Journal of Applied Microbiology Symposium Supplement 2000, 88, 79S–89S

Thermal inactivation of *Escherichia coli* O157:H7

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1. SUMMARY

Verotoxin-producing *Escherichia coli* O157:H7 is a cause of serious foodborne illness. It has a very small infectious dose and so it is vital to eliminate this pathogen from food. As heat treatment is the method of bacterial destruction most frequently used in food processing, accurate prediction of thermal death rates is necessary to achieve desired safety margins whilst minimizing processing. In most studies thermal inactivation has been described using first-order reaction kinetics and *D*-values. Whilst this approach does not seem justified on a theoretical basis, and may increase inaccuracy, there is no doubt that it is convenient and in many cases provides an adequate description of thermal death. A review of published data on the measured thermal inactivation of *E. coli* O157:H7 shows no strong evidence that a heat treatment of 70 °C for 2 min (or equivalent) fails to deliver a 6-decimal reduction in cell numbers.

2. INTRODUCTION

Although many strains of *Escherichia coli* are harmless inhabitants of the gastrointestinal tract, some can cause disease. The pathogenic strains at present of most interest are verotoxin-producing *E. coli* (VTEC). They produce two immunologically distinct types of verotoxin, VT1 and VT2, that are cytotoxic to vero cells (from the kidney of the African Green Monkey). Individual strains produce either one or both verotoxins (Chart 1998). VT1 is immunologically identical to and has the same biological activity as the shiga toxin of *Shigella dysenteriae* (O'Brien and Holmes 1987). VTEC have been associated with a range of human illnesses, varying from mild diarrhoea to serious illness, including severe bloody diarrhoea (haemorrhagic colitis), haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopoenic purpura (TTP) (Coia 1998). Fatality rates can be high in the very young and elderly.

VTEC first came to prominence as a foodborne pathogen in 1982, when serotype O157:H7 was associated with two outbreaks of haemorrhagic colitis linked to the consumption of contaminated undercooked ground beef in the Western USA (Riley *et al.* 1982). Although serotype O157 strains are the most commonly reported cause of VTEC infection in the UK (Simmons 1997), more than 100 serotypes of *E. coli* can produce verotoxins (Coia 1998). Other serogroups associated with human infection include O26, O91, O103, O111, and non-H7 strains of O157 (Goldwater and Bettelheim 1998; Paton and Paton 1998). Food poisoning outbreaks have been associated with minced beef and other beef products, cured and fermented meat products, raw milk, milk products (e.g. cheese, yoghurt), apple juice, and raw vegetables and salads (Coia 1998). Person-to-person contact, environmental exposure (e.g. farm visits) and drinking or swimming in contaminated water are also thought to be important sources of infection. The largest outbreak of food poisoning associated with verotoxin-pro-

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ducing *E. coli* O157:H7 in the UK occurred in central Scotland in 1996. It involved separate but related incidents, including a church lunch, a birthday party and retail sales of contaminated meat. In total, 496 people are known to have been infected, of whom 27 developed HUS or TTP and 20 died (Pennington 1998).

Estimates of the infectious dose of VTEC are as low as 1–100 cells (Paton and Paton 1998). Consequently it is vital to eliminate this pathogen from food, rather than merely to prevent its growth. Heat treatment is the method of bacterial destruction most frequently used in food processing. Accurate information on thermal death rates is important to food processors in order to achieve the desired safety margins whilst avoiding over-processing.

If it is assumed that all cells in a population are identical and that heat acts by destroying a single target, perhaps a single molecule or single site per cell, the rate of death can then be described by first-order reaction kinetics. Here, plotting the logarithm of the number of survivors against heating-time results in a linear relationship, the so-called log-linear plot, in which the rate of destruction is independent of the number of cells or of time. From this line the *D*-value, the time taken to reduce the population by 90%, a one decimal reduction, can be calculated. This approach is straightforward and has been used extensively in calculations of the thermal inactivation of bacterial cells by wet heat. In an alternative approach, termed the 'end-point method', an assumption of first-order reaction kinetics is made, and the *D*-value is calculated from the heating-time required to bring about a defined number of decimal reductions. If the logarithm of the *D*-value against temperature is plotted, it is possible to calculate the *z*-value, the temperature increase required to reduce the *D*-value by a factor of 10. In this review published literature on the thermal inactivation of *E. coli* O157:H7 is summarized.

3. REVIEW OF PUBLISHED DATA

Many authors have studied the thermal destruction of *E. coli* O157:H7 in foods and in culture media. Data in the range of 50–70 °C are summarized in Fig. 1. The best straight line was fitted by linear regression. The equation of the fitted line, the correlation coefficient (R^2), and the *z*-values from the fitted lines are summarized in Table 1. A predictive model of the thermal inactivation of *E. coli* O157:H7 has also been developed as part of the UK Predictive Microbiology Programme and is available in Food MicroModel (Blackburn *et al.* 1997).

