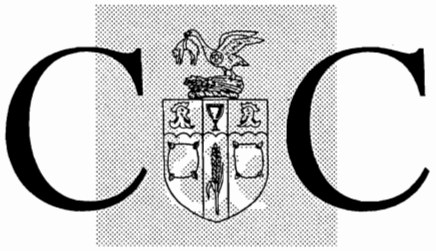
CONFIDENTIAL R&D REPORT NO. 48

The Determination of the Heat Resistance of *Salmonella typhimurium* in High Total Solids Foods

September 1997



Campden & Chorleywood
Food Research Association

Campden & Chorleywood
Food Research Association

Director-General
Prof. C. Dennis
BSc, PhD, FIFST

Chipping Campden
Gloucestershire
GL55 6LD UK
Tel: +44 (0) 1386 842000
Fax: +44 (0) 1386 842100

Confidential R&D Report No. 48
Project No. 20195

The Determination of the Heat Resistance of *Salmonella typhimurium* in High Total Solids Foods

Richard Taylor, Alison Bailie and Joy Gaze

September 1997

SUMMARY

The inactivation kinetics of *Salmonella typhimurium* inoculated into a range of high total solid/ high sugar foods were determined at temperatures of 58, 60, 63,65 and 68°C. The effects of total solids, water activity, protein, carbohydrate and fat content of the products on the heat resistance of *S. typhimurium* were analysed.

All the death curves obtained could be assessed by linear regression in all the heating substrates investigated. The D-values obtained at 58°C ranged from 8.4 minutes in half cream to 1087.2 minutes in cocoa mass. At 68°C, the D-values obtained ranged from 0.3 minutes in custard and half cream to 36.3 minutes in cocoa mass. Cocoa mass and peanut butter had the highest total solids content, 98.3 and 99.0% respectively, and conversely custard and half cream had the lowest total solids content, 23.8 and 21.8% respectively.

Specific associations between the heat resistance characteristics of *S. typhimurium* and product constituents could not be identified by statistical analysis of variance; however, certain trends were observed. It was noted that the heat resistance of *S. typhimurium* in high total solid products, i.e. cocoa mass and peanut butter, was up to sixty times higher than in a low solids substrates such as nutrient broth. Also of note was the differences observed in the heat resistance of *S. typhimurium* in products with similar constituents. The heat resistance in butter for example, was considerably lower than in products with a similar combination of solids and fat content.

The z values obtained in these products ranged from 4.6 in custard to 13.6 in glucose syrup.

The data presented in this study demonstrate the marked increased heat resistance of *S. typhimurium* in products whose constituents contribute to high total solid or low water activity foods. It also demonstrates that to assure safety, constant process evaluation is needed if a product under development changes its composition of solids, fat or water activity.

CONTENTS

INTRODUCTION

Salmonella has been implicated in several outbreaks of disease associated with dairy products with an increased solids or fat content such as cheese (Maguire *et al*, 1992; Barrett, 1986; PHLS, 1996; Ratnam and March, 1986), cream (Barrett, 1986; Evans *et al*, 1996) and ice cream (Hennessy *et al*, 1996; Oemichen, 1995; Buckner *et al*, 1994; Morgan *et al*, 1994; Cowden *et al*, 1989), the majority of which have occurred due to the processing of contaminated raw milk, or pasteurised milk that had been cross contaminated. Data on the survival of *Salmonella* at pasteurisation temperatures is conflicting; for example, Humphrey *et al* (1990) showed that *Salmonella enteritidis* PT4, *Salmonella typhimurium* PT141 and *Salmonella senftenberg* 775W should not survive the pasteurisation of liquid egg, whilst Shah *et al* (1991), demonstrated that pasteurisation of whole liquid egg at 60°C for 2.83 minutes would result in only a 4 log decimal reduction in population.

It has been established that the concentration and growth history of salmonellae can affect the heat resistance of the organism and therefore the chances of survival after pasteurisation. Growth of *Salmonella* above its optimum temperature can lead to thermal tolerance being acquired (Neidhart and Van Bogelen, 1987); in fact Mackey and Derrick (1986) reported a 2-3 fold increase in the heat resistance of *S. typhimurium* under such conditions.

In addition to temperature, the effect of dissolved solutes and fats in the heating menstruum has also been investigated (D'Aoust, 1978; Gomez *et al*, 1973). Reports have shown that the heat resistance of bacteria has an inverse relationship with Aw (Barrile and Cone, 1970; Thomas *et al*, 1966; Goepfert *et al*, 1970 and Archer *et al*, 1997). The complexity of the menstruum also affects the heat resistance as shown in studies using *S. senftenberg* 775W by Thomas *et al* (1966). The effect of solute type on the heat resistance of salmonellae was demonstrated by Goepfert *et al* (1970), who showed that the protective effect afforded by sucrose, sorbitol, fructose and glycerol varied. *Salmonella anatum* heated in solutions of carbohydrates, proteins, salts and gums resulted in an additive increase in heat resistance; however, the protective effect of whole milk greatly exceeded all of these effects (Moats *et al*, 1971).

The implications of these data are that the protective effect observed in experiments using certain food components is complex and not easily quantifiable. In the following studies investigations were concentrated in the areas of high total solid / high sugar foods in anticipation that certain variables such as Aw would be indirectly controlled. By generating heat resistance data of *S. typhimurium* heated in products such as cream, butter, chocolate, dry meat, syrups and spreads, the relationship between levels of fat, protein, carbohydrate and the microorganism could then be analysed and discussed.

The overall objectives of the study were therefore to provide detailed analysis of the food products tested and evaluate any associations with these to explain reasons for enhanced heat resistance and provide a database for these types of product for industry reference. It was anticipated that this study would assist the food industry in understanding how widespread the issues of food composition are and consequently assist with the design and development of safe food products.

MATERIALS AND METHODS

Microorganism

Salmonella typhimurium CRA 1005 (from the CCFRA culture collection) was used in this study. This strain was originally isolated from raw egg.

The heat resistance of other strains of *S. typhimurium* 1627 (CRA), 1009 (CRA), 1007 (CRA) and 1005 (CRA) were tested in Nutrient Broth (NB). Strain 1005 was shown to have the highest heat resistance and was chosen for further work.

Glass beads coated with frozen liquid culture (-80°C) of strain 1005 were suspended in NB and incubated overnight at 37°C. The liquid cultures were used to inoculate Nutrient Agar (NA) slopes on which the strains were maintained throughout the study at refrigeration temperature. The strains were re-streaked every two weeks.

Growth curves

To determine the incubation period of *S. typhimurium* for use as inoculum, the organism was inoculated into NB and incubated at 37°C. At intervals of 0, 3, 6, 23, 25, 28 and 48 hours samples were removed, diluted with Maximum Recovery Diluent (MRD, Oxoid) and plated onto NA. After 48 h incubation at 37°C, typical colonies were enumerated. From a graph of log 10 colony population against time, the time to reach the stationary phase of growth was estimated. This incubation period was used for all *S. typhimurium* inoculum preparations for the study.

Recovery procedure

Different growth media and incubation times at 37°C were evaluated in order to maximise recovery. The media used were: Tryptone Soya Agar plus Yeast Extract (TSAYE), Nutrient Agar (NA), Plate Count Agar (PCA), Yeast Dextrose Tryptone agar plus Starch (YDTAS) and Brilliant Green Agar (BGA). Cultures of *S. typhimurium* 1005 were grown up for 24 h at 37°C and then inoculated onto different media. The plates were incubated at 37°C for 18-48 hours before typical colonies were enumerated.

Heat resistance determinations in broth

S. typhimurium strains CRA 1627, 1009, 1007 and 1005 were inoculated into 10ml NB and grown for 24 hours until the stationary phase of growth was reached. The liquid culture was inserted into glass spheres in 0.1 ml volumes and then heat sealed. The spheres were immersed in a waterbath at the test temperature for pre-determined time intervals. Following heating, the spheres were removed and cooled immediately in iced water. The spheres were

then washed in 5% hydrogen peroxide solution followed by sterile distilled water (SDW) and then crushed into MRD using sterile glass rods. Serial dilutions were prepared and plated on NA. These were incubated at 37°C for 48 hours and the number of colony forming units (CFU) enumerated.

The time taken for a 1 log reduction in population (D-value) was calculated from linear regression on a graph of log10 CFU against time.

Heat resistance determination in product

A loopful of viable *S. typhimurium* 1005 was taken from a NA slope and inoculated into 10g of pureed product and thoroughly mixed to give an initial level of between 10^5 and 10^9 per g of product. In the case of butter and cocoa mass it was necessary to allow these to liquefy at 40°C prior to inoculation to enable the product to mix evenly with the inoculum. Inoculated product was introduced into capillary tubes in 0.05g amounts. These tubes were heat sealed and immersed in a waterbath at the test temperature. The temperatures investigated were 58,60,63,65 and 68°C. The time taken for the product to reach the test temperature was calculated to be between 0.5 and 1.5 seconds. This 'come-up' time would have a negligible effect on the determination of a D-value by linear regression. The tubes were then treated in the same way as the glass spheres as previously described in the broth studies.

Calculation of the D and z values

The log10 CFU of viable survivors following heating was plotted against time of exposure to the test temperature. Linear regression was used to correlate the data points. The reciprocal of the slope of the regression line was used to express the D-value (length of exposure at the test temperature required to achieve a 1 log or tenfold reduction in the viable population). Similarly the reciprocal of the correlation line between the log 10 of the D-value and the temperature was used to express the number of degrees Celsius needed to increase or decrease the D-value tenfold (z-value).

Products investigated

The composition of the products tested were expressed in % weight/weight (w/w) as determined by standard chemical tests (UKAS accredited procedures). Total solids (% w/w) was determined after drying the product in a hot air oven (UKAS accredited procedure) and the results are shown in Table 1.

TABLE 1

The composition of the test products

Product	Carbohyd. (% w/w)	Fat (% w/w)	Protein (% w/w)	Solids (% w/w)	Aw	pH
Nutrient broth	0.5	0.01	2.0	1.4	0.999	7.4
Butter	0	82.7	0.5	83.2	0.952	6.3
Cocoa mass	23.3	55.0	10.8	98.3	0.253	5.5
Glucose syrup	84.7	0	0	85.9	0.612	5.4
Golden syrup	80.5	0	0	69.8	0.61	4.8
Honey	80.0	0	0	80.8	0.851	3.8
Double cream	3.1	55.6	1.9	55.8	0.980	6.7
Custard	16.2	3.0	3.1	23.8	0.977	6.5
Half cream	4.0	12.0	3.0	21.8	0.977	5.5
Peanut butter	12.0	50.5	27.8	99.0	0.368	6.7
Salami	0.4	29.5	20.7	44.9	0.241	5.1

RESULTS

Preliminary work

From the results of the growth curve, *S. typhimurium* 1005 reached stationary phase after approximately 18 hours; therefore, a period of 20 hours was chosen as the incubation period at 37°C to ensure that cells were in the stationary phase of growth.

Recovery of *S. typhimurium* was optimal after a 48 hour incubation period at 37°C. There were no significant differences between recovery on the different agars tested, therefore NA was chosen.

Heat resistance of *Salmonella typhimurium* in broth and products

The death kinetics observed for *S. typhimurium* could be assessed by linear regression in all the heating substrates tested. The results of the heat resistance experiments are given in Table 2. The correlation coefficients (for log 10 survivors against time) ranged from 0.79 to 0.99 (figures 1 - 11.6 in Appendix). The z values obtained for *S. typhimurium* in the test products ranged from 4.61C° in custard to 13.55C° in glucose syrup (Table 3).

The D-values obtained for nutrient broth were comparable to those obtained in half cream, custard and salami. The heat resistance of *S. typhimurium* in cocoa mass and peanut butter was significantly higher than the other products tested. The D-value in cocoa mass and peanut butter was approximately sixty times greater than in nutrient broth irrespective of temperature. The difference in D-values obtained in these products was consistently observed at all test temperatures and was not prominent at any particular temperature.

S. typhimurium heated in golden syrup and butter was shown to have comparable heat resistance characteristics although the products were very different in composition, i.e. butter is 82.7% fat and golden syrup is 84.7% carbohydrate.

In general, products with a high solids and/or fat content (Table 1) resulted in the highest heat resistance to *S. typhimurium*. For example, *S. typhimurium* heated in butter, cocoa mass, glucose syrup and peanut butter gave D-values at 63°C of greater than 19 minutes and all of these products had a total solids content of greater than 69%. Of the other products, the next highest D-value was in custard (6.8 minutes at 63°C) which had a lower solids content of 23.8%. One exception to this trend was honey which has a total solids content of 80.8%, but demonstrated a D-value of only 3.57 minutes at 63°C. The pH for honey was lower than for the other products, i.e. 3.8 compared to the next lowest pH which was for golden syrup (pH 4.8).

TABLE 2

Summary of D-value data obtained for *Salmonella typhimurium* heated in the test products.

Product	D-value (minutes)				
	58°C	**60°C**	**63°C**	**65°C**	**68°C**
Half cream	8.4 (0.98)	6.0 (0.99)	1.9 (0.95)	1.0 (0.99)	0.3 (0.98)
Nutrient broth	14.3 (0.91)	9.2 (0.95)	2.5 (0.87)	2.4 (0.95)	0.4 (0.96)
Salami	14.6 (0.99)	9.2 (0.98)	2.3 (0.91)	2.0 (0.99)	1.2 (0.90)
Double cream	31.3 (0.97)	10.7 (0.98)	5.2 (0.97)	1.6 (0.98)	0.9 (0.90)
Honey	40.1 (0.88)	5.7 (0.96)	3.6 (0.98)	1.8 (0.93)	1.3 (0.95)
Custard	45.2 (0.79)	13.6 (0.98)	6.8 (0.97)	0.9 (0.98)	0.3 (0.97)
Glucose syrup	46.9 (0.98)	36.4 (0.89)	29.9 (0.98)	15.7 (0.99)	8.5 (0.99)
Golden syrup	91.7 (0.90)	42.6 (0.89)	19.1 (0.94)	15.3 (0.88)	7.6 (0.99)
Butter	132.0 (0.98)	75.0 (0.97)	24.9 (0.99)	7.1 (0.99)	4.9 (0.99)
Peanut butter	774.0 (0.86)	526.0 (0.91)	268.5 (0.95)	229.0 (0.90)	32.4 (0.98)
Cocoa mass	1087.2 (0.91)	330.0 (0.94)	209.7 (0.95)	141.5 (0.95)	36.3 (0.96)

Figures in brackets indicate the correlation obtained for calculation of the D-value.

The data also shows difference in heat resistance of *S. typhimurium* within products of similar components. For example the three products with the highest combination of solids and fat content were butter, peanut butter and cocoa mass; however, the D-values obtained in butter were considerably lower than those found in cocoa mass and peanut butter. The major difference in composition of these products appears to be available water (Aw) with butter having a much higher water activity than the other two products.

Differences can also be observed when comparing the D-values for *S. typhimurium* in glucose syrup and honey. Both of these products have similar levels of carbohydrate, fat, protein and total solids and the D-values of *S. typhimurium* at 58°C are similar: 46.9 and 40.1 minutes in glucose syrup and honey respectively; however, as the test temperature increases it can be seen that numbers of *S. typhimurium* were reduced more quickly in the honey than in the glucose syrup. The major difference between the two products is the low pH of the honey.

