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EVALUATING THE EFFECTS OF HEAT ON SALMONELLAE IN FOODS

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There is a substantial amount of detailed information in the literature on the microbiological problems associated with the heat sterilization of canned foods. Thus, although numerous publications provide data on the heat destruction of the spore-forming bacteria, less information is available on the thermal resistances of non-sporing microorganisms and their destruction at pasteurization temperatures. Furthermore, some of the results quoted for the resistances of these non-sporeformers do not assist food manufacturers in designing heat processes sufficient to remove common food pathogens, e.g. salmonellae. For example "most members of this group (i.e. the salmonellae) are killed by exposure to a temperature of 60°C for 15-20 minutes" (1). Although this is generally true, the statement is of limited value to food processors because no mention is made of the numbers of salmonellae involved, the experimental procedure, nor of the relation of this result to death of salmonellae at other times and temperatures.

Certain investigators have determined the heat resistances of non-sporing pathogens in foodstuffs in a way similar to that used in canning technology for sporeformers. Their results are expressed in terms based on those used to define the thermal death of spores of Clostridium and Bacillus. The practical advantage of this "mathematical" approach is that the thermal death of microorganisms can be predicted where process temperatures are not constant. Using this approach the resistance of salmonellae in liquid egg has been extensively studied and reported by such authors as Annelis et al (2) and Osborne et al (3). This work was prompted by a need for a pasteurization process for the elimination of salmonellae in liquid egg and the recommended process is now legalized in U.S.A. and U.K. More recently Bayne et al (4) have determined the heat resistance of salmonellae in chicken meat and Thomas et al (5) have reported on the resistance

of S. senftenberg in four different media. It is useful to note that all the authors mentioned above more or less agree on heat resistance values for salmonella cells suspended in similar substrates.

In our work, in addition to practical inoculation experiments we have also used the "mathematical" approach in investigating the death of salmonellae.

Briefly our investigations consisted of:

1. Screening of the heat resistances of many salmonellae by determination of Decimal Reduction Times at 140°F (60°C) to show whether any of our strains were as heat resistant as the well known resistant S.senftenberg 775W.(Only one of our strains, a S. senftenberg designated S8 and isolated from meat, possessed comparable heat resistance).

2. Determination of the thermal death times of selected strains in various suspending media.

3. Carrying out practical tests of our results by inoculating a meat product with salmonellae and comparing the pattern of salmonella survival with that predicted by our calculations.

In this paper the following symbols are used:

D_{140° - the time in minutes taken for a 90 % reduction in salmonella numbers at 140°F.

F_{140° - the time in minutes taken to completely kill a given salmonella population at 140°F.

It should be noted that the temperature suffix is often expressed as above, i.e. in °F. However it is also common to find the temperature given in °C, thus -

Fahrenheit expression		Centigrade expression
F_{140°	=	F_{60°
D_{140°	=	D_{60°

METHODS

Decimal reduction times

The heat resistances of many salmonellae were assessed by

determining the decimal reduction time at 140°F (60°C) according to the methods of Solowey et al (6) and Katzin et al (7). In this method, 2 ml quantities of the suspensions are carefully injected into cottonwool plugged 3" x 3/8" tubes. The tubes are heated in a waterbath at 60.5°C, without shaking. After the desired heating period the tubes are rapidly transferred to iced water and cooled with vigorous shaking. In these tests the organisms were suspended in 0.1 % peptone water, or in heart infusion broth. Thermocouple readings in a number of such tubes showed that at least $1\frac{3}{4}$ min elapsed before the suspension reached a maximum temperature of 140°F (60°C) and that there was about 10 sec delay before cooling commenced at the end of the heat treatment. For each organism 8 tubes were set up, and 4 withdrawn after $1\frac{3}{4}$ minutes (time t_1) and 4 after $2\frac{3}{4}$ min (time t_2). In some cases t_2 was extended to 3 min, while with Salmonella senftenberg 775W and S. senftenberg S8, t_2 was fixed at 5 min or 10 min.

In each case surface colony counts were carried out on the test suspension and on each of the 8 heated suspensions, using blood agar. All plates were incubated for 48 hours at 37°C. D values were calculated from the colony counts on blood agar using the formula

$$D_{140^\circ} = \frac{t_2 - t_1}{\log_{10}\left(\frac{C1}{C2}\right)}$$

where C_1 and C_2 are the viable counts at times t_1 ($1\frac{3}{4}$ min) and t_2 ($2\frac{3}{4}$, 3, 5 or 10 min) respectively.

Thermal death times

The method used for determining thermal death times of salmonellae was similar to that described by Annellis et al. (2).

