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Calculation of the expected increases of coliform organisms, *Escherichia coli* and *Salmonella typhimurium*, in raw blended mutton tissue

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SUMMARY

Samples of blended mutton tissue in small polyvinyl chloride sachets were incubated in water baths for different times and at varying temperatures. The temperature of each bath was recorded accurately throughout each experiment. Using equations previously derived for the lag and generation times of coliform organisms in blended mutton tissue, the expected increases of these bacteria were calculated from the time/temperature recordings. These were compared with the data obtained from plate counts made on the tissue samples in the sachets before and after incubation. The studies were done with a strain of *Escherichia coli*, one of *Salmonella typhimurium* and the coliform organisms naturally present on sheep carcasses processed in a commercial abattoir. The calculated growth agreed closely with that measured. Therefore, if mutton, after overnight chilling, is warmed again to temperatures within the growth range of these bacteria, the possible increases in the numbers of cells present can be calculated directly from time and temperature measurements.

The implications for the present codes of practice in abattoirs are discussed.

INTRODUCTION

Meat produced for human consumption is sometimes contaminated with food poisoning bacteria such as salmonellas (Hobbs, 1964). To minimize the numbers of these organisms, statutory authorities have imposed codes of practice on abattoirs. These govern the way in which animal carcasses are handled and refrigerated after slaughter and the temperature of the rooms in which subsequent processing (cutting or boning) operations are carried out.

In the United States of America, boning rooms must operate at a temperature not above 10 °C (U.S. Inspected Meat and Poultry Packing Plants, USDA, 1981) while countries within the European Economic Community allow 12 °C as the maximum temperature (EEC Council Directive, 1983). In Australia, the temperature of a boning room must not rise above 12 °C or remain above 10 °C for more than 2 h, otherwise all meat must be removed from the room and placed in a chiller or freezer and operations may not recommence until such time as the boning room temperature has been returned to within the legislated levels (Australian Export

Meat Manual, 1982). No doubt these temperatures effectively inhibit the growth of salmonella bacteria (Mackey *et al.* 1980) but it is possible that such requirements are too stringent and waste energy maintaining an unnecessarily low temperature. Certainly, it is unpleasantly cold for plant operators who have to work in these rooms.

Therefore, the following questions may be asked. What increase could be expected in the numbers of salmonellas on meat produced in a boning room if the temperature rose above 12 °C for a short period or remained above 10 °C for more than 2 h? Is it possible to operate these rooms at higher temperatures, both to save refrigeration energy and to improve the comfort and efficiency of the plant operators working within them, without compromising the microbiological safety of the meat?

In a previous paper (Smith, 1985), equations were derived using the method of Ratkowsky *et al.* (1982) giving lag and generation times of coliform organisms growing in blended mutton tissue at temperatures up to 40 °C. It was proposed this data could be used to estimate the possible increase of coliforms (and, by analogy, of *Escherichia coli* and salmonella bacteria as well) on meat held at temperatures within the range of growth of these organisms.

This paper reports the results of experiments designed to test the validity of this proposition.

METHODS

Preparation of sachets of blended mutton tissue containing coliform organisms, a strain of *E. coli* or a strain of *S. typhimurium*, was described previously (Smith, 1985). The media used and the methods by which these organisms were counted were also outlined in the previous paper.

Three water baths were placed in a refrigerated room (0–2 °C). Each water bath was fitted with a Julaba Exotherm heater with variable temperature thermostat and water pump. This allowed the water in each bath to be adjusted initially to any temperature between about 0 and 40 °C and then to be varied between these temperatures throughout the duration of each experiment. Sachets were placed on a wire rack positioned approximately midway between the surface of the water and the bottom of each bath. Another wire rack was placed on top to keep the sachets submerged in this position. This allowed free circulation of water around the sachets so the temperature of the meat tissue in each would respond quickly to any change in the temperature of the surrounding water.

Four copper–constantan thermocouples (26 gauge) were positioned in each bath immediately next to the submerged sachets to monitor the temperature of the water. The temperature of the water in each of the three baths was recorded during each experiment via these thermocouples through two 10-point Keithley No. 702 automatic scanners connected in series to an Omniscribe recorder (Houston Instruments).

The temperature profile graph for each of the three baths used in each experiment was marked at 15 min intervals. The average temperature at which the sachets of mutton tissue were incubated over each 15 min period was determined.