The z values show a range of rate in change of D-value from 4.6 - 13.6C°. The six products with the highest z values, i.e. salami, cocoa mass, peanut butter, golden syrup, glucose syrup and honey (z =7.3 - 13.6C°), are also the products with the lowest Aw (0.241 - 0.851).

TABLE 3

Summary of z value data obtained from heating *S. typhimurium* CRA 1005 at 58 to 68°C in the test products.

Product	Correlation τ	z value
Nutrient broth	0.97	6.6
Butter	0.98	6.5
Cocoa mass	0.97	7.6
Glucose syrup	0.97	13.6
Golden syrup	0.99	9.5
Honey	0.92	7.3
Double cream	0.98	6.5
Custard	0.98	4.6
Half cream	0.99	6.8
Peanut butter	0.94	7.9
Salami	0.97	8.6

τ Correlation of linear regression applied to all the log10 D-value data points for a particular product.

DISCUSSION

The heat resistance results obtained for *S. typhimurium* CRA 1005 are comparable to some of the data obtained by Goepfert *et al* (1969) in his investigations into the heat resistance of salmonellae at different water activities. The study reported mean $D_{57.2°C}$-values of 26.5 and 61.5 minutes at Aw's of 0.96 and 0.87 in solutions adjusted using sucrose. Heat resistance data obtained in this study showed comparable D-values in some of the products tested, for example the $D_{58°C}$ -value of *S. typhimurium* in half cream (Aw 0.98) was 8.4 minutes and in honey (Aw 0.85) was 40.1 minutes. In general, the strain used in this study was between 5 and 10 times more heat resistant at comparable Aw's; however, most of the products tested were not comparable with the Aw's used in Goepfert's study. The higher heat resistance of CRA 1005 might be explained by its origin, the fact that it was chosen for its high heat resistant properties and the environment it was heated in, i.e. high fat and high total solids.

The increased heat resistance of bacterial spores and vegetative cells when heated in the presence of a high fat environment has been frequently reported (Senhaji and Loncin, 1971; Gaze, 1985; Ababouch *et al*, 1995). The mechanism by which this is thought to happen is by the presence of free fatty acids in the foods which coat the bacteria and exert a stabilising effect (Ababouch *et al*, 1995).

Thomas *et al* (1966) reported heat resistance data for *S. senftenberg* 775W heated in 0.5% sodium chloride solution and green pea soup. $D_{68.3°C - 71.1°C}$ values ranged from 3.7 to 10.0 minutes which is comparable to the mean $D_{68°C}$, for all products obtained in this study, i.e. 8.5 minutes. Some products in this study such as peanut butter and cocoa mass, however, displayed higher $D_{68°C}$-values, 32.4 and 36.3 minutes respectively, which again may be explained by the high fat and solids content of the heating substrate.

The results of the data show that many substances present in foods may significantly protect bacteria from heat. The data shows that products with high levels of solids and/or fats have up to a 10 fold higher heat resistance than simple substrates such as nutrient broth and half cream.

It has been suggested that the reduced water activity of a substrate protects bacteria from heat (Calhoun and Frazier, 1966; Hansen and Riemann, 1963); however, these studies appear to be in agreement with those of Goepfert *et al* (1970) which show that the heat resistance of salmonellae is not dependent on the Aw of the environment alone but more importantly it is dependent upon the product constituent that is affecting the Aw. This is quite clearly demonstrated if the heat resistance of *S. typhimurium* in cocoa mass and salami is considered. Both products have a similar Aw: cocoa mass 0.253 and salami 0.241; however, the D-value is between 31 and 98 times higher in cocoa mass than in salami at all test temperatures. The

major differences between the two products are the solids content and fat content (98.3% and 44.9%, 55.0 and 29.5% for cocoa mass and salami respectively).

Overall, the heat resistance could not be correlated directly to any one of the product components investigated; however, there appeared to be trends, with increased heat resistance in products with low Aw, high fat and high total solids. Statistical analysis of the data showed that heat resistance was product specific but it was unable to identify any specific links between heat resistance and product components.

The mechanism for Aw and solute-dependent heat resistance has not been fully investigated. Theories include plasmolysis and loss of cell water which may alter the structure of cell proteins (Corry, 1974; Gibson, 1973). Other explanations include a stress response to cell starvation as found in osmotic shock, heat shock and oxidative stress (Jenkins *et al,* 1988; Martin *et al,* 1989; Spence *et al*, 1990). The increased heat resistance of *Pseudomonas fluorescens* due to cell starvation was also observed in investigations by Jorgensen *et al* (1994).

If the data is extrapolated from the D-values obtained at 68°C, a pasteurisation process of 70°C for 2 minutes would achieve between 0.1 and 16.5 log reductions of *S. typhimurium* CRA 1005 depending on the substrate. This process would achieve greater than 6 log reductions in nutrient broth, custard and half cream; between 2.7 and 4.5 log reductions in honey, double cream and salami; and less than 1 log reduction in the other products investigated. The serious implications of this data for high level contamination of this type of product with *S. typhimurium* CRA 1005 are obvious and point to the importance of independent evaluation of products of this type. They also give a cautionary warning to those using existing data on the heat resistance of salmonellae in one food product that these may not apply to the safe pasteurisation of a different product.

CONCLUSIONS

The data presented in this study demonstrate the increased heat resistance of *Salmonella typhimurium* when heated in products whose components contribute to a high total solids or low water activity content. The effect of individual components, i.e. fat, carbohydrate or protein, could not be quantified or statistically separated; however, trends in the data suggest that fat and solids content are important in modifying the heat resistance of *S. typhimurium*.

The effects of components in products of this type could have serious implications in terms of thermal pasteurisation processes. It was shown that products with similar Aw could demonstrate up to a 9 fold difference in heat resistance characteristics. The implications of these findings when developing products are that even small changes in product formulation (e.g. solids or fat content) could lead to large changes in the protective effect afforded to pathogens. Independent evaluation during development is therefore required to ensure the production of safe food products.

REFERENCES

Ababouch, L.H., Grimmit, R., Eddafry and Busta, F.F. 1995. Thermal inactivation kinetics of *Bacillus subtilis* spores suspended in buffers and oils. Journal of Applied Bacteriology. **78**: 669-676.

Archer, J., Jervis, E.T. and Gaze, J.E. 1997. The heat resistance of *Salmonella weltevreden* in flour. CCFRA Research and Development Report. In press.

Barrett, N.J. 1986. Communicable disease associated with milk and dairy products in England and Wales: 1983 - 1984. Journal of Infection. **12**: 265-272.

Barrile, J.C. and Cone, F.J. 1970. Effect of added moisture on the heat resistance of *Salmonella anatum* in milk chocolate. Applied Microbiology. **19** (1): 177-178.

Buckner, P., Fergusen, D., Anzalone, F., Anzalone, D., Taylor, J., Hlady, W.G. and Hopkins, R.S. 1994. Outbreak of *Salmonella enteritidis* associated with home made ice-cream - Florida 1993. Morbidity and Mortality Weekly Report. **43** (36): 669-671.

Calhoun, C.L. and Frazier, W.C. 1966. Effect of available water on the thermal resistance of three non-sporeforming species of bacteria. Applied Microbiology. **14**: 416-420.

Corry, J.E.L. 1974. The effect of sugars and polyols on the heat resistance of salmonellae. Journal of Applied Bacteriology. **37**: 31-43.

Cowden, J.M., Lynch, D., Joseph, C.A., O'Mahoney, M., Mawer, S.L., Rowe, B., and Barttlett, C.L.R. 1989. Case-control study of infections with *Salmonella enteritidis* phage type 4 in England. British Medical Journal. **299** (6702): 771-773.

D'Aoust, J.Y. 1978. Recovery of sublethally heat-injured *Salmonella typhimurium* on supplemented plating media. Applied and Environmental Microbiology. **35**(3): 483-486.

Evans, M.R., Tromans, J.P., Dexter, E.L.S, Riberio, C.D. and Gardner, D. 1996. Consecutive *Salmonella* outbreaks traced to the same bakery. Epidemiology and Infection. **116** (2): 161-167.

Gaze, J.E. 1985. The effect of oil on the heat resistance of *Staphylococcus aureus* Food Microbiology. **2**: 227-283.

Gibson, B. 1973. The effect of high sugar concentrations on the heat resistance of vegetative micro-organisms. Journal of Applied Bacteriology. **36**: 365-376.

Goepfert, J.M., Iskander, I.K. and Amundson, C.H. 1970. Relation of the heat resistance of salmonellae to the water activity of the environment. Applied Microbiology. **19** (1): 429-433.

Gomez, R.F., Sinskey, A.J., Davies, R. and Labuza, T.P. 1973. Minimal medium recovery of heated *Salmonella typhimurium* LT-2. Journal of General Microbiology. **74**: 267-274.

Hansen, N.H. and Riemann, H. 1963 Factors affecting the heat resistance of non-sporing organisms. Journal of Applied Bacteriology. **26**: 314-333.

Hennessy, T.W., Hedberg, C.W., Slutsker, L., White, K.E., Besser-Wiek, J.M., Moen, M.E., Feldman, J., Coleman, W.W., Edmunson, L.M., MacDonald, K.L. and Osterholm, M.T. 1996. A national outbreak of *Salmonella enteritidis* infections from ice-cream. New England Journal of Medicine. **334**(20): 1281-1286.

Humphrey, T.J., Chapman, P.A., Rowe, B. and Gilbert, R.J. 1990. A comparative study on the heat resistance of Salmonellas in homogenized whole egg, egg yolk or albumen. Epidemiology and Infection. **104**: 237-241.

Jenkins, D.E., Shultz, J.E. and Martin, A. 1988. Starvation induced cross-protection against heat or H_2O_2 challenge in *Escherichia coli*. Journal of Bacteriology. **170**: 3910-3914.

Jorgensen, F., Nybroe, O. and Knochel, S. 1994. Effects of starvation and osmotic stress on viability and heat resistance of *Pseudomonas fluorescens* AH9. Journal of Applied Bacteriology. **77**: 340-347.

Mackey, B.M. and Derrick, C.M. 1986. Elevation of the heat resistance of *Salmonella typhimurium* by sub lethal heat shock. Journal of Applied Bacteriology. **61**: 389.

Maguire, H., Cowden, J., Jacob, M., Rowe, B., Roberts, D., Bruce, J. and Mitchell, E. 1992. An outbreak of *Salmonella dublin* infection in England and Wales associated with a soft unpasteurised cow's milk cheese. Epidemiology and Infection. **109**: 389-396.

Martin, A., Auger, E.A., Blum, P.H. and Shultz, J.E. 1989. Genetic basis of starvation survival in non differentiated bacteria. Annual Review of Microbiology. **43**: 293-316.

Moats, W.A., Dabbah, R. and Edwards, V.M. 1971. Survival of *Salmonella anatum* heated in various media. Applied Microbiology. **21**: 476-481.

Morgan, D., Mawer, S.L., Harmon, P.L. 1994. The role of home made ice-cream as a vehicle of *Salmonella enteritidis* phage type 4 infection from fresh shell eggs. Epidemiology and Infection. **113** (1): 22-29.

Neidhart, F.C. and Van Bogelen, R.A. 1987. Heat shock response in *Escherichia coli* and *Salmonella typhimurium* : Cellular and Molecular Biology, volume 2, Neidhart, F.C., Ingram, L., Low, K.B., Magasnick, B., Schaechter, M. and Umberger (Eds), p1334. American Society for Microbiology, Washington, D.C.

Oemichen, W.L. 1995 The Schwan's *Salmonella enteritidis* experience. 1995. Journal of the Association of Food and Drug Officials. **59** (2): 48-68.

PHLS. 1996. *Salmonella* in humans, England and Wales: quarterly report. **6** (6): 53.

Ratnam, S. and March, S.B. 1986. Laboratory studies on *Salmonella* contaminated cheese involved in a major outbreak of gastroenteritis. Journal of Applied Bacteriology. **61**: 51-56.

Senhaji, A.F. and Loncin, M. 1977. The protective effect of fat on the heat resistance of bacteria. International Journal of Food Technology. **12**: 203-216.

Shah, D.B., Bradshaw, J.G. and Peeler, J.T. 1991. Thermal resistance of egg-associated epidemic strains of *Salmonella enteritidis*. Journal of Food Science. **56** (2): 391-393.

Spence, J., Cegielska, A. and Georgopoulos, C. 1990. Role of *Escherichia coli* heat shock proteins DnaK and HtpG (c62.5) in response to nutritional deprivation. Journal of Bacteriology. **172**: 7157-7166.

Thomas, C.T., White, J.C. and Longree, K. 1966. Thermal resistance of salmonellae and staphylococci in foods. Applied Microbiology. **14** (5): 815-820.

