Journal of Industrial Microbiology, 12 (1993) 156–161

Published by The Macmillan Press Ltd

Probability models to assess the safety of foods with respect to *Clostridium botulinum*

David A. Baker

Nestec S.A. Centre, de Recherche, Nestle CH-1000, Lausanne, Switzerland

(Received 17 February 1993)

Key words: Predictive microbiology; Modeling; *Clostridium botulinum*; Challenge test; Risk analysis; HACCP; Food safety

SUMMARY

The effectiveness of a preservative system to prevent the growth of *Clostridium botulinum* can be expressed as the probability (P) that not even a single spore will be able to grow and produce toxin. Commercial canning processes for foods have been based upon this principle since the early 1920s. The safety of many current food marketing concepts depends on product formulation, processing, packaging and distribution variables. Direct measurement of *C. botulinum* growth in a food system is difficult. Researchers have relied upon bioassay for botulinum toxin detection and Most Probable Number (MPN) techniques to quantify *C. botulinum* growth in experimental food systems. The methods used to estimate P for a single spore to initiate growth will lead to a discussion on the use of P as a dependent variable in predictive models. Modeling the effects of intrinsic and extrinsic processing variables on food safety will be presented.

INTRODUCTION

The effectiveness of a preservative system to prevent the growth of *Clostridium botulinum* can be expressed as the probability (P) that not a single spore will grow to produce toxin [27]. Commercial processes for canning foods have been based upon this principle since the early 1920s. However, the safety of many foods currently marketed, depends on factors related to product formulation, processing, packaging and consumer use. Since direct measurement of *C. botulinum* growth in foods is difficult, researchers have relied upon time until botulinum toxin is detected and Most Probable Number (MPN) techniques to quantify growth in experimental food systems. A brief review of research based on the estimation of P will lead to a discussion on the use of P as a dependent variable in predictive models and aspects of its application in risk assessment.

The 12-DR concept is applicable to canning

Destruction of bacteria and spores by moist heat is generally accepted as being logarithmic. The D-value describes the time in minutes at a given temperature to cause a decimal reduction in the number of viable organisms:

$$\text{D-value} = \frac{t}{\log N_0 - \log N_t} \quad (1)$$

where t is the duration of heat treatment (min) and N_0 and N_t are the initial and final numbers of viable organisms in samples of equal size. Thus, the first model to assess the risk of *C. botulinum* toxigenesis in canned foods was the thermal death time-curve which shows the mathematical relation between the thermal process and spore survival.

When direct enumeration is not practical the 'Fraction of Negative Units' method can be used to estimate the D-value [25]. In this procedure, a number of replicate units (r) are sampled after the heating process and subsequently incubated to determine the number of units with growth (p) and without growth (q).

$$r = p + q \quad (2)$$

Given the initial number of viable spores per replicate unit (N_0), the number of survivors per replicate unit (N_t) can be calculated by Eqn 3:

$$N_t = \ln(r/q) = 2.303 \log_{10}(r/q) \quad (3)$$

The standard thermal process for shelf-stable, low acid, canned foods requires the reduction of 10^{11} *C. botulinum* spores to less than one. The 12 decimal reductions (DR) of *C. botulinum* spores can also be expressed as the risk of 1 toxic can in 10^{12} units. However, since destruction of spores of some spoilage microorganisms requires even greater heat treatments, the decimal reductions caused to *C. botulinum* spores is normally far greater than 12. Therefore, the 12-DR concept in canning with regard to safety becomes somewhat arbitrary.

