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Physiological and mathematical aspects in setting criteria for decontamination of foods by physical means

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Abstract

In heat processing, microbial inactivation is traditionally described as log-linear. As a general rule, the relation between rate of inactivation and temperature is also described as a log-linear relation. The model is also sometimes applied in pressure and in pulsed electric field (PEF) processing. The model has proven its value by the excellent safety record of the last 80 years, but there are many deviations from log-linearity. This could lead to either over-processing or under-processing resulting in safety problems or, more likely, spoilage problems. As there is a need for minimal processing, accurate information of the inactivation kinetics is badly needed. To predict inactivation more precisely, models have been developed that can cope with deviations of linearity. As extremely low probabilities of survival must be predicted, extrapolation is almost always necessary. However, extrapolation is hardly possible without knowledge of the nature of nonlinearity. Therefore, knowledge of the physiology of inactivation is necessary. This paper discusses the physiology of denaturation by heat, high pressure and pulse electric field. After discussion of the physiological aspects, the various aspects of the development of inactivation models will be addressed. Both general and more specific aspects are discussed such as choice of test strains, effect of the culture conditions, conditions during processing and recovery conditions and mathematical modelling of inactivation. In addition to lethal inactivation, attention will be paid to sublethal inactivation because of its relevance to food preservation. Finally, the principles of quantitative microbiological risk assessment are briefly mentioned to show how appropriate inactivation criteria can be set.

Keywords: Inactivation model; Weibull; Pressure; Temperature; Heat; Resistance; Electric field

1. Scope of the review

Nowadays, consumer demands are more and more towards natural and fresh-like products. On the other hand, foods should be processed in such a way that both the microbiological risk of food poisoning and of food spoilage during the whole shelf life is acceptably low. Although chemical preservatives form an essential part in food preservation, legislation has restricted their use of in different foods (Brul and Coote, 1999). Although physical decontamination techniques are subjected to many food regulations, the legal restrictions are often less severe than for chemical decon-

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tamination of foods. Among physical decontamination techniques, heat is still the single most important decontamination method. Thanks to technological progress, however, physical alternatives such as pressure or high electric fields are becoming more attractive. As will be pointed out below, the required reduction factor should be established, in an ideal situation, within the frame of a quantitative microbiological risk assessment (QMRA). Robust mathematical kinetic inactivation models form an essential element in QMRA. Most inactivation models are mainly empirical but often quite satisfactory. However, mechanistic elements in these models might allow more extrapolation and prediction for unknown conditions. Therefore, the mode of action of some agents such as pressure, heat and pulsed electric field will be addressed in this review. As the chemical factor is important in pressure treatment by supercritical CO_2 (Ballestra and Cuq, 1998; Debs-Louka et al., 1999; Erkmen and Karaman, 2001), it will not be considered here. Other physical decontamination techniques such as ultraviolet, oscillatory magnetic fields and X-rays will not be described here. The reader will be referred to review articles on these subjects and will not be dealt with in this review (Lacroix and Ouattara, 2000; Corry et al., 1995). An extensive discussion of chemical preservatives falls outside the scope of this paper, but occasionally, we will touch upon a few options as the interaction of the composition of the food with the physical agent is of paramount importance (Brul and Coote, 1999).

2. Physiological effects of physical stress

Whereas the effects of temperature and pressure are multi-targeted, it seems generally agreed that the main target of pulsed electric field is the membrane. Hence, the effects of pulse electric field (PEF) will be addressed separately.

2.1. Effect of temperature and pressure on vegetative cells

2.1.1. General aspects

Whereas elevated pressure plays hardly a specific role in thermal processing, temperature plays almost always a role in high-pressure processing. The combined effects of temperature and pressure can be used for a more effective pasteurisation or even sterilisation. Therefore, the two aspects will be discussed simultaneously although the mode of action of pressure is not identical to that of temperature. There are numerous reports on inactivation kinetics with respect to temperature and to a lesser extent with respect to pressure and also of the combined effect of heat and pressure (Ludwig et al., 1992; Sonoike et al., 1992; Patterson and Kilpatrick, 1998). There are also a number of reports of the combined effects of pH and water activity, and the effect of temperature and pressure (Alpas et al., 2000). The latter data are of course very relevant to real food situations. In thermal and in pressure processing, the effect of the environment plays a role during and after treatment. Most of these studies are phenomenological rather than mechanistic. Only scattered data are available with respect to the mechanism of inactivation by the two factors. As will be pointed out in the modelling section, better models can be built when more physiological aspects are taken into account. In this paragraph, these factors will be discussed. It is obvious that both elevated temperature and pressure do not affect one specific site, but that a number of targets are hit. Moreover, the physiological effects are dependent on the temperature and pressure level. For instance, at 64 °C, the membrane seems the main target for damage, whereas at higher temperatures, enzyme inactivation is more pronounced (Earnshaw et al., 1995). It can be expected that the basal heat and pressure resistance may be due to the intrinsic stability of macromolecules, i.e. RNA, ribosomes, nucleic acids, enzymes, proteins in the cell and the membrane, and, in some cases, the cell wall. It seems that many of the targets for temperature and pressure inactivation are the same but that the changes at the molecular level are different. Compression and decompression do not seem to have a specific effect on vegetative cells (Smelt and Hellemons, 2002). The only clear evidence for killing cells by decompression is with gas vacuolated cells such as *Micrococcus aquaticus*. Chastain and Yayanos (1991) found that even barophilic bacteria were fully viable directly after decompression, but they gradually died after long exposure to atmospheric conditions. Experiments with non-vacuolate bacteria reveal virtually complete survival after rapid decompression (Hemmingsen and Hemmingsen, 1978). Although

Palou et al. (1998a) claimed that *Zygosaccharomyces baillii* was more easily inactivated by oscillatory high-pressure treatment, their results show no or only a minor difference between oscillatory and continuous pressure treatment. Aleman et al. (1996) reported that pulse high-pressure treatments of pineapple was more effective than a static pulse treatment. In summary, it can be concluded that observed effect of compression and decompression on vegetative cells is only caused by the integrated effect of time and pressure (Smelt et al., 2002). A brief overview of resistance of various organisms to heat and pressure is given by Smelt (1998).

2.1.2. Gene expression, protein synthesis and stress response

For a detailed review of the mechanisms of stress response, the reader is referred to the review of Abee and Wouters (1999). Microorganisms contain mechanisms to resist and repair the effects of heat and other unfavourable environmental conditions, such as starvation and low pH, which enable them to survive subsequent, potentially lethal temperatures. Generally, there is a narrow temperature range between optimum growth temperature and maximum temperature where stress proteins are induced and where the cell acquires an increased resistance to heat. It is still unclear if a causal relationship exists between the synthesis of heat shock proteins (hsps) and the development of thermotolerance. There are numerous reports of increased thermotolerance after sublethal heat shock in *Escherichia coli* (Tsuchido et al., 1984), *Salmonella* (Mackey and Derrick, 1986, 1987a,b), *Legionella pneumophila* (Lema et al., 1988) and *Listeria monocytogenes* (Fedio and Jackson, 1989; Linton et al., 1990; Appleyard and Gaze, 1993). The optimum growth pressure of most food spoilage and food poisoning organisms is probably near atmospheric conditions. Many biochemical activities of bacteria, such as protein synthesis, glucose utilisation and L-phenylalanine utilisation, are affected by pressures of about 50 MPa (Albright, 1975). Transcription and translation are highly sensitive to pressure and culture pressure can influence the types of proteins that are synthesised (Somero, 1992). Protein synthesis is known to be one of the most pressure-sensitive cell activities (Landau, 1967). In *E. coli* cell-free systems, protein synthesis is completely inhibited at 68 MPa, but the inhibition is completely reversible after pressure release (Schwartz and Landau, 1975; Smith et al., 1975). Elevated hydrostatic pressures between 30 and 50 MPa can influence gene and protein expression. *Yarrowia lipolytica* showed enhanced proteolytic activity after pressure treatment of 80 MPa, presumably not caused by lysis, but rather by activation of proteolytic enzymes by high pressure (Lanciotti et al., 1996). Sato et al. (1995) showed that gene expression initiated from promotors malK-lam and mal-EFG in *E. coli*. They found that the expression of the genes for osmoregulatory membrane proteins was markedly reduced by high pressure, most likely at the transcriptional level. In general, hydrostatic pressure can induce tetraploidy in *Saccharomyces cerevisiae* (Hamada et al., 1992), indicating that high pressure can interfere with replication of DNA.

