Something old, something new:

Hurdle technology - a marriage of preservation techniques

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In 2007, we published a review in Food New Zealand on existing and novel strategies for the biocontrol of foodborne bacteria [22]. In that article we discussed the utility of various biological approaches such as bacteriophages and protective cultures to increase the safety and extend the shelf life of foods. In most cases, these do not in themselves offer a complete food safety solution; hence their application in combination with other preservation options (the hurdle technology concept) holds greater potential. What follows here is a review of this food preservation area and an update on some of the more recent findings published in the literature.

Introduction

The term hurdle technology was first coined by Leistner in 1978 to describe "the deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors (hurdles) that any microorganism present should not be able to overcome" [19]. Originally depicted as a hurdles race where microbes leapt energetically (or otherwise) over variously sized individual hurdles representing each factor, the concept is now considered more accurately as a wall composed of 'hurdle bricks' (Figure 1).

The height of the wall (i.e. the food's ability to inhibit microbial growth) is related to both the number of combined hurdles and

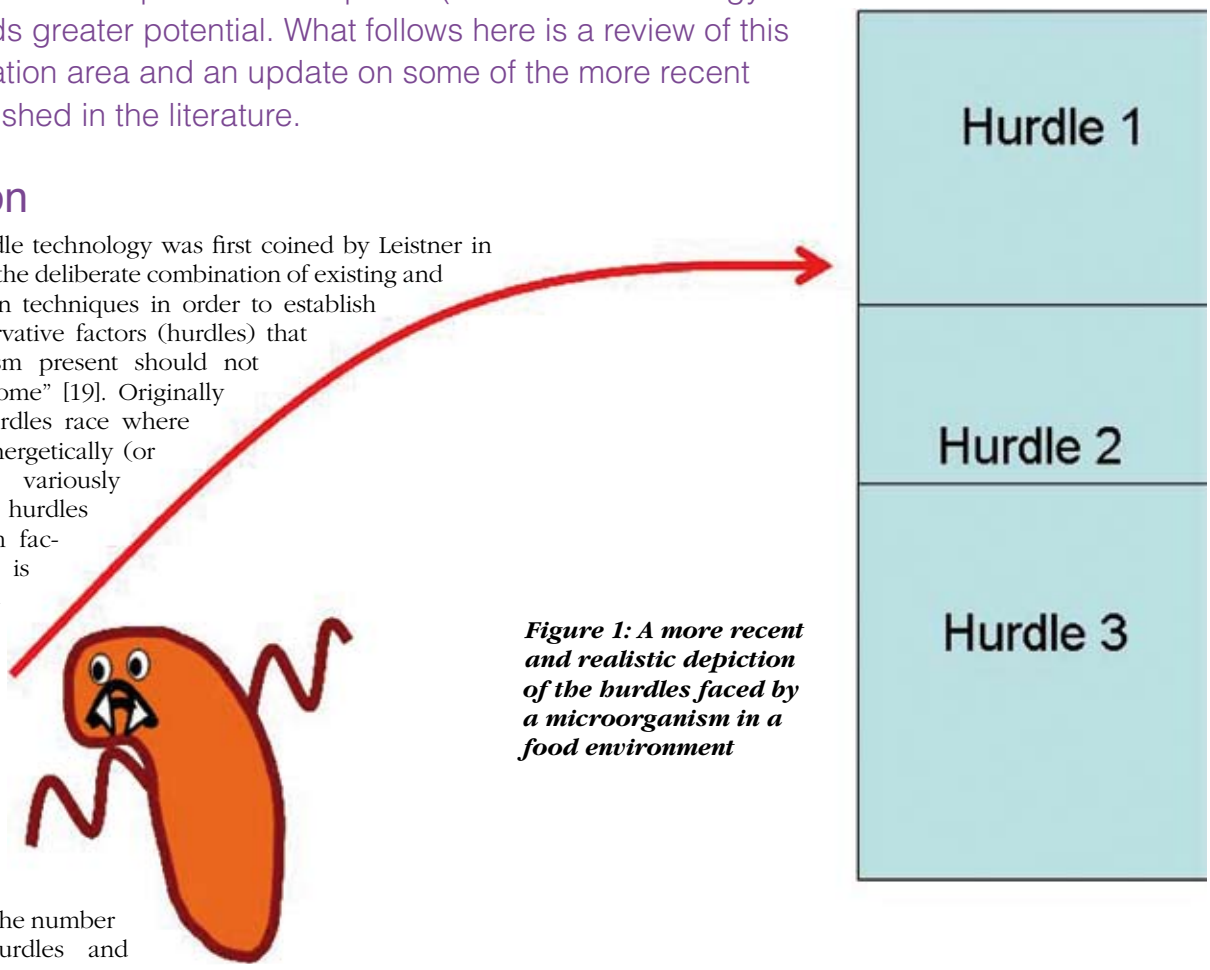

Figure 1: A more recent and realistic depiction of the hurdles faced by a microorganism in a food environment