APPENDIX

Figure 1.1 D-value of *S.typhimurium* 1005 in Nutrient Broth at 58°C

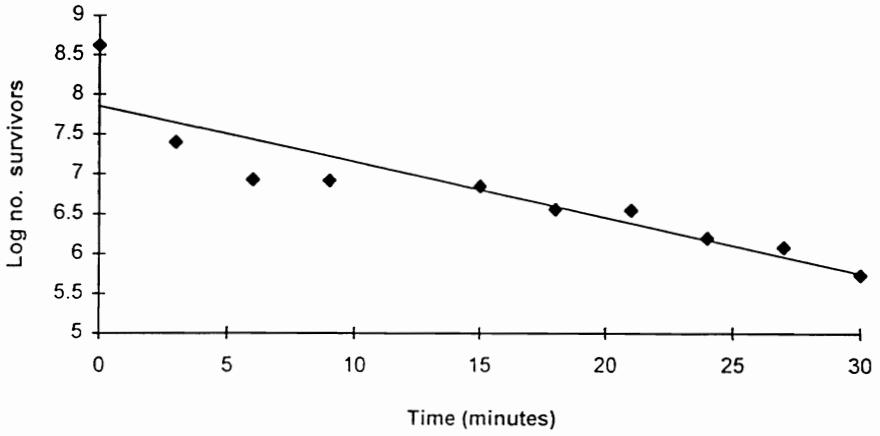


Figure 1.2 D-value of *S.typhimurium* 1005 in Nutrient Broth at 60°C

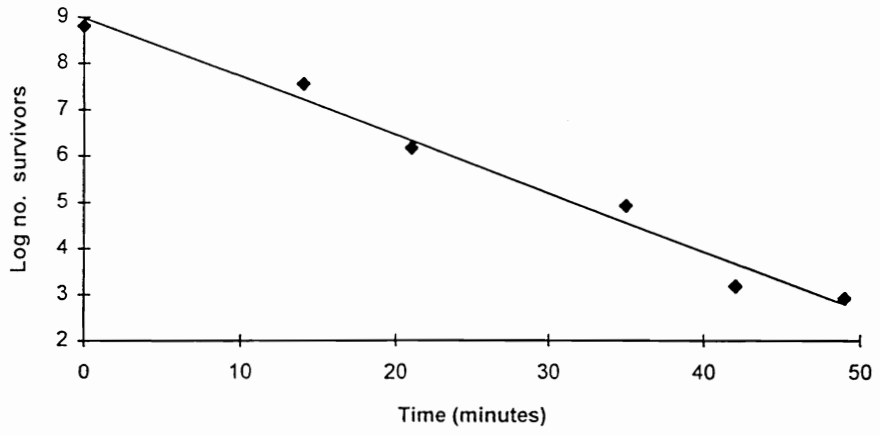


Figure 1.3 D-value of *S typhimurium* 1005 in Nutrient Broth at 63°C

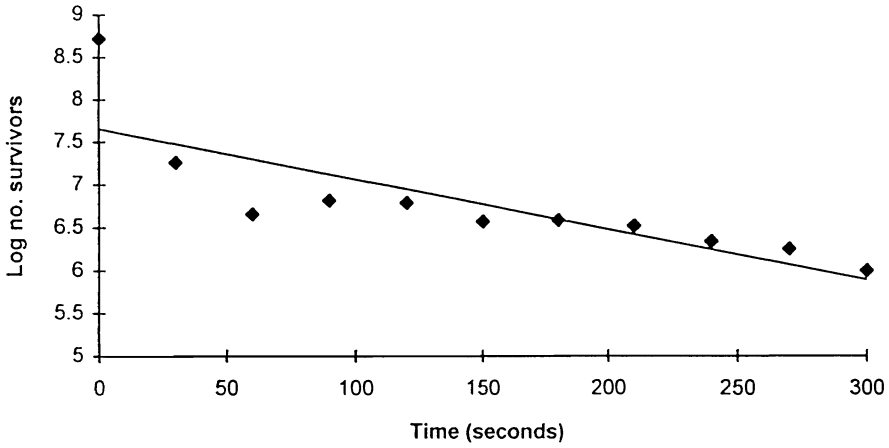


Figure 1.4 D-value of *S.typhimurium* 1005 in Nutrient Broth at 65°C

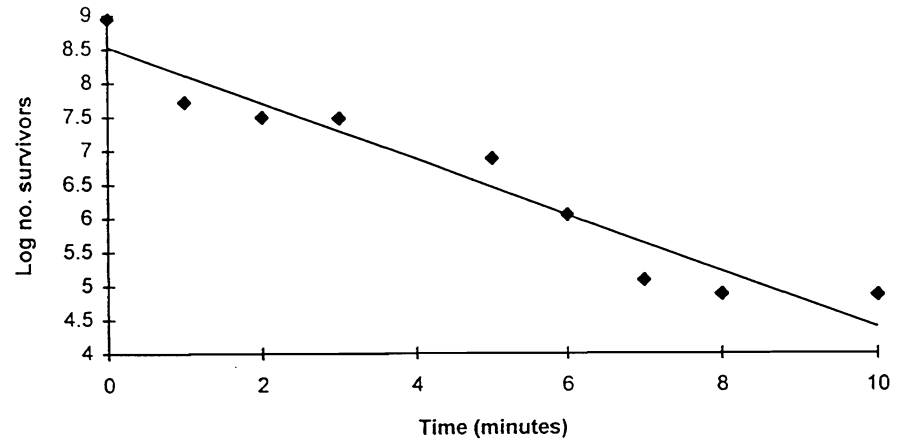


Figure 1.5 D-value of *S.typhimurium* 1005 in Nutrient Broth at 68°C

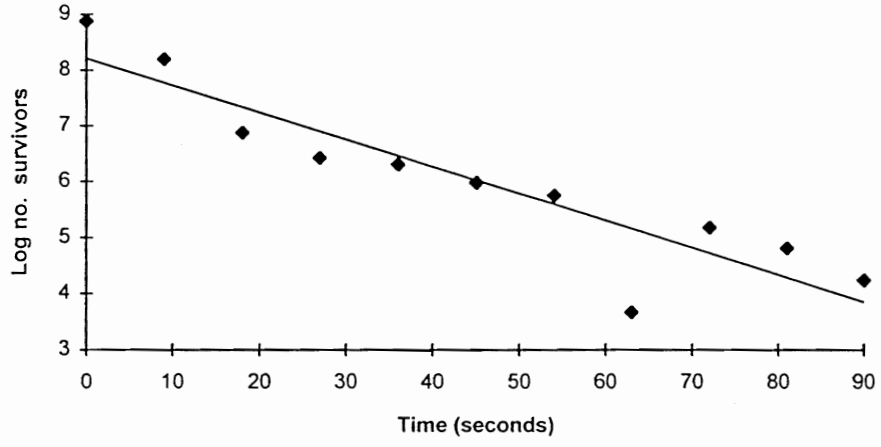


Figure 1.6 z value of *S.typhimurium* 1005 in Nutrient Broth

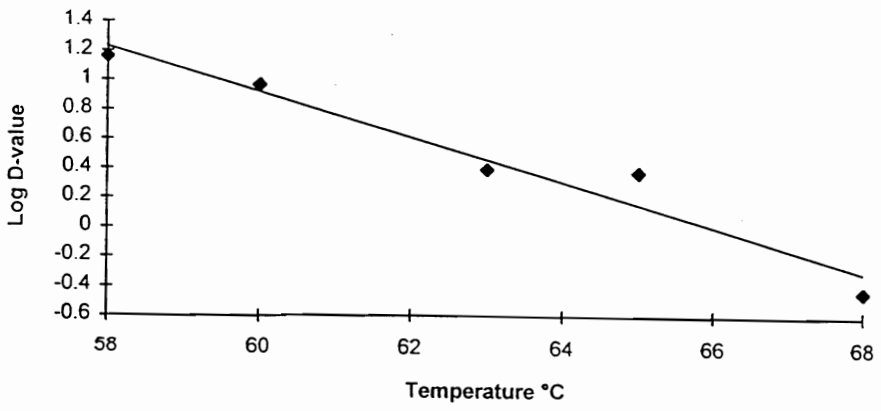


Figure 2.1 D-value of *S.typhimurium* 1005 in Butter at 58°C

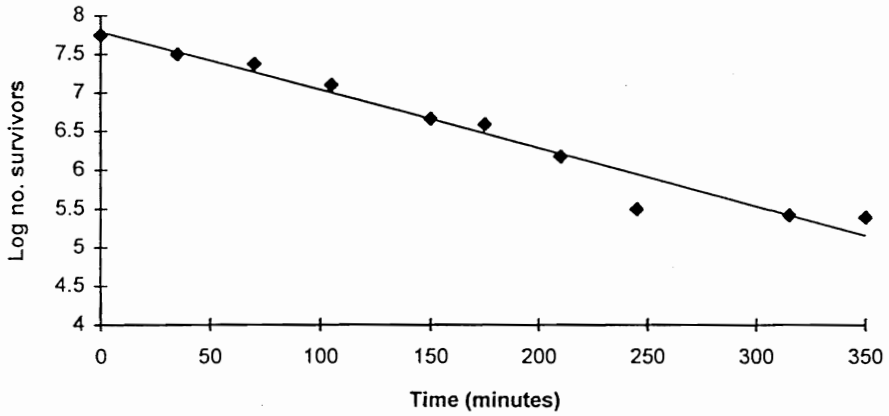


Figure 2.2 D-value of *S.typhimurium* 1005 in Butter at 60°C

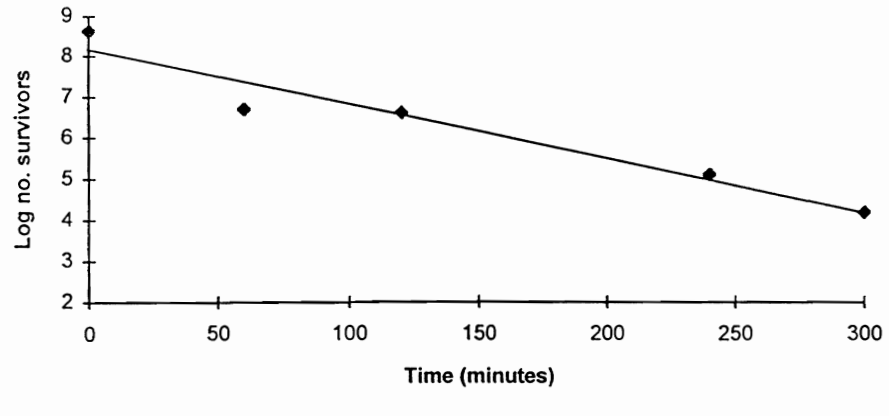


Figure 2.3 D-value of *S.typhimurium* 1005 in Butter at 63°C

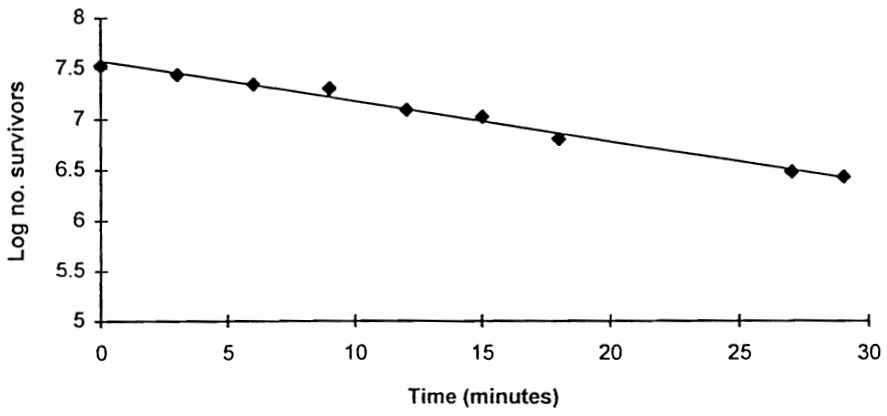


Figure 2.4 D-value of *S.typhimurium* 1005 in Butter at 65°C

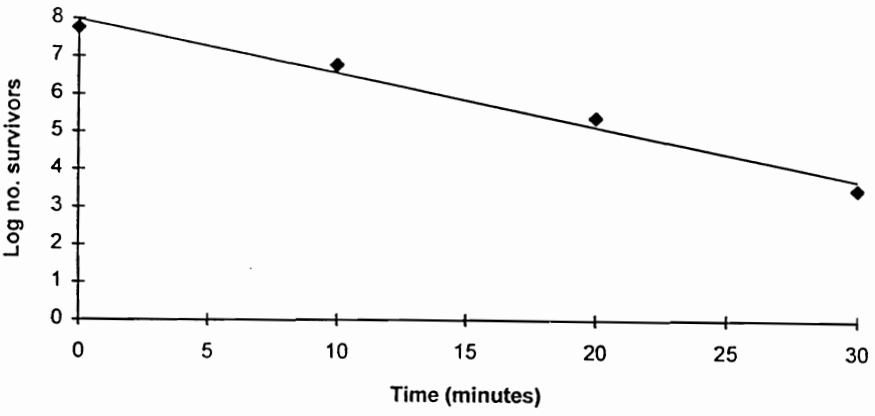


Figure 2.5 D-value of *S.typhimurium* 1005 in Butter at 68°C

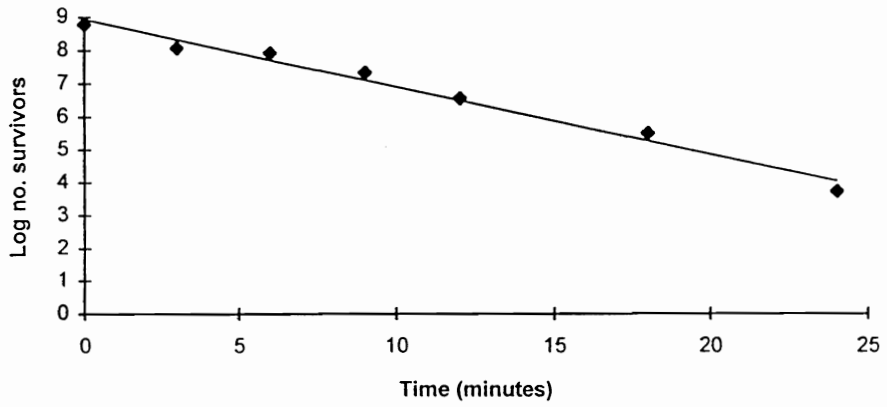


Figure 2.6 z value of *S.typhimurium* 1005 in Butter

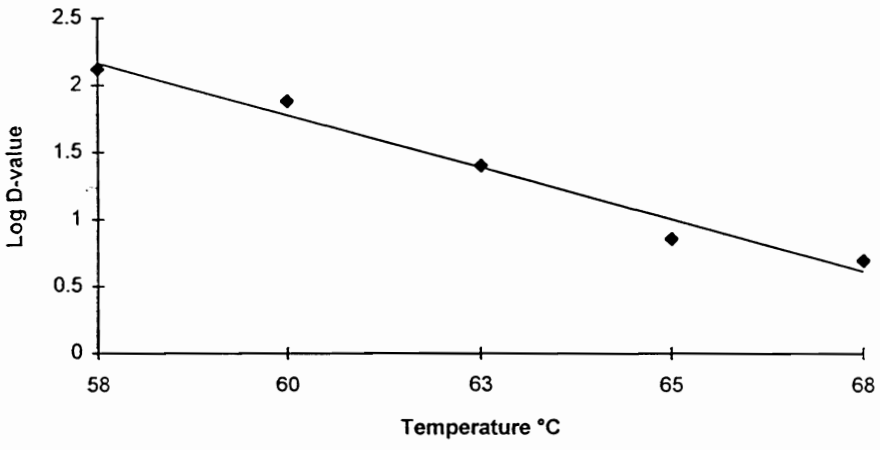


Figure 3.1 D-value of *S.typhimurium* 1005 in Cocoa Mass at 58°C