Pressure inducible proteins have been found in *Methanococcus thermolyticus, Rhodotorula rubra* and *E. coli*, three organisms representing the three domains of life (Bartlett et al., 1995). A pressure of 53 MPa could induce proteins in *E. coli* similar to those found at elevated temperature (Welch et al., 1993). Heat shock proteins induced by elevated hydrostatic pressure have been also found in mammalian cells (Kaarniranta et al., 2001; Elo et al., 2000; Salvador-Silva et al., 2001). Resistance to heat can be brought about by sublethally low pH and vice versa and sublethal heat can enhance pressure resistance in yeasts (Iwahashi et al., 1991; Pagan and Mackey, 2000) and in bacteria (Smelt et al., 1998). So far, increased resistance to heat by sublethal pressure has not yet been observed. When yeasts (Fernandes et al., 1997) or *Lactobacillus plantarum* cells (A.G.F. Rijke and J. Smelt, unpublished results) were subjected to a range of sublethal pressures no increased heat resistance or pressure resistance was found. These observations are in line with our findings (Smelt and Hellemons, 2002) that rate of compression does not alter the reduction rate of *Salmonella enteriditis* other than the effect of the integrated time and pressure. In view of the abovementioned findings that starvation or sublethal pH increases resistance, it is not surprising that exponentially growing cells are more sensitive to pressure than stationary phase cells (Mackey et al., 1995; Smelt et al., 1998). A prolonged stationary phase can even enhance resistance to pressure (Hellemons and Smelt, 2002). Hydrostatic pressure also

inhibits the synthesis of some membrane proteins (Nakashima et al., 1995).

2.1.3. Ribosomes as target for heat and pressure damage

Ribosomes are affected both by heat (Anderson et al., 1991) and pressure (Niven et al., 1999). It may be expected that the death of the individual cell occurs when the number of functional ribosomes has dropped below a critical threshold level, beyond which the cell cannot recover. In the microbial cell, ribosomal RNA (rRNA) ensures that ribosome proteins maintain their specific secondary and tertiary structure, enabling ribosomes to perform their function during protein synthesis. RNA modification has been associated with the early events of heat damage in a cell (Coote et al., 1991). The same seems to hold for pressure damage: Gross et al. (1993) investigated the effects of high pressure and observed ribosome dissociation starting at 40–60 MPa. This in turn can lead to destabilisation of 70S ribosomes. This might be similar for high pressure as Hauben et al. (1998) reported that divalent cations such as Ca^{2+}, Mg^{2+}, Mn^{2+} and Fe^{2+} reduced inactivation by high pressure. It has been demonstrated that ribosomes dissociate in vitro under high pressure (Schultz et al., 1976; Gross and Jaenicke, 1990; Gross et al., 1993). Mackey et al. (1991) demonstrated in vivo studies with differential scanning calorimetry (DSC) that the 50S subunit is more heat stable than the 30S subunit; however, both subunits are more heat labile than the complete 70S particle. By comparing the behaviour of the 30S, 50S and 70S subunits in isolation and in whole cells, they were able to demonstrate that heating in the region between 50 and 80 °C initiated denaturation of the 30S subunit. A relation between the maximum temperature and melting points of the ribosomes would suggest a relationship between ribosome stability and thermal resistance. Niven et al. (1999) carried out a similar study into the change of conformation of ribosomes by pressure in vivo and found a correlation between cell death and ribosome damage.

2.1.4. Susceptibility of proteins and enzymes to heat and pressure

Both pressure and temperature have significant effects on proteins including enzymes. There is an optimum temperature at which proteins are most resistant to pressure. (Hawley, 1971; Fig. 1a) A similar pattern has been observed for the inactivation of microorganisms (Sonoike et al., 1992; Hashizume et al., 1995; Hayakawa et al., 1998; Fig. 1b). Unfortunately, most enzymes have been investigated in isolation and not in whole cells. Hei and Clark (1994) investigated enzymes of extreme thermophiles and they found that these enzymes are not only more heat-resistant but also more pressure-resistant than those from mesophilic microorganisms. Hydrostatic pressure can presumably directly affect enzymes and carriers of transport systems. Lactic dehydrogenase from rabbit muscle and glyceraldehyde-3-phosphate dehydrogenase from baker's yeast were inactivated by pressures of 200 and 100 MPa, respectively. This provides some circumstantial evidence that enzyme

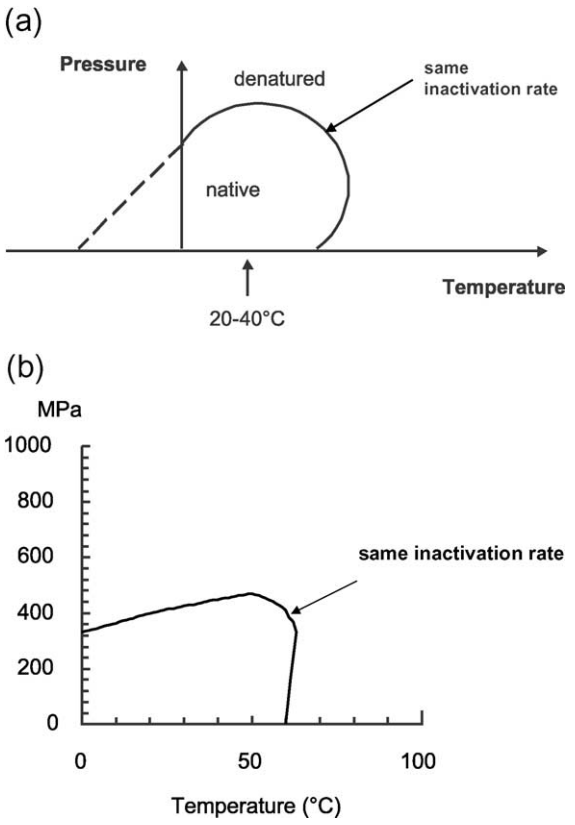

Fig. 1a and 1b. Schematic representation of the combined effect of pressure and temperature on denaturation of enzymes or inactivation of microorganisms (a), and actual behaviour of *Lactobacillus casei* at various pressure/temperature combinations (b) (Sonoike et al., 1992).