(a) Thermal death times of salmonellae suspended in heart infusion broth

250 ml of a standard suspension of the test organism containing approximately 1.0×10^7 cells/ml were distributed aseptically into 12 x 75 mm t.d.t. tubes in 2 ml amounts. The tubes were then plugged with tight fitting rubber bungs and placed in specially constructed racks. Six such racks, holding 12 tubes each, were used for each t.d.t. determination. When the filling operation was complete, serial dilutions were prepared from the original suspension and counts of salmonella per ml determined by plating out 0.1 ml of appropriate dilutions on heart infusion agar. Filling and carrying out of the counts took approximately 20 minutes.

The six racks were immersed simultaneously in a thermostatically controlled water bath. Timing was begun at the moment of

immersion. After selected intervals of time individual racks were removed and immersed in a bath of running tap water. When the temperature of the heating menstruum had fallen to a non-lethal value, the tubes were removed from the cooling bath and incubated for 5 days at 37°C. When less than 12 tubes in a particular rack showed turbidity of the medium after the incubation period, they were plated out on brilliant green agar (B.G.A.). Presence of viable salmonellae was confirmed by typing of suspect colonies with appropriate antisera. Thermal death times were estimated by taking the mean of the end point of survival i.e. the last heating time at which positive tubes were observed and the next observed time at which all tubes were negative. Thermal death time curves were constructed by plotting the experimental end points of survival/kill logarithmically against temperature in °F on a linear scale.

Since the t.d.t. tubes did not reach bath temperature or cool to a non-lethal temperature instantaneously, a correction factor was calculated according to the method of Annellis et al (2), where exposure time corrected = total exposure time in bath minus thermal lag time plus lethality due to come-up and come-down.

The above experiments were repeated with suspensions prepared from salmonella cultures grown up at incubation temperatures other than 37°C, i.e. 25°C, 30°C and 43°C.

(b) Thermal death times in pork homogenate

The blocks of pork used in these experiments were obtained from a local butcher and stored at -20°C when not required.

20 g samples of pork were removed, 170 ml of 0.1 % peptone water added and the mixture homogenised in a M.S.E. Atomix for 3 min at 12.000 r.p.m. The pH of each homogenate sample was measured and recorded. The total number of bacteria/g of pork was estimated by spreading 0.1 ml of suitable dilutions of homogenate on heart infusion agar plates and counting after 2 days incubation at 30°C. By taking pork samples from deep in the block and using strict aseptic conditions the meat could be obtained with extremely low bacterial content.

After measuring the pH and plating out of the homogenate, 10 ml of an 18 h culture of Salmonella was added, and the mixture of cells + 0.1 % peptone water + pork was homogenized for a further 1 min at 12,000 r.p.m. Surface plate counts of the salmonellae were carried out before and after mixing, to estimate the numbers of salmonella/ml of homogenate and to check whether or not homogenizing had any lethal effect on the salmonellae. The salmonella containing homogenate was distributed in 2 ml

amounts into 12 x 75 mm test tubes and the thermal death times of the salmonellae determined at various temperatures as above for heart infusion broth.

(c) Effect of pH on the thermal death time at 140°F (60°C) of salmonellae

Thermal death times were determined at 140°F (i.e. F140°) for Salm. senftenberg (S8) suspended in heart infusion broth at pH 5.5, 6.0, 7.4 and 8.0, and in pork homogenate at pH 5.5, 6.0, 6.9 and 7.4. The natural pH of the homogenate was constant at 6.0, and phosphate buffer was used to adjust pH to the other values after homogenizing.

Frying conditions and temperature records

In our frying tests we used a thermostatically controlled electric frying pan (Sunbeam Inc.), with a 2-3 mm. layer of Covo vegetable oil. The temperature of the oil during frying was recorded with a copper/constantan thermocouple used in conjunction with a "Servoscribe" potentiometer. For this purpose the thermocouple hot junction was soldered onto a thin copper wafer, which could be located in the oil layer.

The increase in temperature in the meat sample with time was recorded using 40 gauge copper/constantan thermocouple connected to a Kent Mark III Electronic recorder. The thermocouples hot junctions were silver soldered into the tips of No. 1 hypodermic needles. Four such thermocouples were prepared, and to obtain a good record two of these were situated in the centre of the sample. The other two were used for spot checks during frying to make certain that the two fixed thermocouples were giving a true record of the "coolest" portion of the sample.

Calculation of lethality of slow, medium and rapid cooks

Bigelow et al's graphical method (8) for calculating total lethality was applied to data derived from the Kent recorder together with previously determined thermal death times of representative salmonellae. The range of lethalities of the various cooks were expressed in terms of equivalent minutes at 140°F (60°C).

Inoculation experiments

These experiments were carried out to determine the relationships of our predicted lethalities to actual kill of salmonellae in similar samples during frying.