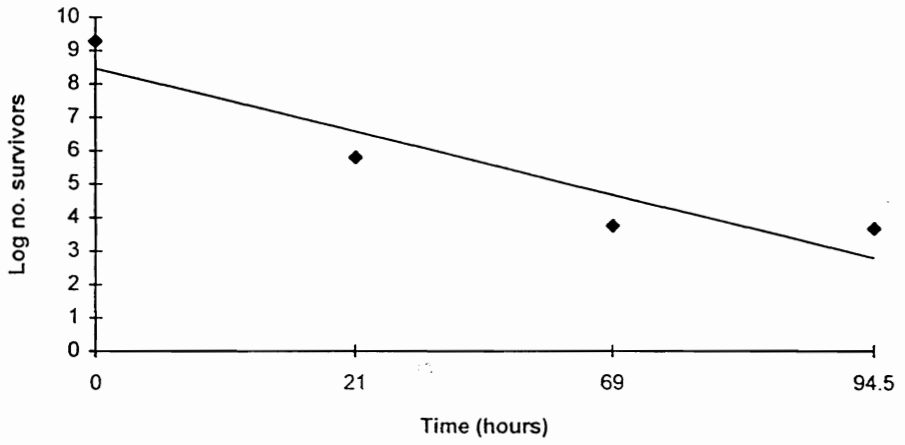


Figure 3.2 D-value of *S.typhimurium* 1005 in Cocoa Mass at 60°C

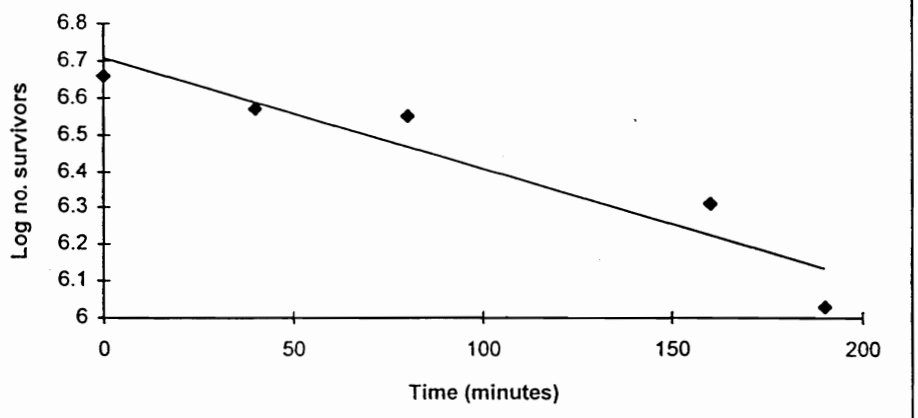


Figure 3.3 D-value of *S.typhimurium* 1005 in Cocoa Mass at 63°C

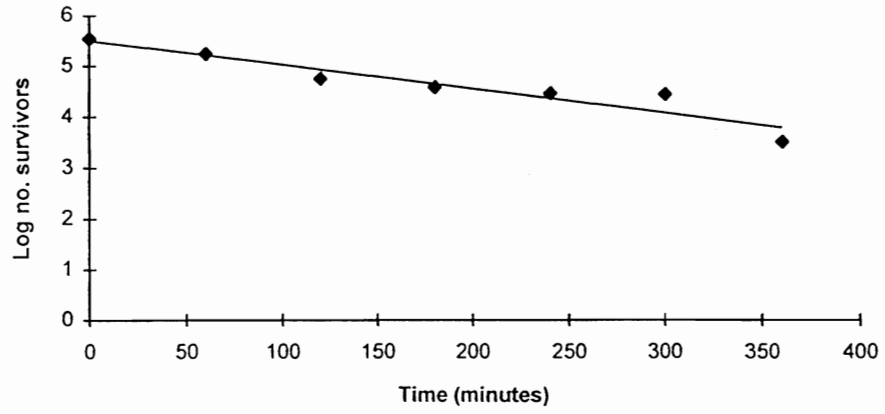


Figure 3.4 D-value of *S.typhimurium* 1005 in Cocoa Mass at 65°C

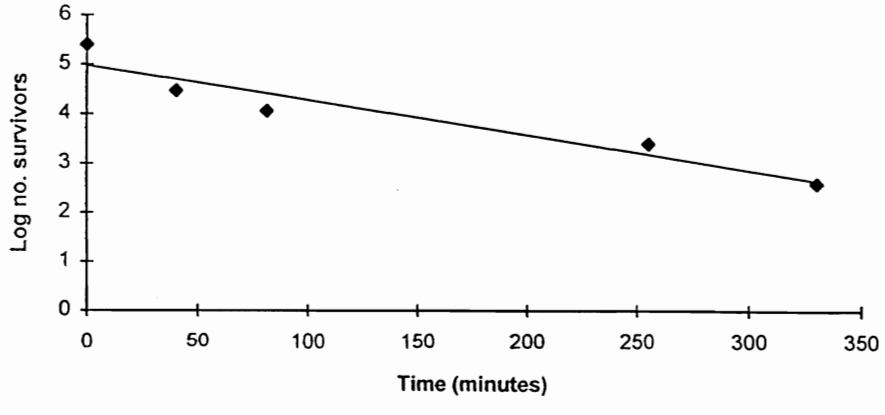


Figure 3.5 D-value of *S.typhimurium* 1005 in Cocoa Mass at 68°C

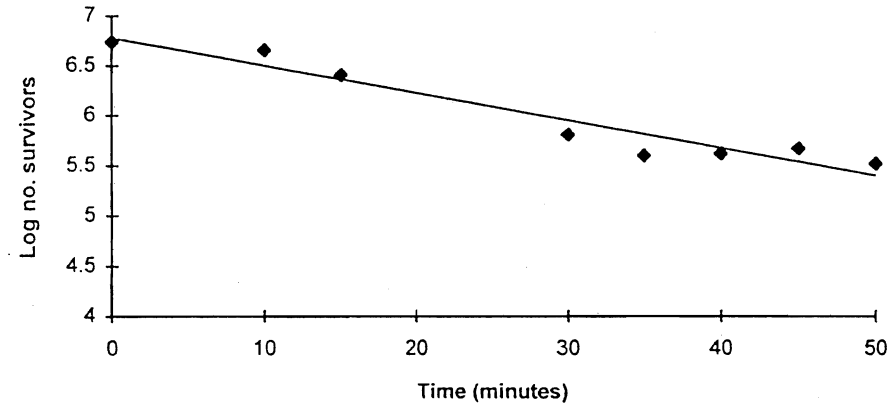


Figure 3.6 z value of *S. typhimurium* 1005 in Cocoa Mass

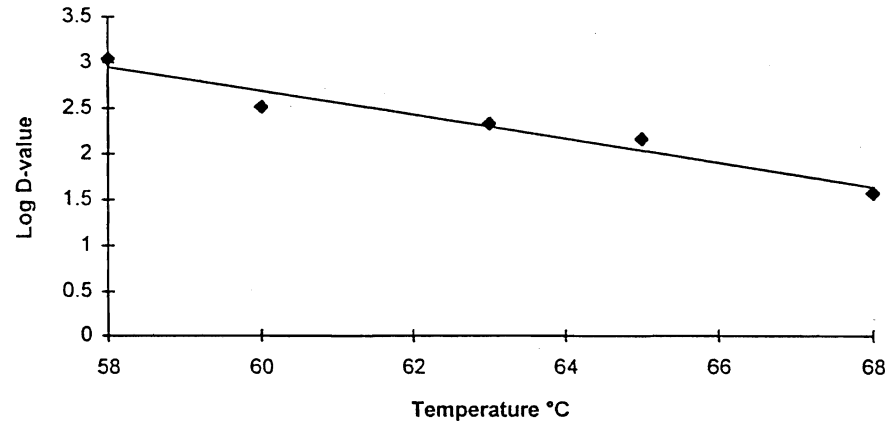


Figure 4.1 D-value of *S.typhimurium* 1005 in Glucose syrup at 58°C

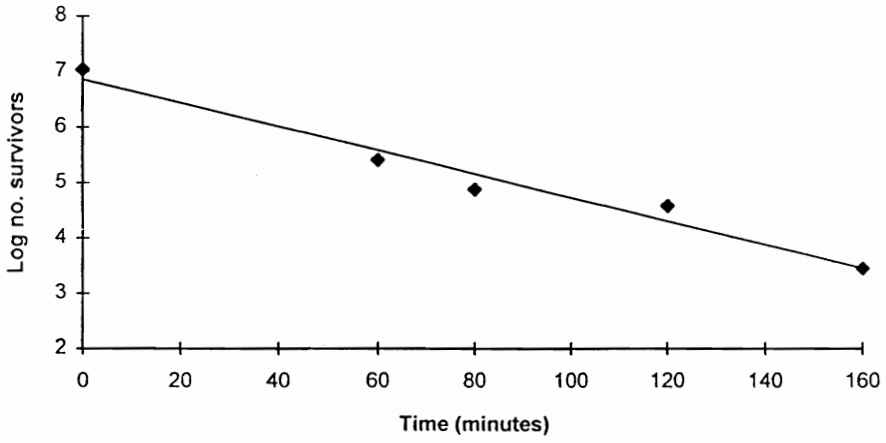


Figure 4.2 D-value of *S.typhimurium* 1005 in Glucose Syrup at 60°C

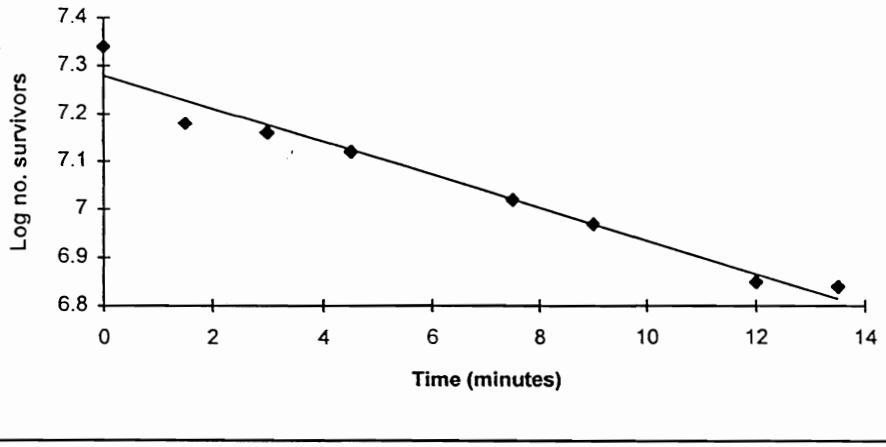


Figure 4.3 D-value of *S.typhimurium* 1005 in Glucose Syrup at 63°C

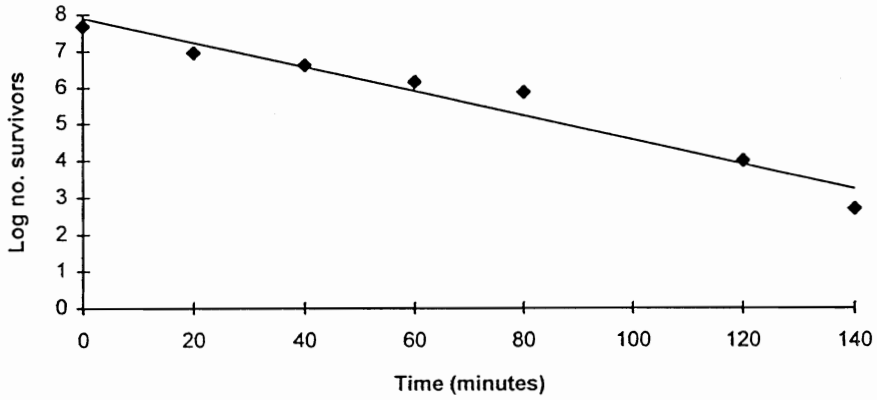


Figure 4.4 D-value of *S.typhimurium* 1005 in Glucose Syrup at 65°C

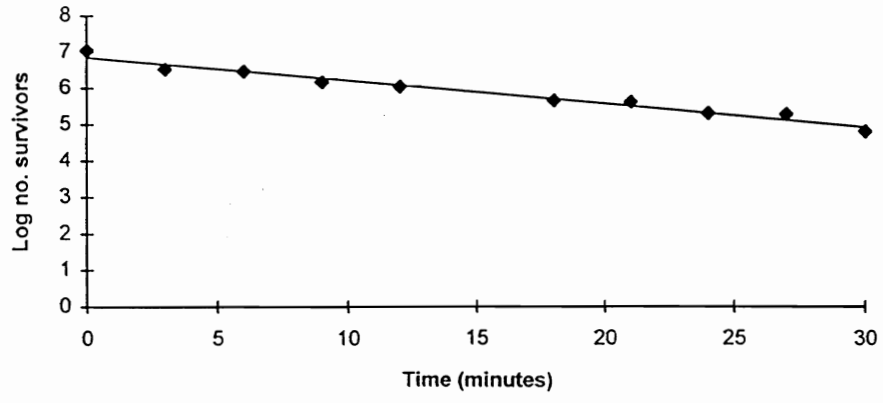


Figure 4.5 D-value of *S.typhimurium* 1005 in Glucose Syrup at 68°C

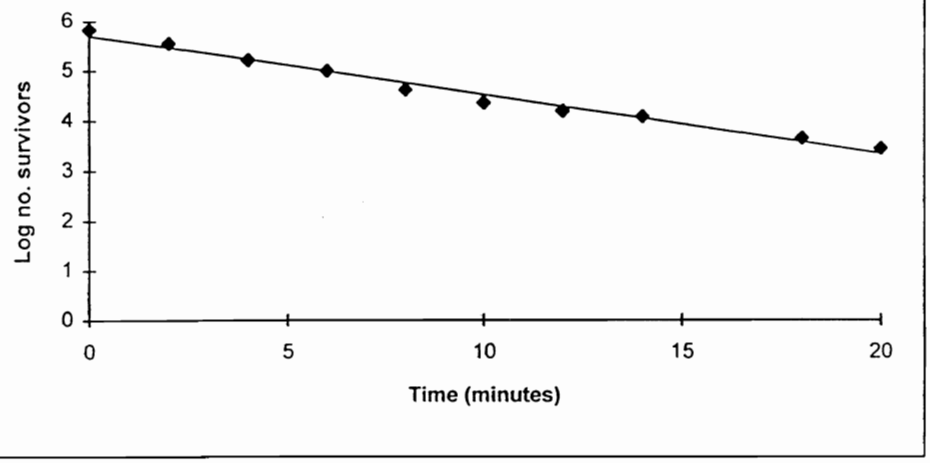


Figure 4.6 z value of *S.typhimurium* 1005 in Glucose Syrup

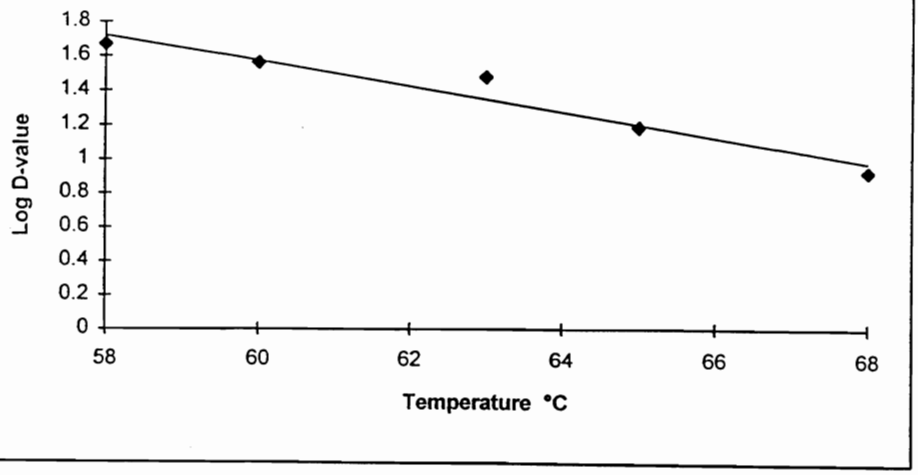


Figure 5.1 D-value of *S.typhimurium* 1005 in Golden Syrup at 58°C

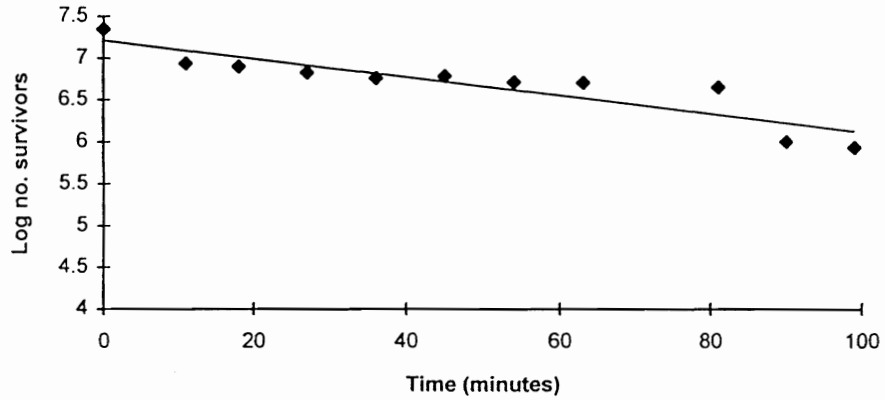