For all menstrua and for culture media and buffer, the correlation between heating temperature and measured *D*-value was weak (Fig. 1a,b, Table 1). Much of the observed variation is due to differences in test conditions and experimental procedures. The effect of factors such as adverse pH, antimicrobials and the use of different enumeration media have been widely tested. For example, the $D_{52\,°C}$-values for *E. coli* O157:H7 heated in Tryptone Soya Broth (TSB) at pH 6·0 and pH 4·8 were 75 and 46 min, respectively (Clavero and Beuchat 1996). Thermal death curve data from apple juice, all meat, poultry meat and red meat samples conform better to a linear relationship (Figs 1c–1f), with an R^2 of 0·75–0·86 (Table 1). This probably reflects a greater uniformity of experimental procedures. From the line of best fit for all meat the $D_{60\,°C}$ is 1·8 min and the *z*-value is 5·5 °C. The range of reported $D_{60\,°C}$ is 0·3–10·0 min (Fig. 1).

The *z*-values reported in the original literature or calculated from papers where thermal destruction was assessed at three or more temperatures in the same heating menstruum are summarized in Fig. 2 and Table 1. The highest *z*-value was 7·29 °C for cells heated in chicken slurry (Betts *et al.* 1993) and the lowest value for serotype O157:H7 was

Table 1 Summary of published data on the effect of different heating menstrua on the thermal destruction of *Escherichia coli* O157:H7

Menstruum	Parameters of lines of best fit*				Mean *z*-value (°C) from published data‡
	Equation for fitted line	R^2	*z*-value (°C)	$D_{60\,°C}$	
All menstrua	Log D = 8·04–0·13t	0·54†	7·6	1·6	4·9 (S.D. = 0·8, *n* = 72)
Broth and buffers	Log D = 5·96–0·09t	0·31†	10·5	1·7	5·5 (S.D. = 1·0, *n* = 11)
Apple juice	Log D = 8·02–0·14t	0·75	7·4	0·8	4·8 (*n* = 1)
All meat	Log D = 11·18–0·18t	0·85	5·5	1·8	4·8 (S.D. = 0·7, *n* = 60)
Poultry meat	Log D = 11·23–0·18t	0·81	5·5	1·7	5·1 (S.D. = 0·9, *n* = 22)
Red meat	Log D = 11·22–0·18t	0·86	5·5	1·9	4·6 (S.D. = 0·5, *n* = 38)

*From the lines of best fit in Fig. 1. †These correlations are weak. The equation for the fitted line and the *z*-value from the fitted line should not be used in thermal death calculations. ‡S.D. = standard deviation; *n* = number of *z*-values reported in the literature. Raw data for all menstrua are plotted in Fig. 2.