The inoculum consisted of 4 g of comminuted meat + 0.1 ml of a suitable dilution of an overnight heart infusion broth

culture of salmonella. The number of salmonella cells mixed with the 4 g of meat was determined by two methods viz.

(a) Surface plate counts on the overnight broth cultures.

(b) A macerate of 1 g of meat + salmonella, with 9 ml of 0.1 % peptone water.

In the above counts brilliant green agar was used as the plating medium.

The meat and salmonella inoculum was loaded into a sterile disposable 10 ml syringe, and with a long probing needle attachment on the syringe this inoculum was forced into the centre of the sample.

Recovery and detection of salmonellae after frying

Immediately after frying, the inoculated sample was homogenized with 250 ml of heart infusion broth using an Atomix bottom drive homogenizer. After incubation of the homogenate for 24 h at 37°C, 250 ml of double strength selenite broth were added. This was incubated for a further 24 h at 37°C during which time loopfuls were removed at 24 and 48 h and plated out on brilliant green agar. The brilliant green agar plates were examined for salmonella colonies after 24 h at 37°C. Salmonellae were confirmed by serological typing. If viable salmonellae were found after frying, the level of salmonella in the inoculum was reduced in stages.

RESULTS

Decimal reduction times

The D_{140}^{o} values of all but two of the salmonella strains tested (including 36S.senftenberg strains) were within the range 0.2 - 2.0 min. The resistant strains were the well known S. senftenberg 775W and an isolate from our own collection namely S. senftenberg S8. Both of these resistant S.senftenberg strains had D_{140}^{o} value of about 10 min (Table 1).

Table 1 Examples of decimal reduction times of salmonellae at 140°F (60°C).

TYPE	D VALUE (MINUTES)
S. carrau	1.2
S. michigan	0.5
S. brandenburg	0.4
S. paratyphi B	0.7
S. lexington	0.6
S. typhimurium	0.6
S. thompson	0.5
S. senftenberg (code 532)	0.7
S. senftenberg (S8)	8.6
S. senftenberg (775W)	10.7

Thermal death times

The thermal death times for salmonellae suspended in heart infusion broth and pork homogenate are shown in Tables 2 and 3 respectively. A thermal death time curve for each organism in pork homogenate is illustrated in Fig. 1.

Table 2 Thermal resistance of salmonellae suspended in Heart Infusion Broth, pH 7.4

(approximate cell load = 2×10^7 per tube)

S. senftenberg (S8)		S. typhimurium (159)	
Temperature °F	Thermal death time (minutes)	Temperature °F	Thermal death time(minutes)
136(57.7°C)	74.0	130 (54.4°C)	65.5
138	52.0	133	23.0
140	36.5	135	13.0
142	27.0	137	9.0
145	8.0	140	3.5
147	6.0	142 (61.1°C)	2,5
149 (65°C)	4.5		

Table 3 Thermal death times of salmonellae suspended in pork homogenate

(approximate cell load = 2×10^7 per tube)

S. senftenberg (S8)		S. typhimurium (159)	
Temperature °F	Thermal death time (to nearest min)	Temperature °F	Thermal death time (to nearest min)
136.5(58.1°C)	103	131.0 (55°C)	73
140.0	40	133.0	35
145.0	14	136.5	16
149.0 (65°C)	3	141.0(60.6°C)	4

*pH of homogenate was 6.0 in all these determinations

When thermal death time curves are constructed (i.e. on semi logarithmic paper) the differences between the resistances of salmonellae suspended in heart infusion broth and pork homogenate are seen to be small.

Table 4 shows the variation in thermal death time at 140°F ($F_{140}°$ value) of S. senftenberg (S8) when suspended in heart infusion broth and pork homogenate at different pH values.

Table 4 $F_{140}°$ (or $F_{60°C}$) values of S. senftenberg (S8) effect of pH heating menstruum

(approximate cell load = 2×10^7 per tube)

Pork Homogenate		Heart Infusion Broth	
pH	$F_{140}°$ value (to nearest minute)	pH	$F_{140}°$ value (to nearest minute
5.5	38	5.5	26
6.0	40	6.0	46
6.9	47	7.4	39
7.4	51	8.0	21

Annellis et al (2) reported that the heat resistance of salmonellae suspended in liquid egg was greater at lower pH values. However the results in Table 4 show that with increasing pH values of the pork homogenate there was an increase in heat resistance of Salm. senftenberg (S8).

The effects of the pre-heating incubation temperature on the $F_{140}°$ values are shown in Table 5.

Table 5

Pre-heating Incubation Temperature °C	$F_{140°}$ (or $F_{60°C}$) value (minutes)	
	S.senftenberg (775W)	S.typhimurium(159)
25	22.0	1.0
30	28.0	1.5
37	36.5	3.5
43	50.0	7.5

For both strains studied it was found that the higher the previous incubation temperature the greater the thermal resistance.

