A QUANTITTATIVE METHOD OF DETERMINING THE HYGIENIC EFFICIENCY OF MEAT THAWING PROCESSES

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SUMMARY

The temperature function integration technique was examined as a means of evaluating the hygienic efficiency of meat thawing processes. Lag phase and growth rate data subsequent to freezing/thawing were obtained for *Escherichia coli* over the temperature range 10 to 40°C. A lag phase model, defined by the equation $Y = 0.00185x^2 - 0.136x + 2.8416$, and a biphasic growth rate model, described by the equations $Y = 0.0514x - 0.171$ and $Y = 0.0323x + 0.341$, were formulated. Application of these models to product temperature histories for both laboratory and commercial thawing gave calculated and observed values for *E.coli* growth that were sufficiently close to directly determined values to be of practical utility in process hygiene assurance.

INTRODUCTION

Meat is handled and processed under conditions selected to limit or preclude the growth of pathogenic bacteria that may contaminate products. For meat thawing processes, control of pathogen growth is sought by stipulating that air temperatures during the process do not exceed 10°C. This temperature is considered sufficiently low to preclude significant growth of *Salmonella*, the pathogen of greatest concern. However, this simple requirement imposes severe restrictions on process flexibility. Factors, other than temperature, which affect pathogen growth during thawing processes, are not taken account of, in particular the effect of the physical properties of the thawing body and the extensive microbial lag that results after freezing/thawing. It would be commercially desirable if these factors were considered, so that processing conditions could be conveniently varied without compromising product hygiene.

Determination of pathogen increases during processing would encompass all factors affecting hygiene. Unfortunately, direct determination is not practicable because the distribution of pathogens is highly irregular and isolation procedures laborious, both factors precluding representative sampling. Alternatively, pathogen increases may be estimated by temperature function integration (TFI), the calculation of bacterial growth from product temperature histories and data relating the lag phase and growth rates of bacteria to temperature. This technique has been satisfactorily applied to evaluation of the hygienic efficiency of commercial processes for offal cooling (Gill and Harrison 1985). Hygienic efficiency was estimated in terms of the growth of *Escherichia coli*, a marker organism for pathogens with similar growth requirements to those of *Salmonella*. The ubiquitous presence of *E.coli* on meat allows the accuracy of growth calculations to be assessed, by comparison with direct counts of the organism.

The evaluation of offal cooling processes involved relatively simple calculations based solely on the growth response of *E.coli* to temperature. For thawing, lag phase duration as well as growth must be considered. The potential application of the TFI technique for hygienic assurance of thawing processes was, therefore, examined for both laboratory and commercial thawing regimes.

METHODS

Determination of E.coli lag phase and growth rates after freezing:

The *E.coli* strain used in this study was isolated from chilled sheep livers. Lag phase and growth rate data were

Table 1. Observed and calculated values for proliferation of E. coli during experimental thawing of meat slices.

Thawing temperature (°C)	E. coli growth (generations)	
	Calculated	Observed(a)
10	4.9	5.4 ± 0.8
15	2.3	2.9 ± 0.6
	8.3	8.4 ± 0.7
20	3.4	2.7 ± 0.6
	4.6	5.0 ± 0.4
30	0.5	0.8 ± 0.5
	3.9	3.6 ± 1.0
35	7.7	7.5 ± 0.9

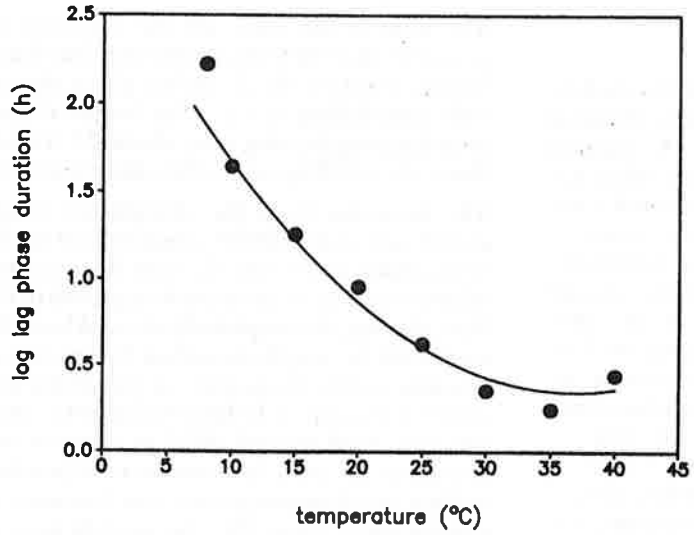
(a) Average and standard deviation of 5 replicate samples.

Table 2 Comparison of directly determined and calculated values for E. coli proliferation on cartoned meat* at several thawing temperatures.

Thawing temperature (°C)	E. coli growth (generations)	
	Calculated	Observed(a)
10	0	0
	0.7	1.6 ± 1.4
15	8.8	7.8 ± 1.6
20	3.1	5.5 ± 0.9
	8.8	8.6 ± 0.5
25	10.3	10.4 ± 1.9

* Temperature profile and samples taken from the top leading corner of the carton.