inactivation plays an important role in pressure inactivation of microorganisms. However, kinetic data on the inactivation of the various sites such as glycolytic enzymes, membrane bound enzymes, ribosomes or membranes are lacking. Moreover, the study of single compounds such as enzymes and nucleic acids can more easily be carried in isolation but most biopolymers behave differently in the cell. Pressure brings about changes in the quaternary structure rather than the tertiary structure because the quaternary structure is mainly maintained by hydrophobic interactions which are pressure-sensitive (Balny and Masson, 1993). As a result, monomeric enzymes are usually more resistant to pressures than multimeric enzymes (Masson, 1992). Schmid et al. (1975) studied the effect of pressure on glycolytic enzymes with coenzymes in vitro and found that rabbit muscle lactic dehydrogenase was more stable against pressure than yeast glyceraldehyde-3-phosphate dehydrogenase. However, hardly any attention has been paid to the extent to which the weakest link in systems are affected, e.g. in glycolysis. Degraeve et al. (1996) studied the effects of pH and temperature on pressure inactivation of *E. coli* β-galactosidase. No inactivation took place below 250 MPa. Cioni and Strambini (1997) studied the dissociation of yeast glyceraldehyde-3-phosphate dehydrogenase. The main effect was due to dissociation of the tetramer. Simpson and Gilmour (1997b) studied the effect of high hydrostatic pressure on the activity of 13 metabolic enzymes of *L. monocytogenes*. They isolated the enzymes before and after pressure treatment of whole cells. Phosphoglucomutase and aconitase were particularly susceptible to pressure and even pressures of around 200 MPa were sufficient to inactivate these enzymes. It is remarkable that *L. monocytogenes* was hardly affected by this pressure, and consequently, these enzymes cannot be considered as critical sites.

Specific adaptations of thermophiles and of deep-sea bacteria might indicate which sites in the organism are extremely sensitive to pressure. As in heat inactivation, it can be expected that there is more than one specific target that can affect the whole organism depending on the pressure level.

2.1.5. Nucleic acids

Nucleic acids, especially DNA, are relatively resistant to heat and very resistant to pressure. However, an extreme condensation of the nuclear material has been found for *L. monocytogenes* and *S. typhimurium* (Mackey et al., 1994) and *L. plantarum* (Wouters et al., 1998). The hypothesis is that at elevated pressures DNA comes into contact with endonucleases, which cleave DNA (Chilton et al., 1997). This condensation has been found in many other instances and it is reversible and, presumably, an enzyme responsible for renaturation is also involved. If this enzyme is deactivated by high pressure, the cell is no longer able to multiply.

2.1.6. Membrane-related events

Pressure treatment almost always involves a perturbation of the bacterial membrane. The same is true for heat treatment, but usually to a lesser extent. Pressure resistance is probably inversely related to the rigidity of the membrane. As an increase in pressure and a decrease in temperature reduce membrane fluidity, it can be expected that cells respond to this stress by altering the composition of the membrane. There is considerable evidence correlating the production of increased proportions of membrane unsaturated acids with bacterial growth at low temperature or high pressure. Fatty acids of barophilic microorganisms become more polyunsaturated with increasing growth pressure (DeLong and Yayanos, 1985; Kamimura et al., 1993). Wirsin et al. (1987) reported that in a psychrophilic and barophilic bacterium of the genus *Alteromonas*, the ratio of unsaturated to saturated fatty acid increased with growth pressure between 1 and 45 MPa at 2 C. The ratio of unsaturated fatty acids was higher when cells were grown at 2 °C than when grown at 10 °C. According to Allen et al. (1999), monounsaturated, rather than polyunsaturated fatty acids, would be required for growth of the deep-sea bacterium *Photobacterium profundum*.

Exponentially growing cells of *L. plantarum* were more resistant to pressure when the cells were grown at suboptimal temperatures (Smelt et al., 1994; Mackey et al., 1995). Under these conditions, the ratio of unsaturated fatty acids was higher in cells grown at optimum temperatures. Lanciotti et al. (1996) obtained similar results with *L. monocytogenes* and *Y. lipolytica* but sometimes, different trends for *E. coli.* Unfortunately, from their study, it is not known whether they used exponentially growing cultures or stationary phase cultures. This could explain the

exception of enhanced pressure resistance in *E. coli* when grown at 30 °C. It has also been shown that when cholesterol is included, the fluidity of cell membranes of eukaryotes decreases and the cells become more sensitive to pressure (MacDonald, 1992), emphasising the importance of this factor in pressure resistance. Cells in the stationary phase seem to be more pressure-resistant when they are grown at optimum temperatures compared to lower temperatures (Ulmer et al., 2002). This unexpected result might be due to the fact that the stationary grown cells are subjected to a number of stress conditions such as starvation and low pH. At lower temperatures, the same degree of stress response is probably attained after much longer incubation than at optimum temperature. The longer duration of the stationary phase at optimum temperature results in increased resistance to pressure (Hellemons and Smelt, 2002). This phenomenon has to be taken into account when stationary phase cells grown at different temperatures are compared.

The protective effect of different carbohydrates on the membrane in general is in the order glycerol < glucose < fructose < sucrose < trehalose and the same order was found for the protective effect of these carbohydrates against pressure (Crowe et al., 1984; Smelt et al., 1998). Propidium iodide and ethidium bromide bind to nucleic acids, but they can only penetrate into the cell when the membrane is damaged. Unlike untreated cells, pressure-treated bacteria can be stained with propidium iodide or ethidium iodide, indicating that membrane damage has occurred (Smelt et al., 1994; Benito et al., 1999). Pressure inactivation is also accompanied by an increase in extracellular ATP, again showing leakage of the membrane (Smelt et al., 1994). Integral and peripheral proteins become more detached from the plasma membrane when the membrane bilayer is sufficiently perturbed by pressure.

2.1.7. Events related to maintenance of intracellular pH

Microbial cells require their internal pH to remain constant in order to maintain essential cell functions. High-pressure treatment can result in reduced intracellular pH, while membrane damage may impair acid efflux.

A decrease in intracellular pH after pressure treatment has been found in *L. plantarum* (Wouters et al., 1998). The change in intracellular pH is accompanied by impaired acid efflux, but this change might also be caused by impaired glycolysis. The observations on membrane damage, protein inactivation, decrease of intracellular pH and the observations on yeasts suggest that membrane bound enzymes associated with efflux of protons might be a target in high-pressure inactivation. Membrane bound F_0F_1 ATPase may be a candidate for this as it may be inactivated or dislocated by pressure. Ulmer et al. (2002) observed that inactivation of *L. plantarum* by pressure coincided with inactivation of HorA, an ATP-binding cassette located in the membrane. As with heat, very severe pressure stress causes considerable damage to the cell and pressure-treated cells become more sensitive to adverse environmental conditions. Abe and Horokishi (1995) observed that a pressure of 40 to 60 MPa reduced the vacuolar pH in yeasts. They concluded that the yeast vacuole served as a sequestrant for protons to prevent the cytosol from acidification. Iwahashi et al. (1997) found that mutants of *S. cerevisiae*, which lacked the ability to accumulate trehalose and/or heat shock protein 104, showed both lower barotolerance and thermotolerance, but trehalose seemed to be more important for barotolerance.

2.2. Effect of temperature and pressure on bacterial spores

Bacterial spores have a compartmentalised structure. The central protoplast is encased by a wide cortex, which consists of peptidoglycan, and is further surrounded by a proteinaceous coat (Gould, 1999). Dehydration of the core guaranteed by this structure is the main factor causing resistance to environmental conditions such as heat, pressure or electric fields heat. Spores can be killed either directly or after a germination step. Once started, germination is an irreversible process. During germination, a number of events occur leading to a vegetative cell. Spores are phase bright and they become phase dark in an early stage of germination. Germination of spores is often preceded by an activation step. Contrary to germination the activation step is reversible. Comparatively high temperatures are required to activate the spore. It is not unlikely that also inactivation of spores is mostly preceded by an activation step. Leuschner and Lillford (2001) observed considerable changes

during activation. Activated spores can be distinguished from dormant spores by gradient density centrifugation (Beaman et al., 1988). Activated spores are less resistant to heat than dormant spores. As the heat resistance of germinated spores is comparable to that of vegetative cells, spores can be easier killed after germination. This two-step process, tyndallisation, has been studied for a more than 100 years, a small fraction survives mostly as it remains dormant during these mild treatments, which makes the treatment less effective. During the last 10 years, there is again more interest in the latter principle, due to interest in high-pressure decontamination.

