THERMAL RESISTANCE OF *YERSINIA ENTEROCOLITICA* AND *LISTERIA MONOCYTOGENES* IN MEAT AND POTATO SUBSTRATES

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ABSTRACT

The heat resistance of a wild type and nalidixic acid resistant strain of Yersinia enterocolitica, *and* Listeria monocytogenes, *was measured in meat (minced beef and minced beef homogenate) and potato substrates over the temperature range 50-60C. Comparisons of heat resistance were determined using D-values calculated using a linear survival model. The results showed that the wild-type strain of* Y. enterocolitica *was more heat resistant than the mutant ($P<0.05$). Under most conditions, the use of a nonselective/overlay recovery medium resulted in higher D-values compared to a selective recovery medium ($P<0.05$). Analysis of the data using a nonlinear survival model (D_1 and D_2-values) suggested the presence of heat resistant subpopulations and was particularly evident for the mutant strain, and in potatoes compared to minced beef.*

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INTRODUCTION

Demographic, social and cultural changes since the Second World War have established a sustained demand for high quality ready to eat meals which can be rapidly and easily prepared for consumption in the home (Conner *et al.* 1989). This consumer demand continues to drive the food production and processing industry towards the development and more extensive application of a range of new technologies capable of providing retail products with these characteristics. One such process is *sous vide* in which food is vacuum packaged, subjected to mild pasteurization and held for extended periods under refrigeration, prior to a further reheating step immediately before consumption. This combination of mild heat treatment and chill storage provides product of very high sensory quality (Light *et al.* 1988) and it has been established that such Refrigerated Foods of Extended Duration (REFEDs) can meet consumer demands for high quality convenience foods (Schellekens and Martens 1992; Armstrong 1996).

However, some concerns have been raised in relation to the overall safety of such processes as they could select for the survival and/or growth of a range of facultative anaerobic psychrotrophic pathogens. This group of organisms which includes such species as *Yersinia enterocolitica* and *Listeria monocytogenes* have emerged as pathogens of major significance in chilled foods in recent years (Schofield 1992). Their well documented psychrotrophic nature coupled with their ability to grow under anaerobic conditions (Gill and Reichel 1989; Walker *et al.* 1990; Hudson *et al.* 1994) mean that, if present in such REFEDs, they could increase to significantly high numbers during extensive chill storage. Thus it is important that *sous vide* products receive heat treatments which are sufficient to eliminate such pathogens, without significant damage to other attributes. It can be difficult to identify the minimum adequate heat treatments to achieve these conflicting objectives.

One major component of such considerations, the heat resistance of bacteria, is modulated by a number of inherent and environmental factors, as extensively reviewed by previous workers (Hansen and Riemann 1963; Stumbo 1973; Jay 1978). In addition to experimental factors, which influence the raw thermal resistance data obtained, the specific nature of the mathematical procedure used in the subsequent analysis of these data, i.e. linear/nonlinear analysis, can have significant effects on the D-values derived (where D_x-value is an estimation of the time in minutes that the organisms must be held at temperature x to achieve a 90% reduction in cell numbers). Although it has been suggested that when a bacterial culture is held at a constant lethal temperature, the pattern of decline is log linear (Stumbo 1973). More recent studies (Mackey *et al.* 1990; Bhaduri *et al.* 1991; Linton *et al.* 1995) have shown that this is not always the case, and that the application of nonlinear models to data which shows deviations from the linear pattern will give a more accurate interpretation. Such deviations include

"shoulders" in the initial part of the survivor curve where cell numbers do not decline or do not decline in a linear pattern. Another type of nonlinearity, "tails", occur at the end of the survivor curve and are thought to represent the most heat resistant parts of bacterial populations (Whiting 1993).

The objective of this study was to calculate the heat resistance of *Y. enterocolitica* and *L. monocytogenes,* two potentially significant psychrotrophic pathogens, in components i.e. meat and potato, and at temperatures, frequently used in the production of *sous vide* products. The study also analyzed thermal resistance data obtained using linear and nonlinear survival models, to identify those models which best represented the dynamics of thermal death of these organisms under such conditions.