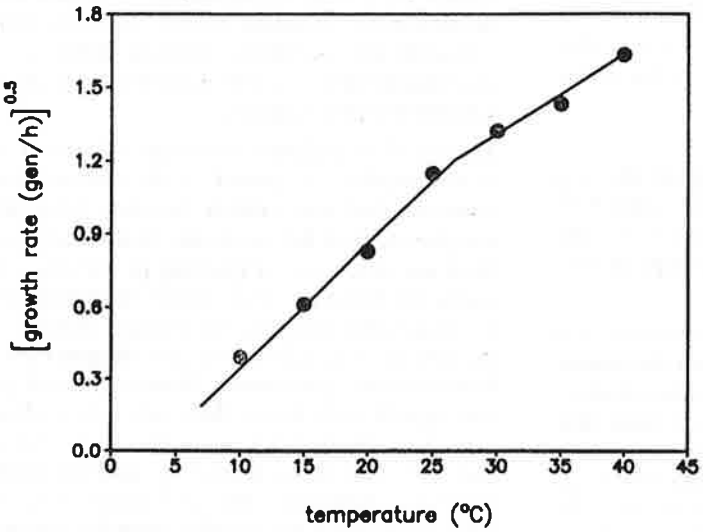
Figure 1. Effect of thawing temperature on lag phase duration of E. coli



(●) experimental lag phase data in SMM.

(—) E. coli lag phase model: $Y = 0.00185x^2 - 0.136x + 2.8416$.

Figure 2. Effect of thawing temperature on E. coli growth rate after lag phase resolution.



(●) experimental growth rate data in SMM.

(—) E. coli growth rate model: $Y = 0.514x - 0.171$; $Y = 0.0323x + 0.341$.

obtained by culturing the organism in a liquid medium that simulated a meat environment. The Synthetic Meat Medium (SMM) consisted of Brain Heart Infusion broth supplemented with 30% haemolysed whole blood and lactic acid (0.9 mg/ml) at a final pH of 5.5. Stationary phase cultures of *E.coli* in SMM (10^4CFU/ml) were frozen to -18°C at a rate approximating 0.1°°C/min, held overnight, then thawed rapidly (approximately 2°C/min) in a shaking waterbath. Once the cultures had thawed completely, the proportion of cells killed upon freezing/thawing was determined by plating directly onto Plate Count Agar (PCA). Lag phase duration and subsequent growth rates, at temperatures between 7 and 40°C, were determined from direct counts made at regular intervals on PCA.

Bench scale meat thawing studies:

Replicate meat slices (pH 5.5-5.7) were inoculated with *E.coli*, frozen at 0.1°C/min and thawed in an environmental cabinet that could be programmed to give a wide range of thawing regimes. Temperature histories of the samples were obtained by means of an electronic data logger. Logger data was transferred directly to a computer for analysis. Growth of *E.coli* was also directly determined from plate counts of five replicate samples taken upon the start of a thawing regime and at appropriate intervals thereafter. Growth was expressed as the number of generations occurring during the process.

Validation tests of E.coli thawing models using cartoned meat:

Cuts of boneless manufacturing grade beef were evenly inoculated with an *E.coli* culture to a final level of 10^4-10^5 CFU/cm^2 then packed into DL 1700 solid fibreboard flat cartons with plastic liners, each containing approximately 27 kg of meat. The cartons were frozen to -18°C, then thawed under prescribed conditions of temperature, air velocity and humidity in an environmentally controlled wind-tunnel. Temperature histories were obtained from thermocouples at 6 points within the carton, including the thermal centre, the top and bottom corners of the leading face and the centre of the top face, by recording data at 30 min intervals directly into a computer. Growth was calculated by application of the thawing models to the temperature histories. Growth of *E.coli* was also directly determined from 6 replicate samples taken at each of the last three sample points at the end of each thawing experiment. Initial counts were obtained immediately surface thawing occurred in inoculated control samples thawed under identical conditions. Growth was expressed as the number of generations occurring during thawing.

RESULTS

E.coli lag phase duration after freezing:

Numbers of *E.coli* were reduced by 37 to 75% upon freezing and thawing in SMM, the variation in lethality being independent of thawing temperature.

The logarithm of the lag phase duration for *E.coli* frozen and thawed in SMM, described a continuous asymptotic curve over the thawing temperature range 8 to 40°C (Fig. 1). At temperatures below 8°C, the lag phase could not be resolved within 7 days.

E.coli growth rate after freezing:

The data for growth of *E.coli* in SMM, following freezing and resolution of lag phase, yielded a temperature-square root of growth rate plot that

conformed to the model proposed by Ratkowsky et al. (1982) across the thawing temperature range 10 to 25°C, but had a distinct change of slope at temperatures above 25°C (Fig. 2). Growth rates were not determined for temperatures below 10°C.