Bacterial spores are extremely resistant to heat and also to pressure. Whereas vegetative cells and most spores of yeasts and moulds are easily inactivated by temperatures far below 75 °C and by pressures of of ~ 400 MPa, bacterial spores can survive temperatures above 80–100 °C and pressures over 1000 MPa. Within the group of spore formers, however, there seems to be no relation between resistance to heat and to pressure. For instance, one very heat-resistant strain of *Bacillus stearothermophilus* was relatively sensitive to pressure, whereas a heat-sensitive strain of *B. megaterium* was not inactivated after a treatment of 40 min, 1000 MPa. On the other hand, it has been shown that low pressures between 50 and 400 MPa can stimulate spores to germinate, followed by a rapid die off at pressures of about 400 MPa. This phenomenon was used in a process similar to tyndallisation. By oscillatory pressure treatments, alternating between 60 and 500 MPa of cycles of 1 min spores of *Bacillus subtilis* could be reduced by a factor $> 10^8$ (Sale et al., 1970; Timson and Short, 1965; Wuytack et al., 1998). Wuytack et al. (2000) studied *B. subtilis* spores and they suggested that pressure of 100 MPa triggers the germination cascades that are introduced by the nutrient L-alanine and by a mixture of asparagines, glucose, fructose and potassium ions (AGFK germination). At 600 MPa, a different kind of germination occurs that is difficult to distinguish from real death. According to the authors, at least part of the Ala and AGFK pathway would play a role in germination at 600 MPa. Therefore, they concluded that pressure treatment at 600 MPa is not merely a physico-chemical process in which water is forced into the spore protoplast. The authors found earlier that at 600 MPa, no germination occurred comparable to that at 100 MPa: small acid-soluble proteins present in the dormant spore that are usually lost during germination are also lost at 100 MPa, but not at 600 MPa. Bacterial spores loose their dipicolinic acid at high temperature and at vary high pressures. In summary, it can be concluded that there are many parallels between the mechanism of inactivation of bacterial spores by heat and by pressure. However, it is unlikely that the mechanisms are the same. It is very likely that activation rather than germination occurs at relatively high pressures and lethal temperatures. In both cases, the spores remain phase bright and they can loose their dipicolinic acid. At lower pressures germination occurs, whereas sublethal temperatures induce activation only.

2.3. Effect of chemical composition of model media and real foods on resistance to temperature and pressure

2.3.1. General aspects

The effects of food constituents on temperature and pressure resistance is complicated. Apart from studies in various foods, many studies in model media have been conducted to evaluate the effect of different food components. Some effects are driven by the effects of pressure on the molecules and especially water (Bridgeman, 1958; Makita, 1992). For instance, high pressure causes water to become solid by formation of different types of ice. The ice formation can be altered by the presence of high concentrations of solutes. Hardly any attention has been paid to the behaviour of complex foods in this respect. Whereas in pressure research much attention has been paid to the fact that the pH shift due to pressure is dependent on the type of buffer, similar effects due to temperature are hardly taken into account. In pressure treatments, the situation becomes even more complicated by the fact that during pressure treatments, temperature changes almost always occur. As mentioned above, the biochemical and molecular basis of the effects of heat and high pressure on microorganisms is poorly understood and for the most part is still empirical data. In real food situations, there are always two effects that determine microbiological safety and stability: the influence of the food during treatment and the fate of microorganisms in the food after treatment. It should also be taken into account that results of studies in buffers or labo-

ratory media cannot directly be extrapolated to real food situations. For instance, milk and cream protects microorganisms against temperature and pressure (Patterson et al., 1995; Carlez et al., 1992; Gervilla et al., 1999). Arroyo et al. subjected lettuce and tomatoes to pressure and they found a decrease in number of colony forming units of about 2 log cycles at a pressure of 350 MPa during 10 min. Shigehisha et al. (1991) subjected pork slurries to pressure and they found pressures as high as 600 MPa to be necessary to inactivate *Micrococcus luteus*, *Staphylococcus aureus* or *Streptococcus faecalis*. Proteins in general seem to protect microorganisms against pressure inactivation. Also, glucose seems to have a protective effect apart from water activity (Simpson and Gilmour, 1997a). Szczawinski et al. (1997) found that *L. monocytogenes* survived in Edam cheese after pressure treatment for 15 min at 500 MPa. Tanaka and Hatanaka (1992) developed an interesting application of hydrostatic pressure by stopping acidification of yoghurt using pressures between 200 and 300 MPa.

2.3.2. Acidity

The change in pH due to temperature is easy to measure in thermal studies but is often ignored. However, although it is known that pressure can alter pH significantly during treatment, this cannot be measured directly. Theoretical calculations can be made to estimate the pH shift in buffers, although each buffer will respond differently. When the activation volume of the buffer is large, the pH change due to pressure will also be large (Kitamura and Itoh, 1987). For instance, pressure causes a large pH shift in phosphate buffer. Despite this fact, Sörensen phosphate buffer is frequently used in microbial inactivation studies. In addition, buffers have their specific effects on the cell physiology. Pressure stable buffers like Tris and imidazol cause more rapid inactivation at the same pH than more physiological buffers such as PIPES and ACES buffers. (Hellemons and Smelt, 2002). Thus, choice of an appropriate buffer is dependent on the purpose of the investigation: whether the specific effect of pH is to be studied or whether a closer relationship with real food situations is studied. For instance, organic acids are relevant for many foods, but as they have several different physiological effects, in particular citric acid, the interpretation of the results can be difficult. Organic acids are antimicrobial due to the effects of pH and of undissociated acid molecules on microbial cells but no specific effect of organic acids during pressure treatment have been observed. This might be due to the fact that pressure favours ionisation of organic acids.

Yeasts and moulds are relatively resistant to low pH and a pH of 4.0 has little effect on these microorganisms when they are subjected to heat or pressure. Vegetative cells of bacteria become more sensitive to pressure and to heat in low pH conditions. (Ritz et al., 1998). In studying effects of physical treatments, the effects during treatment and the effects of the treatment on subsequent recovery should be distinguished. Bacteria surviving heat or pressure treatments become more sensitive to suboptimal pH after treatment. For instance, Garcia-Gaells et al. (1998) observed that a pressure-resistant mutant of *E. coli* was able to survive a pressure treatment of 500 MPa in fruit juice, but contrary to untreated cells, these cells gradually died in the fruit juice. Thus, low pH not only enhances inactivation directly during treatment but can also inhibit outgrowth of cells that have been subjected to heat or pressure.

