Journal of Applied Microbiology 2000, **88**, 626–632

Thermal inactivation of *Listeria monocytogenes* and *Yersinia enterocolitica* in minced beef under laboratory conditions and in sous-vide prepared minced and solid beef cooked in a commercial retort

D.J. Bolton[1] C.M. McMahon[1] A.M. Doherty[1] J.J. Sheridan[1], D.A. McDowell[2] I.S. Blair[2] and D. Harrington[3]

[1]The National Food Centre, Teagasc, Dunsinea, Castleknock, Dublin 15, Ireland, [2]Food Studies Research Unit, The University of Ulster, Jordanstown, Newtownabbey, Northern Ireland, and [3]Statistics Department, Teagasc, Dublin 4, Ireland

7402/09/99: received 28 September 1999, revised 30 November 1999 and accepted 2 December 1999

D.J. BOLTON, C.M. MCMAHON, A.M. DOHERTY, J.J. SHERIDAN, D.A. MCDOWELL, I.S. BLAIR AND D. HARRINGTON. 2000. *D*-values were obtained for *Listeria monocytogenes* and *Yersinia enterocolitica* at 50, 55 and 60 °C in vacuum-packed minced beef samples heated in a laboratory water-bath. The experiment was repeated using vacutainers, which allowed heating of the beef to the desired temperature before inoculation. *D*-values of between 0·15 and 36·1 min were obtained for *L. monocytogenes*. Pre-heating the beef samples significantly affected ($P < 0{\cdot}05$) the D_{60} value only. *D*-values for *Y. enterocolitica* ranged from 0·55 to 21·2 min and all the *D*-values were significantly different ($P < 0{\cdot}05$) after pre-heating. In general, the *D*-values obtained for core inoculated solid beef samples were significantly higher ($P < 0{\cdot}05$) than those generated in minced beef when heated in a Barriquand Steriflow commercial retort.

INTRODUCTION

As changing demographics have increased the market for quality convenience foods, minimal processing technologies have found increasing application (Smith *et al*. 1990; Pralus 1993). One such process, 'sous vide', is defined as "a process whereby foods are vacuum packaged, cooked, chilled and stored refrigerated" (Rhodehamel 1992). This mild processing procedure maintains the intrinsic nutritional quality and culinary value of the food. Several steps are involved, including pre-cook preparation (washing, formulation, rehydration etc.), packaging under vacuum, pasteurization, rapid chilling, storage at 0–3 °C and reheating before consumption (Betts 1992). Preservation is achieved by the combination of vacuum packaging, mild cooking, rapid chilling and chilled storage.

However, pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica* are capable of growth at refrigeration temperatures under anaerobic conditions (Gill and Reichel 1989; Walker *et al*. 1990; Hudson *et al*. 1994) and hence, pose a health risk in sous vide products (Schofield 1992; Smith *et al*. 1990). This is especially true of meat products, as meat is often contaminated with these pathogens (Wendlandt and Bergann 1994; Fransen *et al*. 1996; Logue *et al*. 1996) but receives minimal processing (Schellekens 1996). In the absence of other hurdles, mild cooking may result in the sous vide process becoming selective for these facultative anaerobic psychotrophs.

To date, most research to elucidate the thermal properties of important food-poisoning bacteria has been carried out in the laboratory and little is known about the thermal resistance of these organisms in commercial systems. One potentially important difference between small-scale laboratory and large-scale commercial processes is the significantly longer period necessary to raise the larger amount of product to the sous vide treatment temperature. Such slow gradual heating processes may have effects on the properties of treated foods and the nature and physiological status of the resident microflora. This study also examines the duration of the heating-up phase in laboratory experiments on the subsequent *D*-values, and comments on their commercial significance.

Correspondence to: D.J. Bolton, Food Safety Department, The National Food Centre, Dunsinea, Castleknock, Dublin 15, Ireland (e-mail: d.bolton@nfc.teagasc.ie).

METHODS AND MATERIALS

Micro-organisms

Listeria monocytogenes serotype 4b (NCTC 11994) was obtained from the National Collection of Type Cultures (Central Public Health Laboratory, Colindale, UK). *Yersinia enterocolitica* GER (serotype 0 : 3) was a gift from Dr S. Bhaduri (USDA, ERRC, Philadelphia, PA, USA). Both cultures were stored on cryopreservation beads at −30 °C (Technical Service Consultants Ltd, Lancashire, UK).

