lucas Lopez, Nabil Ben Omar, 2007, Bacteriocin-based strategies for food biopreservation, International Journal of Food Microbiology, Vol. 120, 51–70

Bakes, S.H., Kitis, F.Y.E., Quattlebaum, R.G., Barefoot, S.F., 2004, Sensitization of Gram-negative and Gram-positive bacteria to jenseniin G by sublethal injury, Journal of Food Protection 67, 1009–1013

Barbosa-Canovas GV, Pothakamury UR, Swanson BG, State of the art technologies for the sterilization of foods by non-thermal processes: physical methods, In: Barbosa-Canovas GV, Weln-Chanes J. (Eds) Food Preservation by Moisture Control: Fundamentals and Applications, Lancaster, PA Technomic, 1995: 493-532

Barbosa-Canovas GV, Pothakamury UR, Swanson BG. State of the art technologies for thesterilization of foods by non-thermal processes: physical methods. In: Barbosa-

Canovas GV,Weln-Chanes J. (Eds) Food Preservation by Moisture Control: Fundamentals and Applications.Lancaster, PA Technomic, 1995:493-532

Beard, B.M., Sheldon, B.W., Foegeding, P.M., 1999, Thermal resistance of bacterial spores in milk-based beverages supplemented with nisin, Journal of Food Protection 62, 484–491

Bendicho, S., Barbosa-Cánovas, G.V., Martín, O., 2002, Milk processing by high intensity pulsed electric fields, Trends in Food Science & Technology 13, 195–204

Black, E.P., Kelly, A.L., Fitzgerald, G.F., 2005, The combined effect of high pressure and nisin on inactivation of microorganisms in milk, Innovative Food Science and Emerging Technologies 6, 286–292

Boziaris, I.S., Humpheson, I., Adams, M.R., 1998, Effect of nisin on heat injury and inactivation of *Salmonella enteritidis* PT4, International Journal of Food Microbiology 43, 7–13

Calderón-Miranda, M.L., Barbosa-Canovas, G.V., Swanson, B.G., 1999a, Inactivation of *Listeria innocua* in liquid whole egg by pulsed electric fields and nisin, International Journal of Food Microbiology 51, 7–17

Calderón-Miranda, M.L., Barbosa-Canovas, G.V., Swanson, B.G., 1999b, Inactivation of *Listeria innocua* in skim milk by pulsed electric fields and nisin, International Journal of Food Microbiology 51, 19–30

Calderón-Miranda, M.L., Barbosa-Canovas, G.V., Swanson, B.G., 1999c, Transmission electron microscopy of *Listeria innocua* treated by pulsed electric fields and nisin in skimmed milk, International Journal of Food Microbiology 51, 31–38

Church, N., 1994, Developments in modified-atmosphere packaging and related technologies, Trends in Food Science & Technology 5, 345–352

Costa JL, Hoffman GA Malignancy treatment, US Patent 1987, 4,665,898

Devlieghere, F., Debevere, J., van Impe, J., 1998, Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms, International Journal of Food Microbiology 43, 105–113

Fang, T.J., Lin, L.-W., 1994a, Growth of *Listeria monocytogenes* and *Pseudomonas fragi* on cooked pork in a modified atmosphere packaging/nisin combination system, Journal of Food Protection 57, 479–485

Fang, T.J., Lin, L.-W., 1994b, Inactivation of *Listeria monocytogenes* on raw pork treated with modified atmosphere packaging and nisin, Journal of Food and Drug Analysis 2, 189–200

Farber, J.M., 1991, Microbiological aspects of modified-atmosphere packaging technology—a review, Journal of Food Protection 54, 58–70

Farkas, D.F., Hoover, D.G., 2000, High pressure processing, Journal of Food Science 65, 47–64 (Supplement)

Farkas, J., Andrassy, E., Meszaros, L., Simon, A., 2003, Increased salt- and nisin sensitivity of pressure injured bioluminescent *Listeria monocytogenes*, Acta Microbiologica et Immunologica Hungarica 50, 331–337

Gallo, L.I., Pilosof, A.M.R., Jagus, R.J., 2007, Effect of the sequence of nisin and pulsed electric fields treatments and mechanisms involved in the inactivation of *Listeria innocua* in whey, Journal of Food Engineering 79, 188–193

Gould, G. W. 1995, Homeostatic mechanisms during food preservation by combined methods, In Barbosa-Cánovas, G. W. and J. Welti-Chanes (Eds.). Food Preservation by Moisture Control-Fundamentals and Application.Technomic, Lancaster, PA, 397-410

Grahame W Gould, 2000, Preservation: Past, Present and Future, British Medical Bulletin,56 (No 1), 84-96

Grande, Ma.J., Lucas, R., Abriouel, H., Valdivia, E., Ben Omar, N., Maqueda, M., Martínez-Bueno, M., Martínez-Cañamero, M., Gálvez, A., 2006, Inhibition of toxicogenic *Bacillus cereus* in rice-based foods by enterocin AS-48, International Journal of Food Microbiology 106, 185–194