Table 1: Examples of food-related hurdles (adapted from [19])

Hurdles	Examples
Physical	Thermal (high, low temperature) Non-thermal (e.g. irradiation, ultrahigh pressure, pulsed electric and magnetic fields, mano-thermo- sonication, etc.) Packaging (films, edible coatings, modified atmospheres, aseptic packaging)
Physicochemical	Addition/alteration of: water activity (aw), pH, redox potential (Eh), salt, nitrites, nitrates, oxygen, carbon dioxide, ozone, smoke, ethanol, acids, spices, herbs, essential oils, lysozyme, Maillard browning compounds, etc.
Microbial	Background microflora, protective cultures, bacteriophages, bacteriocins

their applied magnitude. Theoretically, applying more hurdles will produce a greater additive effect, but only if these hurdles have different modes of action capable of affecting, for example, microbial DNA, cell membranes and their ability to respond and adapt to environmental changes (termed homeostasis) [18]. Taking aim at multiple targets also improves the food's capacity to control a more diverse range of different microbes.

For a microbe to scale such a wall requires various physiological responses and modifications to occur which expend cellular energy [16]. Ideally therefore, a multiple-hurdle-preserved food will deplete this energy to cause 'metabolic exhaustion'. Even under conditions where no growth can occur, the greater the energy needed for the cell to survive, the faster the rate of death among the pathogen population. If initial microbial numbers are low, or sub-lethal injury has occurred as a result of a previous exposure or treatment, scaling this wall will be particularly difficult. If, on the other hand, high contamination levels are present, getting over the wall may be a slightly easier proposition. Whether genuinely synergistic relationships occur (as suggested by Leistner) to boost the additive effects of each hurdle is debatable.

The growing list of potential hurdles that can be employed to preserve foods is summarised in Table 1. These are generally considered in three main groups - physical, physicochemical and microbial. Many, including heating, drying, salt and sugar addition, acidification, smoking, etc. have been used in food preservation for centuries but have typically been the major hurdle.

It should of course be pointed out that producing a microbiologically stable food (i.e. where microbes are unable to proliferate) does not necessarily equate to a safe food. This will only occur when high quality (ideally pathogen-free) raw materials are used, or alternatively when any contamination present is below the threshold for disease to occur which in practice may be difficult to reliably achieve. Hurdles such as thermal and non-thermal processes capable of inactivating pathogens therefore play an important role in maintaining the safety of foods, particularly those of animal origin, and extending their shelf life.

When applied in combination with other factors, often only sub-optimal levels of a hurdle may be necessary to create the desired effect. This offers a number of advantages, particularly in terms of the development of 'consumer-friendly' foods low in, for example, chemical preservatives, without radical changes in sensory characteristics. Additionally, eliminating the need for refrigeration or frozen storage and reducing the severity of thermal processes, potentially offer the additional bonus of cost savings as a result of lower energy requirements [18]. This has been exploited in particular by developing countries in Asia to manufacture shelf-stable yet safe traditional meat products [3, 13].

Table 2: Examples of the diversity of foods preserved by hurdle technology

Food	Hurdles employed
Grated fresh coconut	Refrigeration, sodium chloride, citric acid, sodium citrate, butylated hydroxyanisole
Shelf-stable seaweed	Pasteurisation, water activity, pH, potassium sorbate
Steamed bread (dumpling-like)	Water activity (glycerol), mild heat treatment, packaging
Tortellini	Water activity, mild heating, modified atmosphere packaging
Fruit	Mild heat treatment, pH, water activity, sorbate, sulphite
Shelf-stable guacamole	Acids, antimicrobials, antibrowning agents, vacuum packaging, high pressure, mild heat processing
Gnocchi	Sorbate, lactic/citric acids, temperature
Shelf-stable military bread	Water activity and pH
Canned peas	Heat treatment, pH and nisin
Fish	Superchilling (core -1.5°C), natural additives, modified atmosphere packaging, refrigeration
Pickled vegetables	Acetic acid, salt, heat treatment

While foods of animal origin have been the main focus of hurdle technology (and will likewise be in this review), a large variety of different food products has been developed based on this multifactorial preservation approach (Table 2).