MATERIALS AND METHODS

Beef and Potatoes Preparation

All beef was obtained from the on-site facility in the Industrial Development Unit at The National Food Centre, Dunsinea, Castleknock, Dublin 15, Ireland. Meat from the neck region was removed from the carcass 24 h postmortem and trimmed of all visible fat.

Minced beef was produced by double mincing lean neck beef pieces (50-100 g) through a 5 mm plate using a Crypto-Peerless mincing machine (Model EB12F, Crypto-Peerless Ltd., London, England). The pH of the minced beef was measured as described by Bendall (1973). Proximate analyses were performed using standard AOAC procedures (Methods 968.06; 985.14 and 985.15) (AOAC 1990).

Minced beef homogenate was produced by homogenizing 50 g of minced beef in 100 mL of distilled water for 10 min in a Robot Coupe blender (Model R301 ultra, Robot Coupe (UK) Ltd., London).

Minced beef filtrate was prepared by centrifuging a 100 mL aliquot of minced beef homogenate at 3,019 g for 10 min at 4C, and filtering the resultant supernatant through a 12.5 cm glass micro-fiber filter (GF/A, Whatman®, Whatman International Ltd., Maidstone, England).

Potatoes, variety Pentland Dell, were peeled manually and cut to 3 mm slices using a Krups Universal Super slicer (Krups Sales Ltd., Limerick, Ireland).

Organisms and Culture Conditions

Y. enterocolitica GER serotype 0:3 was obtained from Dr. S. Bhaduri, USDA, ERRC, Philadelphia, PA, USA. To facilitate isolation of the organism from mixed indigenous microbiota, nalidixic acid resistant mutant of the above *Y. enterocolitica* strain was prepared at The National Food Centre according to the method of Park (1978). *L. monocytogenes* serotype 4b (NCTC 11994) was obtained from the

National Collection of Type Cultures at the Central Public Health Laboratory, Colindale, London.

Each culture was grown on tryptone soya agar (TSA, Oxoid, Unipath Ltd., Basingstoke, Hampshire, England), for 18 h at 25C for *Y. enterocolitica*, and at 30C for *L. monocytogenes*. Stock cultures were prepared by transferring derived cell suspensions to cryopreservation beads as recommended (Protect, Technical Service Consultants Ltd., Lancashire, England) and stored at -34C.

Inoculum Preparation

One bead containing *Y. enterocolitica* or *L. monocytogenes* was added separately to 30 mL of brain heart infusion broth (BHI; Oxoid) and incubated for 24 h at 25C or 30C, respectively, to give a stationary phase culture. A 1 mL aliquot from each resultant stationary phase culture was transferred to 300 mL of BHI and incubated at 25C for 16 h. The derived cultures were centrifuged at 3019 g for 10 min at 4C and resuspended in 300 mL of BHI to give inocula containing 10^8-10^9 cfu/mL *Y. enterocolitica* or *L. monocytogenes.*

Inoculation and Heating of Vacuum Packaged Samples

Fifty-100 g lean neck beef pieces were immersed in 300 mL of BHI inoculum containing *Y. enterocolitica* or *L. monocytogenes*. After 5 s, excess liquid was drained off and the inoculated meat pieces were minced as described above. The derived minced beef had an initial *Y. enterocolitica* or *L. monocytogenes* count of 10^7-10^8 cfu/g. Ten gram samples of this material were vacuum packaged in Cryovac BB4L bags (approximately 7 cm × 7.5 cm) (W.R. Grace Ltd., Co. Dublin, Ireland) using a Swissvac machine (Model 380, Swissvac (GB) Ltd., Berkshire, England).

Potato slices were immersed in 300 mL of BHI inoculum containing *Y. enterocolitica* or *L. monocytogenes,* supplemented with 200 ppm sodium metabisulphite (May & Baker Ltd., Dagenham, England), a preservative widely used in commercial potato processing to maintain acceptable potato color. Preliminary studies (results not shown) confirmed that the presence of this sodium metabisulphite, at concentrations of up to 800 ppm had no effect on test organism numbers recovered from inoculated potato slices. After 5 s, excess liquid was drained to yield inoculated slices containing initial *Y. enterocolitica* or *L. monocytogenes* counts of 10^7-10^8 cfu/g. Individual potato slices were vacuum packaged as described above.