Figure 5.2 D-value of *S.typhimurium* 1005 in Golden Syrup at 60°C

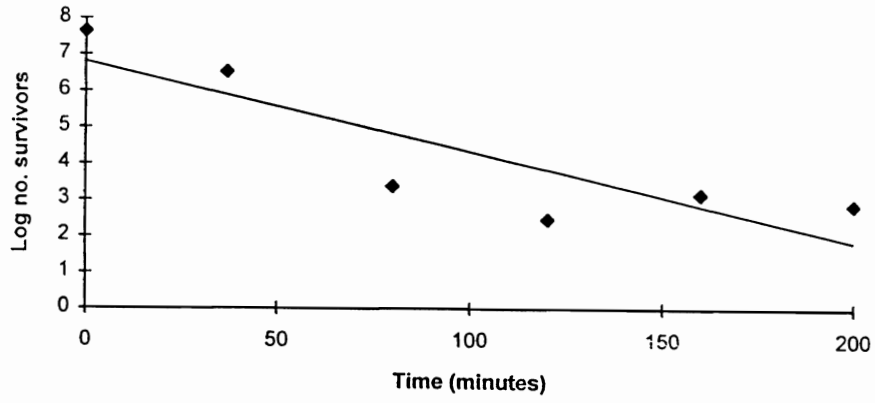


Figure 5.3 D-value of *S.typhimurium* 1005 in Golden Syrup at 63°C

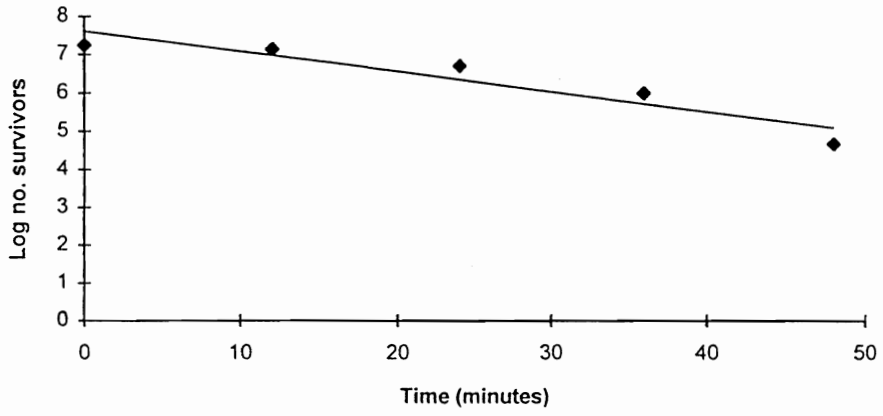


Figure 5.4 D-value of *S.typhimurium* 1005 in Golden Syrup at 65°C

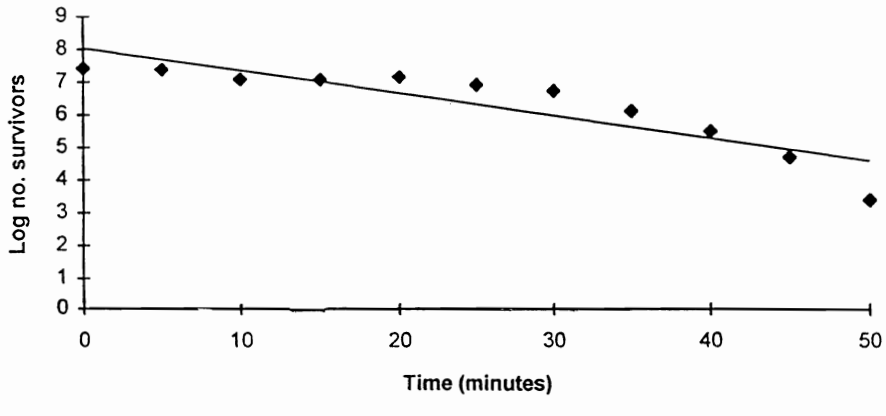


Figure 5.5 D-value of *S.typhimurium* 1005 in Golden Syrup at 68°C

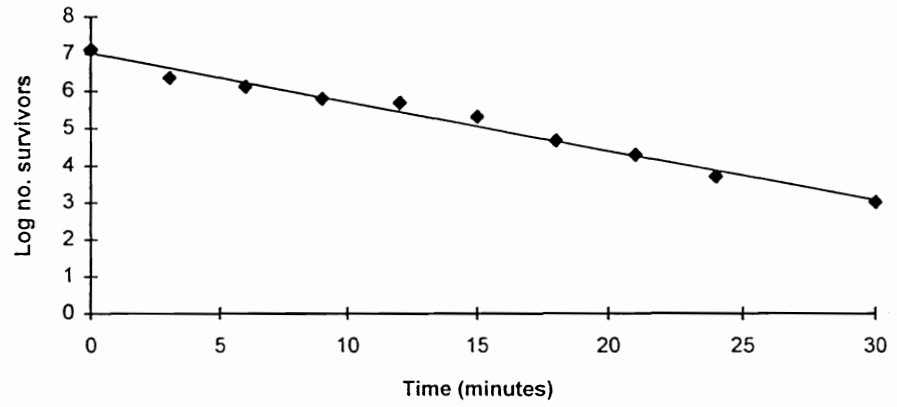


Figure 5.6 z value of *S.typhimurium* 1005 in Golden Syrup

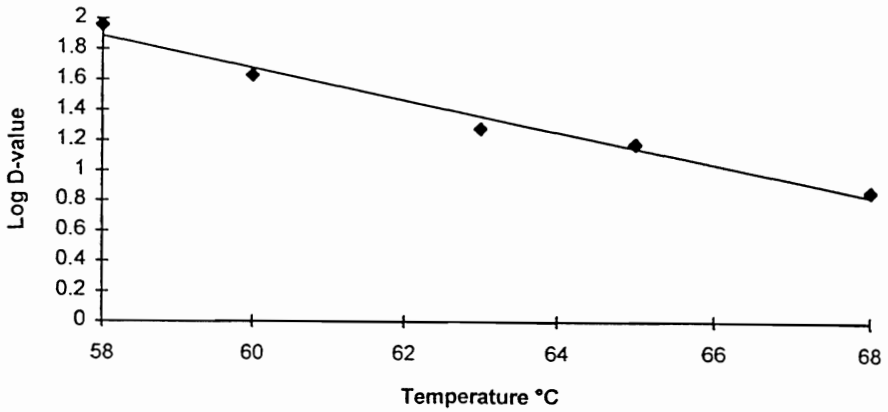


Figure 6.1 D-value of *S.typhimurium* 1005 in Honey at 58°C

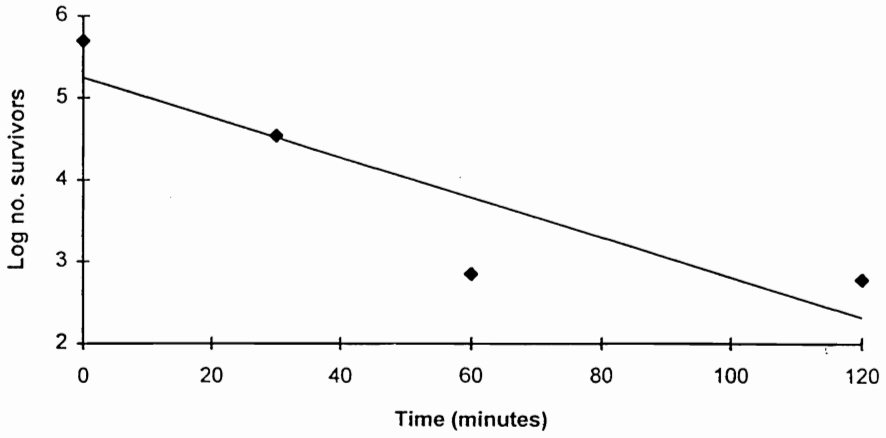


Figure 6.2 D-value of *S.typhimurium* 1005 in Honey at 60°C

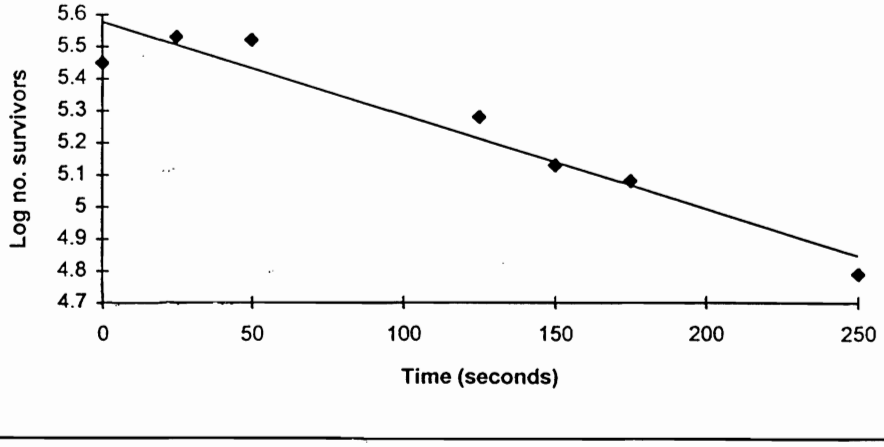


Figure 6.3 D-value of *S.typhimurium* 1005 in Honey at 63°C

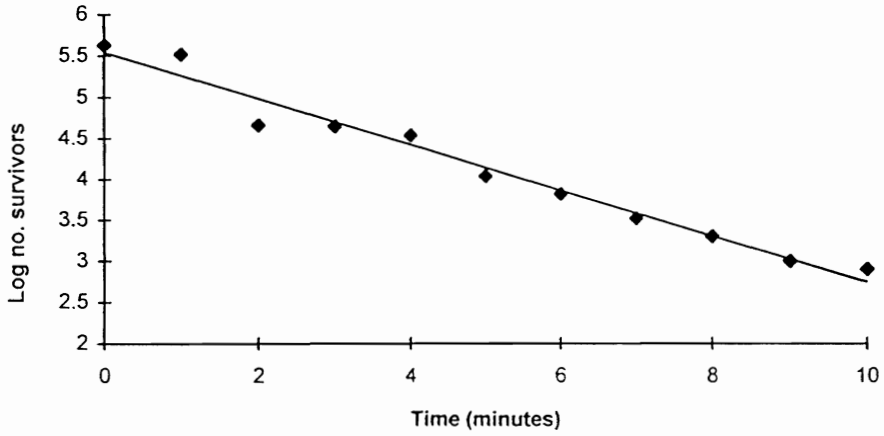
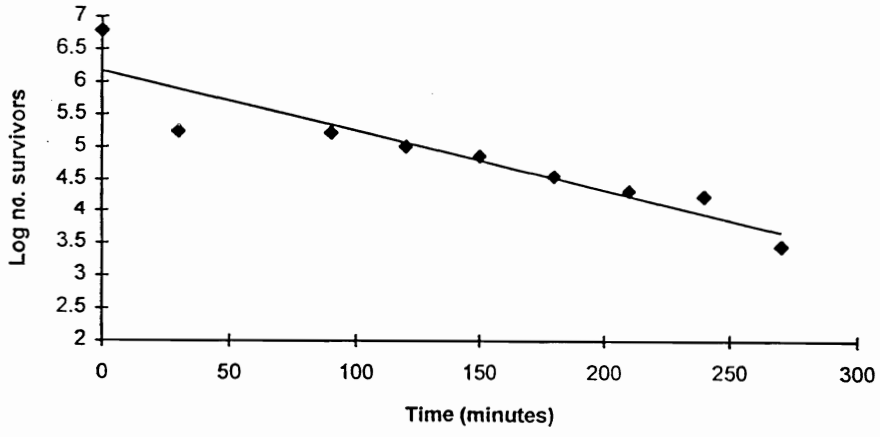


Figure 6.4 D-value of *S.typhimurium* 1005 in Honey at 65°C



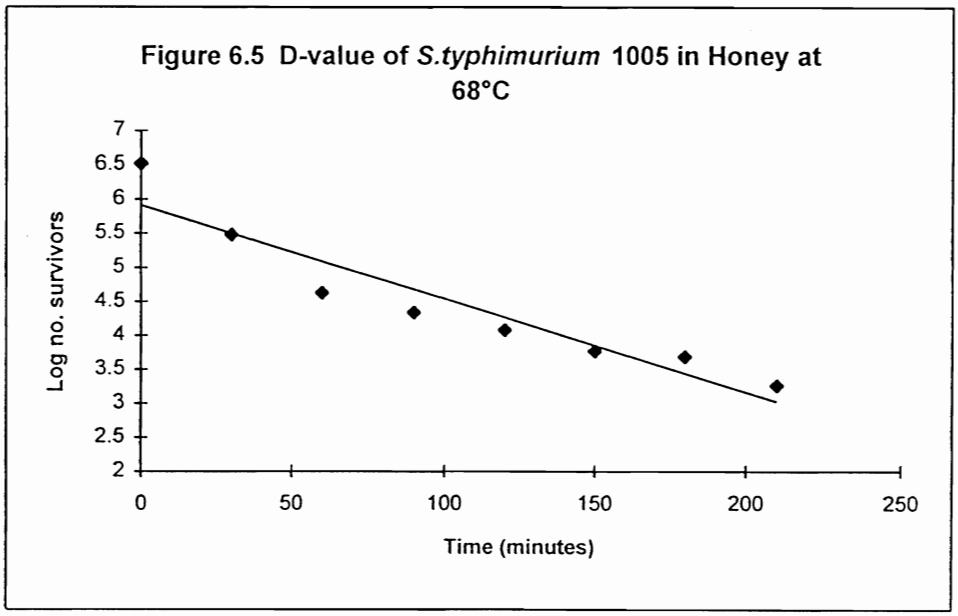


Figure 6.5 D-value of S.typhimurium 1005 in Honey at 68°C
Log no. survivors
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
0
50
100
150
200
250
Time (minutes)

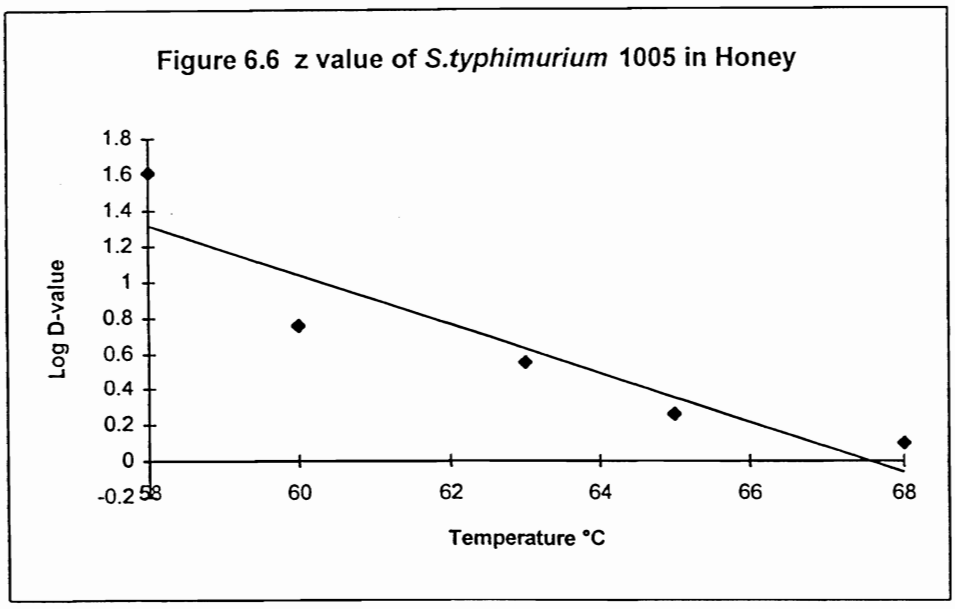


Figure 6.6 z value of S.typhimurium 1005 in Honey
Log D-value
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
58
60
62
64
66
68
Temperature °C

