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Review

Quantifying the hurdle concept by modelling the bacterial growth/no growth interface

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Abstract

The hurdle concept described eloquently over many years by Professor Leistner and his colleagues draws attention to the interaction of factors that affect microbial behaviour in foods. Under some circumstances these effects are additive. Under others the implication is that synergistic interactions lead to a combined effect of greater magnitude than the sum of constraints applied individually. Predictive modelling studies on the combined effects of temperature and water activity and temperature and pH suggest that the effect of these combinations on growth rate is independent. Where the effect of the two factors is interactive rather than independent is at the point where growth ceases — the growth/no growth interface. An interesting and consistent observation is that a very sharp cut off occurs between conditions permitting growth and those preventing growth, allowing those combinations of factors to be defined precisely and modelled. Growth/no growth interface models quantify the effects of various hurdles on the probability of growth and define combinations at which the growth rate is zero or the lag time infinite. Increasing the stringency of one or more hurdles at the interface by only a small amount will significantly decrease the probability of an organism growing. Understanding physiological processes occurring near the growth/no growth interface and changes induced by moving from one side of the interface to the other may well provide insights that can be exploited in a new generation of food preservation techniques with minimal impact on product quality.

Keywords: Growth/no growth interface; Hurdle concept; Quantitative description; Microbial interactions, Additive and synergistic; Food preservation developments

1. Introduction

For many years Professor Leistner and his colleagues at the Federal Centre for Meat Research in Kulmbach, Germany, have advocated food preservation by combined methods (the Hurdle Concept). The essence of this approach is that foods can remain stable and safe even without refrigeration, and are acceptable organoleptically and nutritionally due to the mild process applied (Leistner, 1978).

The mode of action of combined hurdles may be additive or even synergistic with the latter deserving particular attention as a means to select constraints that best achieve microbial stability and safety

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(Leistner, 1992). That synergism is anticipated derives from the effect of hurdles on separate targets within the cell which disturb homeostasis by different mechanisms.

In this paper we address the question of additive or synergistic interaction of combined factors by examining the underlying physiological mechanisms by which temperature, water activity and acid pH exert their effects. We shall also describe the concept of growth/no growth (G/NG) interface modelling which demonstrates synergism between various factors by providing a quantitative description of the hurdle concept.

2. Interactions during growth of microbial populations

Kinetic models for the dependence of microbial growth rate on temperature have been proposed based on Arrhenius-type kinetics (Schoolfield et al., 1981) or on Belehrádek (square-root)-type kinetics (Ratkowsky et al., 1982, 1983). Extensions of the models describing temperature effects to include water activity were developed by McMeekin et al. (1987) and Davey (1989).

McMeekin et al. (1987) described the combined effects of temperature and water activity on the growth rate of a halotolerant organism, *Staphylococcus xylosus*, isolated from salted, dried, Indonesian fish. This strain grew well over the water activity range 0.848–0.996 with an optimum water activity (a_w) value of 0.976 with NaCl as the humectant. When examined across a temperature range from 3 to 28°C a square-root model provided a good description of experimental data at each water activity examined. Extrapolation of individual regression lines to zero rate to estimate the theoretical minimum temperature for growth (T_{min}) indicated that this was constant at 275.9 K (Fig. 1). The form of the equation was:

$$\sqrt{k} = C(T - T_{min})\sqrt{a_w - a_{w,min}}$$

The result was non-intuitive as the researchers had hypothesised that both the T_{min} value and the rate of growth would decrease with reduced water activity. However, as only the slope (b) of the square-root plot varied with water activity it was sufficient to substitute b by a term to model the effect of water activity in addition to temperature. For *S. xylosus* grown in NaCl or glycerol adjusted media a plot of