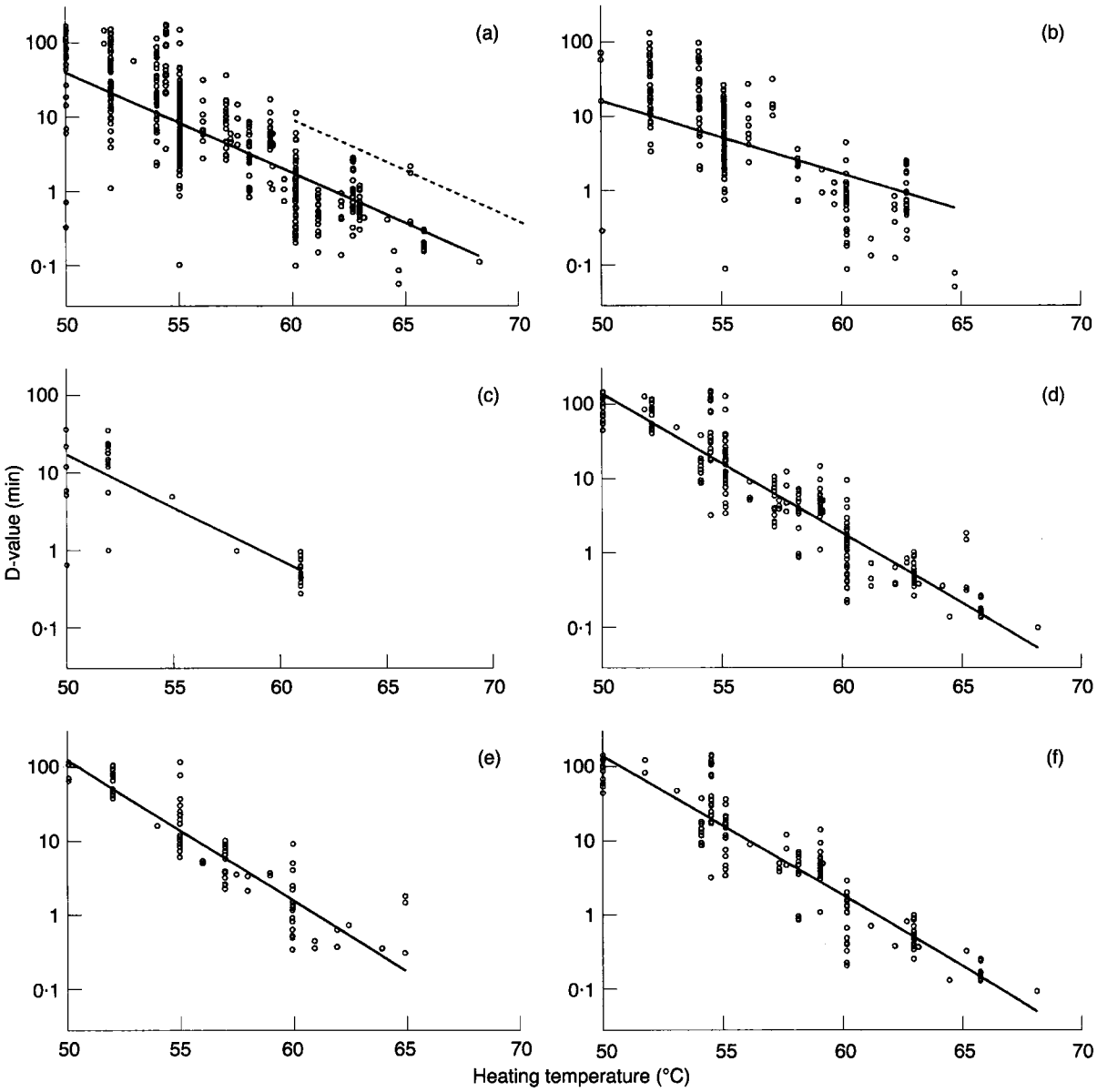

Fig. 1 Summary of published heat resistance data for *Escherichia coli* O157:H7 heated in all menstrua (a), broth and buffer (b), apple juice (c), all meat (d), poultry meat (e) and red meat (f). The solid lines indicate the line of best fit (see Table 1 for parameters of these lines), and the broken line on (a) indicates the 'line of safety' corresponding to a 1D reduction from the recommendations of the ACMSF (1995). Data are from: Doyle and Schoeni 1984; Line *et al.* 1991; Murano and Pierson 1992, 1993; Abdul-Raouf *et al.* 1993; Betts *et al.* 1993; Nishikawa *et al.* 1993; Todd *et al.* 1993; Ahmed and Conner 1995; Ahmed *et al.* 1995; Clavero and Beuchat 1995, 1996; Czechowicz *et al.* 1996; Jackson *et al.* 1996; Splittstoesser *et al.* 1996; Teo *et al.* 1996; Blackburn *et al.* 1997; Juneja *et al.* 1997a; Kotrola and Conner 1997; Kotrola *et al.* 1997; Orta-Ramirez *et al.* 1997; Williams and Ingham 1997; Clavero *et al.* 1998; Ingham and Uljas 1998; Juneja *et al.* 1998; Kaur *et al.* 1998; Quintavalla *et al.* 1998; Semanchek and Golden 1998; Williams and Ingham 1998; Veeramuthu *et al.* 1998; Duffy *et al.* 1999; George *et al.* 1999; Stringer *et al.* 2000

3·9 °C for cells heated in ground beef and enumerated on MEMB (Clavero *et al.* 1998). A *z*-value of 3·5 °C was reported for a VTEC strain of serotype O157:NM in beef (Todd *et al.* 1993). Of the 72 reported *z*-values, three were below 4·0 °C, 58 were 4·0–5·9 °C, nine were 6·0–6·9 °C, and two were 7·0 °C or above (Fig. 2). The mean *z*-value for all menstrua was 4·9 °C, with a standard deviation of 0·8 °C (Table 1). The mean *z*-value for each heating men-

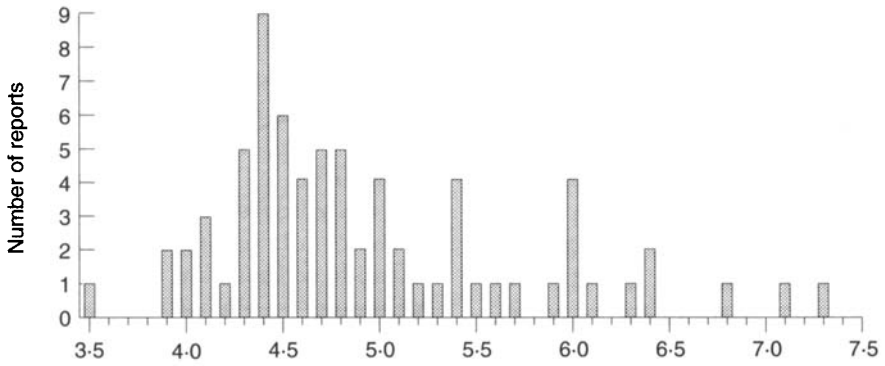

Fig. 2 Summary of published *z*-values for *Escherichia coli* O157:H7. Data are from references in Fig. 1

struum was in the range 4·6–5·5 °C (Table 1). It is noteworthy that whilst an average *z*-value seems to be in the region of 5 °C, the Advisory Committee on the Microbiological Safety of Food (ACMSF) used a *z*-value of approximately 7 °C (ACMSF 1995).

Although measured heat resistance can vary considerably with test conditions, none of the published data suggests that *E. coli* O157:H7 is unusually heat-resistant as compared with other nonspore-forming foodborne pathogens such as *Listeria monocytogenes* (Mackey and Bratchell 1989; George *et al.* 1998). In the UK, the ACMSF report on VTEC recommends that minced beef and minced beef products, including beefburgers, should be heated to an internal temperature of 70 °C for 2 min or its equivalent (Table 2). Similar recommendations have been made by the Food Safety Authority of Ireland (1999). These heat treatments were considered to be sufficient to give a 6D (six decimal) reduction in numbers of VTEC (ACMSF 1995). By dividing the times to a 6D reduction by six, the maximum safe *D*-value can be calculated for each temperature, and this is plotted as a "line of safety" in Fig. 1(a). From this figure, it is apparent that almost all of the reported *D*-values in the range 60–70 °C lie below the line of safety (Fig. 1a). The three points that lie above the line are from the study of Veeramuthu *et al.* (1998) who reported a $D_{60\,°C}$ of 10·0 min and $D_{65\,°C}$ of 1·7 min and 2·1 min in ground turkey. High heat resistance at temperatures above 70 °C has been reported. There was an estimated 5D reduction in *E. coli* O157:H7 in pepperoni on a frozen pizza baked in an oven at 191 °C (375 °F) for 20 min or at 246 °C (475 °F) for 15 min (Faith *et al.* 1998). It is difficult to compare these results with the data below 70 °C, because the temperature of the pepperoni was variable and did not reach that of the oven, and the bacterium may have been protected by a low water activity due to a high fat content and drying of the pepperoni.