Vacuum bags containing inoculated minced beef or potatoes were completely submerged in a water bath pre-adjusted to 50, 55 or 60C. The temperature of the substrate was continuously monitored throughout the course of the experiment using thermocouples inserted into the center of the substrate through a silicone

rubber seal on the vacuum bag. The thermocouples were attached to a temperature microprocessor system (ELLAB UK Ltd., King's Lynn, Norfolk, England) which gave a continuous printout of the temperature measurements. Processing times were measured from the time the core temperature of the test substrate reached the target temperature. In all cases the substrate core reached the target temperature within 2 min. When sample temperatures had achieved the target treatment temperature, one sample was removed as the time zero sample. Test substrates inoculated with *Y. enterocolitica* were removed every 10 min at 50C, 0.5 min at 55C and 4 s at 60C. Test substrates inoculated with *L. monocytogenes* were removed every 30 min at 50C, 5 min at 55C and 0.5 min at 60C. At the selected time intervals, samples were removed and cooled in iced water (0±1C) to 5C, within 3 min. Duplicate samples were processed at each time interval and the experiment was replicated three times.

Inoculation of Preheated Samples

Inocula were prepared as described above except that a 0.1 mL aliquot was transferred to 30 mL of BHI after the initial incubation. After centrifugation, the pellet was resuspended in 30 mL of minced beef filtrate to form inocula containing 10^8-10^9 cfu/mL *Y. enterocolitica* or *L. monocytogenes*.

Samples (9.9 g) of minced beef or minced beef homogenate were placed in vacutainers (100 mm × 16 mm) which were sealed with rubber injection caps. Two sterile needles were inserted through the cap of the vacutainer. The tip of one (inoculation) needle (14 G, 6.35 cm) was adjusted to the geometric center of the substrate. A second (venting) needle (20 G, 3.81 cm) was inserted into the air space above the sample to allow air to escape during preheating, inoculation and heat treatment.

Vacutainers containing minced beef or minced beef homogenate were completely submerged in a water bath preadjusted to 50, 55 or 60C. The temperature of the substrate was continuously monitored throughout the course of the experiment using thermocouples inserted into the center of the substrate through the rubber cap of the vacutainer, as described above. When the temperature of the test substrate reached the desired temperature, samples were inoculated with a 0.1 mL aliquot of the test suspension. Samples were removed immediately and cooled in iced water (0±1C) to 5C, within 8 min. As soon as the first vacutainer was inoculated, all others were inoculated in turn, and removed to iced water at the selected time intervals as described above. Duplicate samples were processed at each time interval and the experiment was replicated three times.

Enumeration of Organisms in Treated Samples

Cooled samples were homogenized in 10 mL of maximal recovery diluent (MRD, BBL Becton Dickinson Microbiology Systems, Cockeysville, MD) for 1

min in a Colworth stomacher (Model BA6021, A.J. Seward & Company Ltd., London). Bacterial numbers were estimated by drop and spread plating, of 1 mL or 0.1 mL aliquots of the homogenate or successive ten fold dilutions in MRD, onto appropriate selective agar plates.

The wild type strain of *Y. enterocolitica* was enumerated by direct plating on Yersinia selective agar containing 15 mg/L cefsulodin, 4 mg/L irgasan and 2.5 mg/L novobiocin (Oxoid) incubated for 24 h at 37C and then at 25C for 24 h.

Numbers of uninjured and injured cells of the mutant strain of *Y. enterocolitica* were enumerated using selective and nonselective/overlay recovery techniques (Speck *et al.* 1975). Uninjured organisms were recovered as described above on Yersinia selective agar supplemented with 50 mg/L nalidixic acid (Sigma, St. Louis, Missouri). Uninjured and injured organisms were detected using a recovery overlay technique in which samples were plated out onto TSA, incubated at 25C for 6 h, and overlaid with 10 mL of Yersinia selective agar supplemented with 50 mg/L nalidixic acid. When the overlay agar had set, the plates were incubated for 24 h at 37C and then at 25C for 24 h.

L. monocytogenes was enumerated by direct plating on Palcam agar containing 10 mg/L polymixin B, 5 mg/L acriflavine hydrochloride and 20 mg/L ceftazidime (Oxoid), incubated at 30C for 48 h.