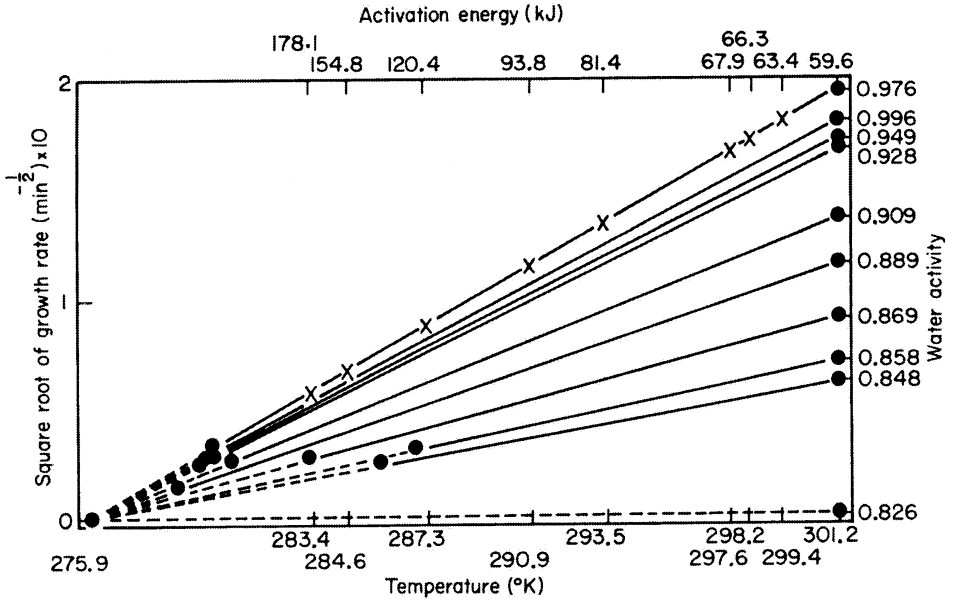

Fig. 1. Square-root plot of the effect of temperature and water activity on the growth rate of *Staphylococcus xylosus*. T_{min} is constant at 275.9 K but the actual minimum temperature for growth (●) increases with decreasing water activity (reproduced from Chandler, 1988).

b^2 versus a_w was linear and from that $a_{w,min}$ (the theoretical minimum a_w for growth) was estimated by extrapolation of the regression line. The general response was similar with NaCl or glycerol as the humectant but a specific solute effect was evident with $a_{w,min}$ in NaCl = 0.838 and $a_{w,min}$ in glycerol = 0.908.

The general response shown in Fig. 1 indicates that the effect of the combined factors is additive and not synergistic as sequential reductions in water activity at any temperature have proportionally the same effect on growth rate. If a synergistic interaction was occurring one would anticipate increased magnitude of the water activity effect at lower temperatures.

This conclusion was supported by Davey (1989) using a modified Arrhenius model

$$\ln k = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 a_w + C_4 a_w^2$$

in which the absence of a cross-product term involving a_w and temperature indicates that their effects are independent. Using data from the literature, additive interactions were inferred for *Pseudomonas*, '*Aerobacter*' *aerogenes*, *Pediococcus cerevisiae*, *Clostridium botulinum* type E, '*Microbacterium*' *thermosphactum* and *Salmonella typhimurium*.

A similar conclusion was reached in respect of pH, acidulant and temperature on the growth rate of *Yersinia enterocolitica* by Adams et al. (1991), who proposed a model of the same general form as the square-root model in which the a_w term was replaced with a pH term. At every combination of pH and acidulant the response was well described by the square-root model. The mean T_{min} value was 269.0±0.4 K and the constancy of T_{min} was unaffected by any of the combinations of acidulant and pH level examined.

3. Combined effects of temperature and water activity on the observed minimum temperature for growth and the energy diversion hypothesis

Despite the fact that the combined effect of temperature and water activity on growth rate is additive and the theoretical minimum temperature for growth (T_{min}) was unaffected by water activity, the actual minimum temperature at which growth was observed increased with decreasing a_w levels (Fig. 1). This suggests that temperature and water activity act synergistically at the point where growth ceases.

Microbial responses to stressful conditions, such as those encountered at low a_w values, may constitute a drain on the energy resources of the cell as it attempts to maintain cytoplasmic homeostasis, e.g., by accumulating or synthesising compatible solutes (Csonka, 1989). Subsequently the energy diversion hypothesis was supported by McMeekin et al. (1993) and by Knochel and Gould (1995).