A couple of recent publications have made significant contributions to advancing the understanding of the mathematics behind hurdle technology. The gamma hypothesis suggests that the effects of different antimicrobial factors are independent and do not act in a synergistic fashion, and the authors analysis of the data tends to support this [1]. While some publications report significant statistical interactions, this paper cautions that statistical interactions may not be representative of biological interactions. Further experimental work is required to investigate this further. In the second paper evidence is presented to support the assertion that most of the inactivation occurring under growth-preventing conditions is determined by the temperature of incubation, as opposed to other factors such as pH and salt concentration [27]. These considerations are of importance to food safety as they indicate that the primary drivers behind pathogen reductions in foods that rely on hurdles for their safety are time and temperature. It is suggested that other factors, such as pH, may be manipulated to prevent growth but they have little effect on the rate of inactivation.

Meat Products

Shelf-stable salami-style sausages are probably the best recognised example of hurdle technology applied to the preservation of meats. Multiple hurdles are created during processing and ripening including the use of preservatives (salt, nitrites and nitrates), the addition of a competitive microflora (in the form of lactic acid bacteria cultures) which also lowers the pH (through the production of lactic acid) and the redox potential, and reduced water activity due to drying. Smoking may also be used in semi-dry sausage production, adding another hurdle. A fermented meat product manufactured in this way will be microbiologically stable when it has achieved both a pH value in the range of 4.6 - 5.3 and a water activity of <0.95 [10]. Alternatively, either reducing the pH to <4.5 or reducing a_w to <0.91 can achieve a similar effect [26].

The use of organic acids such as lactic acid is common in hurdle technology. The acids serve to lower the pH, but they exert an additional effect. In low pH conditions, undissociated molecules form which can diffuse into the microbial cell. Once inside, the molecule dissociates in the neutral cytoplasm to form an acid which the cell must then deal with to prevent acidification. The cell thus has to expend energy pumping the acid out

in a futile attempt to maintain its pH.

Leistner's research group was influential in the development of a range of German sausages termed "shelf stable products (SSP) storable without refrigeration" [17]. Four different approaches were developed, all of which focused on differing combinations of F (heat), a_w, pH, E_h and packaging (Table 3).

Since then, developments in preservation technologies such as high hydrostatic pressure (HHP) and the increased availability of bacteriocins and other novel antimicrobials have resulted in additional approaches to improving the safety of meat products. Researchers at IRTA in Spain have been particularly active in investigating the application of microbially-derived hurdles in combination with HHP to improve the safety and quality of low acid fermented sausage which lacks the pH hurdle.

Table 3: Combinations of hurdles used to produce shelf-stable sausage products

Product	Hurdles employed	Shelf life
F-SSP e.g. liver, blood, Bologna-type sausages	Heat treatment (F value >0.4) a^w<0.96-0.97 pH <6.5 E_h low (air-tight casings)	At least 6 weeks
a^w-SSP e.g. Italian Mortadella, German Brühdauerwurst	a^w≤0.95 E_h low Heated to 72°C internal temperature May be vacuum packed and repasteurised (45 min at 85°C)	15 - 18 months
pH-SSP e.g. brawns (jelly sausages)	pH<5.2 (acetic acid) Heated to 72°C -<80°C internal temperature	6 days at 30°C
Combi-SSP e.g. Brühwurst	a^w≤0.965 pH <5.8 Heated to >72°C internal temperature Vacuum packed and repasteurised (45-60 min at 82-85°C)	6 days at 30°C

In experiments to determine the effectiveness of enterocins A and B in combination with pressure processing, HHP and ripening (a_w<0.94) was sufficient to reduce *Salmonella* levels to <1 $\log_{10}$ cfu g^{-1} in low acid sausage after 30 days of storage [11]. *Listeria monocytogenes* was controlled only by the presence of both hurdles, where populations were maintained below 1 $\log_{10}$ cfu g^{-1} under room temperature storage conditions, and progressively declined to similarly low levels at 7°C. However, due primarily to its greater resistance to HHP, *Staphylococcus aureus* maintained post-ripening levels of ~6 $\log_{10}$ cfu g^{-1} throughout storage, numbers sufficient to be a food safety concern. Employing a starter culture in conjunction with HHP was shown to reduce *Enterobacteriaceae* and enterococci significantly in low acid fermented sausage without impacting on other quality attributes [20]. HHP in combination with enterocins or lactate-diacetate has also been demonstrated to control *L. monocytogenes* on cooked ham [21]. The most effective set of hurdles employed (HHP, enterocins and storage at 1°C) reduced populations to 4 MPN g^{-1} after three months, despite a simulated cold chain break at day 60 which exposed samples to room temperature abuse for 24 h.