Figure 7.1 D-value of *S.typhimurium* 1005 in Double Cream at 58°C

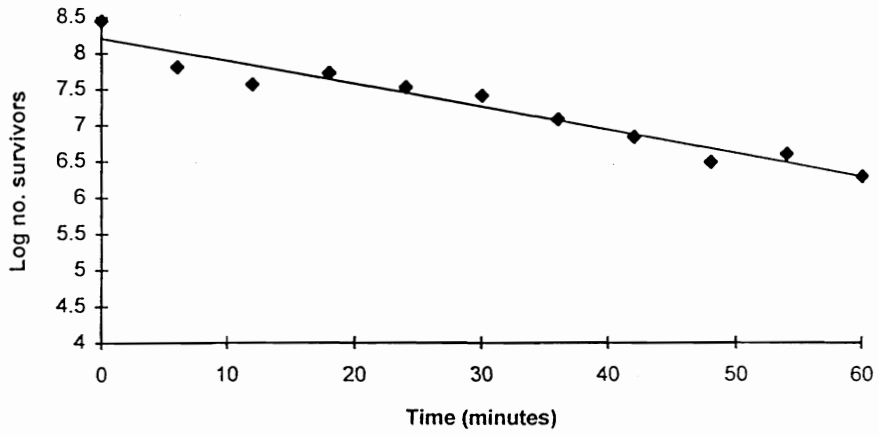
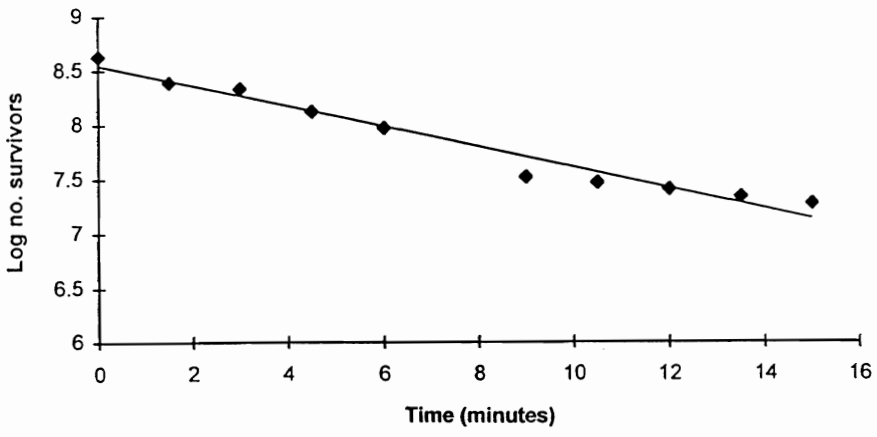


Figure 7.2 D-value of *S.typhimurium* 1005 in Double Cream at 60°C



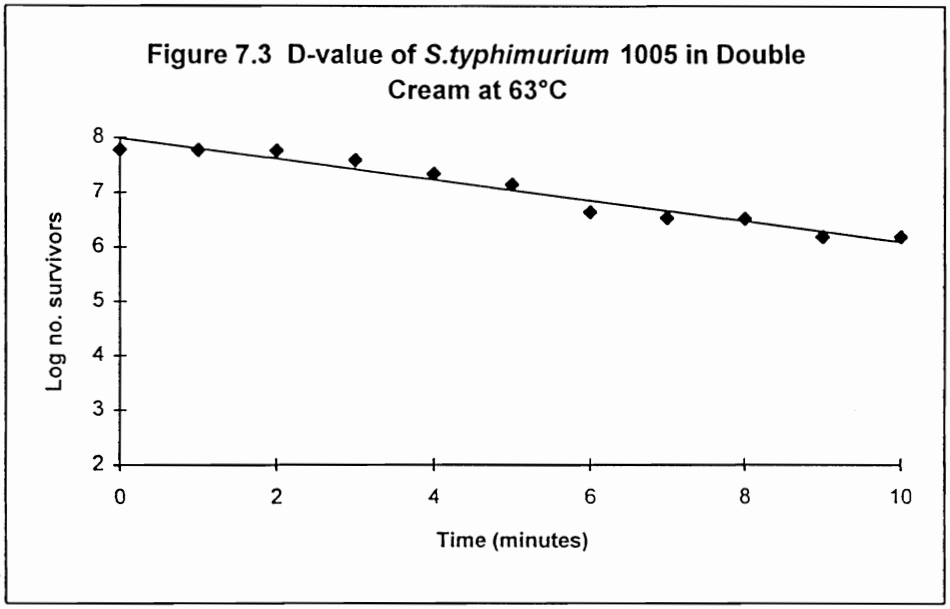


Figure 7.3 D-value of *S.typhimurium* 1005 in Double Cream at 63°C

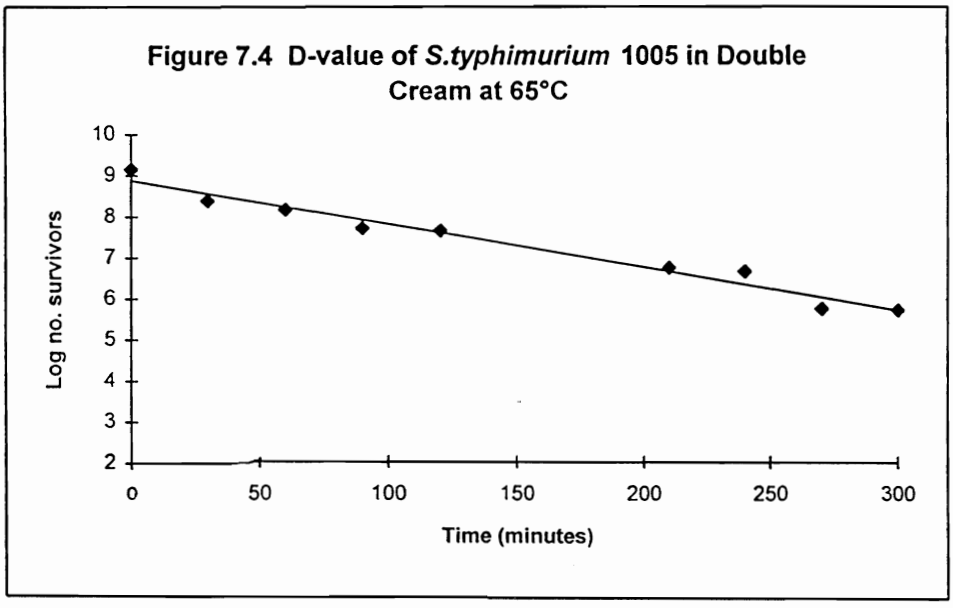


Figure 7.4 D-value of *S.typhimurium* 1005 in Double Cream at 65°C

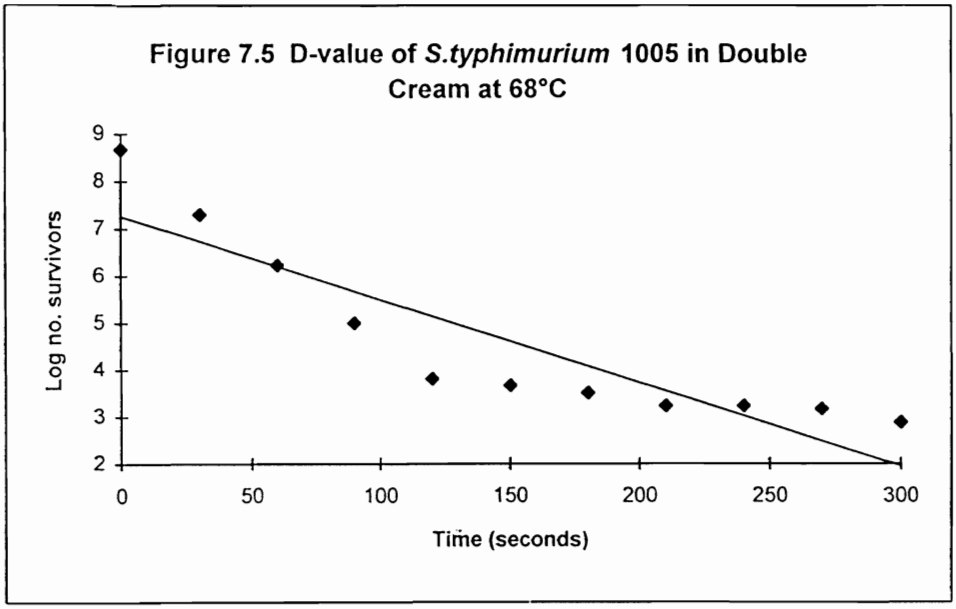


Figure 7.5 D-value of S.typhimurium 1005 in Double Cream at 68°C
Log no. survivors
9
8
7
6
5
4
3
2
0
50
100
150
200
250
300
Time (seconds)

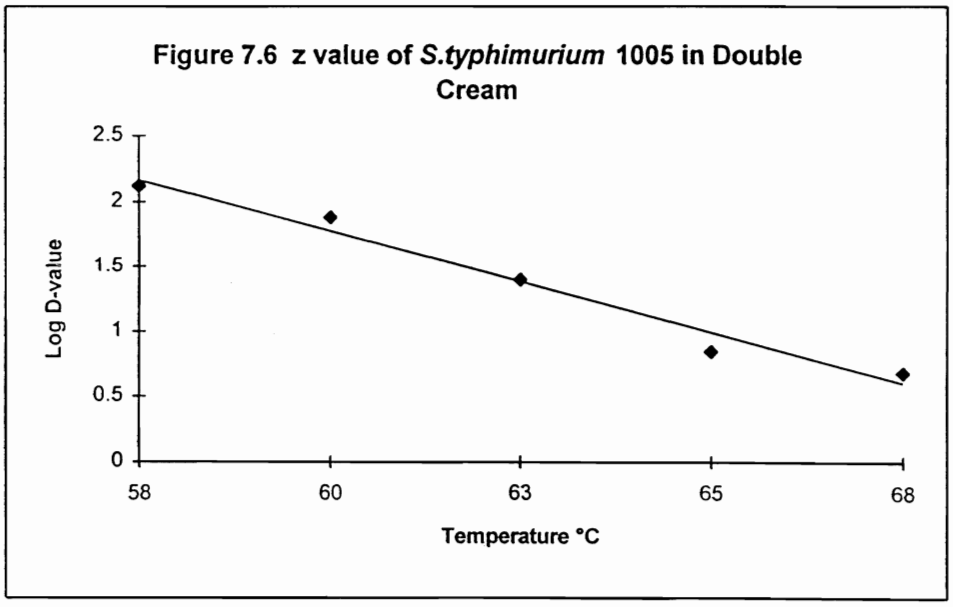


Figure 7.6 z value of S.typhimurium 1005 in Double Cream
Log D-value
2.5
2
1.5
1
0.5
0
58
60
63
65
68
Temperature °C

Figure 8.1 D-value of *S.typhimurium* 1005 in Custard at 58°C

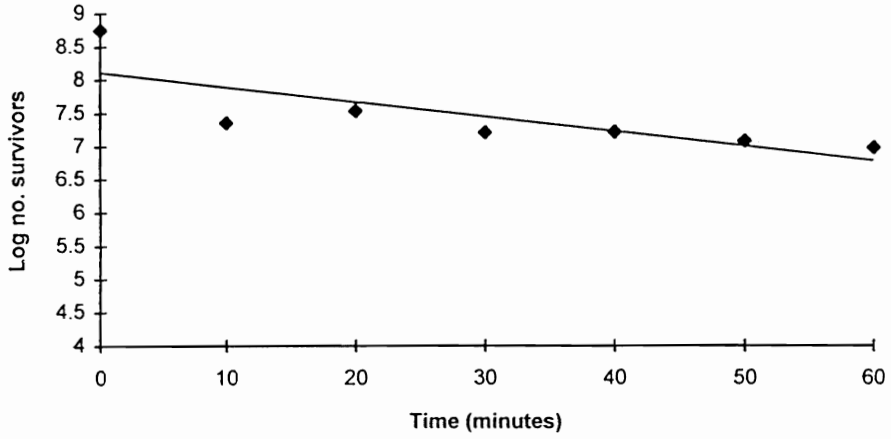
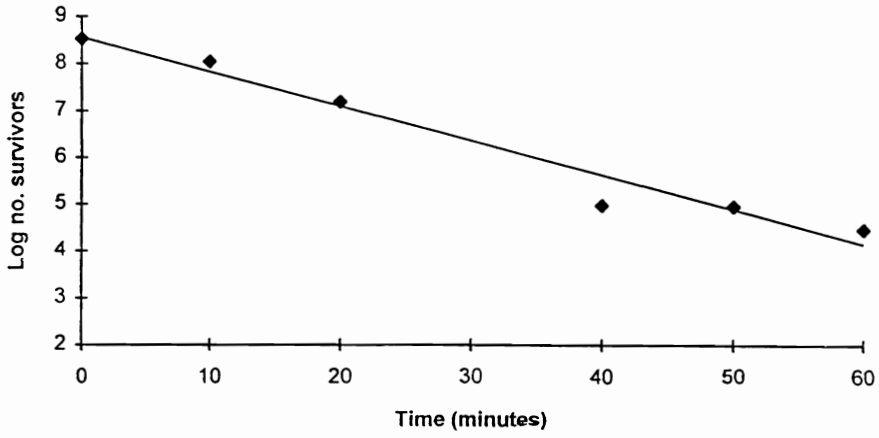


Figure 8.2 D-value of *S.typhimurium* 1005 in Custard at 60°C



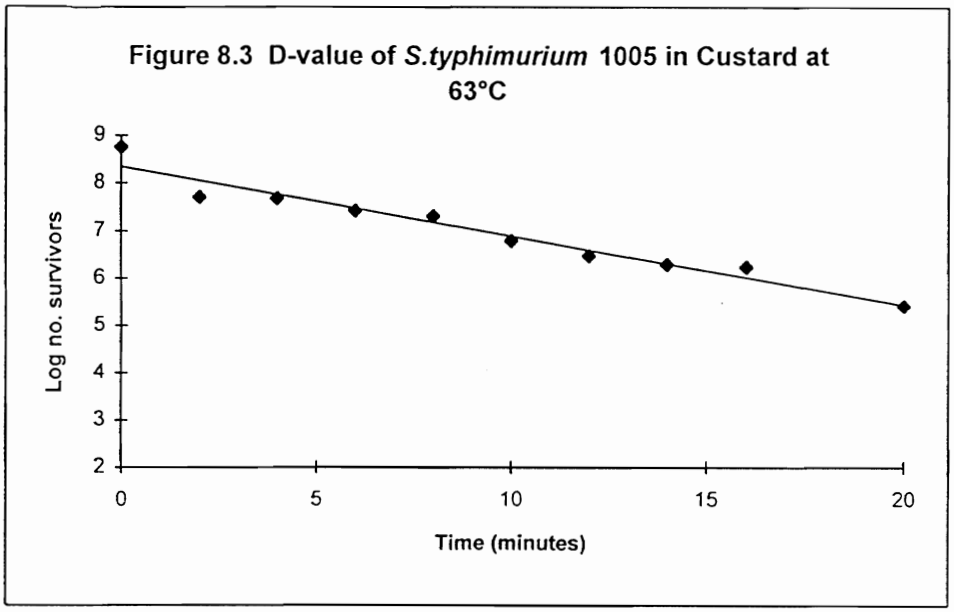


Figure 8.3 D-value of S.typhimurium 1005 in Custard at 63°C
Log no. survivors
9
8
7
6
5
4
3
2
0
5
10
15
20
Time (minutes)