Guidelines for the application and management of Hurdle Technology, Draft Of Annex Ii, Part A, To the draft code of hygienic practice for milk and milk products

Heinz, V., Alvarez, I., Angersbach, A., Knorr, D., 2002, Preservation of liquid foods by high intensity pulsed electric fields-basic concepts for process design, Trends in Food Science & Technology 12, 103–111

Hoffman GA, Inacavation of microoganisms by an oscillating magnetic field, US Patent 1985, 4,524,079 and Int Patent 1985 WO85/02094

Hoffman GA. Inacavation of microorganisms by an oscillating magnetic field, US Patent 1985, 4,524,079 and Int Patent 1985 WO85/02094

Hoover, D.G., 1993, Pressure effects on biological systems, Food Technology 47, 150–155

Kalchayanand, N., Dunne, C.P., Sikes, A., Ray, B., 2004a, Viability loss and morphology change of foodborne pathogens following exposure to hydrostatic pressures in the presence and absence of bacteriocins, International Journal of Food Microbiology 91, 91–98

Kalchayanand, N., Dunne, C.P., Sikes, A., Ray, B., 2004b, Germination induction and inactivation of *Clostridium* spores at medium-range hydrostatic pressure treatment, Innovative Food Science and Emerging Technologies 5, 277–283

Kalchayanand, N., Frethem, C., Dunne, P., Sikes, A., Ray, B., 2002, Hydrostatic pressure and bacteriocin-triggered cell wall lysis of *Leuconostoc mesenteroides*, Innovative Food Science and Emerging Technologies 3, 33–40

Kalchayanand, N., Hanlin, M.B., Ray, B., 1992, Sublethal injury makes Gram negative and resistant Gram-positive bacteria sensitive to the bacteriocins, pediocin AcH and nisin, Letters in Applied Microbiology 16, 239–243

Kalchayanand, N., Sikes, A., Dunne, C.P., Ray, B., 1994, Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins, Applied and Environmental Microbiology 60, 4174–4177

Kalchayanand, N., Sikes, A., Dunne, C.P., Ray, B., 1998b, Interaction of hydrostatic pressure, time and temperature of pressurization and pediocin AcH on inactivation of foodborne bacteria, Journal of Food Protection 61, 425–431

Kato, M., Hayashi, R., 1999, Effects of high pressure on lipids of biomembranes for understanding high-pressure-induced biological phenomena, Bioscience, Biotechnology and Biochemistry 68, 1321–1328

L. Leistner, 2000, Basic aspects of food preservation by Hurdle Technology, International Journal Of Food Microbiology 55, 181 -186

Leistner L, 1995, "Principles and Applications of Hurdle Technology" In Gould Gw (Ed.),New Methods Of Food Preservation, Springer, 1-21

Leistner L. (1992), Food preservation by combined methods, Food Research International 25, 151-158

Leistner, L., 1994a, Further developments in the utilization of hurdle technology for food preservation, J. Food Engineering 22, 421–432

Leistner, L., 1995b, Emerging concepts for food safety, In: 41st International Congress of Meat Science and Technology, 20– 25 August 1995, San Antonio, Texas, 321–322

Leistner, L., 1999b. Use of combined preservative factors in foods, of developing countries. In: Lund, B.M., Baird-Parker, A.C., Gould, G.W. (Eds.), The Microbiological Safety of Foods, Aspen Publishers, Gaithersburg, Maryland, in press

Lothar Leistner and Leon G.M. Gorris, 1995, Food Preservation by Hurdle Technology,Trends In Food Science &Technology February, 6

M. Walkling-Ribeiro, O. Rodríguez-González, S. Jayaram, M.W. Griffiths,2011, Microbial inactivation and shelf life comparison of 'cold' hurdle processing with pulsed electric fields and microfiltration, and conventional thermal pasteurisation in skim milk, International Journal Of Food Microbiology, 144, 379-386

M.C. Pina-Pérez, A. Martínez-López, D. Rodrigo, 2012, Cinnamon antimicrobial effect against Salmonella typhimurium cells treated by pulsed electric fields (PEF) in pasteurized skim milk beverage, Food Research International 48, 777-783

Mahadeo, M., Tatini, S.R., 1994, The potential of nisin to control *Listeria monocytogenes* in poultry, Letters in Applied Microbiology 18, 323–326

Maisnier-Patin, S., Tatini, S.R., Richard, J., 1995, Combined effect of nisin and moderate heat on destruction of *Listeria monocytogenes* in milk, Lait 75, 81–91

Modi, K.D., Chikindas, M.L., Montville, T.J., 2000, Sensitivity of nisin-resistant *Listeria monocytogenes* to heat and the synergistic action of heat and nisin, Letters in Applied Microbiology 30, 249–253