Enumeration methods

Cell numbers in cultures and samples withdrawn at various stages of the experiment were examined as follows. Meat samples were stomached in maximal recovery diluent (MRD) using a Colworth stomacher (Model BA 6024, A.J. Seward & Company Ltd, London, UK). Samples and cultures were serially diluted in MRD.

Direct counts

Direct counts were obtained by plating in duplicate from the dilution series onto appropriate selective and non-selective agars. Samples inoculated with *Y. enterocolitica* were plated on *Y. enterocolitica* selective agar (Oxoid) plus selective supplement (Oxoid) and incubated at 37 °C for 24 h, with further incubation at 25 °C for 24 h. Samples inoculated with *L. monocytogenes* were plated out on Palcam agar (Oxoid) plus selective supplement (Oxoid) and incubated at 30 °C for 48 h. Incubated plates were examined for typical colonies of *Y. enterocolitica* or *L. monocytogenes*. The number of colony forming units (cfu) on the agar plates were counted and converted to $\log_{10}$ cfu g^{-1} counts.

Overlay counts

Injured cells were enumerated by plating onto TSA (Oxoid) and incubating at 25 °C for 6 h. TSA plates from samples inoculated with *Y. enterocolitica* were overlaid with 10 ml *Y. enterocolitica* selective agar (Oxoid) plus selective supplement (Oxoid), incubated and enumerated as described above. TSA plates from samples inoculated with *L. monocytogenes* were overlaid with 10 ml Palcam agar (Oxoid) plus selective supplement (Oxoid), incubated and enumerated as described above.

Inoculum preparation

Listeria monocytogenes and *Y. enterocolitica* were grown in 30 ml brain heart infusion broth (BHI, Oxoid) for 24 h at 25 °C and 30 °C, respectively. A 1·0 ml aliquot of each was sub-cultured in 300 ml BHI at 25 °C for 16 h. The resultant stationary phase cells were recovered by centrifugation at 3019 *g* (Kontron Instruments, Milan, Italy) for 10 min at 4 °C. They were resuspended in either (i) 300 ml BHI or (ii) 30 ml minced beef homogenate, a 1:2 mixture of minced beef and distilled water homogenized in a robot coupe blender (Model R301 ultra, Robot Coupe Ltd, London, UK) and centrifuged at 3019 *g* for 10 min at 4 °C before filtration through a 12·5 cm glass microfibre filter (GF/A, Whatman).

Measurement of beef pH, protein, fat and moisture content

The pH of the beef used in these experiments was measured according to the method described by Bendall (1973). Protein, fat and moisture contents were estimated using standard AOAC (1990) procedures.

Preparation of inoculated vacuum packaged beef

Minced beef samples were prepared and inoculated by dipping strips (approximately 5 × 10 cm) of forequarter beef (*M. sternomandibularis*) in 300 ml of a bacterial suspension and mincing (Crypto-Peerless Model EB12F, Crypto-Peerless Ltd, UK). Lots (10 g) of inoculated, minced beef were vacuum-packaged in Cryovac BB4L bags (7 × 7·5 × 0·3 cm) (W.R. Grace Ltd, County Dublin, Ireland) using a Swissvac machine (Model 380, Swissvac (GB) Ltd, Berkshire, UK).

Solid pieces of beef were prepared by freezing strips of forequarter beef overnight at −5 °C, and cutting them into 50 g pieces (5 × 5 × 2 cm). Pieces were inoculated by injecting 1·0 ml from the 30 ml of inoculum prepared in beef homogenate into the geometric core using a 2 mm glass syringe and needle (B-D Yale Microlance, 18G $1\frac{1}{2}$ 40/12). Inoculated mince and beef pieces were then packaged in 12 × 7 cm vacuum bags (BB4L, Cryovac).

Pre-heating and inoculation of minced beef in vacutainers

Uninoculated minced beef was placed in vacutainers and allowed to equilibrate in water-baths pre-adjusted to 50, 55 or 60 °C (McMahon 1997). The core temperature was continuously monitored using thermocouples (Ellab A/S, Denmark). Once the desired temperature was achieved, 0·1 ml of the same 30 ml bacterial suspension as used to inoculate the solid samples was injected into the core of the sample.

Heat treatment

In the laboratory, heating temperatures of 50, 55 and 60 °C were chosen to facilitate comparison of results with the thermal resistance data reported in the literature. Both inoculated vacuum packs and vacutainers were placed in laboratory water-baths at 50, 55 or 60 °C. Samples were removed periodically and immediately placed in ice-water.