2.3.3. Water activity

A low water activity protects proteins and whole organisms against heat and pressure. There is a plethora of data showing the protective effect of low water activity against heat. There are also a number of reports showing the protective effects of low water activity against pressure (Oxen and Knorr, 1993; Palou et al., 1997). Apart from the general osmotic effect of water activity on the cell, there are also specific effects. Salts confer less protection than carbohydrates against thermal or pressure damage. At the same water activity, thermotolerance and barotolerance is lower in glycerol than in solutions of monosaccharides and disaccharides. Trehalose seems to be particularly effective (Smelt et al., 1998). There are a number of explanations for the role of trehalose as a particular effective saccharide against environmental stress. According to Shinsuke et al. (1996), macromolecules are stabilised against heat and pressure, and barotolerance and thermotolerance seem to be linearly related to the number of equatorial OH groups. Although low water activity protects cells against environmental stress during treatment, the surviving microorganisms that have been injured by

heat or pressure are more difficult to recover at water activity values suboptimal for growth. For instance, Patterson et al. (1995) showed that recovery of pressure-treated cells was much lower when 2% salt was added to the medium. In conclusion, it can be stated that the net effect of lower water activity is not always easy to predict.

2.3.4. Other antimicrobial compounds

Sorbic acid, which acts as an organic acid, but also interferes with the microbial membrane, is more active in combination with pressure. Palou et al. (1997) showed that sorbate, whether or not combined with reduced water activity, could be used as an extra 'hurdle' to obtain pressure-treated foods. Microbes are particularly sensitive to nisin during or after pressure treatment. Kalchayanand et al. (1994) reported that sublethal injury sensitised not only *L. monocytogenes*, but also *E. coli* and *S. typhimurium* to nisin when pressure-treated. Apparently, Gram-negatives such as *E. coli* and *Salmonella* that are normally resistant to nisin can be sensitised to the agent when pressurised (Kalchayanand et al., 1994). This might be explained by the specific action of nisin. Nisin interacts with the cell membrane and it might be possible that it can penetrate to the inner cell membrane. The specific synergistic action of nisin and high pressure might occur via local immobilisation of phospholipids resulting in enhanced susceptibility to pressure (Russell et al., 1995; ter Steeg et al., 1999). During pressure treatment, *E. coli* was found to be sensitive to lysozyme, nisin and ethylenediaminetetraacetic acid (EDTA) separately and even more so in a combination of these compounds (Hauben et al., 1997). Propyl hexobenzoate and sodium ascorbate did not influence pressure resistance of *L. monocytogenes*. Ogawa et al. (1990) observed that the addition of 5–10 g/l allyl isothiocyanate increased the reduction of *Salmonella* at 200 MPa by 5 log cycles. Papineau et al. (1991) showed that chitosan salts had an additive effect on pressure inactivation. Kalchayanand et al. (1998a,b) investigated the combined effect of hydrostatic pressure, temperature and pediocin AcH (3000 U/ml). They found that the presence of pediocin during pressure resulted in an additional inactivation of 2 log cycles. In many studies on the effect of antimicrobials, the agent was only present during treatment. In real food situations, the agent is mostly still always present after treatment, which can contribute to an additional effect. Ponce et al. (1998a,b) studied the combined effect of nisin and high pressure on the destruction of *L. monocytogenes* and *E. coli* in liquid whole egg. An additional effect of nisin was found after a relatively severe pressure treatment (400 MPa for 15 min at 20 °C).

2.4. Pulsed electric field

The action of pulsed electric field (PEF) will be only briefly outlined here. More information can be found in the review articles by Wouters and Smelt (1997). Whereas the mode of action of temperature and pressure seems to be multi-targeted, in PEF inactivation, permeabilisation of the cytoplasmic membrane seems to be the single most important target. This feature is also applied as a recombinant DNA technique. Temporary holes in the membrane allow transformation of cells with foreign DNA. Although it cannot be ruled out that enzymes are affected in situ, studies of isolated enzymes have shown that PEF had no effect under the conditions usually applied (Van Loey et al., 2001). According to Weaver and Chizmadzhev (1996), cells have two possibilities for irreversible electroporation: (1) rupture of a portion of the membrane and (2) lysis as a consequence of chemical imbalance caused by molecular transport through transient pores. Wouters et al. (2001a,b) investigated cells of *L. plantarum* with propidium iodide (PI) staining and they investigated the cells afterwards with flow cytometry and image analysis. Larger cells seem to be more susceptible than smaller cells. Contrary to thermal and pressure inactivation, sublethal damage due to PEF does not seem to exist, but it seems an all or nothing effect (Simpson et al., 1999; Russell et al., 1995; Dutreux et al., 2000). Bacterial spores do not seem to be susceptible to PEF (Wouters et al., 2001a,b).

3. Development of inactivation models

3.1. Microbiological aspects

The choice of test strain(s) is of paramount importance and no single answer can be given to the question whether cocktails or single strains should

be used. As cocktails always contain the strain that is most resistant in a particular situation and thus a fail-safe situation is better guaranteed. The problem is that the interpretation of survivor curves is difficult. A satisfactory solution to the problem is a careful selection of one single test strain on resistance, followed by validation in a later stage. There are several reports on variation of strains with respect to their resistance to heat or to pressure (Robey et al., 2001; Whiting and Golden, 2002; Hellemons and Smelt, 2002) As for spores, resistance of vegetative cells to one physical agent does not necessarily imply high resistance to another physical agent (Hellemons and Smelt, 2002). Therefore, an extensive screening of the effect of the various factors determining resistance is necessary. Next to the selection of strains, the preparation of the inoculum is important. Exponentially growing cells are more susceptible than stationary phase cells to heat, pressure and possibly high electric fields. (Smelt et al., 1998; McClements et al., 2001; Pothakamury et al., 1996). Stationary phase cells are presumably stressed by starvation or toxic metabolites. A prolonged stationary phase can even result in an increased resistance to PEF (Wouters et al., 1999). It is a well-known observation that the heat resistance of vegetative cells and bacterial spores is enhanced at higher sporulation temperature (Rowan et al., 1999; Palop et al., 1999; Cazemier et al., 2001). On the other hand, there seems to be an inverse relation between cultivation temperature as shown above of vegetative cells and sporulation temperature and pressure resistance (Raso et al., 1998). Thus, problems arise in finding the cultivation conditions when the combined effects of heat and pressure are investigated. Finally, recovery conditions are important. Recovery is mostly conducted under optimum conditions, but it is certain that models based on results obtained with optimum recovery conditions will be unnecessary conservative to predict safety of real foods.

3.2. Selection of mathematical models

3.2.1. Temperature and pressure, primary models

3.2.1.1. Empirical models. Traditional thermal process models are often built on experience rather than systematic investigation of the inactivation kinetics of the relevant organism(s). These models do not take into account physiological effects such as specific sensitive target and stress response. More recent models take into account the shape of the inactivation curve, but although the models are based on some biological assumptions, most of these models are empirical. The traditional model describes thermal inactivation of microorganisms as exponential decrease of number of cells analogous to inactivation of many pure enzymes (Ludikhuyze et al., 1997; Weemaes et al., 1998a,b) As shown in Fig. 2a, the D value is used in microbiology to describe log-linear decrease. The D value is the time needed to kill 90% of the microbial population and is independent of the number of the population. Microbial inactivation is mostly expressed by the following equation:

$$\log(N_t) = \log(N_0) - (1/D)t$$

in which N_t is the number of microorganism at time t, N_0 is the number of microorganism at time 0, and D the decimal reduction time or D value.