Equations for first-order kinetic reactions describing thermal destruction can also be used applied to survivor curves. This mechanistic concept has been successfully

Correspondence to: D.A. Baker, Nestec S.A. Centre, de Recherche, Nestle CH-1000, Lausanne 26, Switzerland.

applied to model the P of *C. botulinum* to overcome hurdles inhibitory to growth [16]:

$$P = \text{MPN spores outgrowing / MPN spores inoculated} \quad (4)$$

P relates the most probable number (MPN) of spores initially inoculated in each sample, to the MPN of spores able to survive the heat process and outgrow despite the presence of hurdles. Probability expressed as $\log(1/P)$ represents the number of spores or cells (in log units) required to initiate growth. The decimal reductions (DR) caused by thermal destruction are analogous to those resulting from inhibition, providing that the hazard represented by a contaminating microorganism is related to its potential for growth. Thus, $\log(1/P)$ has been used for the design of quantitative experiments and employed as a common denominator for the re-analysis of existing inoculated pack data in the literature [11].

In studies where the inoculation was made at a single level, the MPN of outgrowing spores may be calculated [10]:

$$\text{MPN} = \ln(n/q) \quad (5)$$

where n is the number of inoculated packs, and q is the number of non-toxic packs after processing and incubation. This method assumes that the probability of a single spore to grow and initiate toxigenesis is not affected by the presence of other spores. Dividing the MPN calculated by the number of spores inoculated (s), yields a calculation of the P of toxin development from a single *C. botulinum* spore:

$$P = \ln(n/q)/s \quad (6)$$

A probability statement that incorporates experimental results with an estimate of product contamination is obtained when P is multiplied by the natural prevalence of *C. botulinum* spores expected per sample (i). This estimate of P has been applied in product risk analyses [11,13].

$$P_i = (\ln(n/q)/s) \times i \quad (7)$$

Similarly, $\log 1/(P \times i)$ can express the decimal number of units produced for each unit which may be expected to become toxic, based on the expected prevalence of contaminating *C. botulinum* spores per unit [12].

Risk assessment and multiple growth hurdles

The margin of safety attributed to the 12-DR process seems to have become an idealistic goal, as the need to establish realistic safety criteria for new types of products has emerged. Most of these product types receive a minimal thermal process. Assessment of the safety of shelf-stable, canned, cured meats required industry to incorporate factors other than heat into the risk assessment for *C. botulinum* growth and toxigenesis. Probability was expressed to quantify the safety of canned, cured meats as a function of the interactions between food preservatives and thermal processes. Experiments demonstrated the number of initial spores which would be required to overcome a preservation system. Comparison of P to the low initial number of *C. botulinum* spores expected, presented a sound argument to reduce the need for 12 DR (12,26,27].

Riemann calculated that the MPN of putrefactive anaerobic spores in meats (beef, pork and ham) ranged from 0.02–0.54 g^{-1}, assuming a log normal distribution [26]. Though this prevalence seemed low, a comment warned that individual samples have contained up to 51 anaerobic spores g^{-1}, and one sample contained 15 clostridial spores g^{-1}. Data suggest that the spore ratio of putrefactive anaerobes to *C. botulinum* is 10^4 to 1 in raw meats [12]. The numbers of *C. botulinum* spores in meats have generally not been reported to exceed 4 per kg [7]. However, *C. botulinum* spore numbers have been found to be 100–1000-fold higher in fish, and their prevalence in raw foods which have had soil contact is not well characterized.

Riemann [27], Roberts et al. [28] and Hauschild [11] were among the first investigators to estimate the P of a single spore to initiate growth and toxigenesis (Eqn 4). The interactions between thermal process, pH and NaCl and $NaNO_2$ were shown to affect the number of spores able to outgrow over a six-month incubation period at 30 °C. The increased effects of combined hurdles could be quantified. For example: at $F_0 = 0.4$, 1% of inoculated spores outgrew, while only 0.1% outgrew at $F_0 = 1.0$; 150 ppm $NaNO_2$ was not more effective than 50 ppm at $F_0 = 0.4$, but inhibited 10 times more spores at $F_0 = 1.0$ [26]. Application of P calculations allowed product formulations to be adjusted with a predictable level of inhibition. The effect of 4.5% and 5.5% NaCl caused approximately 5 and 6 DR, respectively. A 6-DR level of protection could be maintained by balancing the NaCl concentration (0–4.67%), with the pH (5–6.5) [26]. Formulation of canned luncheon meats could be manipulated to cause between 5 and >9 DR of *C. botulinum* spores through the presence of salt (3.6–5.8% brine) and nitrite (0–150 ppm) [12].