Vacuum-packed minced beef was also heated in the commercial retort, as were vacuum-packed solid samples. Preliminary work in this commercial system established that treatment at or below 48 °C caused little or no reductions in cell numbers, while at temperatures of 56 °C or above, cell death was practically instantaneous, i.e., no cells were detected in sub-samples removed as soon as the core of the minced beef had reached this target temperature. Treatment temperatures of 48, 52 and 56 °C were therefore used in the elements of this study which used the commercial retort system. Samples to be processed by the commercial system were placed on the top tray of a Barriquand Steriflow retort (type: Steroflow 1311 EAG.OG, single basket with a working volume of 1700 cm^3) programmed to heat the samples of beef as rapidly as possible to the target temperature of 48, 52 or 56 °C and to maintain that temperature for the duration of the experiment. The core and surface temperatures of the beef samples, as well as the chamber temperature of the retort, were continuously monitored using Ellab A/S thermocouples.

Calculation of *D*- and *z*-values and statistical analysis

Each experiment was carried out in duplicate and repeated three times at each temperature. The slope (b) and standard error (SE) were obtained for each plot of $\log_{10}$ of surviving cells ml^{-1} against time using linear regression analysis (Genstat 5, Statistics Department, Rothamsted Experimental Station, UK). *D*-values for *Y. enterocolitica* and *L. monocytogenes* were calculated using the average slope for a given treatment. Differences between *D*-values were determined by analysing differences between the average slopes using the *t*-test $(b_1 - b_2/v\ (SE_1)^2 + (SE_2)^2$, with degrees of freedom $(n_1 + n_2) - 4)$. Significant differences between treatments were set at the 5% level of significance ($P < 0{\cdot}05$). The *z*-values for *L. monocytogenes* were obtained by linear regression of the plots of $\log_{10}$ *D*-values against temperature. The Mann–Whitney test was used to compare *z*-values. If all the slopes for a given treatment were greater or less than all the corresponding slopes for the comparative treatment, these differences were considered significant at the 5% level.

RESULTS

The average pH of the beef used was 5·8. The average protein, fat and moisture contents were 20·4, 2·8 and 76%, respectively. Although the retort was programmed to reach the target temperature in each case as quickly as possible, there were, in some cases, considerable delays before samples of beef pieces achieved the target temperatures. Thus, the core of beef piece samples did not reach 48 °C within 32 min, 52 °C within 21 min, or 56 °C within 18 min. The core temperatures of minced beef samples increased much more rapidly under similar conditions. Thus, the core of all minced beef samples achieved the target temperature within 2 min, regardless of the heating system used or target temperature.

In the water-bath, there was no significant difference ($P < 0{\cdot}05$) between the *D*-values obtained on the selective and overlay agars, so only the results for TSA overlaid with the relevant selective agar are given. The *D*-values obtained for *L. monocytogenes* and *Y. enterocolitica* in the minced beef (pre-heated in vacutainers) and in vacuum bags (and therefore not pre-heated) are given in Table 1. There was a significant difference ($P < 0{\cdot}05$) between the pre-heated (vacutainer) and non-pre-heated (vacuum bag) samples for *L. monocytogenes* at 60 °C and for *Y. enterocolitica* at all three temperatures.

In the commercial retort, with the exception of the D_{56}-value for *L. monocytogenes* on TSA, the *D*-values obtained in solid beef were significantly greater ($P < 0{\cdot}05$) than those recorded in minced beef samples (Table 2).

A similar finding was observed with *Y. enterocolitica,* which was significantly more thermoresistant when inoculated into the geometric core of a solid beef piece than when minced into the beef sample (Table 3). With the exception of *L. monocytogenes* in mince, there was no significant difference ($P < 0{\cdot}05$) between the *D*-values obtained with the direct (selective) and resuscitation counts.

The *z*-values calculated for *L. monocytogenes* in the sous vide beef heated in the retort, and in the minced beef heated in the water-bath, are presented in Table 4. These ranged from 5·5 to 5·9 °C for the former and from 4·2 to 4·9 °C for the latter. A comparison of the *z*-values obtained with these different heating methods, using the Mann–Whitney test, showed that there was a significant difference ($P < 0{\cdot}05$) between water-bath- and retort-generated data.