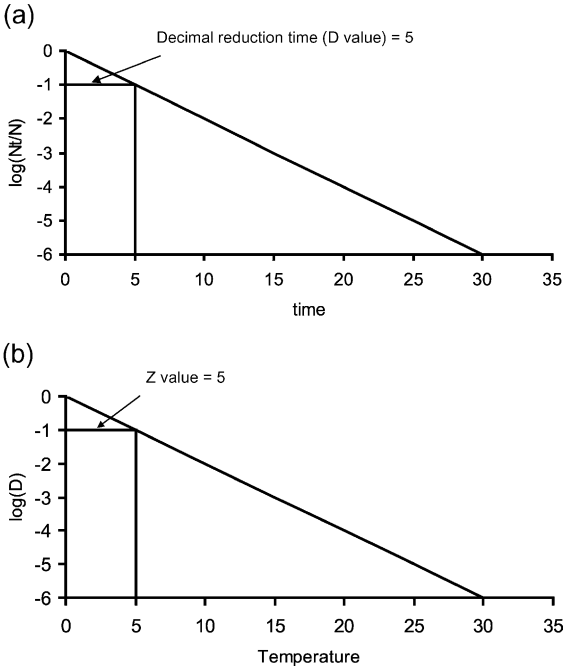

Fig. 2a and 2b. The traditional D (a) and z (b) model (log-linear modelling). D value is the time to reduce 90% of the population; z value is the temperature change corresponding with a change in D value of a factor 10.

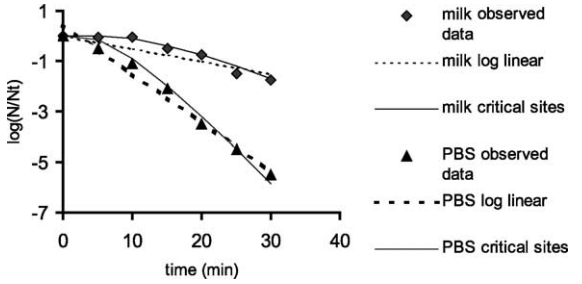

Fig. 3. Effect of pressure (375 MPa) on the inactivation of *L. monocytogenes* in phosphate buffer and milk (Patterson et al., 1995). The data were modelled according the traditional model (Fig. 2a and b) and according the critical sites model as explained in Section 3.2.1.2.

The D value is directly related to the inactivation rate k by the following relation:

$$D = (\ln(10))/k$$

As will be explained in the section on secondary models, a similar relation is assumed between. The first aim of the model was to ensure absence of *Clostridium botulinum* in sterilised foods and the excellent safety record of canned foods over 80 years has shown the value of this model. However, deviations from linearity of survivor curves have been observed too frequently to ignore. Curves are often characterised by 'shoulders' or 'tails' (Figs. 3 and 4). Especially spoilage problems due to 'tailing' can be a serious risk. In the last 10 years, a number of models have been developed to describe non-linearity. One group of models is empirical, e.g. a mirror image of a growth curve (Linton et al., 1995; Baranyi et al., 1996). Another empirical model was presented by Cole et al. (1993) and Anderson et al. (1996). Despite the excellent fit of the models of Linton et al. (1995), the models of Cole et al. (1993) and of Anderson et al. (1996), it is questionable whether extrapolation is justified. A general objection to the abovementioned models is the use of a parameter describing a final lower asymptote. An (upper) asymptote makes sense for description of growth, but what is questionable is whether a (lower) asymptote describes tailing in inactivation processes correctly. Instead, tailing might be better characterised by a curve descends at a continuously slower rate. The latter problem is elegantly solved by distribution models that are discussed below.

3.2.1.2. Critical sites model. Although Moats (1971) did not present a model in a mathematical form, his concept needs still attention. According to Moats (1971), a large number of the same critical sites, or a large number of different critical sites, need to be inactivated before the cell dies. These sites might be ribosomes (Anderson et al., 1991) or enzymes. Also, membrane damage could be considered as damage of critical sites (Earnshaw et al., 1995). The concept is in line with the observations on gradual increase of sublethal damage during treatment. Sublethal treatment is reflected by an increased sensitivity to adverse environmental conditions such as the presence of antibiotics, high

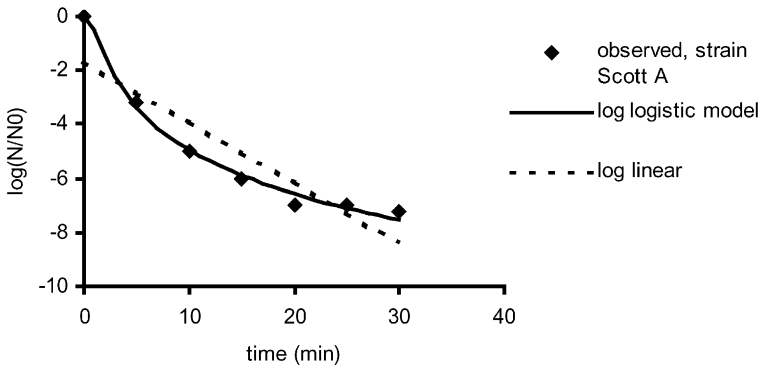

Fig. 4. Example of a fit with the traditional log-linear model and a distribution (log-logistic) model.

osmolarity or low pH, and also by a longer lag of sublethally damaged cells, when the cell resumes growth after treatment. As was mentioned above, there is a lack of knowledge of the target sites of heat and pressure inactivation let alone more quantitative data. We have worked out the idea of Moats (1971) further by assuming a minimum number of critical molecules or critical sites that must always remain intact for survival and that the minimum number would be dependent on the recovery conditions. Under more inimical recovery conditions, the minimum number would be higher. Here, we propose a possible numerical expression of the model: The cell contains n critical sites. Each critical site has a certain probability to survive (N_{ct}/N_{c0}). N_{ct} is the number of intact critical sites at time t and N_{c0} the number of intact critical sites at time 0.A first order inactivation of the critical sites is assumed, which means that the probability p of survival of a critical site is $(N_{ct}/N_{c0})e^{-kt}$, k is the inactivation rate of a critical site. When the number of critical sites per cell is equal or below a certain minimum number ($m+1$, $m<n$), the cell is dead. The probability of a cell to be dead (N_{bt}/N_{b0}) is the cumulative probability of a cell having $<m$ critical sites or a cumulative binomial distribution:

$$F(m) = \sum \binom{n}{i} p^i (1-p)^{n-i} \quad (1)$$

Note: summation goes from $i=0$ to $i=m$.

By substitution of p $\exp(-k_1 t)$, the distribution becomes:

$$(N_{bt}/N_{b0}) = F(m) = \sum \binom{n}{i} (\exp(-k_1 t))^i \times (1-(\exp(-k_1 t)))^{n-i} \quad (2)$$

Hence, the probability of a cell to be survive is:

$$1-(N_{bt}/N_{b0}) = 1 - \sum \binom{n}{i} (\exp(-k_1 t))^i \times (1-(\exp(-k_1 t)))^{n-i} \quad (3)$$

$$\log(1-(N_{bt}/N_{b0})) = \log\left(1 - \sum \binom{n}{i}\right) \times (\exp(-k_1 t))^i \times (1-(\exp(-k_1 t)))^{n-i} \quad (4)$$

An example of a fit with this model is given in Fig. 3.

A model that is numerical similar to this model is the possibility that organisms are present as clumps containing cells of the same resistance and where each clump results in one colony.

3.2.1.3. Other models. Another hypothesis put forward by Han (1975, 1976) is the assumption that increased resistance occurs during heating. This hypothesis would lead to tailing. At that time, less was known of stress response and it might be possible that a cell can be damaged or can become more resistant during exposure to stress. The model could be described as a probability model: Cells have a small probability per time unit (preferably in an infinitely small time period) to enter a heat-resistant state or another probability to die also in an infinitely small time period. Once the cells had become resistant, there is a certain probability to die but this probability is much lower than for sensitive cells in the same time period. Another possibility would be a gradual decrease of the inactivation rate (or increase of the D value) to an asymptotic value. This model could be combined with the critical sites' model by assuming that the critical sites become more resistant, e.g. by stress response.