Each of the three baths was subjected to a different temperature regime (Fig. 1). The actual times and temperatures of incubation of the sachets in each bath were deliberately varied to some extent between experiments so that exact repli-

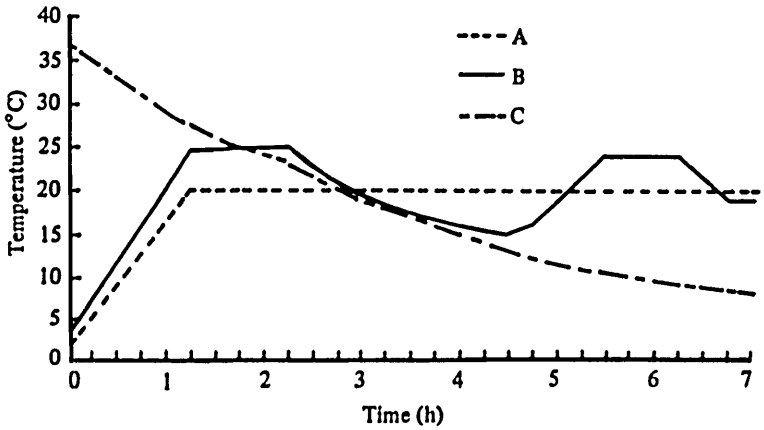

Fig. 1. Typical time–temperature regimes for the three water baths in which sachets of blended meat were incubated.

Table 1. *Calculation of the expected increase in log_{10} number of coliform cells per g of blended meat in experiment 1 in bath A*

Time (h)	Temperature (°C)	Average temperature (0·25 h intervals)	Calculated growth per 0·25 h interval	
0	2	4	—	Lag time
0·25	6	7·5	—	
0·5	9	11·5	0·02	
0·75	14	15	0·04	
1	16	18	0·07	
1·25	20	20	0·09	
1·5	20	20	0·09	
1·75	20	20	0·09	
2	20	20	0·09	
2·25	20	20	0·09	
2·5	20	20	0·09	
2·75	20	20	0·09	
3	20	20	0·09	
3·25	20	20	0·09	
3·5	20	20	0·09	1·03
3·75	20	20	0·2	Generation time
4	20	20	0·2	
4·25	20	20	0·2	
4·5	20	20	0·2	
4·75	20	20	0·2	
5	20	20	0·2	
5·25	20	20	0·2	
5·5	20	20	0·2	
5·75	20	20	0·2	
6	20	20	0·2	
6·25	20	20	0·2	
6·5	20	20	0·2	
6·75	20	20	0·2	
7	20	20	0·2	
7·25	20	20	2·8 generations	

2·8 generations × 0·3 = log_{10} 0·84 increase.

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Table 2. *Calculation of the expected increase in $\log_{10}$ number of coliform cells per g of blended meat in experiment 1 in bath B*

Time (h)	Temperature (°C)	Average temperature (0·25 h intervals)	Calculated growth per 0·25 h interval	
0	3	5	—	Lag time
0·25	7	9	0·01	
0·5	11	13	0·03	
0·75	15·5	18	0·07	
1	20	22	0·11	
1·25	24	24·5	0·14	
1·5	25	25	0·14	
1·75	25	25	0·14	
2	25	24	0·13	
2·25	23	22·5	0·11	
2·5	22	21·5	0·10	0·98
2·75	21	20·5	0·21	Generation
3	20	19	0·18	time
3·25	18	17·5	0·14	
3·5	17	16·5	0·12	
3·75	16	15	0·10	
4	14·5	14	0·08	
4·25	13·5	13	0·07	
4·5	13	14	0·08	
4·75	15	17·5	0·14	
5	20	20·5	0·21	
5·25	21	21·5	0·24	
5·5	22	22	0·25	
5·75	22	23	0·28	
6	24	24	0·31	
6·25	24	22·5	0·26	
6·5	21	20	0·20	
6·75	19		2·87 generations	

2·87 generations × 0·3 = $\log_{10}$ 0·86 increase.

cation of results did not occur, but the general outline of the recorded graphs was always the same for each particular bath.

In each experiment, counts ($\log_{10}$ number of cells) were made on two sachets at zero time and on another two sachets from each bath at the end of the incubation period.