Table 2 Equivalent heat treatments recommended for minced beef products (ACMSF 1995)

Temperature (°C)	Heating time
60	45 min
65	10 min
70	2 min
75	30 s
80	6 s

If all the published data are taken into account, there is no strong evidence that a heat treatment of 70 °C for 2 min, or its equivalent, is insufficient to give a 6D reduction of *E. coli* O157:H7. From Fig. 1 it would, however, seem unsafe to extrapolate the ACMSF recommendations (ACMSF 1995) to temperatures below 60 °C.

The heat treatments recommended by the ACMSF for the UK differ from those specified in the USA Food Code published in 1997 (FDA 1997) and updated advice issued in 1998 (FSIS 1998). The advice was that, in restaurants, ground beef patties should be heated to an internal temperature of 68·3 °C (155 °F) for 15 s, and in the home burgers should be cooked to an internal temperature of 71·1 °C (160 °F), as measured using a food thermometer (FSIS 1998). In tests with a mixture of five strains, heating ground beef patties to an internal endpoint temperature of 68·3 °C (155 °F) was sufficient to give a 4D reduction of *E. coli* O157:H7 (Juneja *et al.* 1997b).

4. FACTORS THAT AFFECT THE MEASURED THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7

The measured thermal resistance of a species can be influenced by many factors, including the growth conditions, such as the growth phase of the cells, composition, pH and water activity of the growth medium, growth temperature, holding period before heat treatment, heat shock, the heating method, for example use of open heating system and rate of heating, the heating menstruum, including its composition, pH, water activity and choice of humectant, and the recovery conditions (Olsen and Nottingham 1980). Heat resistance may also be affected by physical interactions such as attachment to solid surfaces. For example, the $D_{58\,°C}$ of *Salmonella typhimurium* attached to muscle was higher than that for free cells (Humphrey *et al.* 1997) and the presence of filterable pulp in apple juice enhanced the measured thermal tolerance of two strains of *E. coli* O157:H7 (Ingham and Uljas 1998). Several authors have quantified the effect of the experimental procedure on measured heat resistance and the conclusions from some of these studies are presented below.

4.1. Effect of growth conditions

The ability of *E. coli* O157:H7 to tolerate heat is strain-dependent (Betts *et al.* 1993; Ahmed and Conner 1995; Clavero and Beuchat 1995; Blackburn *et al.* 1997; Clavero *et al.* 1998; Quintavalla *et al.* 1998; Duffy *et al.* 1999; Fig. 3). A comparison of the heat resistance of seven strains of *E. coli* O157:H7 gave $D_{60\,°C}$ from 0·44 to 1·29 min, with strain 30-2C4 showing the highest heat resistance and strain W2-2 the lowest (Fig. 3). Clavero *et al.* (1998) compared the heat resistance of 10 strains at four temperatures. The ranking of the strains, in terms of heat resistance, varied with heating temperature. $D_{58·9\,°C}$ ranged from 4·9 to 15·0 min and $D_{62·8\,°C}$ ranged from 0·56 to 1·14 min.

Heat resistance was greater when cells were in the stationary rather than the logarithmic growth phase (Todd *et al.* 1993; Jackson *et al.* 1996; Kaur *et al.* 1998), and when cells were grown at 37 or 40 °C rather than 10, 23, 25 or 30 °C (Jackson *et al.* 1996; Kaur *et al.* 1998; Semanchek and Golden 1998; Fig. 3). For example, the heating time at 55 °C required for a 5D reduction was 7·3 min for exponential-phase cells grown at 23 °C, and 106·4 min for stationary-phase cells grown at 37 °C (Jackson *et al.* 1996). Storage conditions can also affect heat resistance. Whilst Jackson *et al.* (1996) reported that storage at 3 °C decreased heat resistance, and that the heat resistance of cells in meat that had been stored frozen was greater than for equivalent samples stored under refrigeration, Stringer *et al.* (2000) found that holding cells in TSB at 2 °C for 10 d brought about a small increase in heat resistance (Fig. 3). The thermal tolerance of two strains of *E. coli* O157:H7 decreased when they were held in apple juice (pH 3·4) at 4 °C for 24 h, while storage at 21 °C for 2 h decreased the thermal tolerance of only one of the strains (Ingham and Uljas 1998).