Pagán et al. (1997) studied the effect of previous heating during different times and different temperatures on the inactivation kinetics, and they showed that both the duration of previous heat treatment had an effect on the subsequent heat resistance. This model can be built based on the abovementioned hypothesis and Pagan's data. These data show that an inactivation model can be developed that incorporates the effect of slow heating. So far, the inactivation models are based on the assumption of a homogeneous population, where each cell has the same probability to die. The probability can be a result of inactivation of a cell or a number of critical site(s) per cell, the probability of inactivation might also change during treatment. Although it will be difficult to test such a model in a rigorous way with biological data, it

must possible to gather circumstantial evidence for testing such a model. Cerf (1977) reviewed a number of articles and proposed a two-fraction model. The population is characterised by a large heat-sensitive fraction and a small heat-resistant fraction, and each subpopulation follows first kinetics. Xiong et al. (1999) developed a model by combining the idea of Cerf (1977) and implicitly the idea of Moats (1971). Their model can produce both a shoulder and a tail. The idea of inactivation of a heterogeneous population has been more generalised in a series of articles by Peleg and Cole (1998), Peleg (1996, 2000) and Peleg and Penchina (2000), and by Kilsby et al. (2001). Peleg (1996) proposed the Fermi equation, which is in fact equal to a (log)logistic distribution. They consider a cell population as intrinsically heterogeneous. Indeed, apart from some small genetic variation due to mutation, considerable phenotypic variation can be expected. McAdams and Arkin (1997) collected strong arguments for stochastic mechanisms in gene expression leading to phenotypical heterogeneity of a population. It might be possible that even the resistance of synchronised cells is heterogeneous. Given a heterogeneous cell population, these models can be considered as deterministic. Peleg (1996) worked out this idea and they assumed that the distribution of the resistance of the cell in a population follows a log-logistic or a log-normal distribution. The mathematical expression of this model is given in Table 1. Peleg and Cole (1998) investigated a large number of curves and they also concluded that a number of curves that were considered as semi-logarithmic were in fact slightly curved. This curvature can have a significant effect on inactivation when determined by extrapolation. If this is true, it can be expected that models based on heterogeneous populations are not limited to inactivation by heat. Indeed, Peleg and Cole (1998) could describe inactivation by heat and by pressure by assuming that resistance was distributed by a Weibull distribution. The idea of a Weibull distribution in pressure inactivation was also put forward by Heinz and Knorr (1996). Their model has some resemblance to the model of Moats (1971) as it assumes that there is a transient stage before the organisms are killed. It should be borne in mind that the distribution models do not take into account specific mechanisms of inactivation apart from the assumption of heterogeneity. More recently, Kilsby et al. (2001) proposed a distribution model based on the Prentice distribution. The model contains more parameters than most other models, but for their data, the fit was better than for a normal distribution. As in the model of Cole et al. (1993), this model contains an equation with log time; hence, time zero cannot be plotted as log 0 is minus infinite. This problem is mostly solved by choosing a very short time period assuming that after this period of time the number is equal to that of time 0. Peleg and Cole (1998) and Van Boekel (2002) investigated thoroughly the use of the Weibull distribution to describe thermal inactivation of microbial vegetative cells, and they concluded that the Weibull model performs much better than the classical first order approach. Van Boekel (2002) investigated 55 data sets of which 33 showed a convex decline and 18 a concave decline. So far, the models mentioned above describe mainly lethal inactivation. As was pointed out above, it is very unlikely that lethal inactivation is a single hit event. In many instances, sublethal inactivation can be sufficient either when the pH or water activity is not optimal for the organism or for products with limited shelf life. To enhance the flexibility of a model, the gradual transition from partly damaged cells to completely inactivated cells should be described. There are several options to include sublethal damage in an inactivation model: According to the concept of Moats, a transient stage exists between complete inactivation and undamaged cells. Another option would be a change in distribution of the resistance of cells depending on the time of treatment. Many aspects dealt with in the previous paragraph apply equally well for pressure. Lower recovery under suboptimum conditions and prolonged lag times after sublethal pressure treatments suggest that inactivation by pressure is not caused by a single hit either. There is no reason that heterogeneity of the cell population would play no role in pressure inactivation.

The number of nonlinear inactivation curves seems to occur more frequently in pressure inactivation than in temperature inactivation (Metrick et al., 1989; Ludwig et al., 1992; Patterson et al., 1995). These curves are often characterised by tailing, although log-linear inactivation is also found in pressure inactivation (Cheng and Tseng, 1997; Mussa and Ramaswamy, 1997; Mussa et al., 1999). The initial drop in pressure inactivation can partly be explained by the pressure come-up time. The coming up time is often

ignored in pressure studies (Palou et al., 1998b). In conclusion, it can be stated that nonlinear inactivation is in line with several hypotheses. It might be that it depends on the situation which factors can be ignored. Experimental evidence to corroborate the models is badly needed. It can be obtained by recovery studies, single cell studies in image analysis or flow cytometry. Knowledge of the numbers of ribosomes and their inactivation in the cell might substantiate the critical sites' model. Also, DNA micro-array studies would offer new opportunities. Studies with nisin during recovery could be a tool to quantify membrane damage. So far, the models discussed above apply to vegetative cells rather than to bacterial spores. The mechanism of spores is fundamentally different from that of vegetative cells.

Many of the considerations for vegetative cells apply equally well for spores: Inactivation of a number of critical sites resulting in sublethal damage prior to death, clumping, phenotypical heterogeneity—presumably even more significant for spores than vegetative cells. On the other hand, contrary spores have specific features to be able to cope with environmental stress, as present in vegetative cells, no mechanism is known by which spores become more resistant during exposure to stress. A clear difference has to be made between activation followed either by germination or by direct inactivation At relatively low pressure, i.e. 50–200 MPa, spores can be killed by initial germination followed by death of germinated spores. Tailing of spores might also be caused by a heterogeneity of the *activation* rate during heating. Spore specific traits such as activation and germination have been discussed in a previous chapter. A model describing the effect of activation has been proposed by Sapru et al. (1993). The model of Sapru was criticised by Geeraerd et al. (2000) that a flat shoulder can hardly be described by this model. However, by assuming very low k values for activation combined with the concept of Moats (1971), flat shoulders can be described. This problem is possibly solved by a simple modification of the equation. Rodriguez and Smerage (1996) developed an equation with one term equal to the classical exponential model and similar term describing the transition from a dormant spore to an activated spore.

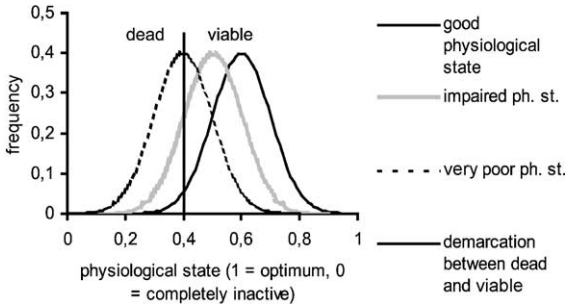

Fig. 5. Hypothetical model describing the effect of physical stress on a gradual impairment of the physiological state of a population of cells.