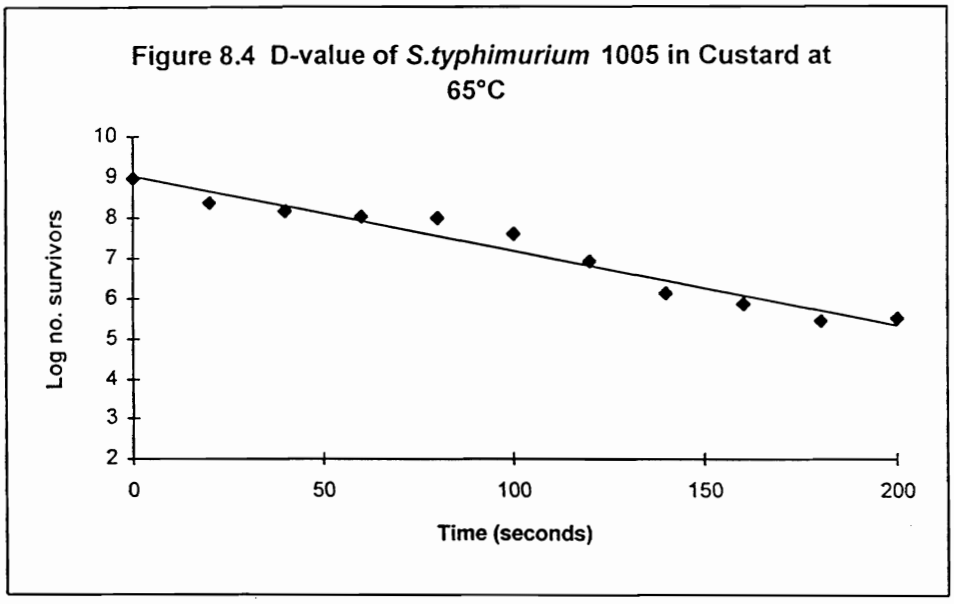


Figure 8.4 D-value of S.typhimurium 1005 in Custard at 65°C
Log no. survivors
10
9
8
7
6
5
4
3
2
0
50
100
150
200
Time (seconds)

Figure 8.5 D-value of *S.typhimurium* 1005 in Custard at 68°C

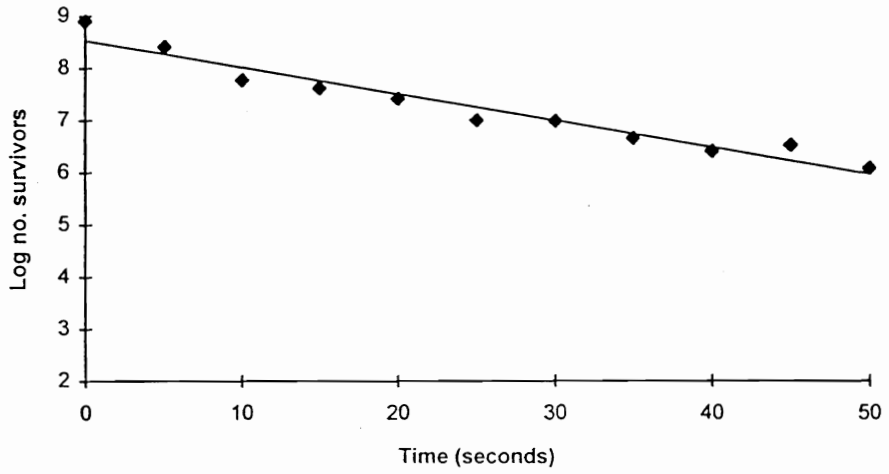


Figure 8.6 z value of *S.typhimurium* 1005 in Custard

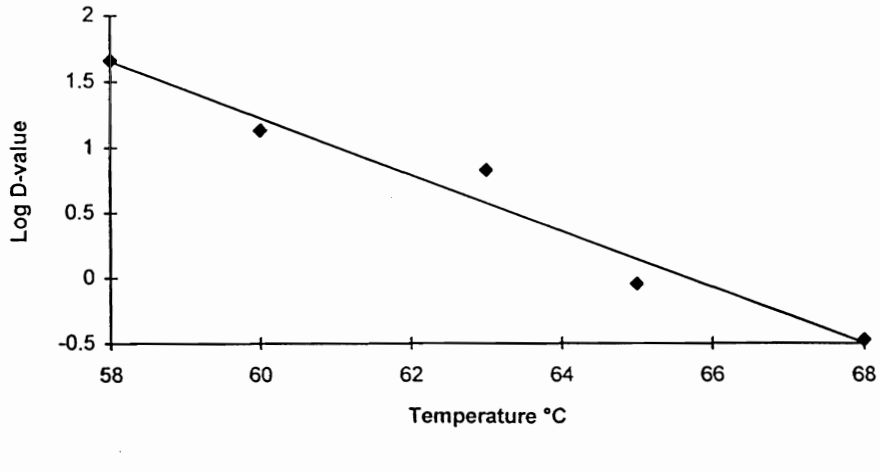


Figure 9.1 D-value of *S.typhimurium* 1005 in Half Cream at 58°C

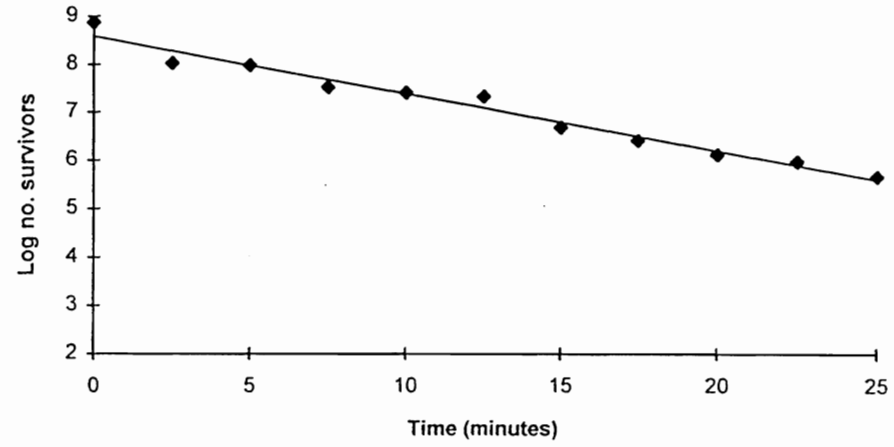
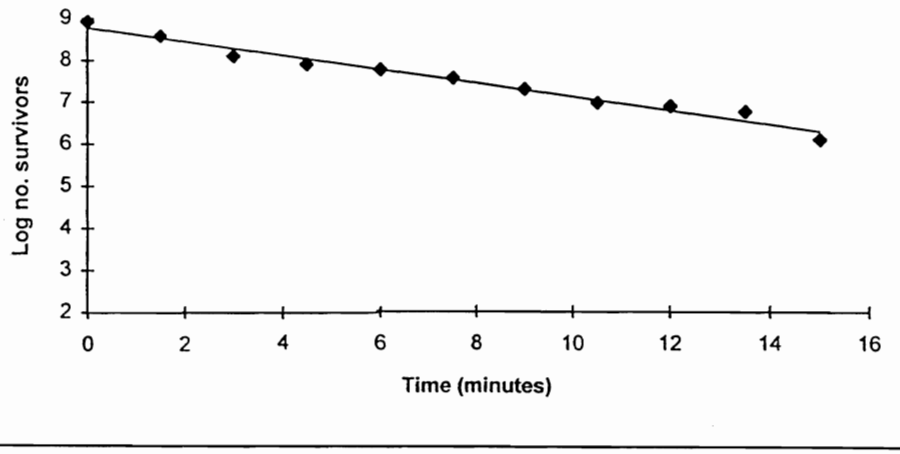


Figure 9.2 D-value of *S.typhimurium* 1005 in Half Cream at 60°C



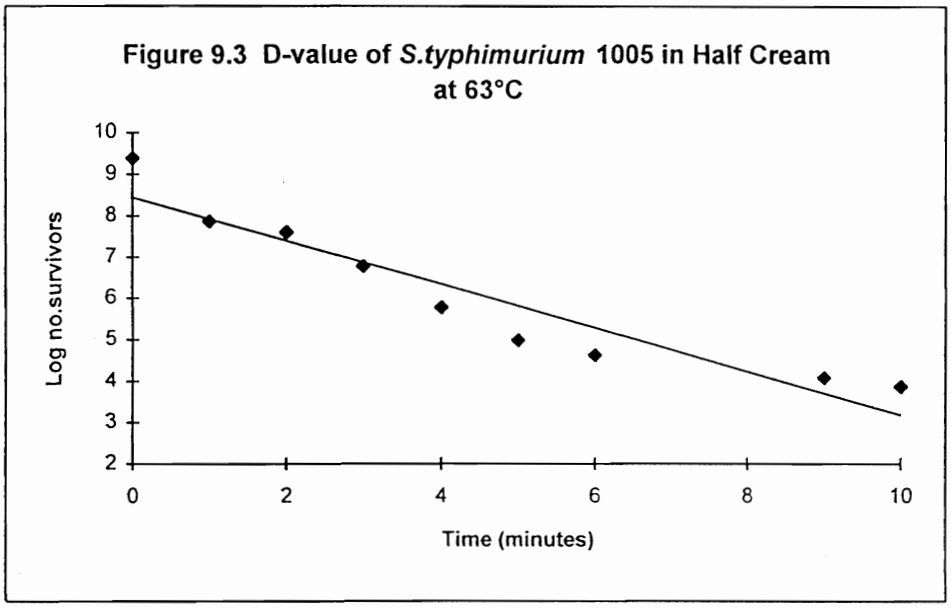


Figure 9.3 D-value of S.typhimurium 1005 in Half Cream at 63°C
Log no.survivors
10
9
8
7
6
5
4
3
2
0
2
4
6
8
10
Time (minutes)

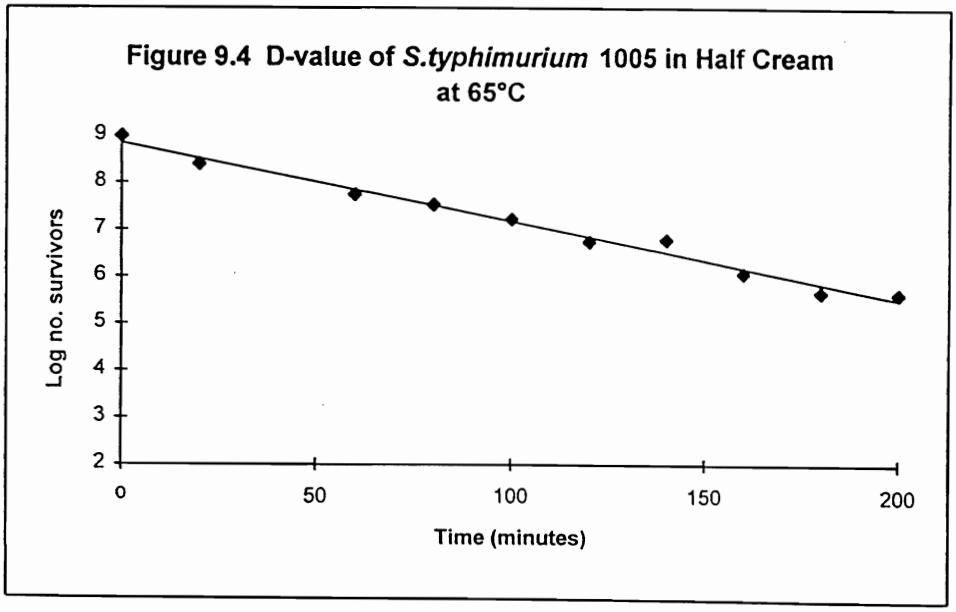


Figure 9.4 D-value of S.typhimurium 1005 in Half Cream at 65°C
Log no. survivors
9
8
7
6
5
4
3
2
0
50
100
150
200
Time (minutes)