DISCUSSION

The *D*-values obtained in this study for both organisms were considerably lower than those reported in the literature (Gaze *et al.* 1989; Sorqvist 1989; Mackey *et al.* 1990; Toora *et al.* 1992). These differences may be due to a number of factors previously reported to influence heat resis-

Table 1 *D*-values for *Listeria monocytogenes and Yersinia enterocolitica* in minced beef using a laboratory water-bath

Temperature (°C)		*D*-value (min)	Slope	S.E.*	*n*†
L. monocytogenes					
	Vacutainer	32·7	−0·0306	0·0016	17
50	Vacutainer	32·7	−0·0306	0·0016	17
	Vacuum pack	36·1	−0·0277	0·0015	21
55	Vacutainer	3·4	−0·2918	0·0290	17
	Vacuum pack	3·2	−0·3128	0·0233	24
60	Vacutainer	0·31	−3·2753	0·310	11
	Vacuum pack	0·15	−6·5579	0·3691	24
Y. enterocolitica					
	Vacutainer	17·4	−0·0576	0·0046	20
50	Vacutainer	17·4	−0·0576	0·0046	20
	Vacuum pack	21·2	−0·0471	0·0021	17
55	Vacutainer	1·96	−0·5102	0·0330	20
	Vacuum pack	1·06	10·937	0·1016	17
60	Vacutainer	0·97	−1·0277	0·1668	20
	Vacuum pack	0·55	−1·8027	0·2486	17

*S.E., standard error of the slope.
† *n*, number of observations.

tance, including variations between strains (Gaze *et al.* 1989) and the differences in the extent of heat protection afforded by the environment in which heating takes place (Fain *et al.* 1991; Embarek and Huss 1993).

The significant differences ($P < 0·05$) in *D*-values obtained in retorted solid compared with retorted minced beef were attributed to variations in the heating rates. Indeed, slow heating rates (less than 5 °C min^{-1}) are known to significantly increase the *D*-values for *L. monocytogenes* (Quinvalla and Campanini 1991; Kim *et al.* 1994) and *Y. enterocolitica* (Shenoy and Murano 1996), and this is thought to be due to the formation of heat-shock proteins (Stephens *et al.* 1994).

With the exception of *L. monocytogenes* in retorted minced beef, the media used did not significantly effect *D*-values. This was an unusual finding as the number of sur-

Table 2 *D*-values for *Listeria monocytogenes* in solid and minced beef when heated in a commercial retort

Temperature (°C)	Beef sample	Culture medium	D-value (min)	Slope	S.E.*	*n*†
48	Solid	Palcam	88·6	−0·0113	0·0007	18
48	Solid	TSA	92·6	−0·0108	0·0011	18
48	Mince	Palcam	74·1	−0·0135	0·0011	18
48	Mince	TSA	65·8	−0·0152	0·0008	18
52	Solid	Palcam	26·7	0·0375	0·0030	18
52	Solid	TSA	29·0	−0·0345	0·0032	18
52	Mince	Palcam	16·3	−0·0612	0·0039	18
52	Mince	TSA	21·3	−0·0469	0·0027	18
56	Solid	Palcam	3·1	−0·3220	0·0180	18
56	Solid	TSA	3·3	−0·3033	0·0215	18
56	Mince	Palcam	2·3	−0·4385	0·0264	18
56	Mince	TSA	3·0	−0·3340	0·0203	18

*S.E., standard error of the slope.
†*n*, number of observations.

Table 3 *D*-values for *Yersinia enterocolitica* in solid and minced beef when heated in a commercial retort

Temperature (°C)	Beef sample	Culture medium	*D*-value (min)	Slope	S.E.*	*n*†
48	Solid	YSA	70·9	−0·0141	0·0008	18
48	Solid	TSA	75·8	−0·0132	0·0006	17
48	Mince	YSA	44·2	−0·0226	0·0007	17
48	Mince	TSA	44·8	−0·0223	0·0017	15
52	Solid	YSA	9·6	−0·1040	0·0061	18
52	Solid	TSA	9·7	−0·1030	0·006	18
52	Mince	YSA	6·1	−0·1638	0·0080	18
52	Mince	TSA	7·1	−0·1415	0·0083	18
56	Solid	YSA	2·0	−0·4963	0·0595	17
56	Solid	TSA	1·4	−0·7411	0·2064	15
56	Mince	YSA	1·2	−0·8295	0·0654	1
56	Mince	TSA	1·2	−0·8482	0·0597	18