Calculation of D-values

Survivor curves of mean bacterial counts ($\log_{10}$ cfu/g) versus heating time were plotted at each temperature for all the substrates. D-values were calculated using (1) linear regression analysis of the linear portions of the survivor curve and (2) a nonlinear model (Whiting 1993) using the Gauss-Newton curve fitting programme (ABACUS Software Programme, ERRC, USDA, Philadelphia, PA). The nonlinear model included an initial shoulder and two populations (a major population, comprising the main portion of the curve, and a subpopulation, comprising the tail of the curve). In this model, D_1 and D_2-values were calculated to describe the heat resistance of the major and subpopulations, respectively. In the present study, D_1 and D_2-values were presented if 50% or more of the six repetitions (three replicates in duplicate) demonstrated a tailing effect.

Statistical Analysis

Single, parallel and individual lines were fitted to the data and the system that best fitted the results indicated significant differences between the slopes at the 5% level ($P<0.05$) (Genstat™ 5, Statistics department, Rothamsted Experimental Station, Hertfordshire, UK).

RESULTS AND DISCUSSION

The pH of the minced beef used in this study was in the range 5.6-5.8. Proximate analysis established that the minced beef had average protein, moisture and fat contents of 20.8%, 75.5% and 2.35%, respectively.

D-values obtained by linear and nonlinear analysis of selective counts for the wild-type strain of *Y. enterocolitica*; selective and nonselective/overlay counts for the mutant strain of *Y. enterocolitica*, and selective counts for *L. monocytogenes*, are presented in Tables 1, 2 and 3. Typical survivor curves for the wild-type strain of *Y. enterocolitica* heated in minced beef in vacutainers at 50, 55 and 60C are shown in Fig. 1.

The heat resistance of the wild-type strain of *Y. enterocolitica* was compared with the nalidixic acid resistant mutant strain of the organism. Examination of the results for minced beef in vacutainers indicated that the D-values obtained from selective counts were significantly higher for the wild type strain compared to the mutant ($P<0.05$) (Tables 1 and 2). This was evident at 50, 55 and 60C.

Generally, D-values calculated when survivors were enumerated using the nonselective/overlay technique were consistently higher than those obtained using the selective recovery technique. Specifically, significantly higher D-values were obtained for the mutant strain of *Y. enterocolitica*, enumerated on nonselective compared to selective agar, in minced beef in vacutainers at 55 and 60C, in minced beef in vacuum bags at 50C, and in minced beef homogenate in vacutainers at 50 and 55C ($P<0.05$) (Tables 1 and 2). This can be attributed to the enumeration of both uninjured cells and heat damaged survivors using the nonselective/overlay technique. The use of selective and non-selective media for the recovery of heat stressed *Y. enterocolitica* has been previously investigated (Hanna *et al.* 1977b). The results of the present study are in agreement with those reported by Hanna *et al.* (1977b) who noted a lower number of *Y. enterocolitica* survivors on bismuth sulphite agar (selective) compared to tryptic soy agar (nonselective), after heating in roast beef to an internal temperature of 51C. The difference between the recovery agars was attributed to the detrimental effects of components of the bismuth sulphite agar on the development of injured cells.

Comparison of mutant *Y. enterocolitica* D-values from minced beef samples inoculated (1) before heating (vacuum bags) and (2) after heating (vacutainers), showed that, in general, vacuum packaged samples had higher D-values than vacutainer samples ($P<0.05$). This pattern was observed in counts derived from selective and nonselective/overlay counts and at each of two temperatures, i.e. 50 and 55C (Tables 1 and 2). This greater degree of heat resistance may be due to the formation of heat shock proteins in the minced beef which was inoculated before heating. Under this scheme bacteria would have had a longer interval to produce such heat shock proteins during heating of the minced beef (<2 min) than the

TABLE 1.
LINEAR (D) AND NONLINEAR (D_1 AND D_2) D-VALUES (MIN) FOR *YERSINIA ENTEROCOLITICA* AND *LISTERIA MONOCYTOGENES* HEATED AT 50C AND RECOVERED UNDER VARYING CONDITIONS