RESULTS

Calculations of the expected increases in the numbers of organisms present in the sachets were made from the average temperature of each water bath over each 15 min interval using the equations published previously (Smith, 1985) for the lag and generation times of coliform bacteria in mutton tissue. The equation relating the lag time to temperature is

$$T = 3{\cdot}00 + 29{\cdot}09\ \sqrt{(1/L)}$$

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Table 3. *Calculation of the expected increase in $\log_{10}$ number of coliform cells per g of blended meat in experiment 1 in bath C*

Time (h)	Temperature (°C)	Average temperature (0·25 h intervals)	Calculated growth per 0·25 h interval	
0	37	36	0·32	Lag time
0·25	35	34	0·28	
0·5	33	32	0·25	
0·75	31	30	0·22	1·07
1	29	28	0·44	Generation
1·25	27	26·5	0·39	time
1·5	26	25·5	0·35	
1·75	25	24·5	0·32	
2	24	23·5	0·29	
2·25	23	22·5	0·26	
2·5	22	21	0·22	
2·75	20	19·5	0·19	
3	19	18·5	0·17	
3·25	18	17·5	0·14	
3·5	17	16·5	0·12	
3·75	16	15·5	0·11	
4	15	14·5	0·09	
4·25	14	13·5	0·07	
4·5	13	12·5	0·06	
4·75	12	12	0·05	
5	11·5	11	0·04	
5·25	11	11	0·04	
5·5	10·5	10	0·02	
5·75	10	10	0·02	
6	10	10	0·3	
6·25	9	9	0·02	
6·5	9	9	0·02	
6·75	8·5	8	0·02	
7	8	8	0·02	
7·25	8		3·52 generations	

3·52 generations × 0·3 = $\log_{10}$ 1·06 increase.

and the equation relating the generation time to temperature is

$$T' = 3.40 + 18.58\sqrt{R},$$

where T' is temperature (°C), L is the lag time (h) and R is generations/h.

When bacteria are incubated at temperatures at which they can initiate growth, the cells must first pass through a lag phase before they enter the generation phase and begin to divide. Examples of the way the calculations were made for the first experiment for each water bath (A, B and C) are shown in Tables 1, 2 and 3.

As the temperature of incubation was not constant, the average lag phase for a given time interval is a complicated function involving the temperature/time relation. The value of interest is the time required to give a lag phase of one during the temperature regimes recorded. To simplify the calculation of this time, the lag phase corresponding to the average temperature over 15 min intervals was determined. The time required for a lag phase to be obtained with a value as close as

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Table 4. *Increase of log_{10} number of cells per g in sachets of blended mutton tissue in seven experiments in Bath A*

Highest temp obtained in bath (°C)	Increase calculated	Increase found				
		Coliform	*E. coli SF*		*Salmonella typhimurium*	
		Mac	Mac	TYSG	BGA	TYSG
20	0·84	0·07	0·69	0·90	0·65	0·66
20·5	0·48	0·34	0·41	0·40	0·38	0·15
19·5	0·85	0·63	0·77	0·75	1·03	1·02
30	1·57	1·83	1·53	1·45	1·38	1·25
23	1·17	1·20	1·15	1·13	1·33	1·27
25	1·22	1·08	1·47	1·40	0·92	0·83
25·2	1·65	1·54	1·60	1·53	1·46	1·54
Average	1·11	1·08	1·09	1·08	1·02	0·96

Mac, MacConkey agar No. 3 (Oxoid).

BGA, Brilliant Green agar (Oxoid) containing sulphadiazine (May and Baker) 80 μg/ml and with pH adjusted to 7·2.

TYSG, Tryptone Soya agar (Oxoid) with the addition of 2 g glucose and 2 g yeast extract (Oxoid) per 1000 ml.

Table 5. *Increase of log_{10} number of cells per g in sachets of blended mutton tissue in seven experiments in Bath B*

Highest temp obtained in bath (°C)	Increase calculated	Increase found				
		Coliform	*E. coli SF*		*Salmonella typhimurium*	
		Mac	Mac	TYSG	BGA	TYSG
25	0·86	0·67	0·81	0·96	0·81	0·92
24	0·57	0·31	0·60	0·58	0·42	0·48
24	0·92	0·80	0·67	0·75	1·02	1·05
28	1·97	1·92	2·13	2·02	2·12	1·94
23	0·54	0·27	0·47	0·40	0·65	0·45
36	2·07	1·62	1·47	1·65	1·74	1·70
34	1·71	1·56	1·70	1·62	1·79	1·69
Average	1·23	1·02	1·12	1·14	1·23	1·19

possible to one was calculated by accumulating sufficient partial lag values over successive 15 min intervals. The use of smaller intervals of time had little effect on the estimation of this value.