The use of multiple levels of spore inoculation yields a more precise estimation of P. As the number of spores inoculated decreased, the effects of brine (approx. 4%) and nitrite concentrations (0–150 ppm) delayed toxigenesis longer at abusive storage temperatures [13]. Acceptable protection ($P < 2\times10^{-6}$) justified the consistent use of 150 ppm nitrite.

In the 1970s, the concern for nitrosamines prompted the evaluation of the safety of cured meats with reduced additions of nitrite. In a major study, probability was used as the dependent variable to model the minimum effective nitrite concentration needed to inhibit *C. botulinum* growth in pork meat media. Experiments were based on interactions between the factors: meat pH (5.5–6.3 or 6.3–6.8), NaCl (2.5, 3.5, 4.5% w/v in water), sodium nitrite (0, 100, 200, 300 ppm), sodium nitrate (0, 500 ppm), sodium isoascorbate (0, 1000, etc. ppm), polyphosphate (0, 0.3% w/v slurry), potassium sorbate (0, 0.26% w/v), heat process {(0, to 70 °C internal temperature (low), 70 °C IT for 1 h (high)}, and storage at

15, 17.5, 20 and 35 °C for up to 6 months of incubation [8, 9,28,29,30,31]. Logistic regression was used to model P:

$$P_{ijk...rst} = 1/(1 + e^{-\mu}) \quad (8)$$

where, $P_{ijk...rst}$ is the probability of toxin production under the combination of treatments defined by the subscripts $_{ijk...rst}$. The linear predictor, μ, is expressed by an equation which includes the individual terms of regression on factors, and their interactions. Combinations and interactions of intrinsic and extrinsic factors which minimize the probability of toxigenesis, will be reflected by reducing the value of μ.

Fitting a model to the experimental variables allowed predicted estimates for P to be calculated within the range of each parameter tested. The effects of treatments were reported as the probability (%) of toxin production by *C. botulinum* type A and B, in pork slurries. Low pH pork inoculated with 10^3 spores per bottle, at the lowest NaCl level, and given the high heat treatment required the highest level of $NaNO_2$ to keep the average $P < 10\%$. The highest NaCl treatment further lowered P as did increasing $NaNO_2$ levels and decreasing storage temperatures.

Other environmental factors which influence the P of *C. botulinum* growth were studied. The interaction of Eh, O_2 and NaCl on P was quantified in a model system [18,22, 23]. Media with 3.25% NaCl at Eh −400 mV resulted in 2 DR, while raising the Eh to between +62 and +122 mV caused 6 DR. As the partial O_2 pressure (pO_2) increased, P decreased reaching 4 to >5 DR at pO_2 1.1–1.6 × 10^{-2} atm (Eh +271–294 mV). Combinations of storage temperature (8–20 °C) and pH (≤5.1) inhibited the growth of *C. botulinum* over an 8-week incubation period, P was $<3 \times 10^{-6}$.

A relationship between pH, undissociated sorbic acid and storage temperature on the P of *C. botulinum* growth in broth was demonstrated [19,20,21]. Regression analysis was used to fit models relating the range of independent variables to characteristic points on a curve describing the increase of P over time. The model predicted the minimum number of *C. botulinum* cells necessary to initiate growth. Since the increase in P is approximated by a logistic curve, many of the methods published fitting growth data to sigmoidal curves could also be applied to model P [2,8].