		Selective Recovery		Nonselective/Overlay Recovery	
Yersinia enterocolitica (wild type)			se*		se
Minced beef in vacutainers	D	26.3	0.002	ND	
	D_1	-	-		
	D_2	-	-		
Yersinia enterocolitica (mutant)					
Minced beef in vacutainers	D	18.9	0.003	18.5	0.006
	D_1	14.1	-	15.4	-
	D_2	91.0	-	85.7	-
Minced beef in vacuum bags	D	20.8	0.002	25.0	0.002
	D_1	16.7	-	-	-
	D_2	39.0	-	-	-
Minced beef homogenate in vacutainers	D	9.6	0.10	16.1	0.06
	D_1	-	-	-	-
	D_2	-	-	-	-
Listeria monocytogenes					
Minced beef in vacutainers	D	36.1	0.12	ND	
	D_1	-	-		
	D_2	-	-		
Minced beef in vacuum bags	D	43.5	0.09	ND	
	D_1	-	-		
	D_2	-	-		

*se= standard error of the negative reciprocal of the D-value; ND = not determined

almost instantaneous heating of cells injected into preheated minced beef in vacutainers. The more gradual heating over a period of minutes afforded to bacteria preinoculated into vacuum packaged minced beef would provide an adequate opportunity for the production/expression of heat shock proteins. Thus for example, Neidhardt *et al.* (1984) has reported for *Escherichia coli* that increased production of heat shock proteins occurs within seconds following an appropriate rise in temperature. The presence of higher levels of heat shock proteins in gradually heated *Y. enterocolitica* cultures would explain the differences in thermotolerance at 50 and 55C, observed in this study.

The pattern of heat resistance at 60C observed in this study was different from the patterns observed at 50 and 55C. It was not possible to calculate a D_{60} value for *Y. enterocolitica* in vacuum packaged minced beef because the organism was rapidly inactivated during the heat-up time before the minced beef reached the target temperature of 60C. This observation is in agreement with Hanna *et al.* (1977a, b)

TABLE 2.
LINEAR (D) AND NONLINEAR (D_1 AND D_2) D-VALUES (MIN) FOR *YERSINIA ENTEROCOLITICA* AND *LISTERIA MONOCYTOGENES* HEATED AT 55 AND 60C AND RECOVERED UNDER VARYING CONDITIONS

		55C				60C			
		Selective Recovery		Nonselective/ Overlay Recovery		Selective Recovery		Nonselective/ Overlay Recovery	
Yersinia enterocolitica (wild type)			*se		se		se		se
Minced beef in vacutainers	D	1.24	0.06	ND		0.11	0.03	ND	
	D_1	-	-			-	-		
	D_2	-	-			-	-		
Yersinia enterocolitica (mutant)									
Minced beef in vacutainers	D	0.65	0.17	0.95	0.06	0.07	0.03	0.1	0.01
	D_1	-	-	-	-	0.04	-	-	-
	D_2	-	-	-	-	0.36	-	-	-
Minced beef in vacuum bags	D	1.15	0.08	1.28	0.11	ND		ND	
	D_1	-	-	-	-				
	D_2	-	-	-	-				
Minced beef homogenate in vacutainers	D	0.56	0.14	0.9	0.08	0.09	0.02	0.1	0.02
	D_1	-	-	0.6	-	-	-	0.1	-
	D_2	-	-	3.9	-	-	-	0.4	-
Listeria monocytogenes									
Minced beef in vacutainers	D	3.53	0.04	ND		0.33	0.01	ND	
	D_1	-	-			-	-		
	D_2	-	-			-	-		
Minced beef in vacuum bags	D	3.14	0.01	ND		0.24	0.01	ND	
	D_1	-	-			-	-		
	D_2	-	-			-	-		

*se=standard error of the negative reciprocal of the D-value; ND=not determined

TABLE 3.
LINEAR (D) AND NONLINEAR (D_1 AND D_2) D-VALUES (MIN) FOR *YERSINIA ENTEROCOLITICA* AND *LISTERIA MONOCYTOGENES* HEATED AT 50, 55 AND 60C ON POTATO SLICES

		50C		55C		60C	
			se*		se		se
Yersinia enterocolitica (wild-type)	D	17.9	0.003	0.95	0.24	ND	
	D_1	13.2		-			
	D_2	342.4		-			
Listeria monocytogenes	D	30.6	0.08	3.3	0.02	0.51	0.01
	D_1	24.7		3.7		-	
	D_2	210.0		63.4		-	

*se=standard error of the negative reciprocal of the D-value
ND=not determined

who examined the heat resistance of this organism in milk and meat and concluded that the organism was unable to survive in foods held at 60C or above for several minutes.