It has been reported that heat shock increases heat resistance, but its effectiveness depends on the environmental conditions. Heat shock increased the heat resistance of cells in TSB but not in beef slurry (Williams and Ingham 1997); heat shock at 42, 45, 48 or 50 °C had little effect on exponential-phase cells grown in nutrient broth (Kaur *et al.* 1998); heat shock at 42 °C for 5 min induced larger increases in heat resistance in cells grown aerobically than those grown anaerobically (Murano and Pierson 1992); heat shock at 45 °C for 5 min increased approximately 10-fold the number of cells recovered after heating at 59 °C for 5 min, while heat shock at 42 °C for 5 min had little effect (Bromberg *et al.* 1998). The increased thermotolerance gained by heat shock at 46 °C was maintained during a period of storage (Juneja *et al.* 1998).

4.2. Effect of heating method

To obtain good thermal inactivation data it is important to use a method of heat treatment that avoids local temperature variations, because a subpopulation of cells subjected to a lesser heat treatment will appear to be more heat resistant and tailing will occur. Teo *et al.* (1996) used an open system to examine the effects of high pH on the thermal death of *E. coli* O157:H7. Initially experiments were performed with heated flasks and the level of the water bath 2·0 cm above the surface of the heating menstruum and the cells mixed into the menstruum by hand. The resulting survivor curves had pronounced tails, and it is likely that the subpopulation in the tail did not receive the full heat treatment. Tailing was prevented by using a magnetic stirrer in the flask and increasing the level of the water bath to 5·0 cm above the heating menstruum. With these two methods, the heating times at 50 °C to give a 6D reduction were approximately 20 min and 5 min, respectively. Although some authors who used open heating systems have published log-linear survivor curves, it is best to avoid controversy and use closed systems. Even in closed systems, the heating method may affect measured heat resistance. A slow rate of heating may induce heat shock and so increase heat resistance. Careful measurement of the actual heat treatment is also required. It is not uncommon for reports to fail to take account of the come-up time.

4.3. Effect of heating menstruum

The medium in which the cells are heated can strongly influence the heat resistance of *E. coli* O157:H7. Heat resis-

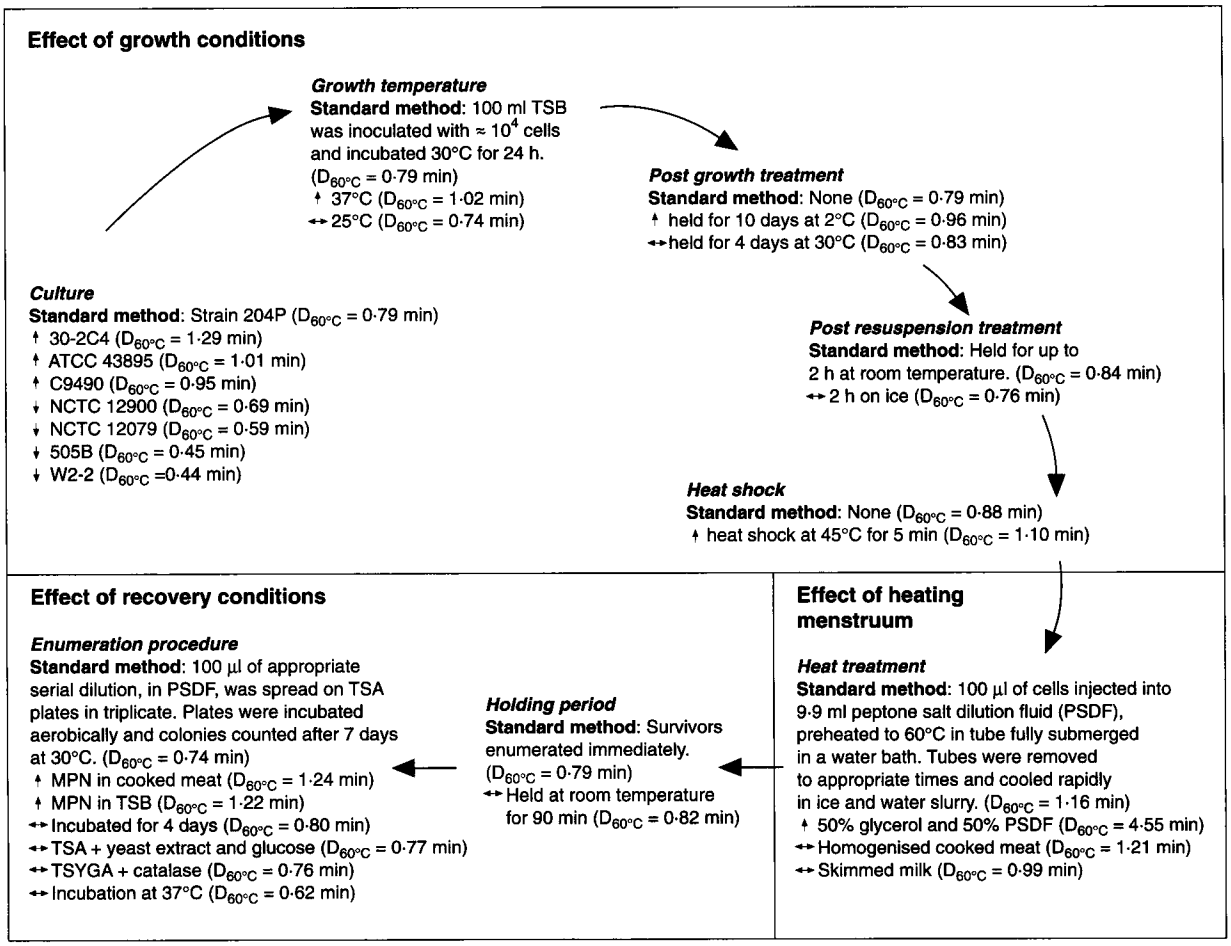

Fig. 3 Effect of procedural changes on the measured heat resistance of *Escherichia coli* O157:H7 at 60 °C. The arrows indicate whether changes significantly increased (↑), decreased (↓) or had no effect (←→) on measured heat resistance when compared to the standard method. Significant differences at the 95% level were determined using the *F*-test. From Stringer *et al.* (2000)