The energetic burden imposed by acid conditions to maintain favourable pH levels by actively pumping protons from the cytoplasm is well established (Booth and Kroll, 1989). This effect was confirmed by Krist et al. (1998) who examined growth rate and yield of glucose-limited cultures of *E. coli* under acid stresses. Whilst the rate was maintained across a wide pH range, cell yield declined linearly with increased H^+ concentration.

This contrasted with the response to lowered water activity where rates decreased proportionally but yield was maintained until a critical point beyond which it declined rapidly to zero. Thus, as all of the limited substrate was eventually converted to the same level of biomass at a_w values >0.97, it was argued that the energy burden imposed by osmoregulatory processes in this range is not severe (Krist et al., 1998).

The a_w rate/yield response is similar to that observed with temperature, i.e., over a wide range rate declines progressively with temperature but yield is maintained, except beyond critical high and low points at which a sudden decline is observed. Similar results were reported by Senez (1962).

For both temperature and water activity the implication is that cessation of growth is not due to exhaustion of available energy options. This is consistent with the mechanistic model of Ross reported in McMeekin et al. (1993) in which the proportion of a key enzyme in its catalytically active form defines the high and low temperature limits for growth. A similar range of growth rates as a result of temperature or water activity constraints provides further support for a common mechanism as does the cryo- and osmoprotective effects of compatible solutes (Ko et al., 1994).

4. Growth/no growth (G/NG) interface modelling

The genesis of 'modern' predictive microbiology involved modelling the probability of toxin production by *C. botulinum* by the Genigeorgis group in the USA and the Roberts group in the UK in the early 1970s. In the 1980s the emphasis in modelling switched to developing kinetic models describing the rate of growth of microbial populations as influenced by environmental factors. Several kinetic models for food-borne pathogens and spoilage organisms were incorporated into publicly available applications software such as the Pathogen Modeling Program (developed by the USDA), Food Micromodel (developed by the UK MAFF) and Food Spoilage Predictor (developed by the University of Tasmania).

Kinetic models are adequate when dealing with spoilage or with pathogens where some growth may be tolerated. However, the situation changed dramatically with the emergence of food-borne pathogens with very low infective doses, such as *E. coli* 0157:H7 where quantitative information on growth limits may be more valuable than describing rates of growth.

A procedure to develop G/NG interface models was first reported by Ratkowsky and Ross (1995) by which a growth rate model for the effect of temperature, pH, water activity and nitrite concentration was modified to predict the probability of growth or no growth of *Shigella flexneri*.

The modification involved taking the logarithm of both sides of the equation and replacing the left-hand side with a logit term (logit p) where p is the probability of growth occurring. This approach was subsequently used by Presser et al. (1998) to define the interface of *E. coli* as influenced by temperature, pH, lactic acid concentration and water activity. G/NG models are also now available for a toxigenic *E. coli* strain (temperature and NaCl concentration) (Salter, 1998), *L. monocytogenes* (temperature, pH, lactic acid and NaCl concentrations) (Tiengunoon, 1998) and *Klebsiella oxytoca* (temperature, water activity, pH and lactic acid concentration) (Ross, 1999). An example is shown in Fig. 2.

Defining the G/NG interface has a number of important practical and scientific implications which arise from several common features of the interfaces thus far defined.

(i) The interface can be characterised very closely in terms of the factors preventing growth. For