Figure 9.5 D-value of *S.typhimurium* 1005 in Half cream at 68°C

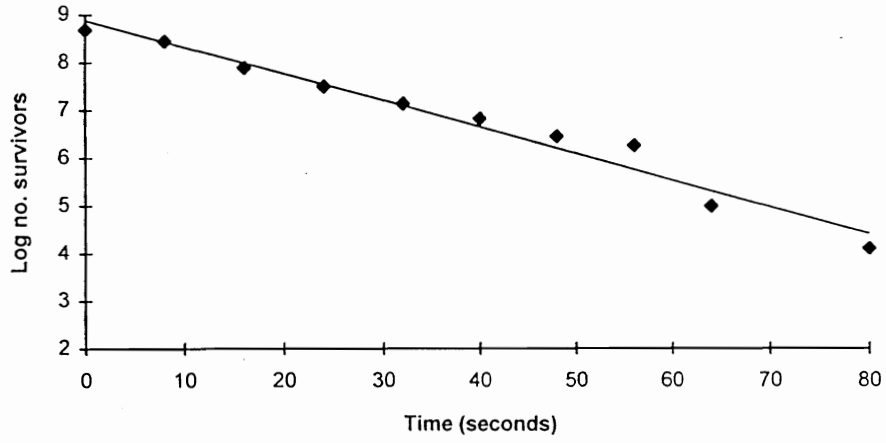


Figure 9.6 z value of *S.typhimurium* 1005 in Half Cream

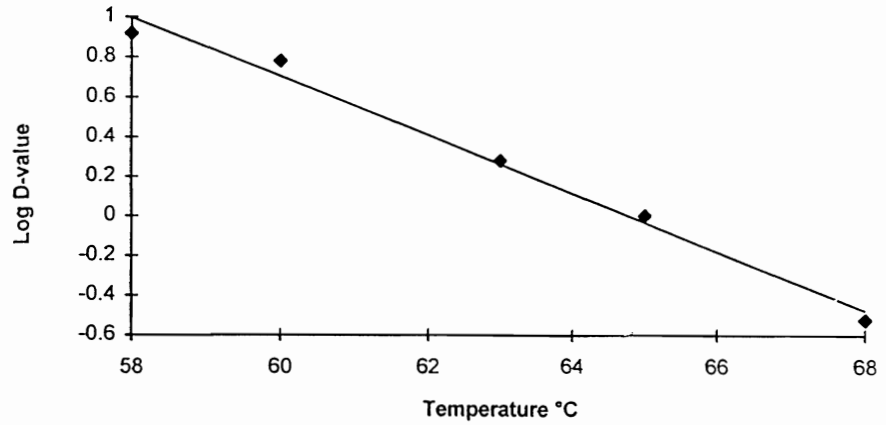


Figure 10.1 D-value of *S.typhimurium* 1005 in Peanut Butter at 58°C

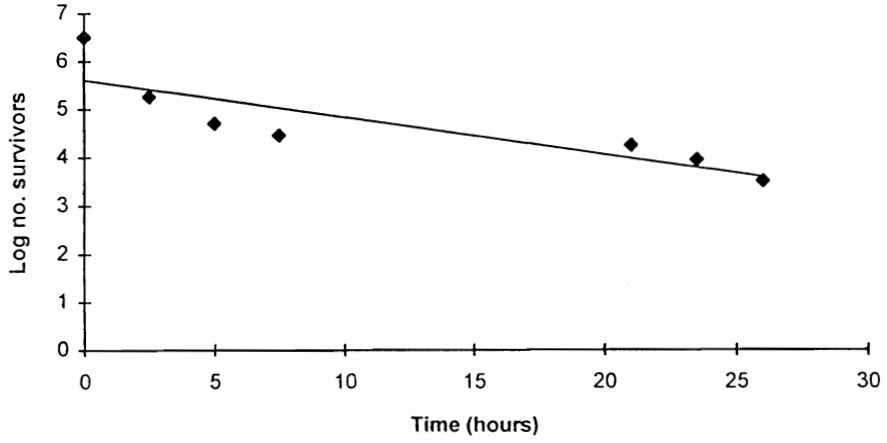


Figure 10.2 D-value of *S.typhimurium* 1005 in Peanut Butter at 60°C

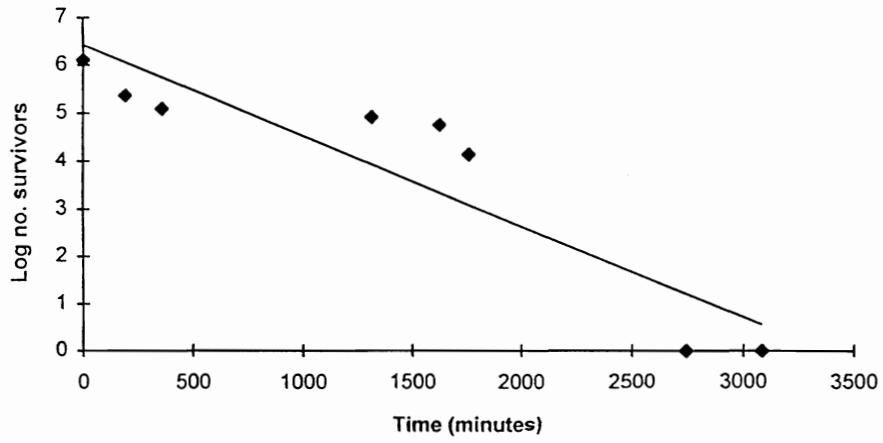


Figure 10.3 D-value of *S.typhimurium* 1005 in Peanut Butter at 63°C

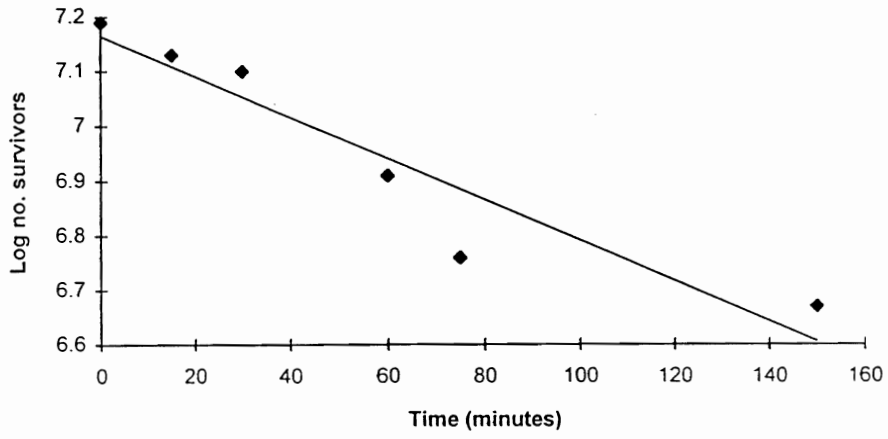


Figure 10.4 D-value of *S.typhimurium* 1005 in Peanut Butter at 65°C

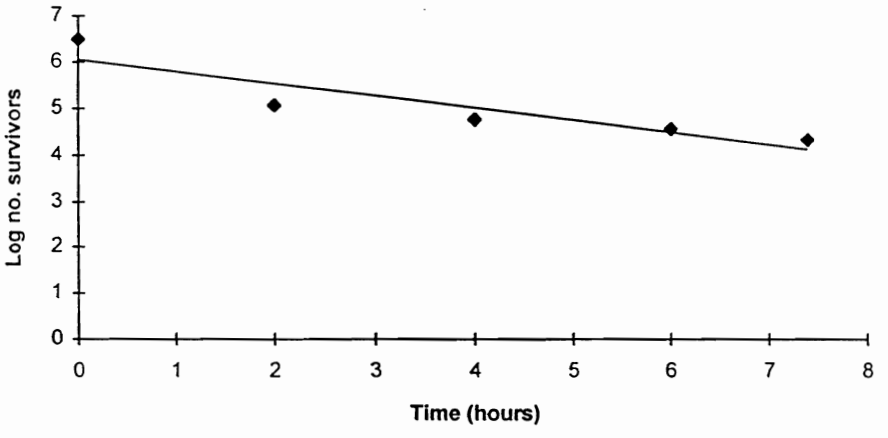


Figure 10.5 D-value of *S.typhimurium* 1005 in Peanut Butter at 68°C

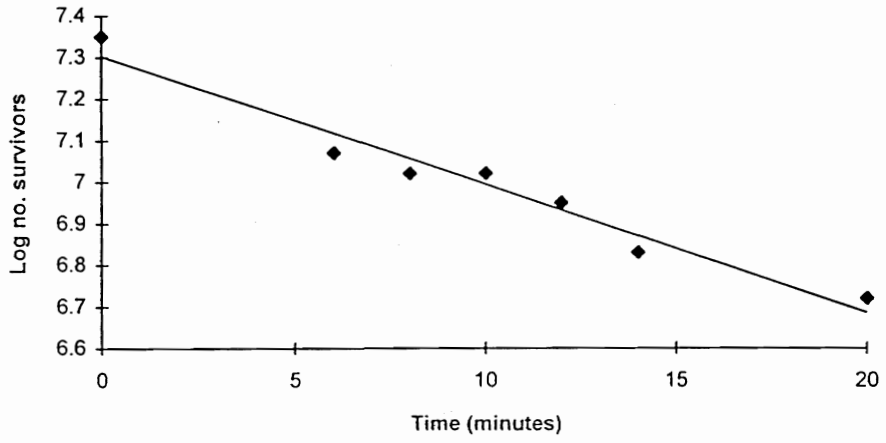


Figure 10.6 z value of *S.typhimurium* 1005 in Peanut Butter

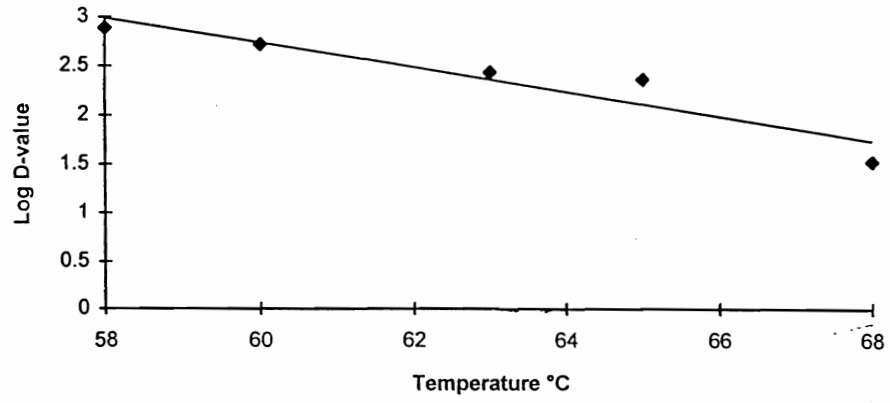


Figure 11.1 D-value of *S.typhimurium* 1005 in Salami at 58°C

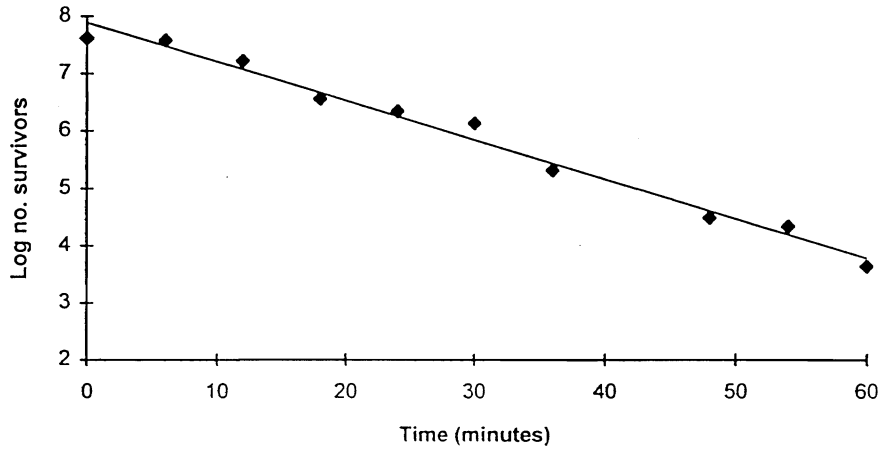


Figure 11.2 D-value of *S.typhimurium* 1005 in Salami at 60°C

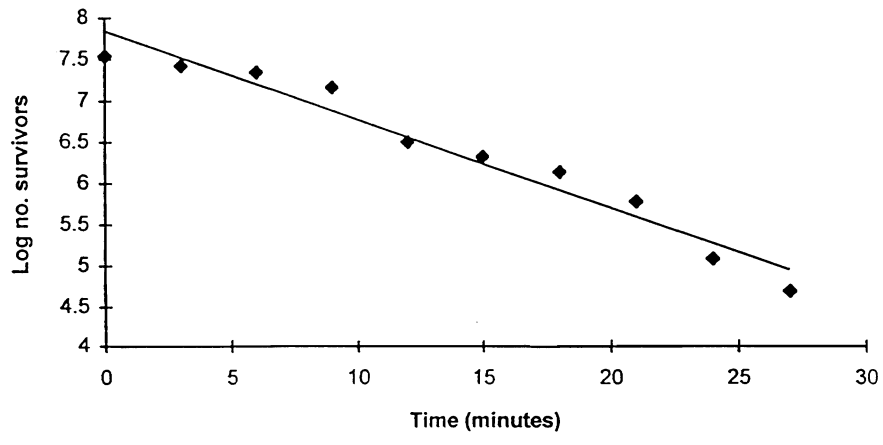


Figure 11.3 D-value of *S.typhimurium* 1005 in Salami at 63°C

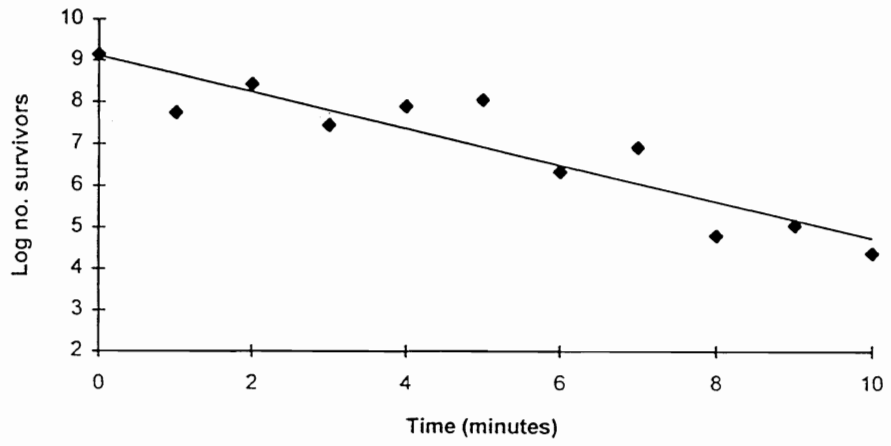


Figure 11.4 D-value of *S.typhimurium* 1005 in Salami at 65°C

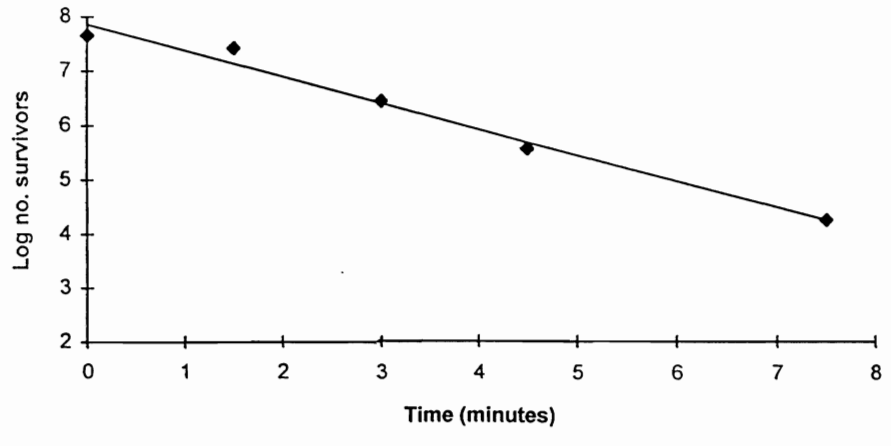


Figure 11.5 D-value of *S.typhimurium* 1005 in Salami at 68°C

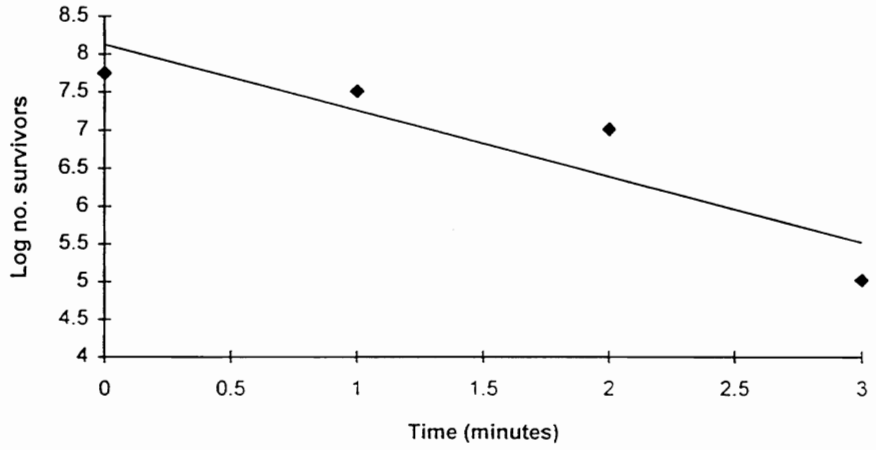


Figure 11.6 z value of *S.typhimurium* 1005 in Salami