5:14 Prevention of Early Spoilage of Livers

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Introduction

There is a widespread belief in the meat industry that livers are inherently prone to rapid spoilage, with a chill-temperature shelf life of only two or three days (Patterson & Gibbs, 1979). In fact, livers can remain acceptable for between one and two weeks when stored in air at 5°C (Gill & De Lacy, 1982). When vacuum packaged, the storage life at that temperature can exceed one month (Patterson & Gibbs, 1979).

The short commercial shelf life of livers held at chiller temperatures is apparently due to inadequate handling of livers between their removal from carcasses and chilling to 5°C. Generally, livers are packed while still warm into plastic tubs of 2 kg capacity. These tubs are themselves packaged, four to a cardboard carton, before any cooling process is applied. With such handling, initial temperatures within tubs are invariably above 30°C and can reach 39°C. If a batch process is used, cooling may be delayed for several hours, during which time liver temperatures will exceed 30°C. Even when cooling commences, 8 to 10 h are required for temperatures at the centres of tubs to fall to 5°C (Gill & Penney, 1984).

Livers packed in tubs closed with self-sealing lids will rapidly produce an anaerobic environment. If livers are cooled to chiller temperatures shortly after packaging, they will develop a flora of lactobacilli and have a shelf life similar to that of vacuum packaged produce. However, if cooling is delayed, a flora composed predominantly of Escherichia coli develops and the shelf life is greatly reduced (Gill & Penney, 1984). There is thus an obvious need for some method of determining the extent of bacterial proliferation during cooling of livers so that the hygienic efficiency of cooling processes can be evaluated and improved.

Direct determination of increases in bacterial contamination is time consuming and, with continuous freezing processes, appropriate sampling may be difficult. As an alternative, it should in principle be possible to estimate bacterial growth for any meat product from the temperature history and appropriate growth rate vs temperature data for the organisms of greatest concern. Packaged livers offer an excellent model for this approach because of the dominance of warm-temperature floras by a single potentially pathogenic species, E. coli. Experimentation was therefore undertaken to determine whether E. coli proliferation during cooling could be calculated with an accuracy sufficient to allow estimated growth values to be used for quality control and regulatory evaluation of liver cooling processes.

Materials and Methods

Growth rate determinations

The variation of growth rate with temperature was determined for six strains of E. coli isolated from commercially packed livers. Strains were maintained on nutrient agar slopes and cultivated in Difco nutrient broth supplemented with glucose at 2 g/l. The growth medium was dispensed in 50 ml volumes in 100 ml conical flasks. Cultures were incubated in a shaking water bath controlled to within ± 0.05°C of the required temperature.

Flasks were inoculated from stock slopes and grown overnight at 30°C. These cultures were used to inoculate further flasks, sufficient inoculum (about 2 ml) being added to give cultures with an initial optical density at 550 nm of about 0.05. The freshly inoculated cultures were replaced in the water bath and a slow stream of oxygen-free nitrogen directed into each flask. The temperature control of the water bath was adjusted to that at which growth was to be monitored. After 15 to 30 minutes the bath temperature had stabilized and monitoring of growth commenced. Growth was monitored from the increase in $O.D_{550}$ of 3 ml samples withdrawn at time intervals suited to the growth temperature. Each sample was discarded after the $O.D_{550}$ was recorded. At least five readings at optical densities below 0.6 were obtained for each growth rate determination.

The growth rate for each strain at each temperature was obtained from the slope derived by least square linear regression analysis of data computed as log $O.D_{550}$ vs time.

Direct estimation of E. coli proliferation in tub-packed livers

For each temperature profile, 10 livers were collected at a commercial abattoir within 15 min of their removal from the animals. Each liver was placed in a separate plastic bag and transported to the laboratory within 30 min. The livers were washed with running water at 30°C and placed in individual plastic tubs of 650 ml capacity. Twenty ml of sterile 0.1% peptone water was added to each tub. The tubs were then closed with self-sealing lids and shaken before samples of the bathing fluids (5 ml) were removed for enumeration of E. coli.

The filled tubs were placed in a climatic cabinet (Fisons, Loughborough, U.K.) which was programmed to simulate the required cooling curve. Thermistor thermometers were inserted through the pierced lids of three tubs and immensed in the bathing fluid. Readings from these thermometers were recorded by a data logger at 3.75 min intervals. After completion of the cooling cycle, further samples of bathing fluids were removed for enumeration of E. coli.