Modeling the P for *C. botulinum* is appropriate for describing the safety of products protected by multiple hurdles. Inoculated pack studies have shown that the safety of shelf-stable cured meat products depends on: a) a mild cook to kill vegetative cells and injure spores; b) the action of nitrite, NaCl, ascorbate and other curing agents to inhibit heat-damaged spores; and c) a low initial concentration of *C. botulinum* spores in the raw product. Proper refrigeration can replace the need for some of the preceding safety factors in perishable luncheon meats. The literature indicates that a hurdle system causing inhibition equivalent to 4–6 decimal reductions offers a substantial safety margin with respect to the low *C. botulinum* spore numbers typically found in cured meat and pasteurized cheese spreads [3,12,26,33,35]. This experimentally defined margin of safety is in contrast to the 12-DR concept, however, a record of safety for cured meat products supports the risk assessment.

Risk has been related back to the observed safety of individual commercial products on a volume basis over the time of their marketing history [12]. Recalling that log $1/(P \times i)$ represents the expected rate at which a unit would become toxic based on an assigned prevalence of *C. botulinum* per unit. Safety units (SU) were described as an industrial interpretation relating the decimal number of units marketed per number of units causing illness, e.g. if $10^{8.5}$ units were marketed safely, then the SU would be >8.5. Hauschild and Simonsen [12] applied production data for shelf-stable, canned, cured meats to estimate minimum safety in terms of SU. The margins of safety for luncheon meat, ham and shoulder and sausages ranged from >6.6 to >9.5 SU depending on the source and volume of production data available. The authors caution that SU estimates are less conservative than experimentally derived log $(P \times i)$ values. Commercial turn-over of each unit of product may be much faster than defined experimental periods.

When safety is a function of low *C. botulinum* spore contamination, product formulation and processing, then the severe storage abuses needed for *C. botulinum* to initiate growth would indeed be a rare event. Given that the record of safety and production rates continued unchanged for the products described by Hauschild and Simonsen [12], then >1500 years of data would be necessary to justify a SU >12.

Risk assessment when refrigeration is the primary hurdle

Knowledge of the level of *C. botulinum* spore contamination is more important in food products that are not optimal *C. botulinum* growth substrates, or for which a system of preservative hurdles can be demonstrated. When refrigeration is the only growth hurdle, the question of how to assess the risk posed by the occurrence of a *C. botulinum* spore becomes much more difficult. Determination of how infrequently a *C. botulinum* spore may be found in raw materials is costly and subject to insensitivity when sampling for a rare event. Smelt [34] applied a Poisson distribution to 40 samples (25 g each) of strawberry. Assuming uniform spore distribution, >5 spores kg^{-1} were needed to reduce the probability of not detecting a positive to <1%. In fact, there is no basis to assume *C. botulinum* spores are randomly distributed in nature, or that 5 spores kg^{-1} can be expected.

Shorter times to *C. botulinum* toxigenesis have been attributed to increases in spore inoculum [4,5,11,17]. However, when a food presents a conducive environment, such that a single *C. botulinum* spore may initiate growth, then inoculum size will have little effect on the time to toxigenesis [1,14]. Under this scenario, the number of *C. botulinum* spores in raw ingredients becomes less important, since their eventual presence could lead to catastrophic failure. As the P of one spore to grow approaches 1, the need to conduct experiments with multiple *C. botulinum* inoculation levels decreases. Modeling the relationship of storage temperature to *C. botulinum* lag time (LT) will become more essential in a risk assessment.

A formula for prediction of the shortest LT was constructed based on the analysis of the compiled experiments with modified atmosphere packaged fish [1].

$$\log LT = 0.974 - 0.042(\text{Temp. °C}) + (2.74/\text{Temp. °C}) - 0.091(\log \text{spore inoculum}) + 0.035(\text{initial log aerobic plate count})$$

The analysis demonstrated that increased spore loads have the greatest effect on shortening the length of LT at the lower refrigeration temperatures, i.e. below 8 °C. Temperature alone accounted for 74.6% of the total explained variation, while initial spore inoculum accounted for an additional 7.4% ($r^2 = 0.883$). The effect of spore inoculum diminished at refrigeration temperatures of 10–12 °C and the P of growth from a single *C. botulinum* spore approached unity. This model of lag time could be used to set limits for the static time and temperature conditions not to be exceeded, assuming natural levels of *C. botulinum* spore contamination.