*S.E., standard error of the slope.
†*n*, number of observations.

viving cells detected after heating is often dependent on the enumeration media used (Embarek and Huss 1993) and hence, the choice of media has a considerable effect on the *D*-values subsequently calculated (Fain *et al.* 1991). This is especially true of selective media, which often contain substances which do not inhibit undamaged cells but which do prevent the growth of injured cells. This has been shown to be the case with selective media for *L. monocytogenes* (Golden *et al.* 1988) and *Y. enterocolitica* (Hanna *et al.* 1977; Fukushima and Gomyoda 1986; Sheridan *et al.* 1998). It would therefore appear that, under the conditions used in these experiments, cells were either destroyed completely or not effected at all.

The *z*-values obtained for *L. monocytogenes* ranged from 4·2 to 5·9 °C and are well within those reported in the literature for the organism, ranging from 3·39 °C in chicken

Table 4 *z*-values for *Listeria monocytogenes* in the retort

Heating method	Beef sample	Culture medium	*z*-value	Slopes	Average slope
Retort	Solid	Palcam	5·5	−0·1965 −0·1663 −0·1852	−0·1827
Retort	Solid	TSA	5·6	−0·1882 −0·1824 −0·1690	−0·1799
Retort	Mince	Palcam	5·3	−0·1824 −0·1941 −0·1901	−0·1888
Retort	Mince	TSA	5·9	−0·1765 −0·1599 −0·1706	−0·1690
Water-bath bag	Mince	TSA	4·2	−0·2280 −0·2546 −0·2276	−0·2368
Water-bath vacutainer	Mince	TSA	4·9	−0·2019 −0·2014 −0·2016	−0·2017

gravy (Huang *et al*. 1992) to 13·2 °C (Fain *et al*. 1991) in fatty ground beef. However, analysis of the *z*-values suggested that thermal resistance data obtained for *L. monocytogenes* in the laboratory were not applicable in a commercial setting. In other words, laboratory-generated data may not always give an accurate portrayal of how pathogens behave in commercial systems, and only data obtained using these systems should be used to model commercial applications and predict adequate cooking time–temperature combinations.

ACKNOWLEDGEMENT

This research was performed as part of the EEC Agro-Industrial Research (AIR) project No. CT 93–1519

REFERENCES

AOAC (1990) *Official Methods of Analysis* 15th edn Virginia, USA: Association of Official Analytical Chemists Inc.

Bendall, J.R. (1973) Post-mortem changes in muscle. In *The Structure and Function of Muscle* 2nd edn, Vol. II, ed. Browne, G.H. pp. 275–279. New York, NY: Academic Press.

Betts, G.D. (1992) *The Microbiological Safety of Sous-Vide Processing*. Technical Manual no. 39. Campden: Campden Food & Drink Association.

Embarek, P.K.B. and Huss, H.H. (1993) Heat resistance of *Listeria monocytogenes* in vacuum packaged pasteurized fish fillets. *International Journal of Food Microbiology* **20**, 85–95.

Fain, A.R., Line, J.E., Moran, A.B. et al. (1991) Lethality of heat to *Listeria monocytogenes* Scott A: *D*-value and *z*-value determinations in ground beef and turkey. *Journal of Food Protection* **54**, 756–761.

Fransen, N.G., van den Elzen, A.M.G., Urlings, B.A.P. and Bijker, P.G.H. (1996) Pathogenic microorganisms in slaughterhouse sludge — a survey. *International Journal of Food Microbiology* **33**, 245–256.

Fukushima, H. and Gomyoda, M. (1986) Growth of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* biotype 3B serotype O:3 inhibited on cefsulodin-irgasan-novobiocin agar. *Journal of Clinical Microbiology* **24**, 116–120.

Gaze, J.E., Brown, G.D., Gaskell, D.E. and Banks, J.G. (1989) Heat resistance of *Listeria monocytogenes* in homogenates of chicken, beef steak and carrot. *Food Microbiology* **6**, 251–259.

Gill, C.O. and Reichel, M.P. (1989) Growth of the cold-tolerant pathogens *Yersinia enterocolitica, Aeromonas hydrophila* and *Listeria monocytogenes* on high pH beef packaged under vacuum or carbon dioxide. *Food Microbiology* **6**, 223–230.

Golden, D.A., Beucht, L.R. and Brackett, R.E. (1988) Inactivation and injury of *Listeria monocytogenes* as affected by heating and freezing. *Food Microbiology* **5**, 17–23.