E. coli numbers were determined using Violet Red Bile (VRB) agar with a pour plate technique. Samples of bathing fluid were suitably diluted with 0.1% peptone water when necessary. Each fluid aliquot of 1 ml was mixed with 12 ml of VRB agar tempered to 45°C. The agar was then poured into a sterile Petri dish. When the agar had set it was overlayed with a further 5 ml of VRB agar. Plates were incubated at 37°C for 24 h when all colonies within the agar were counted. Three sequential ten-fold dilutions were plated at each sampling, each dilution being plated in triplicate. Counts were made from plates bearing 10 to 100 colonies.

Results

Growth rates

At most temperatures, the values for growth rates of the six strains of E. coli were within 10% of the average value (Table 1). Wide divergences between the growth rates of the strains occurred only at the extremes of the growth temperature range. Growth rates at temperatures above 44°C could not be determined because the rates declined with time. Growth ceased after about two generations at 46°C and did not occur at 47°C. At the lower end of the growth temperature range, growth declined abruptly below 8°C. Two strains did not grow at 6°C, and none grew at 5°C.

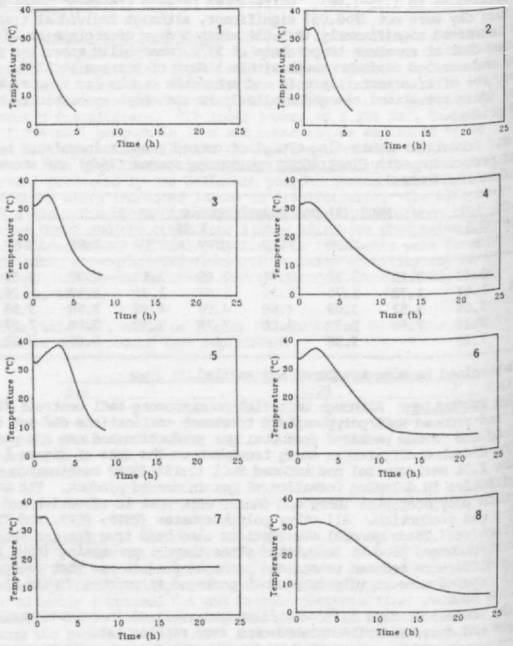

Figure 1. Temperature profiles for cooling livers. Proliferation of Escherichia coli was directly determined with 10 livers for each profile. The log values of the average increase in numbers for each profile were: (1) 0.7, (2) 1.1, (3) 1.8, (4) 2.2, (5) 3.3, (6) 3.8, (7) 3.8, (8) 4.0.

E. coli proliferation by direct enumeration

Initial numbers of E. coli in the fluid bathing the livers were generally between 10 and 10^2/ml, although in a few cases numbers approached 10^3/ml. The values obtained for increase in numbers of E. coli varied widely between individual livers subjected to the same cooling regime. Average increases for individual cooling regimes vaired from less than one log cycle to 4 log cycles (Fig. 1).

Table 1. Average growth rates for six strains of Escherichia coli isolated from lamb livers.

Temperature (°C)	Growth rate, r (gen/h)	Standard deviation
44	1.43	0.269
40	1.77	0.060
35	1.52	0.112
30	1.37	0.071
25	0.83	0.046
20	0.52	0.033
15	0.23	0.028
10	0.10	0.008
8	0.04	0.012
7	0.02	0.009

E. coli proliferation by calculation

As suggested by Ratkowsky et al. (1982), a plot of the square root of growth rate (vr) against temperature (T) gave a straight line relationship over much of the growth temperature range. However, a distinct change of slope occurred above 30°C. At 44°C, the growth rate had declined from the maximum value observed at 40°C. For computational purposes, a plateau was assumed for rates between 40 and 45°C and the simple three phase plot terminated at maximum and minimum temperatures of 45°C and 6°C (Fig. 2). The lines for values between 8°C and 30°C and between 30°C and 40°C were fitted by least square linear regression analysis of the data.

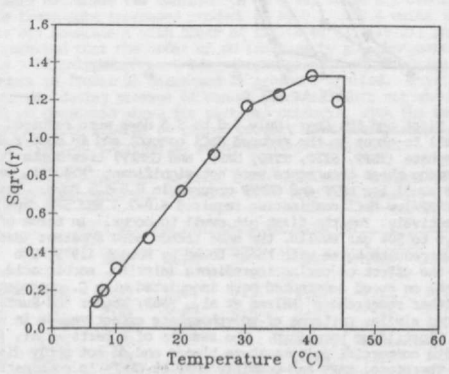

Figure 2. The plot of square root of growth rate [Sqrt(r)] against temperature for Escherichia coli used to derive growth rate values for calculation of E. coli proliferation.