E.coli lag phase and growth rate models:

The lag phase and growth rate data were used to calculate *E.coli* proliferation from temperature histories obtained in bench scale meat thawing experiments. Calculated values were in good agreement with observed values for both lag phase durations and subsequent growth rates between 15 and 30°C, but significant errors occurred above and below these thawing temperatures. Data from the bench scale thawing experiments were used to modify the lag phase and growth rate data, at the two temperature extremes, to obtain predictive models describing *E.coli* behaviour during thawing at temperatures between 7 and 40°C. The best-fit lag phase model, plotted as log of the lag period vs temperature, is described by the quadratic $Y = 0.00185x^2 - 0.136x + 2.8416$ (Fig. 1). The best-fit growth rate model, plotted as the square root of growth rate vs temperature, is a biphasic plot described by the linear equations $Y = 0.0514x - 0.171$ and $Y = 0.0323x + 0.341$ (Fig. 2). The break point for this model is 26°C.

These models were applied to the temperature histories obtained from the bench scale thawing studies to calculate *E.coli* growth. The calculated values agreed well with the observed values, the extent of growth differing by less than one generation in each case (Table 1). In all bench scale thawing experiments the proportions of surviving cells were very similar those obtained for *E.coli* in SMM.

Carton thawing studies:

Calculated values for *E.coli* proliferation at the top leading corner of cartoned meat generally agreed to within one generation of average observed values over the thawing temperature range 10 to 25°C, except in one experiment (Table 2).

A similar agreement between calculated and observed *E.coli* growth was obtained for samples from the centre surface of the meat mass. *E.coli* growth at this sampling point was always at least 10 generations less than that observed at the top corner. In all instances where zero growth was calculated no growth of *E.coli* was observed. When calculated values for *E.coli* growth exceeded 15 generations, observed values were only 50-64% of the calculated values. For all thawing experiments, the observed growth of *E.coli* at the sample point on the bottom leading corner of the carton averaged only 40% of calculated growth. In some experiments, cartons were maintained at the thawing temperature for periods beyond the thaw point of the thermal centre of the cartons.

DISCUSSION

The limited reduction in numbers observed for *E.coli* during freezing/thawing in SMM and on meat (37-75%) indicates that any Gram-negative pathogens present on meat must be expected to survive freezing in significant numbers. The observed *E.coli* death was significantly less than 90- 99% kill suggested for Gram-negative mesophilic species on meat (Georgala and Hurst 1963). This probably reflects a difference in response between the 'wild- type' strain used in this study and more commonly used 'laboratory-maintained' strains.

The form of the curve for the logarithm of *E.coli* lag period vs thawing temperature matches that obtained by Smith (1985) for the *E.coli* lag phase duration on meat following chilling to 0°C. The length of the lag periods after freezing/thawing were about 2.5 times longer than those after chilling across the entire temperature range.

The deviation from the straight-line relationship for growth of *E.coli* in SMM, when plotted by the method of Ratkowsky et al (1982), may be attributable to an inhibitory effect of lactic acid on growth of the organism. Nevertheless, the empirically derived best fit for the data could still be simply described by two linear equations allowing ready integration of the temperature history curves with respect to bacterial growth. Neither model was extended beyond 40°C as thawing temperatures above this are unlikely in commercial practice. Although neither lag phase nor growth rate data were obtained for temperatures below 8°C, the models were extended to 7°C to take account of the widely accepted 7°C minimum growth temperature for *E.coli* and Salmonella.

The variability inherent in direct enumeration techniques for *E.coli* was minimized in the bench scale studies by controlling inoculum level and sampling methods. Therefore, the almost complete agreement of calculated values for *E.coli* proliferation to within one standard deviation of observed values indicates that, provided suitable temperature history data are obtained, calculated values can be used for hygienic assurance of various thawing regimes.

The good correlation between calculated and directly determined *E.coli* growth in the carton thawing studies confirms that temperature function integration offers a simple method for accurate routine evaluation of the hygienic efficiency of thawing processes. Although, as expected, there was wide variation in growth determined for individual samples, the average directly determined growth and the calculated growth generally differed by less than one generation. However, *E.coli* proliferation was significantly lower than calculated when predicted growth exceeded 15 generations, because with that increase, the flora would be approaching maximum numbers. Similarly, observed values were smaller than calculated values for samples from the bottom corners of cartons. This deviation from predicted values is probably a result of rapid development of anaerobic conditions in drip from thawed surfaces that accumulates at this point. Although in a few cases calculated growth was 2-3 generations lower than observed growth, such deviations probably resulted from slight misplacements of the temperature probes. Any errors of placement would take the probe away from the meat surface and therefore underestimate the temperature history curve with respect to the meat surface.

In application of the technique for assessing the hygienic efficiency of thawing processes, the point from which temperature histories are recorded must represent the area in which maximal proliferation can be expected. For

meat cartons, this point was the top leading corner of the carton. Provided the worst case situation can be defined for a process, and appropriate temperature histories obtained, temperature function integration appears to offer a convenient means for evaluation of process hygiene. Such evaluation would also allow identification of operationally convenient and hygienically acceptable variations between procedures.

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