If definitive critical control points are not incorporated to eliminate *C. bolutinum* spores, then a hazard analysis of critical control points (HACCP) plan can only address the risks associated with a product's raw materials, processing, distribution, marketing, and use by the consumer. Smelt [34] applied a general model for risk assessment to the minimum number of *C. botulinum* needed to initiate growth in jellied milk with fruit stored under refrigeration. Risk was assessed in the following terms:

$$Q = e^{-N_f R} \quad \text{or} \quad N_f = -(\ln Q)/R \tag{10}$$

where

- Q = the probability that at no time will a given number of susceptibles be exposed to a minimum number of *C. botulinum* capable of growth;
- N_f = number of spores able to grow per unit, assuming a random distribution of *C. botulinum* spores;
- R = $V \times I$, where V = size of the population at risk, and I = number of units annually produced, given a limited storage life.

N_f must therefore be estimated based upon the normal range of contamination, lethality of processing, and the probability of outgrowth by survivors. The probability of outgrowth at 20 °C was $<0.4 \times 10^{-6}$ up to 24 days. The risk assessment was based on initial contamination levels, chance of exposure to temperatures >10 °C, and the associated probability of outgrowth after processing. Consequently, this risk assessment estimated a probability of 10^{-12} that *C. botulinum* spores could be expected to grow in each unit of jellied milk with fruit, given that the intended refrigerated storage is 24 days.

Risk is the potential to realize an unwanted event. It is characterized by a probability of occurrence, a pathway to a manifestation, and a magnitude of the consequence. Every technology involves a gamble [32]. Unlike the probabilities in a game of chance, the outcomes faced by the food industry are multi-faceted. Therefore, a risk assessment should highlight critical issues and limitations, while separating value judgments from quantitative parameters. The understanding developed will help evaluate each component of risk individually, which then must either be accepted or rejected prior to effective planning and decision making. To accept that refrigerated products are not shelf-stable, will be the hardest decision of all.

The preceding assessment by Dr Smelt is perhaps the only published probability estimate relating *C. botulinum* safety to development and marketing of a refrigerated product. The minute degree of risk (log P) estimated by Smelt [34] was additive. The P of one *C. botulinum* spore to grow (4×10^{-7}) given a minimum temperature abuse of 24 days at 20 °C was the greatest contribution to the risk estimate, the initial spore load and thermal process (approx. 10^{-2}), and a combination of storage factors which if considered as independent variables, contribute to reduce the P of toxigenesis by 6×10^{-4}. However, these product storage factors may not all be independent. One could suspect that retailers with the worst temperature control (particularly small shops), may also be those whose stock rotation practices may allow products to reach their maximum shelf-life, and have customers most likely to further temperature-abuse products.

A second consequence of this additive risk assessment is the dilemma to explain the relevance of the P achieved. Most refrigerated products, in particular the new trend toward minimally processed meal components, will not be able to achieve the same level of safety (12 DR) as demonstrated for the jellied-milk product. Though a P of growth may seem exceedingly small, e.g. 10^{-7}, when re-expressed as the expected number of deaths or illnesses per quantity of product sold, it becomes an unacceptable criteria to base decisions in a safety assessment. Therefore, risk assessment should adopt a more mechanistic and distributed approach toward assuring the development, processing and distribution of safe refrigerated products. Documentation of an offensive attitude toward designing safety into food manufacturing will be the best defence of 'Due Diligence' in the future [6]. ('A good offence is the best defence' (reference unknown).)