Ratkowski et al. (1983) have offered a modified equation relating vr and T which is designed to encompass the upper end of the growth temperature range. This formula was applied to the data for E. coli, but offered no obvious advantage over the simple vr vs T plot as it did not fit well to growth rate values at the higher temperatures.

Proliferation of E. coli was calculated both manually and by computer. For manual calculation, average growth rates for 5°C temperature intervals between 5°C and 45°C were obtained from the vr vs T plot (Table 2). The times that the cooling livers were within each of the temperature zones were multiplied by the appropriate growth rate values and the resultant products summed to give the total growth. Growth below 10°C was omitted from these calculations as the growth rate was too slow for proliferation in the lowest temperature zone to have a significant effect upon the calculated total growth.

Table 2. Average growth rates within 5°C temperature increments used for manual calculation of growth of Escherichia coli from cooling rate data for livers.

Temperature range (°C)	Average growth rate (gen/h)
40-45	1.80
35-40	1.69
30-35	1.46
25-30	1.12
20-25	0.66
15-20	0.37
10-15	0.16
5-10	0.03

With computer calculation, growth rates from the vr vs T plot for average temperatures in sequential 3.75 minute periods were used to calculate growth within each period, and total growth was obtained by summation.

Comparison of experimental and calculated values for E. coli proliferation

Bacterial proliferation is conveniently expressed as the number of generations occurring during a cooling period. For all cooling regimes, values for the number of E. coli generations calculated by computer or by hand were within 0.5 generation of each other. High standard deviations were obtained for most data sets of direct determinations of E. coli proliferation. All calculated values for proliferation differed by less than one standard deviation from the average values for direct determinations (Table 3).

Table 3. Experimental and calculated values for replication of Escherichia coli during cooling of livers.

Temperature profile (see Fig. 1)	Observed replication		Calculated replication	
	Average No. of generations	Standard deviation	No. of generations calculated by computer	by hand
1				
2	2.4	0.9	2.3	2.1
3	3.6	0.9	4.3	3.8
4	5.9	4.2	6.3	6.3
5	7.3	3.1	7.5	7.3
6	10.8	2.6	8.8	9.3
7	12.6	2.5	10.7	10.8
8	12.6	3.1	11.7	11.6
	13.3	3.7	12.7	12.3

Discussion

During the past 15 years there has been increasing regulatory activity aimed at improving the hygienic standards of abattoir products. It is therefore somewhat surprising that the general mishandling of livers and other offals has passed largely unremarked. As those concerned with meat hygiene are well aware that bacteria of concern for public health can proliferate rapidly at warm temperatures, the apparent failure to appreciate the likely microbiological consequences of packaging warm offals must be due in part to the absence of a simple method for quantification of bacterial proliferation when product is cooling from body to storage temperatures.

The elucidation by Ratkowsky et al. (1982) of a generally applicable linear relationship for variation of bacterial growth rate with temperature should greatly simplify the problem of calculating the microbiological effects when product must move through a temperature range permissive of microbial growth. In the case of the simple liver-cooling system, calculated and observed values for bacterial proliferation are sufficiently close for the calculated values to be of practical utility. In most abattoirs there would be severe restriction of the number of samples that could be processed for direct estimation of microbial proliferation. Variations of an order of magnitude can be expected for replicate plate counts so, unless a large number of samples can be routinely enumerated, the calculated values are probably a better practical guide to microbial proliferation during cooling than direct estimation.

The hygienic efficiency of any liver-cooling process can thus be quantified in terms of the likely bacterial proliferation. Any liver-cooling procedure could be assigned a Bacterial Growth Number, this being the number of bacterial generations occurring during the process. Use of the Bacterial Growth Number concept should simplify regulatory and quality control supervision as, provided the Number was below a tolerable maximum and generally acceptable procedures were employed, the exact form of the cooling profile would be irrelevant.

However, caution must be exercised in extension of this method of process evaluation to processing of products other than livers. In the case of livers, an organism of concern is readily identified, any lag before growth commences can be discounted, and growth will not be inhibited by surface drying or other factors. Other offals undergoing cooling may well provide similar growth conditions for bacteria, but this requires to be clearly demonstrated. With other product and processes an obvious organism of concern may not be readily identifiable (although Salmonella is probably an appropriate species to consider for any fresh meat product), the time of growth onset may be delayed and growth rates may be reduced. In general, it would be unwise to apply calculation of bacterial growth to products and processes unless the factors affecting bacterial growth in each particular system are demonstrably understood.

Further study of the use of temperature function integration in meat processing would be merited as, when used appropriately, the technique seems to offer a simple quantitative method of process evaluation, regulation and control.

Acknowledgements

I thank Mr J.C.L. Harrison and Mr D.M. Phillips for their skilled technical assistance.

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