After the theoretical lag phase was complete, the possible increase in the number of cells present was calculated in the same way using the equation relating the generation time to temperature of incubation, and accumulating all the values obtained over 15 min intervals for the remaining incubation period. Again, the use of shorter time intervals did not significantly change the total value obtained.

It was also found that if calculations were made directly from the tables published previously (Smith, 1985), the results agreed closely with those shown in Tables 1, 2 and 3.

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Table 6. *Increase of $\log_{10}$ number of cells per g in sachets of blended mutton tissue in seven experiments in Bath C*

Highest temp obtained in bath (°C)	Increase calculated	Increase found				
		Coliform	*E. coli SF*		*Salmonella typhimurium*	
		Mac	Mac	TYSG	BGA	TYSG
37	1·06	1·22	0·94	1·01	1·08	1·18
33	0·64	0·64	0·83	0·74	0·61	0·56
34	0·99	0·99	1·04	1·03	0·83	1·00
38	1·23	0·99	1·36	1·19	1·29	1·18
35	0·92	0·90	1·28	0·97	1·02	1·11
36	1·07	0·96	0·78	0·89	1·33	1·32
37·2	1·09	1·10	1·24	0·84	1·36	1·21
Average	1·00	0·97	1·07	0·95	1·07	1·08

The expected increases obtained by the method shown and the increases actually obtained by plate counts in seven experiments for each water bath (A, B and C) are given in Tables 4, 5 and 6.

Statistical analysis using *t* tests showed no significant differences between the results obtained by calculation and those obtained by plate counts in any one experiment in each water bath, or between the means of the seven experiments in any one bath.

DISCUSSION

The results indicate that the equations published previously (Smith, 1985) can be used to estimate the increase of coliforms, *E. coli* and salmonellas on meat allowed to warm again after overnight chilling to temperatures within the range of growth of these organisms. Although in some experiments there was a difference between the calculated increase and the increase actually found by plate counts of more than $\log_{10}$ 0·3 (one generation), good agreement was obtained overall. Temperatures were measured accurately and the equations were derived originally from the results of a large number of experiments. The discrepancies within experiments probably are due mostly to errors inherent in the plate count technique.

There was no detectable difference between the counts obtained using selective MacConkey agar and those found with a non-selective nutrient medium (TYSG) for the pure culture *E. coli* SF inoculated into the blended mutton. Thus, it can be inferred that the coliform counts obtained in mutton tissue taken from carcasses in a commercial abattoir and containing unknown numbers of these organisms would also reflect closely the actual number of coliform bacteria present. The increases found in the sachets containing these coliform organisms were the same as the increases obtained using pure cultures of *E. coli* and *S. typhimurium* indicating that, under similar conditions to those outlined in these experiments, similar increases in the numbers of each of these types of bacteria would occur on sheep carcasses or on boned out mutton processed in commerical abattoirs.

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The highest increase found was approximately two log cycles i.e. for each viable cell originally present there were about 100 cells at the end of the experiment. This is between six and seven generations or doublings of the original number of cells present and predictions were accurate to this level. Accurate predictions for higher increases may be possible but this has not yet been attempted. It must be emphasized, however, that these predictions are made on the basis that temperature is the only factor affecting the growth of cells. No account has been taken of the effect of drying on the cells on the surface of carcasses or on boned-out meat. Such an effect would tend to decrease considerably the growth of these organisms (Scott, 1936; Scott & Vickery, 1939).

The experiments described indicate the maximum growth that can occur when chilled meat is subsequently warmed again and, provided that the temperature regime is known, this can be calculated accurately. The work establishes that valid predictions can be made and this information can be used to assess the maximum risk from the growth of salmonella bacteria which will arise if meat is subjected to abnormal or unusual temperature regimes. For example, if chilled meat is allowed to warm to 12 °C, it would have to be held at this temperature for 15 h before one division of any salmonella cells that might be present could take place. Even if the room ran constantly at 15 °C, one cell division could not occur in less than 8·45 h, and at 20 °C it would require 4·18 h.

This paper is not meant to be taken as a criticism of the reasons for the imposition of regulations governing the production of meat for human consumption, or of the statutory authorities whose duty it has been to enforce them. Nevertheless, a better understanding of the time and temperature relationships which allow the growth of food poisoning organisms on meat should enable alterations to be made to the present regulations so that they are no more stringent than is necessary to ensure that the microbiological safety of the meat produced is not compromized in any way.

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