Morgan, S.M., Ross, R.P., Beresford, T., Hill, C., 2000, Combination of hydrostatic pressure and lacticin 3147 causes increased killing of *Staphylococcus* and *Listeria*, Journal of Applied Microbiology 88, 414–420

Nilsson, L., Chen, Y., Chikindas, M.L., Huss, H.H., Gram, L., Montville, T.J., 2000, Carbon dioxide and nisin act synergistically on *Listeria monocytogenes*, Applied and Environmental Microbiology 66, 769–774

Ohlsson T and Bengtsson N, 2002, "The Hurdle Concept", Minimal Processing Technologies In The Food Industry, Woodhead Publishing, 175-195

Oscar Rodriguez Gonzalez, 2011, Hurdle Technologies: Microbial inactivation by pulsed electric fields during milk processing, Proquest Dissertations And Thesis

Patterson, M., 2000, High pressure treatments of foods. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Encyclopedia of food microbiology, Academic Press, San Diego, 1059–1065

Pol, I.E., Mastwijk, H.C., Bartels, P.V., Smid, E.J., 2000, Pulsed-electric field treatment enhances the bactericidal action of nisin against *Bacillus cereus*, Applied and Environmental Microbiology 66, 428–430

R. Tabla ,B. Martínez, J.E. Rebollo, J. González, M.R. Ramirez, I. Roa, A. Rodríguez, P. Garcia,2012, Bacteriophage performance against *Staphylococcus aureus* in Milk is improved by High Hydrostatic Pressure treatments, International JournalOf Food Microbiology, 156, 209–213

Ray, B., 2002, High hydrostatic pressure: microbial inactivation and food preservation, In: Britton, G. (Ed.), The Encyclopedia of Environmental Microbiology, Wiley, New York, 1552–1563

Santi, L., Cerrutti, P., Pilosof, A.M., de Huergo, M.S., 2003, Optimization of the conditions for electroporation and the addition nisin for *Pseudomonas aeruginosa* inhibition, Revista Argentina de Microbiología 35, 198–204

Sobrino-Lopez, A., Martin Belloso, O., 2006, Enhancing inactivation of *Staphylococcus aureus* in skim milk by combining high-intensity pulsed electric fields and nisin, Journal of Food Protection 69, 345–353

Sun-Young Lee, 2004, Microbial safety of pickled Fruits and Vegetables and Hurdle Technology, Internet Journal Of Food Safety, 4, 21-32

ter Steeg, P.F., Hellemons, J.C., Kok, A.E., 1999, Synergistic actions of nisin, sublethal injury pressure, and reduced temperature on bacteria and yeast, Applied and Environmental Microbiology 65, 4148–4154

Terebiznik, M.R., Jagus, R.J., Cerrutti, P., De Huergo, M., Pilosof, A.M., 2000, Combined effect of nisin and pulsed electric fields on the inactivation of *Escherichia coli*, Journal of Food Protection 63, 741–746

Terebiznik, M.R., Jagus, R.J., Cerrutti, P., De Huergo, M., Pilosof, A.M., 2002, Inactivation of *Escherichia coli* by a combination of nisin, pulsed electric fields, and water activity reduction by sodium chloride, Journal of Food Protection 65, 1253–1258

Trujillo, A.J., Capella, M., Saldo, J., Gervilla, R., Guamis, B., 2002, Applications of high-hydrostatic pressure on milk and dairy products: a review, Innovative Food Science and Emerging Technologies 3, 295–307

Ueckert, J.E., ter Steeg, P.F., Coote, P.J., 1998, Synergistic antibacterial action of heat in combination with nisin and magainin II amide, Journal of Applied Microbiology 85, 487–494

Vega-Mercado, H., Martín-Belloso, O., Qin, B.-L., Chang, F.J., Góngora-Nieto, M.M., Barbosa-Cánovas, G.V., Swanson, B.G., 1997, Non-thermal food preservation: pulsed electric fields, Trends in Food Science & Technology 8, 151–157

Vogel, R. F., W. P. Hammes, M. Habermeyer, K. H. Engel, D. Knorr, and G. Eisenbrand, 2011, Microbial food cultures - opinion of the Senate Commission on Food Safety (SKLM) of the German Research Foundation (DFG), Mol. Nutr. Food Res., 55, 654-662

Wandling, L.R., Sheldon, B.W., Foegeding, P.M., 1999, Nisin in milk sensitizes *Bacillus* spores to heat and prevents recovery of survivors, Journal of Food Protection 62, 492–498

Wouters, P., Álvarez, I., Raso, J., 2001, Critical factors determining inactivation kinetics by pulsed electric field food processing, Trends in Food Science & Technology 12, 112–121

Young, L.L., Reverie, R.D., Cole, A.B., 1988, Fresh red meats: A place to apply modified atmospheres, Food Technology 42, 64–66, 68–69