INTEGRATION OF PREDICTIVE MODELING INTO HACCP PROGRAMS

Comparison of the hurdle system to inhibit *C. botulinum* growth in a new product using data from the literature and with eventual access to microbial growth databases, will help determine whether the number of DR should be quantified, because large P would be expected. If P is small, i.e. perhaps >4–5 DR, then it should be possible to establish product safety in a manner similar to that used for cured meats. However, when product safety is primarily based on refrigeration, challenge test data should support the diligence of a marketing strategy.

Inclusion of inoculated pack studies as part of a comprehensive HACCP [15] quality assurance program will allow processors to establish maximum time/temperature storage conditions for their products, based on worst case scenarios with respect to contamination with *C. botulinum* spores, either in the raw product or resulting from in-plant contamination and potential consumer abuse.

The upper graph in Fig. 1 (A) demonstrates that a marketing strategy based on refrigeration can be divided into: 1) ideal storage conditions, 2) abusive storage conditions which must be accepted and 3) gross temperature abuse resulting from negligence. A safety margin between anticipated abuse and absolute abuse can be envisioned, as shown by the area to the left of the dashed line. This dashed line should coincide with the boundary between the safe limits for storage of inoculated products and the time/temperature relationships necessary for *C. botulinum* growth demonstrated in the lower portion of Fig. 1 (B). Providing that product safety is developed in conjunction with its marketing requirements and labeling, the associated risk(s) with worst case scenario can be accepted or rejected by the prospective producer. In other words, those conditions which would be necessary for a contaminated product to become toxic during handling by a distributor, retailer or consumer can be specified. Given an ample margin of safety, the decision to market with Due Diligence could be substantiated.

For example, Mass et al. [24] inoculated turkey rolls containing sodium lactate with up to $10^{3.7}$ *C. botulinum* spores per pack. The lag time before detectable toxicity, at 27 °C, was extended from 2 to 6 days with increasing levels of sodium lactate (0–3.5%). The safety implied by the need to incubate inoculated turkey rolls for >2 days at 27 °C demonstrates how badly these products must be treated before toxin can develop. Advantage of additional hurdles should be employed whenever possible. This challenge study, supported by the low prevalence of *C. botulinum* spores in poultry products, yields solid data to compare risk with a market strategy for the refrigerated product. The validity of this assessment must be continually maintained by a stringent HACCP plan designed to: (a) prevent raw materials from becoming contaminated in the production environment, (b) guarantee thermal processing is performed to specifications, (c) ensure products are properly labeled, and (d) require proper storage temperatures be maintained and documented throughout distribution.

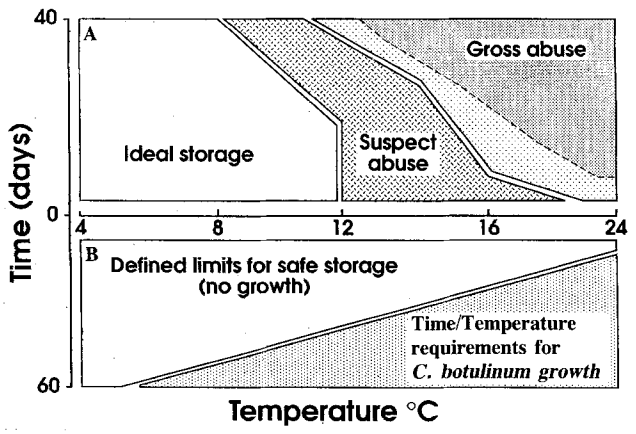

Fig. 1. Challenge test data demonstrates diligence of market strategy.

CONCLUSION

Modeling strategies should aim towards consolidation of mathematical and experimental approaches, thus providing the food industry with rational guidelines for design and routine safety testing. Standardization of factorial and other design experimentation would allow the refrigerated foods industry to develop a *C. botulinum* growth (*LT*, *P*) database. Manufacturers would then be able to design general formulae including nutritional, physical and chemical factors pertinent to their specific product. Access to such a database would greatly reduce the cost of conducting product challenge studies by providing *C. botulinum* growth expectations. It would also improve the reliability of challenge studies through tighter bracketing of sampling times and temperatures.