tance was greater for cells heated in lean ground beef than in lean ground chicken (Juneja *et al.* 1997a) and greater in beef and pork slurry than in TSB (Williams and Ingham 1997; Quintavalla *et al.* 1998; George *et al.* 1999). For three strains of *E. coli* O157:H7 heated in TSB and in pork slurry with curing agents, $D_{60\,°C}$ ranged from 0·28 to 0·33 min and from 0·94 to 1·05 min, respectively (Quintavalla *et al.* 1998). Cells can be more heat-resistant in fatty than in lean meat (Line *et al.* 1991), but this effect is not always observed. For example, cells were no more heat-resistant in turkey meat with 11% fat than in meat with 3% fat (Kotrola and Conner 1997). Ahmed *et al.* (1995) reported that heat resistance was greater in meat with a high fat content than in meat with a lower fat content, but they stated that as the moisture content decreased and the fat content increased, any affect could be related to water activity and not necessarily to the fat content. Sodium chloride concentrations up to 8·5% protected cells of *E. coli* O157:H7 from thermal inactivation in TSB (Blackburn *et al.* 1997) and *D*-values were increased when 8% NaCl was added to ground turkey breast (Kotrola and Conner 1997). However, Clavero and Beuchat (1996) found $D_{52\,°C}$ decreased when 8·6% or 16·8% NaCl was added to TSB. When 50% glycerol was added to the peptone salt dilution fluid used as the heating medium, $D_{60\,°C}$ increased from 1·2 min to 4·6 min (Fig. 3). Addition of a mixture of sucrose, glucose and fructose to apple juice to increase the Brix from 11·8° to 16·5° had no effect on heat resistance (Splittstoesser *et al.* 1996).

Heat resistance is also affected by the pH of the heating menstruum. Blackburn *et al.* (1997) found heat resistance was highest at pH 5·2–5·9 for cells heated in TSB. The pH

optimum depended on the heating temperature and NaCl concentration. Greater heat resistance was observed for cells in apple juice at pH 4·4 than at pH 3·6 or 4·0 (Splittstoesser *et al.* 1996), and in sodium carbonate buffer at pH 7 than in buffer at pH 10 or 11 (Teo *et al.* 1996). In these experiments adverse pH, either high or low, undoubtedly contributed to the kill. Cells were more sensitive to heat in the presence of sorbic, benzoic, acetic, citric or lactic acid (Abdul-Raouf *et al.* 1993; Splittstoesser *et al.* 1996; Blackburn *et al.* 1997) and a commercial mixture of polyphosphates (Kaur *et al.* 1998). The combined effect of water activity and pH were included in a predictive model of the thermal inactivation of *E. coli* O157:H7. The model provided fail-safe predictions of the effect of heating conditions, that is temperature (54·5–64·5 °C), NaCl (0–8·5%) and pH (4·2–9·8), on the thermal inactivation of *E. coli* O157:H7, and was suitable for use with many foods (Blackburn *et al.* 1997).

4.4. Effect of recovery conditions

The conditions under which cells are incubated after heat treatment and the incubation period can affect the measured heat resistance of cells. Measured heat-inactivation was increased when heat-treated cells were enumerated with pour plates rather than spread plates (Czechowicz *et al.* 1996), when cells were held on ice rather than at 20 °C before enumeration (Williams and Ingham 1997) and when incubation was at 20 or 25 °C rather than 30 °C (George *et al.* 1998). The measured heat resistance has been widely shown to depend on the media used to enumerate heat-treated cells (Abdul-Raouf *et al.* 1993; Murano and Pierson 1993; Ahmed and Conner 1995; Clavero and Beuchat 1995; McCleery and Rowe 1995; Clavero and Beuchat 1996; Clavero *et al.* 1998; Fisher and Golden 1998; Quintavalla *et al.* 1998; Duffy *et al.* 1999). It was usually lower if heat-treated cells were enumerated on media selective for *E. coli* O157:H7 rather than on non-selective media. For example, low counts were obtained with modified sorbitol MacConkey agar (Clavero and Beuchat 1995, 1996; Clavero *et al.* 1998). Heat-treated cells were also sensitive to 2% NaCl during enumeration (Semanchek and Golden 1998).