Other successes of hurdle technology have included increasing the acceptable shelf life of *keema*, an Indian ground meat and spices product, at ambient storage temperature by up to four additional days [13]. Reduced water activity and pH adjustments, in combination with vacuum packaging, preservatives (ascorbic acid, sorbic acid and sodium nitrite) and partial frying were employed. Similarly at ambient storage temperature, shelf-stable intermediate moisture mutton kebabs have been developed using reduced water activity and vacuum packaging [12] which do not support the growth of *S. aureus, Clostridium sporogenes* and *Bacillus cereus* for up to three months [3]. Applying irradiation at a level of 2.5 kGy successfully reduced both *S. aureus* and *B. cereus* to undetectable levels within one month of storage, but even after a 10 kGy treatment, viable *C. sporogenes* were detectable at a level of 1.84 $\log_{10}$ cfu g^{-1} after three months.

Cheese

Cheese is another fermented food that uses hurdle technology to prevent the growth of pathogens but, like salami, the use of hurdles was not applied by design but occurred by good fortune. Similarly to salami production, the key starter culture organisms concerned are the lactic acid bacteria which ferment sugar (lactose in the milk) to produce lactic acid, so lowering the pH (achieving pH 5.5-5.2 within 24 hours is desirable) and producing the bactericidal effects of undissociated fatty acids described above. Salt may be added at levels high enough to cause an inhibitory effect (for example 11.5% in the water phase for blue cheese [24]), and cheeses may be matured for long periods which, in some cases, results in an increase in the salt concentration through water loss by evaporation. The curd cooking temperature may also be high enough to bring about a thermal kill of pathogens (e.g. in cottage cheese [29]) and in some varieties, for example Mozzarella [14], there is a thermal step that is a very effective control of pathogen numbers.

In most cheese, most of the time, pathogens present at the start of ripening either do not grow or gradually decrease in numbers during storage/ripening. Exceptions are those cheeses where there is a surface ripening, for example, Camembert. Here the fungus grows on the surface of the cheese and metabolises the lactic acid, so reducing its concentration and causing the pH to rise. For example, the pH of camembert may rise from 4.5 on the day of manufacture to 6.4 at day 50 [30], with the effect more pronounced at the surface compared to the core of the cheese [31]. These cheeses will allow the growth of pathogens such as *L. monocytogenes* because of this effect.

Some cheeses do not rely on a lactic acid fermentation, for example whey cheeses, and so do not benefit from its preservative effect. Given the general rule of thumb that if vegetative bacterial cells are not growing then they are dying, higher ripening temperatures can bring about faster rates of inactivation than those achieved at lower temperatures [15], although in practice this may not always occur [28]. This is reflected by US regulations concerning cheese made from thermised milk where a maturation period and *minimum* storage temperature are used [5].

If lactic acid bacteria which produce bacteriocins (e.g. pediocin and nisin) are used, the control of pathogens is increased when compared to the use of non-bacteriocin-producing strains [25], with between 1 (for *S. aureus*) and 3 (for *L. monocytogenes*) $\log_{10}$ differences being recorded. Other bacteria associated with cheese can also exert an inhibitory effect, for example the natu-

ral flora used to produce red smear cheese [6] where the activity may also be mediated, at least in part, by bacteriocin production. A novel addition to the hurdle controls available for cheese production is the use of bacteriophages [9, 22], and papers have now described the control of *Salmonella* [23], *Staphylococcus* [7] and *L. monocytogenes* [2].

A nice study of hurdle technology in cheese [32] looked at the manipulation of conditions needed to ensure that *C. botulinum* was unable to grow in cheese spread. They used data from 304 treatment combinations (lots) and varied the salt concentration, pH, a_w and disodium phosphate concentration. Storage of the cheese was at 30°C for 28 days. At water activity values <0.944 no toxin was produced, while when the water activity exceeded 0.957 all lots developed toxin. The authors were able to produce contour maps of moisture content plotted against the pH and total concentration of sodium phosphate and sodium chloride to show the boundary that delimited conditions where toxin was and was not produced. Thus, the model was predictive for conditions not included in the original study.

Despite the many hurdles present in cheese manufacture outbreaks of foodborne disease have been linked to its consumption. As a gross generalisation, pathogens are able to grow during cheese manufacture and may survive subsequent ripening and storage despite the fact that their numbers may decline under these conditions. Therefore the risks associated with cheese consumption depend on the concentration of the inoculum, the amount of growth that might occur during manufacture and the change in numbers during ripening and storage. Obviously, the higher the concentration of pathogens in the cheese milk the greater the risk of disease when the cheese is consumed. There is little doubt that cheese made with raw milk is "riskier" than the equivalent cheese made with pasteurised milk.