Much more thought, discussion and data will be necessary before any single concept for risk assessment for refrigerated food can gain wide acceptance. Why the marketing of these foods to date has been so safe is unknown; however, the chance associated with this safety is the source of our concern and disturbance. A mechanistic risk assessment and challenge test data, should be able to support diligence in the development products for the expanding minimally processed, refrigerated foods market.

REFERENCES

1 Baker, D.A. and C. Genigeorgis. 1990. Predicting the safe storage of fresh fish under modified atmospheres with respect to *Clostridium botulinum* toxigenesis by modeling length of the lage phase of growth. J. Food Protect. 53: 131–140.

2 Buchanan, R.L. and J.G. Phillips. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Protect. 53: 370–376.

3 Cerveny, J.G. 1980. Effects of changes in the production and marketing of cured meats on the risk of botulism. Food Technol. 34: 240–243.

4 Eklund, M.W. 1982. Significance of *Clostridium botulinum* in fishery product preserved short of sterilization. Food Technol. 3612: 107–112, 115.

5 Eyles, M.J. and A.D. Warth. 1981. Assessment of the risk of botulism from vacuum-packaged raw fish: a review. Food Technol. Aust. 33: 574–580.

6 Fidler, D.G. 1990. Due Diligence and quality assurance in the UK. Food Control (April): 117–121.

7 Genigeorgis, C.A. 1976. Quality control for fermented meats. JAVMA 11: 1220–1228.

8 Gibson, A.M., N. Bratchell and T.A. Roberts. 1988. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. Int. J. Food Microbiol. 6: 155–178.

9 Gibson, A.M., T.A. Roberts and A. Robinson. 1982. Factors controlling the growth of *Clostridium botulinum* type A and B