Measured heat resistance is higher if anaerobic (reduced) conditions, rather than aerobic (oxidized) conditions, are used for enumeration (Murano and Pierson 1992, 1993; Blackburn *et al.* 1997; Bromberg *et al.* 1998; George and Peck 1998; George *et al.* 1998, 1999). The measured heat resistance of *E. coli* O157:H7 was highest when anaerobic gas mixtures were used (time at 59 °C for a 6D reduction, 19–24 min), moderate when low oxygen concentrations (0·5–1%) were included (time for a 6D reduction, 13–17 min) and lowest when higher oxygen concentrations (2–40%) were included (time for a 6D reduction, 3–5 min) (George *et al.* 1998). In this study the oxidation–reduction (redox) potential of the media was altered by the use of different partial pressures of oxygen, but in a subsequent study in which the redox potential was manipulated with the oxidants potassium ferricyanide and 2,6-dichloroindophenol and the reductant dithiothreitol, the measured heat resistance was affected by the redox potential independently of the oxidant used to adjust it and therefore independently of the oxygen concentration (George and Peck 1998). When cells were heated so as to give a 6D reduction when enumerated in aerobic TSYGB, only a 3D reduction was due to thermal inactivation with the other 3D reduction related to sensitivity to oxidized conditions (Bromberg *et al.* 1998). Maintenance of these cells under anaerobic conditions at 30 °C for 4 h resulted in repair of sublethal damage and recovery of the ability of the bacteria to grow in oxidized media. Cells held at 5 °C did not recover in 816 h (Bromberg *et al.* 1998). Some authors have reported that catalase (McCleery and Rowe 1995) or pyruvate (Czechowicz *et al.* 1996) added to the enumeration medium increased recovery of heat-damaged cells, whilst others found little effect (Clavero and Beuchat 1995; Fig. 3).

To determine the true extent of thermal inactivation, it is important to use procedures that recover the greatest number of cells. For example, counting colonies after too short an incubation period, the use of certain selective media, or the use of aerobic counting techniques may overestimate the effectiveness of the heat treatment and hence the margin of safety that has been achieved. Failure to take full account of anaerobic recovery may be particularly important with regard to the margin of safety for foods that are packed under vacuum or a modified (low oxygen) atmosphere, have a low redox potential in spite of exposure to air, or support growth of other microorganisms that might lower the redox potential.

5. THERMAL INACTIVATION KINETICS OF *ESCHERICHIA COLI* O157:H7

5.1. Functions to describe thermal inactivation kinetics of bacterial cells

Although first-order reaction kinetics and *D*-values have been used extensively in calculations of thermal inactivation, the literature is full of examples where first-order reaction kinetics are not followed. Indeed Pflug and Holcomb (1991) estimated that only one-third of thermal destruction data follow such kinetics. Deviations take the form of shoulders or tails, and this subject has been reviewed (Moats *et al.* 1971; Cerf 1977). There are two basic explanations why first-order kinetics are not followed. The first is that shoulders or tails are artefacts that can be

Table 3 Possible explanations for shoulders or tails in thermal inactivation curves

	Explanation
Shoulders and/or tailing are artefacts and due to limitations in the experimental procedure	•Treatment heterogeneity (e.g. heating method, heating menstruum not uniform, sticking to the walls of vessels) •Use of mixed populations (mixtures of strains with an unequal resistance, cells at different stages of growth) •Clumping or clump separation during heat treatment or enumeration of survivors •Protective effect of dead cells •Method of enumeration (e.g. use of selective media, too short recovery period) •Poor statistical design (e.g. use of low counts)
Shoulders and/or tailing are a normal feature of the mechanism of inactivation	•Multiple hit mechanism (not single hit) •Natural distribution of heat sensitivity within the population (genetical or physiological heterogeneity) •Heat adaptation or heat shock/activation during heat treatment

attributed to limitations of the experimental procedure (Table 3). The second explanation is that shoulders or tails are a natural feature bound to the mechanism of heat inactivation or resistance of the population (Table 3).

For convenience, deviations from first-order kinetics are often ignored when calculating thermal resistance values, with straight lines fitted to all of, or sections of, curves. In these circumstances the use of log-linear plots is practical rather than theoretical and may be misleading. In an attempt to describe thermal death data more accurately, a number of alternative mathematical functions have been adopted. Some are based on understanding the physiological mechanism of inactivation. For example, Moats *et al.* (1971) assumed that targets are clumped at critical sites within the cell, Körmendy and Körmendy (1997) assumed that there is a distribution of resistance within the population, and Smerage and Teixeira (1993) assumed that heterogeneity occurs by adaptation to heat during heating. Other models involve empirical curve fitting (e.g. Pruitt and Kamau 1993; Stecchini *et al.* 1993; Whiting 1993). Although one function may appear to fit a thermal inactivation curve better than another, it is often difficult to prove that the model performance is better.

5.2. Discussion of the most appropriate function to describe the thermal inactivation kinetics of *Escherichia coli* O157:H7

When describing the thermal death of *E. coli* O157:H7, first-order reaction kinetics have generally been fitted to all or part of the curve. However, Blackburn *et al.* (1997) obtained curves that showed tailing and were poorly described by first-order reaction kinetics. These curves were better described by the log-logistic function (Cole *et al.* 1993). Predicted time to a 5D reduction was greater from the log-logistic model than the log-linear (*D*-value) model (Blackburn *et al.* 1997). An empirical approach developed by Whiting (1993) permits the application of up to three straight lines to thermal inactivation data giving different rate constants for the shoulder, exponential region and tail. Juneja *et al.* (1997a) used this model to determine *D*-values for subpopulations of *E. coli* O157:H7 heated in beef and chicken. Herremans *et al.* (1997) introduced a dynamic model to take account of the experimentally observed shoulder and tail in the thermal death curve of *E. coli* O157:H7 heated at 60 °C.