Examination of D-values for the mutant strain of *Y. enterocolitica* in minced beef and minced beef homogenate in vacutainers, generally showed that there were no differences between the heating substrates on either of the recovery media at 50, 55 or 60C (Tables 1and 2). Two exceptions were noted. The D_{50} value of 18.9 min recorded from selective counts for minced beef was significantly higher than the corresponding D_{50} value of 9.6 min for minced beef homogenate ($P<0.05$). At 60C, a comparison of selective counts showed a significantly higher D-value for minced beef homogenate (0.09 min) compared to minced beef (0.07 min) ($P<0.05$).

The *Y. enterocolitica* D_{60} values (0.07-0.11 min) obtained in this study are lower than those obtained in other studies at higher heating temperatures (62-62.8C). It is however difficult to make direct comparisons with other reports as the majority of previous workers have investigated liquid systems, most frequently milk (Hanna *et al.* 1977a; Francis *et al.* 1980; Lovett *et al.* 1982; Sörqvist 1989; Toora *et al.* 1992). Such systems have been suggested to give lower heat resistance values than the meat based systems, probably due to the lower levels of solids, protein and fats in such liquid media (Boyle *et al.* 1990; Batish *et al.* 1991). Thus it is somewhat surprising to note that milk/liquid based studies of *Y. enterocolitica* have returned D-values higher than those noted in this study i.e. $D_{62.8}$ values of 0.01-0.29 min (Francis *et al.* 1980); $D_{62.8}$ values of 0.24-0.96 min (Lovett *et al.* 1982); D_{62} values of 0.15-0.19 min (Sörqvist 1989) and $D_{62.8}$ values of 0.1725-0.1755 min (Toora *et al.* 1992).

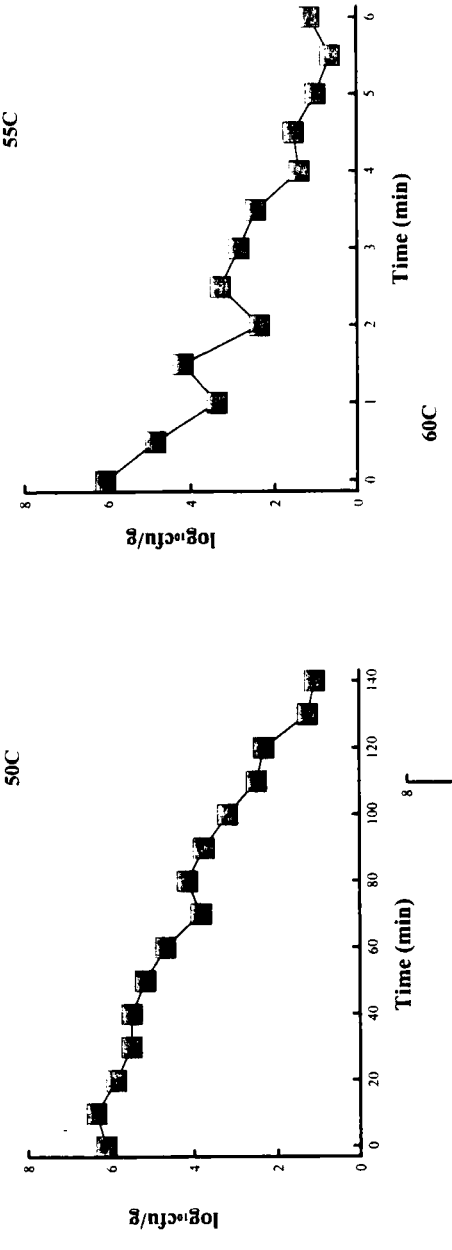

FIG. 1. SURVIVOR CURVES FOR A WILD TYPE STRAIN OF *YERSINIA ENTEROCOLITICA* HEATED AT 50, 55 AND 60C IN MINCED BEEF IN VACUTAINERS AND RECOVERED ON SELECTIVE AGAR