Hanna, M.O., Stewart, J.C., Carpenter, Z.L. and Vanderzant, C. (1977) Effect of heating, freezing and pH on *Yersinia enterocolitica*-like organisms from meat. *Journal of Food Protection* **40**, 689–692.

Huang, I.D., Yousef, A.E., Marth, E.H. and Matthews, M.E. (1992) Thermal inactivation of *Listeria monocytogenes* in chicken gravy. *Journal of Food Protection* **55**, 492–496.

Hudson, J.A., Mott, S.J. and Penny, N. (1994) Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on vacuum and saturated carbon dioxide controlled atmosphere packaged sliced roast beef. *Journal of Food Protection* **57**, 204–208.

Kim, K., Murano, E.A. and Olson, D.G. (1994) Heating and storage conditions affect survival and recovery of *Listeria monocytogenes* in ground pork. *Journal of Food Science* **59**, 30–32.

Logue, C.M., Sheridan, J.J., Wauters, G., McDowell, D.A. and Blair, I.S. (1996) *Yersinia* spp. and numbers, with particular reference to *Y. enterocolitica* bio/serotypes, occurring on Irish meat and meat products, and the influence of alkali treatment on their isolation. *International Journal of Food Microbiology* **33**, 257–274.

Mackey, B.M., Pritchet, C., Norris, A. and Mead, G.C. (1990) Heat resistance of *Listeria*: strain differences and effect of meat type and curing salts. *Letters in Applied Microbiology* **10**, 251–255.

McMahon, C.M. (1997) Pathogen Control in Sous Vide Processing by Thermal Inactivation and Hurdle Technology. Thesis, The National Food Centre/ University of Ulster.

Pralus, G. (1993) 'Sous vide' cooking. In *Proceedings of the FLAIR First European 'sous vide' Cooking Symposium*. pp. 143–150. Leuven: Alma Sous Vide Competence Centre.

Quintavalla, S. and Campanini, M. (1991) Effect of rising temperature on the heat resistance of *Listeria monocytogenes* in meat emulsion. *Letters in Applied Microbiology* **12**, 184–187.

Rhodehamel, E.J. (1992) FDA's concerns with sous vide processing. *Food Technology*, **46**, 73–76.

Schellekens, M. (1996) New research issues in sous-vide cooking. *Trends in Food Science and Technology* **7**, 256–262.

Schofield, G.M. (1992) Emerging food-borne pathogens and their significance in chilled foods. *Journal of Applied Bacteriology* **72**, 267–273.

Shenoy, K. and Murano, E.A. (1996) Effect of heat shock on the thermotolerance and protein composition of *Yersinia enterocolitica* in Brain heart infusion broth and ground pork. *Journal of Food Protection* **59**, 60–364.

Sheridan, J.J., Logue, C.M., McDowell, D.A., Blair, I.S. and Hegarty, T. (1998) A study of the growth kinetics of *Yersinia enterocolitica* serotype 0:3 in pure and meat culture systems. *Journal of Applied Microbiology* **85**, 293–301.

Smith, J.P., Toupin, C., Gagnon, B., Voyer, R., Fiset, P.P. and Simpson, M.V. (1990) A Hazard Analysis Critical Control Point Approach (HACCP) to ensure the microbiological safety of sous vide processed meat/pasta product. *Food Microbiology* **7**, 177–198.

Sorqvist, S. (1989) Heat resistance of *Campylobacter* and *Yersinia* strains by three methods. *Journal of Applied Bacteriology* **67**, 543–549.

Stephens, P.J., Cole, M.B. and Jones, M.V. (1994) Effect of heating rate on thermal inactivation of *Listeria monocytogenes*. *Journal of Applied Bacteriology* **77**, 702–708.

Toora, S., Budu-Amoaka, E., Ablett, R.F. and Smith, J. (1992) Effect of high temperature short-time pasteurization, freezing and thawing and constant freezing, on the survival of *Yersinia enterocolitica* in milk. *Journal of Food Protection* **55**, 803–805.

Walker, S.J., Archer, P. and Banks, J.G. (1990) Growth of *Listeria monocytogenes* at refrigeration temperatures. *Journal of Applied Bacteriology* **68**, 157–162.

Wendlandt, A. and Bergann, T. (1994) *Listeria monocytogenes*. Occurrence in a factory for slaughtering, carving and meat processing. *Fleischwirtschaft* **74**, 1329–1331.