in pasteurized, cured meats. IV. The effect of pig breed, cut and batch of pork. J. Food Technol. 17: 471–482.
10 Halvorson, H.O. and Ziegler, N.R. 1933. Application of statistics to problems in bacteriology. J. Bacteriol. 25: 101.
11 Hauschild, A.H.W. 1982. Assessment of botulism hazards from cured meat products. Food Technol. 36: 95–104.
12 Hauschild, A.H.W. and B. Simonsen. 1985. Safety of shelf-stable canned cured meats. J. Food Protect. 48: 997–1009.
13 Hauschild, A.H.W., R. Hilsheimer, G. Jarvis and D.P. Raymond. 1982. Contribution of nitrite to the control of *Clostridium botulinum* in liver sausage. J. Food Protect. 45: 500–506.
14 Huss, H.H., I. Schaeffer, E. Rye Peteresen and D.C. Cann. 1979. Toxin production by *Clostridium botulinum* type E in fresh herring in relation to the measured oxidation–reduction potential (Eh). Nord. Vet. Med. 31: 81–86.
15 International Commission on Microbiological Specifications for Foods. 1988. Microorganisms in Foods, Vol. 4, Application of the Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality. Blackwell, Oxford, UK.
16 Leistner, L. and W. Rödel. 1976. The stability of intermediate moisture foods with respect to micro-organisms. In: Intermediate Moisture Foods (Davies, R., G.G. Birch and K.J. Parker, eds), pp. 120–134, Applied Science Publishers, London.
17 Lindsay, R. 1981. Modified atmosphere packaging systems for refrigerated fresh fish providing shelflife extension and safety from *Clostridium botulinum* toxigenesis. In: Proceedings of the First National Conference on Modified and Controlled Atmosphere Packaging of Seafood Products (Martin, R.E., ed.), pp. 30–51, National Fisheries Institute, Washington, DC.
18 Lund, B.M. and G.M. Wyatt. 1984. The effect of redox potential, and its interation with sodium chloride concentration, on the probability of growth of *Clostridium botulinum* type E from spore inocula. Food Microbiol. 1: 49–65.
19 Lund, B.M., S.M. George and J.G. Franklin. 1987a. Inhibition of type A and type B (proteolytic) *Clostridium botulinum* by sorbic acid. Appl. Environ. Microbiol. 53: 935–941.
20 Lund, B.M., A.F. Graham and J.G. Franklin. 1987b. The effect of acid pH on the probability of growth of proteolytic strains of *Clostridium botulinum*. Int. J. Food Microbiol. 4: 215–226.
21 Lund, B.M., A.F. Graham, S.M. George and D. Brown. 1990. The combined effect of incubation temperature, pH and sorbic acid on the probability of growth of non-proteolytic, type B *Clostridium botulinum*. J. Appl. Bacteriol. 69: 481–492.
22 Lund, B.M., M.R. Knox and A.P. Sims. 1984. The effect of oxygen and redox potential on the growth of *C. botulinum* type E from a spore inoculum. Food Microbiol. 1: 277–287.
23 Lund, B.M., G.M. Wyatte and A.F. Graham. 1985. The combined effect of low temperature and low pH on survival of, and growth and toxin formation from, spores of *Clostridium botulinum*. Food Microbiol. 2: 135–145.
24 Mass, M.R., K.A. Glass and M.P. Doyle. 1989. Sodium lactate delays toxin production by *Clostridium botulinum* in cook-in-bag turkey products. Appl. Environ. Microbiol. 55: 2226–2229.
25 Pflug, I.J. and R.G. Holcomb. 1983. Principles of thermal destruction of microorganisms. In: Disinfection, Sterilization and Preservation, 3rd ed (Block, S.S., ed.), pp. 751–810, Lea and Febiger, Philadelphia.
26 Riemann, H. 1963. Safe heat processing of canned cured meats with regard to bacterial spores. Food Technol. 1: 39–42, 44, 46, 49.
27 Riemann, H. 1967. The effect of the number of spores on growth and toxin formation by *C. botulinum* type E in inhibitory environments. In: Botulism 1966. Proceedings of the 5th International Symposium on Food Microbiology, Moscow, July 1966 (Ingram, M. and T.A. Roberts, eds), pp. 150–168, Chapman & Hall, London.
28 Roberts, T.A., A.M. Gibson and A. Robinson. 1981a. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. I. Growth in pork slurries prepared from 'low' pH meat (pH range 5.5–6.3). J. Food Technol. 16: 239–266.
29 Roberts, T.A., A.M. Gibson and A. Robinson. 1981b. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. II. Growth in pork slurries prepared from 'low' pH meat (pH range 6.3–6.8). J. Food Technol. 16: 267–281.
30 Roberts, T.A., A.M. Gibson and A. Robinson. 1981c. Prediction of toxin production by *Clostridium botulinum* in pasteurized pork slurry. J. Food Technol. 16: 337–355.
31 Robinson, A., A.M. Gibson and T.A. Roberts. 1982. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. V. Prediction of toxin production: non-linear effects of storage temperature and salt concentration. J. Food Technol. 17: 727–744.
32 Rowe, W.D. 1986. Identification of risk. In: Risk and Reason: Risk Assessment in Relation to Environmental Mutagens and Carcinogens (Brogger, A. and P. Oftedal, eds), pp. 3–22, Alan R. Liss, New York.
33 Silliker, J.H., R.A. Greenberg and W.R. Schack. 1958. Effect of individual curing ingredients on the shelf stability of canned comminuted meats. Food Technol. 12: 551.
34 Smelt, J.P. 1980. Heat resistance of *Clostridium botulinum* in acid ingredients and its significance for the safety of chilled foods. Thesis, University of Utrecht, Holland.
35 Tanaka, N., E. Traisman, P. Plantinga, L. Finn, W. Flom, L. Meske and J. Guggisberg. 1986. Evaluation of factors involved in antibotulinal properties of pasteurized process cheese spreads. J. Food Protect. 49: 526–531.