The differences between the results obtained in this study and those of previous studies may be due to inter-strain variations. The fact that many of the isolates used in previous studies were recovered from milk and environmental sources, rather than from a clinical source as in the present study, could explain the observed differences. Toora *et al.* (1992) reported that the D-values obtained for clinical isolates of *Y. enterocolitica* heated in milk at 62.8C, were lower than those recorded in other studies using milk isolates (Francis *et al.* 1980; Lovett *et al.* 1982). They suggested this was due to the lower heat resistance of clinical isolates compared to milk or environmental isolates (Toora *et al.* 1992).

In the present study, there were no significant differences between the D-values recorded for *L. monocytogenes* in minced beef in vacutainers and vacuum bags at 50, 55 or 60C (Tables 1 and 2). The values obtained are lower than previously reported D-values for *L. monocytogenes* in ground meat. The average D_{50} value of 39.8 min observed in this study is lower than the D_{50} value of 85.0 min obtained by Mackey *et al.* (1990). Similarly, the average D_{55} value of 3.3 min is below the previous reported values of D_{56} of 13.18 min (Farber 1989); D_{55} of 21 min (Mackey *et al.* 1990) and $D_{54.4}$ of 19.3 min (Schoeni *et al.* 1991). At 60C, the average D-value of 0.29 min is also lower than previously reported D_{60} values of 3.12-3.8 min (Farber 1989; Mackey *et al.* 1990; Schoeni *et al.* 1991).

Thus, there are considerable variations among reports on the heat resistance of *L. monocytogenes*. There are several factors, such as differences among strains, inoculum history and preparation, substrate specific effects, experimental conditions and protocols, and recovery media and methods, which may affect heat resistance. Thus, direct comparisons between studies are difficult, although it is reasonable to accept that at least some of these factors underlie the observed variations in heat resistance. This uncertainty highlights the need for caution in presenting D-values as precise, rather than indicative values.

The D-values derived in this study for organisms heat-treated in potatoes are presented in Table 3. Heat resistant subpopulations were detected in *Y. enterocolitica* cultures heat treated in potatoes at 50C, and in *L. monocytogenes* cultures heat treated in potatoes at 50 and 55C. The heat resistant subpopulations calculated were considerably more heat resistant than the main population i.e. D_2-values were between 7 and 19 times greater than D-values obtained from the linear model. In contrast, heat resistant subpopulations were not evident when these organisms were heated in minced beef. This heat resistant subpopulation, detected using the nonlinear method, may be due to the higher carbohydrate content of potatoes (Holland *et al.* 1991) compared to minced beef which contains little or no carbohydrate. This high carbohydrate content may have had a protective effect on the bacterial cells against the lethal effects of the thermal treatment. Several researchers have reported that the presence of carbohydrates in foods causes an increase in the thermal resistance of microorganisms (Hansen and Riemann 1963; Jay 1978).

Analysis of data from the nonlinear method revealed that D_1 and D_2-values were

also recorded for the mutant strain of *Y. enterocolitica* in minced beef and minced beef homogenate. It was noted that two of the D_2-values for the mutant strain occurred on the selective agar at 50 and 60C in minced beef in vacutainers. This is particularly interesting considering that significantly higher D-values (calculated using the linear method) were recorded for the wild-type strain compared to the mutant under these conditions (see above). Thus, the increased heat resistance of the subpopulation was masked when the linear method was used to calculate the D-values. In the recovery procedure for the mutant strain, nalidixic acid was incorporated in the recovery medium. Based on the results of other studies (Niedhardt *et al.* 1984; Lindquist 1986; McCallum and Inniss 1990), the increased heat resistance of the subpopulation may be due to the effect of this antibiotic. These workers reported the induction of heat shock proteins in cells treated with nalidixic acid. In the present study, the presence of nalidixic acid in the recovery medium may have induced the production of heat shock proteins. The ability of heat shock proteins to provide protection from the toxic effects of stress (Lindquist 1986), could have allowed the cells to survive for longer periods than usual, thus accounting for the heat resistance of the subpopulation.

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