Maori foods

Many traditional foods (which would probably include cheese and salami) use hurdle technology in their preparation. Some traditional Maori foods are no different. Kanga Kopiro (fermented maize) is produced in a process whereby organic acids are elaborated [34]. More detailed studies have been made into the processes behind fermented Kina and Tiroi (preserved Puha) production [8]. The fermentation of Kina is unusual as the result is an increase in the pH of the food; we recorded increases in the pH in each of the preservation methods studied, although sometimes the increase was not large. This was accompanied by an increase in organic acid contents under some conditions. With Tiroi, a reduction of pH could be measured in food produced by one of the methods tested, along with an increase in the lactic acid concentration. This food, therefore, appears to be produced by a process similar to that of Sauerkraut and Kimchi.

Validation of Hurdle Technology-derived foods

The ideal means of validating the ability of a set of hurdles in a given food to control foodborne pathogens is to carry out challenge testing. However, this can be a problem as using a pathogen in a processing plant is not wise (!). It may be possible to use a surrogate organism, for example *L. innocua* for *L. monocytogenes*, but even here it will be difficult for food businesses to carry out the test satisfactorily, and the surrogate itself would need to be validated to ensure that it behaved in the same way as the pathogen of interest. There would be considerable expense involved and the results would only be good for the range covered; if you changed a parameter outside of the original study it would need to de done again.

Alternatively, it is possible that there is a paper in the literature that has examined exactly the same hurdles as those needing testing. One such paper, for example, examines the effects of water phase salt and phenolic compounds on the behaviour of *L. monocytogenes* in smoked salmon [4]. Another looks at the effects on *Salmonella* of oregano essential oil concentration, temperature and pH (adjusted with lemon juice) in Taramasalata [15]. Unfortunately it is unlikely that a study will be found that matches any particular set of hurdles, and most of these papers are focused on *L. monocytogenes*. Validation of a set of hurdles would, of course, need to consider all hazards relevant to the food in question.

A more convenient place to start would be the use of predictive models. Some of these are available for free, for example there is a CD produced by Meat and Livestock Australia (Level 1, 165 Walker Street, North Sydney, NSW 2060, Australia) which predicts the growth of *Escherichia coli* in fermented meats. The input required is a time/temperature history of product manufacture and ripening. Other models exist, but these have generally been produced using sterile bacteriological broths and so may or may not match well inactivation in "real life". The Pathogen Modeling Program (http://www.ars.usda.gov/Services/docs.htm?docid=6786) produced by the USDA contains many models to predict growth, but rather fewer that predict non-thermal inactivation. It does, however, contain a useful bibliography. ComBase (http://ifrsvwwwdev.ifrn.bbsrc.ac.uk/CombasePMP/GP/CBToolbox_About.aspx) has some growth models available, but few model inactivation.

For the more adventurous, there are many published models available in the scientific literature, and many of those have been produced by our colleagues across the Tasman in Hobart. As an example a model has been produced which predicts whether *L. monocytogenes* can grow or not given certain parameters [33]. Many models are, again, based on broth systems and it is undoubtedly preferable to use models produced in food as they should cover parameters not explicitly accounted for by broth-based models, such as the effect of the natural microflora.

While the use of models provides some useful guidance and should allow for hurdle parameters to be narrowed down in terms of controlling pathogens in new food formulations there is no substitute for challenge testing!

Some guidelines on validation have been proposed by the Codex Alimentarius Commission (www.codexalimentarius.net/download/standards/11022/cxg_069e.pdf). This document includes the provision of some definitions, tasks that need to be done prior to validation, and the validation process itself. It also provides some examples, one of which is the validation of a process to meet a performance objective for Shiga- toxigenic *E. coli* (like *E. coli* O157:H7) in hard raw milk cheese. It will be apparent from this example that this is quite an involved process.

Conclusions

Hurdle technology is a commonly used and widely proven technique which can offer a number of different solutions to the age old problems of food spoilage and safety. It is a particularly exciting area given recent advancements in non-thermal processing, packaging and the application of novel antimicrobials such as essential oils and bacteriophages which offer more 'natural' alternatives to existing traditional approaches. Developing new hurdle technology foods to cater for consumer

needs is, however, not without its challenges and care must be taken to ensure that the hurdles being applied are both appropriate and of sufficient vigour to ensure their safety, particularly for those stored at ambient temperatures. Validation of these hurdles is therefore a crucially important aspect of the product development process. Understanding the science behind the interaction of hurdles is gaining in momentum but more research is required to elucidate and quantify effects to further improve the usefulness of predictive models.

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