Although mentioned briefly when the concept of Moats discussed, inactivation models for vegetative cells and for spores do generally not include sublethal damage. Apart from applying the concept of Moats, in principle, distribution models can be easy adapted to include sublethal damage, as is shown in Fig. 5. In this figure, a distribution of physiological state is assumed. Physiological state is not yet clearly defined but it might refer to the lag time, number of critical molecules per cell. In this example, physiological state of 1 means optimum physiological state and 0 the poorest physiological state possible. When the physiological state impairs, there is a moment when cells can no longer divide but still perform some physiological function. In the figure, a demarcation line is given between dead and alive. The figure also shows that, thus, sublethal damage and death can be described as 'moving' distribution function.

3.2.2. Temperature and pressure, secondary models

As there is a need for a dynamic description when dealing with time-varying (and spatially varying) temperatures within a food product, the relation between inactivation rate and temperature should be known. In reaction kinetics of enzymes, Arrhenius or Eyring equations are used to describe inactivation. This approach is often followed in microbiology, but is questionable whether the constants of these equations remain the same over a wide temperature range. In thermal processing, an even more empirical approach is generally followed: the relation between inactivation rate is usually described as log-linear. The inverse of the slope is usually designated as z value. As shown in Fig. 2b, there is a direct relation between

the slope of the curve and the z value. In food processing, the following equation is mostly used:

$$\log(D) = a - (1/z)T$$

D is D value; a is a constant; z is the z value, i.e. the temperature difference corresponding to D values differing by a factor 10; T is temperature mostly expressed in °C.

Instead of a log-linear model, a log-polynomial model was also proposed. As was shown by Peleg and Cole (1998) and Van Boekel (2002), the Weibull distribution seems to describe inactivation very well. Van Boekel showed that one of the two Weibull parameters was strongly dependent on temperature, whereas the dependence of the other parameter was not very large. From his results, it can be concluded that the Weibull distribution is also very suitable to describe dynamic situations. For secondary models, the traditional z concept has been also applied to pressure (Ludikhuyze et al., 2000; Smelt and Rijke, 1992; Reyns et al., 2000). Equations analogous to the z concept have been proposed: z_P is the increase in pressure (mostly in MPa) that would produce a reduction in D of a factor 10. Whereas the use of D and z values in thermal processes is useful, although an oversimplification, the use of similar parameters in pressure processing is even more questionable. Within a relatively narrow pressure range at isothermal conditions, this model is satisfactory, but the relation between log(D) and pressure is no longer linear at a wider pressure range. Instead of z values, sometimes, equations analogous to Arrhenius or Eyring equations have been proposed (Table 2). As temperature always plays a role in pressure inactivation, a combined temperature pressure model has also been proposed. Sonoike et al. (1992) used an equation that contained a quadratic term for temperature. This is necessary as there exists an optimum temperature at which enzymes and microorganisms are most resistant (Hawley, 1971; Sonoike et al., 1992). Besides, a quadric term has been introduced for pressure as well. Although pressure acts instantaneous, temperature gradients always arise both spatial and time-wise. These temperature gradients arise due to a combination of adiabatic heating and cooling of the pressure vessel. In temperature inactivation, a relatively small temperature range (e.g. 20–30 °C) is relevant for the target organism. Whereas in pressure inactivation, a wide temperature range varying from subzero temperatures to temperatures up to 100 °C can be relevant. Although there is some knowledge of the mechanism of pressure inactivation, it is not sufficient to develop mechanistic models. As stated before, there seems to be good reason to neglect the effect of compression and decompression for vegetative cells apart from the effect of the lethality caused by the integrated time and pressure. This facilitates the development of a dynamic model.

3.2.3. Inactivation models for pulsed electric fields

Contrary to temperature and pressure, the physiological effects of electric fields seem to be unequivocal. However, modelling PEF inactivation is complicated by the large number of different parameters that are involved. It is not always easy to separate the effect of different parameters. As for pressure, temperature effects play a role during PEF treatment since the temperature increases. The following process parameters determine the effectiveness of PEF treatment: electric field strength, pulse wave-shape, pulse length and number of pulses and temperature. The effects of temperature are difficult to separate from the electric effects. Although it is clear that the electric effects are the main inactivation factors at low temperature, there is almost always an interaction between temperature and the electric effects. A good curve fit is obtained by using the log-logistic model (Cole et al., 1993). Various models have been proposed to describe the kinetics of microbial inactivation by PEF. A model developed by Abram et al. (submitted for publication) incorporates all parameters, but it should be noticed that more investigations are necessary to solve the problem of large confidence intervals.

4. Setting inactivation criteria, quantitative microbiological risk assessment

Although there seems to be consensus with respect to the required inactivation factors, these standards are probably based on empirical grounds rather than sound data. So far, these standards are, however, good guidelines as shown by the good safety record with respect to sterilisation and pasteurisation. It might be that a combination of some over processing and poor

understanding of the exact inactivation kinetics results in a safe product. It is possible that over-processing of a few °C does not impair the product quality in traditional heating. However, as a consequence, the same lethality may be required for novel processes and this unnecessary over processing might become a problem when novel decontamination processes are applied. To overcome this problem, not only accurate and robust inactivation models are necessary, but also a better estimate of the required inactivation factor. To obtain that factor, a formal risk assessment can be of great help. A formal quantitative risk assessment requires a considerable effort and the outcome of it will contain mostly a high degree of uncertainty. Yet, a quantitative risk assessment will help to set consistent inactivation criteria for different situations. The structure of the risk assessment procedure has been discussed elsewhere (Whiting and Buchanan, 1997; Anonymous, 1997; Hoornstra and Notermans, 2001) and uncertainty and variability should be taken into account. A simplified example will show the principle of the procedure to set a decontamination criterion. To ensure safety of pâté de foie gras with respect to *L. monocytogenes* pressurisation of pate de foie gras would be an option. Let us assume that (i) *L. monocytogenes* can grow on foie gras, (ii) contamination by *L. monocytogenes* is 10/g immediately before pressure treatment (Nichols et al., 1998; Dominguez et al., 2001), (iii) the maximum tolerable concentration for healthy people is 10^4/g (Lindquist and Westlöö, 2000). As *L. monocytogenes* can grow to numbers far above 10^4, the product should be free from *L. monocytogenes*. Ideally, the risk should be zero. In practice, zero-risk can never be realised. Hence, a very low risk should be accepted, e.g. 10^{-8}. To ensure this risk, a reduction factor of 10^9 should be realised. We realise that the examples are grossly simplified as uncertainty and variability of contamination, growth behaviour before decontamination and, finally, estimation of dose–response plays an important role.

5. Concluding remarks

Log-linear inactivation models are among the first mathematical models used food microbiology. The models are simple and have proven their value in heat processing over 80 years, although this may be due to systematic over-processing. As serious deviations from log-linearity occur too frequent to ignore, in the last 10 years, a number of polynomial or nonlinear models have been developed that describe the inactivation curve adequately. Many of these models are purely based on statistical principles and therefore do not allow extrapolation. Extrapolation is relevant for flexibility in food processing, but is only possible when adequate knowledge of the physiology of the microbial cell population is available. Hence, mechanistic models are necessary that are based on hypotheses that can be tested. In this review, an inventory has been made of the current knowledge on the mechanisms of inactivation by heat, pressure and electric fields. This knowledge should be used when developing realistic inactivation models. In the second part, the review describes existing models that were based on various physiological assumptions. Ideally, the first and second parts of the review are matched for future modelling exercises. Tailing of survival curves is among the first problems to be solved. Next to knowledge on the physiology of whole populations, the behaviour of individual cells in the population should be known. Thus, stochastic elements, particularly heterogeneity of the population, should be investigated. Moreover, relatively little attention has been paid to the continuum varying from mild sublethal damage to lethal inactivation. Models that describe kinetics of various degrees of damage are probably of great help in food industry.

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