First-order reaction kinetics assume that the population is homogeneous and that heat acts by destroying a single target. From this, it may be expected that cells will exist in one of two states; either the target is undamaged and the cell is healthy or the target has been inactivated and the cell is dead. This theory does not allow the possibility of sublethally damaged cells. There are, however, many examples of heated *E. coli* O157:H7 cells showing sublethal damage. For example, delays have been reported in time to growth and of sensitivity to growth on media containing selective agents, sensitivity to 2% NaCl, and sensitivity to high redox potential and oxygen concentration during enumeration (as described earlier). Bromberg *et al.* (1998) identified two distinct periods of repair of sublethal injury during recovery and subsequent growth of heated cells of *E. coli* O157:H7. Cells were grown under strictly anaerobic conditions in TSYGB at 30 °C, heated in anaerobic TSYGB for 5 min at 59 °C, cooled in ice-water and held at 30 °C. At timed intervals samples were removed and counts performed in aerobic and anaerobic TSYGB by a MPN procedure (Fig. 4). Immediately after heating for 5 min and cooling, the aerobic count was 3×10^3 cells ml^{-1} and the anaerobic count was 3×10^6 cells ml^{-1}. After incubation for 4 h the aerobic and anaerobic counts were similar, indicating that all the cells had regained their ability to grow in the aerobic media. The anaerobic count did not change during this period (Fig. 4). There was no increase in cell numbers for approximately 13 h, when the aerobic and

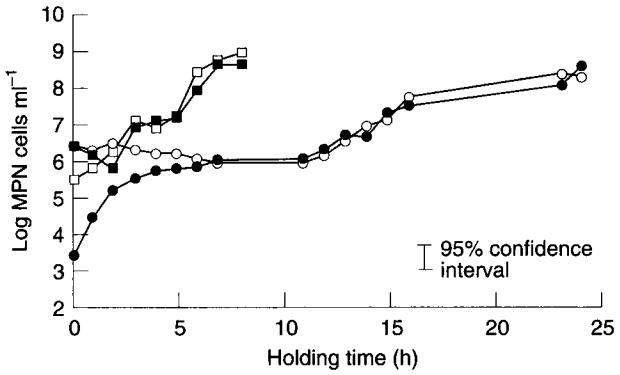

Fig. 4 Recovery and growth of sublethally heat-injured cells of *Escherichia coli* O157:H7 strain 204P. Cells were heated at 59 °C for 5 min, held at 30 °C in anaerobic TSYGB medium, and enumerated in aerobic TSYGB (●) and anaerobic TSYGB (○), or cells were unheated (but diluted), held at 30 °C in anaerobic TSYGB medium and enumerated in aerobic TSYGB (■) and anaerobic TSYGB (□). Results are the mean of four experiments (heated cells) and duplicate experiments (unheated cells). The 95% confidence interval was derived from the average of the individual confidence intervals. Data for heated cells are from Bromberg *et al.* (1998) and data for unheated cells are unpublished results of George and Peck

anaerobic counts increased at the same rate and reached 10^8–10^9 cells ml^{-1} within 24 h (Fig. 4). In a control test, cells that were diluted instead of heated, but were otherwise subjected to the same treatment, gave similar initial counts in aerobic and anaerobic TSYGB, and the cell numbers increased after 3 h, with both counts reaching 10^8–10^9 cells ml^{-1} after 7 h (Fig. 4). Heated cells of *E. coli* O157:H7 were therefore sublethally injured, and had two distinct periods of cell repair. The first period of repair took 4 h at 30 °C during which time sublethally injured cells regained their ability to grow in oxidized media. The second period of repair took a further 9 h at 30 °C, after which cell division commenced.

Sub-lethal injury has been studied in many bacteria, for example similar observations of the effect of heat treatment have been made with *L. monocytogenes* (e.g. Mackey and Bratchell 1989; George *et al.* 1998). These reports cast doubt on the validity of the mechanism that underlies the use of first-order reaction kinetics (and *D*-values) to describe thermal inactivation of *E. coli* O157:H7. Many of the thermal inactivation data for *E. coli* O157:H7 have, however, been adequately described by first-order reaction kinetics.

6. CONCLUSIONS

Accurate prediction of the thermal death rate of *E. coli* O157:H7 is important so food processors can ensure that appropriate safety margins are attained. Although there is some variation in the published heat inactivation data for *E. coli* O157:H7, there is no strong evidence that a heat treatment of 70 °C for 2 min or the equivalent, as recommended by the ACMSF, fails to deliver a 6D reduction in cells of *E. coli* O157:H7.

In most studies thermal inactivation has been described by first-order reaction kinetics and *D*-values. While this does not seem to be justified on a theoretical basis, there is no doubt that it is convenient, and in many cases provides an adequate description of thermal death. An important step forward would be to develop a mathematical function to describe thermal death that can be justified on a theoretical basis, that is one based on understanding the process of thermal death at the cellular level. A more accurate description of thermal death would benefit industry, regulators and consumers because it would allow a tighter control of safety margins.

7. ACKNOWLEDGEMENTS

The authors are most grateful to Dr József Baranyi, Dr Gary Barker, Dr Bernard Mackey and Dr Peter Wilson for many helpful discussions. This work was funded by the Ministry of Agriculture Fisheries and Food.

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