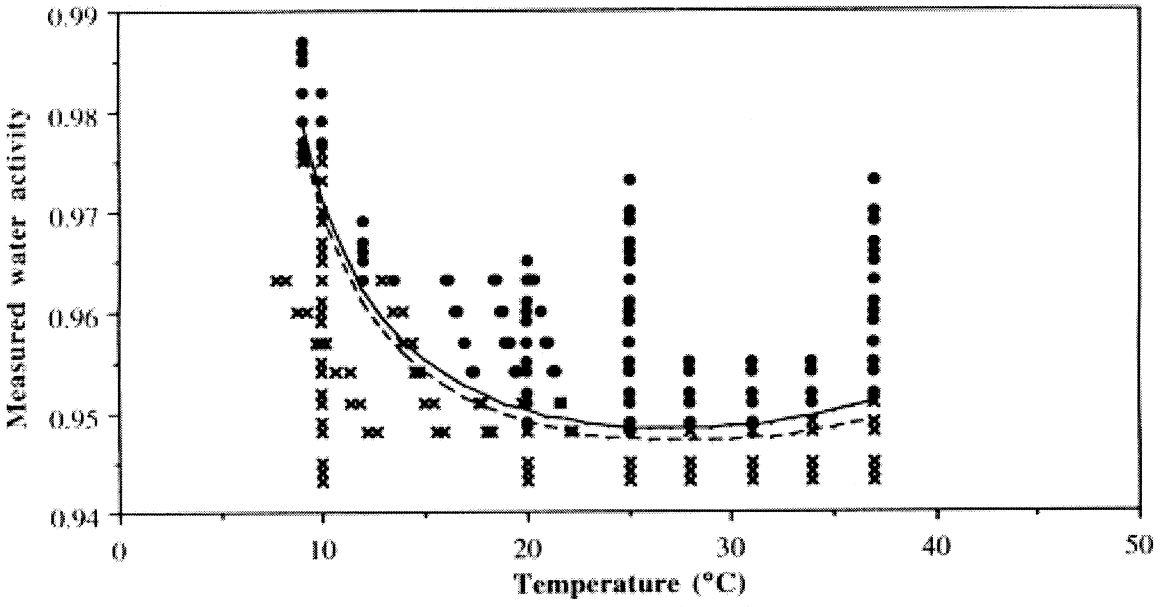

Fig. 2. Growth/no growth data for combinations of temperature and water activity for a toxigenic *E. coli* strain compared with predictions from a regression model. (●) Growth; (×) no growth; solid line, model ($P=0.5$); broken line, model ($P=0.1$) (adapted from Salter, 1998).

example for water activity the interface is typically defined across 0.01–0.03 units which is equivalent to the resolution of most water activity meters. For pH the definition is 0.1–0.2 pH units. Further evidence that a sharp cut-off occurs between growth and no growth conditions comes from the similarity of combinations describing 50% probability and 90% probability of growth (Fig. 2).

(ii) Tolerance to low water activity and pH is not optimal near the optimum temperature for growth rate. For example, with *E. coli* growth at the lowest a_w (0.948) occurred at 25–30°C with a minimum a_w of 0.951 at 37°C.

(iii) The synergistic interaction of temperature and water activity and/or pH can be quantified and combinations preventing growth specified. For example, with *E. coli* at 20°C a water activity of ~0.95 is required but at 10°C a water activity of ~0.97 will suffice to prevent growth.

The practical implications of G/NG interface modelling lie in the accurate description of conditions which can be applied to control a process or specify a formulation to ensure that no growth of a dangerous food-borne pathogen will occur. In this context the important adjective is accurate as the technique provides a mechanism to limit risk to the consumer and to the food processor by approaching the edge of 'Cole's Cliff'.

The scientific implications are also fascinating as we can now examine physiological mechanisms close to either side of the interface. Many of the events occurring in this region will not be expected and may well be reversed as the interface is crossed. As an example of the unexpected, we have observed that nisin is effective against *L. monocytogenes* at 20°C when the medium is poised at pH 4.9 (growth side of the interface), but is ineffective at pH 4.8 (no growth side of the interface) (D. Miles, pers. comm.).

Reversal of consequences is illustrated by the effect of compatible solutes. For growing cultures of *E. coli* these increase the rate and temperature range for growth but in non-growing cultures the death rates may be enhanced (Krist, 1998). Presumably this occurs because uncoupled biosynthetic and energy-yielding enzymes continue to operate in an uncoordinated way as a result of the protective effect of compatible solutes.

Considerable attention has been attributed recently to the effect of oxidative bursts as a result of uncoupling biosynthetic and catabolic processes (Imlay, 1995; Dodd et al., 1997; Bloomfield et al., 1998) and it would be of interest to study the effect of sequentially moving across the G/NG interface to ascertain if it resulted in cumulative damage to the cell and its ultimate death. Thus, definition of the G/NG interface, i.e., quantifying the hurdle concept, provides a precise set of conditions upon which a new generation of mild food preservation techniques may be based.

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