Application of thermal death time information

The following example shows how the above thermal death time information was used in investigating the lethal effects of a cooking process applied to a particular type of sample - in this case a Shallow frying of a small comminuted pork product.

We first obtained a temperature record at the centres of the samples (weight about 50g, thickness about 2.5 cm) during a short hot fry, and during a slow fry at moderate temperature (Figs 2 and 3). The total heat treatments at the centres of these samples are expressed in Table 6 in terms of "equivalent minutes at 140°F (60°C)". It should be noted that these figures relate only to the heating-up period. Depending on circumstances, additional "equivalent minutes" could be recorded during the cooling of the sample.

The figures given in Table 6 are only examples of several determinations on this particular product. When more samples were examined, we obtained a range of values for "equivalent minutes at 60°C". For example, in the short hot fry the value varied from 1 to 8 minutes. These variations were probably due to differences in sample dimensions. Another important factor which could influence the heat treatment is the initial temperature of the sample before heat is applied (our samples started at 32°F - 0°C).

Table 6 Total lethality of two frying treatments, expressed as equivalent minutes at 140°F (60°C).

Treatment	Maximum oil temperature	Frying time	Equivalent minutes at 140°F (60°C).
Slow, moderate fry	120°C	25 min	Approx. 70,000
Short hot fry	170°C	10 min	Approx. 6 (range 1-8)

The results in Table 6 were then used to predict the effect of these treatments on any salmonellae which might be present in the centres of such samples. It was clear that the 70,000 "equivalent minutes at 60°C" of the slow fry would guarantee destruction of any salmonellae present, as the most resistant type known has a thermal death time at 60°C of ± 40 minutes (Fig 1). In contrast, the 1-8 "equivalent minutes at 60°C" of the short hot fry could not guarantee the destruction even of sensitive Salm. typhimurium (thermal death time at 60°C of about 4½ min - see Fig 1.), and certainly would not ensure destruction of the heat resistant Salm. senftenberg (S8).

Inoculated samples were used to test these predictions. The salmonellae in these samples were always located in the central "coolest" portion of the sample. The slow fry at moderate temperature did in fact completely destroy large numbers of Salm. typhimurium and Salm. senftenberg S8. And also as expected, the short hot fry did not destroy even moderate numbers of either serotype.

SUMMARY AND CONCLUSIONS

Out of over 100 strains of salmonellae tested by $D_{140°}$ determinations, only one strain, i.e. S. senftenberg S8, was found to possess comparable heat resistance to S. senftenberg 775W. It therefore seems likely that heat resistant salmonellae strains are rare (9).

Although we have only touched lightly on the factors which influence heat resistance, it is well recognized that thermal resistance of microorganisms may vary according to the pre-heating conditions, the physico-chemical properties of the heating menstruum and the recovery method for the heated cells. Therefore, when estimating the effect on bacteria of a heat process, the experimental conditions should approximate as near as possible to those found in commercial practice and also, results obtained should be reproducible from one experiment to another.

In our investigations, the lethality for salmonellae of a shallow frying process was calculated by an integration of thermal resistance data for the organisms with heating characteristics of a particular food sample. Our results have shown that this concept can be applied to predicting the fate of salmonellae during shallow frying and we feel that our results can also be used for estimating the effect of heat on salmonellae in other cooking procedures and in other foods. The concepts and experimental procedure described here should also be applicable to problems associated with the fate of other non-sporing pathogens in cooked foods.

ZUSAMMENFASSUNG

Von mehr als 100 Salmonellenstämmen, die durch $D_{140°F}$ ($D_{60°C}$) Bestimmungen untersucht worden waren, zeigte S. senftenberg S8 als einziger eine Hitzeresistenz vergleichbar mit der des Stammes S. senftenberg 775W. Es scheint daher, dass hitzebeständige Salmonellenstämme selten sind.

Obwohl wir die Faktoren, welche die Hitzeresistenz von Mikroorganismen beeinflussen, nur im Vorübergehen erwähnt haben, hängt diese bekanntlich von den Bedingungen des Vorerhitzens, den physisch-chemischen Eigenschaften des Milieus, in dem das Erhitzen erfolgt, und der zur Isolierung der erhitzten Zellen angewandten Technik ab. Will man die Wirkung eines Wärmevorganges auf Bakterien beurteilen, sollen die Versuchsbedingungen den in der industriellen Praxis vorherrschenden Bedingungen möglichst genau angenähert werden. Die erhaltenen Ergebnisse müssen ausserdem gut reproduzierbar sein.

Die letale Wirkung eines Bratvorganges wurde durch Zusammenfassung der Hitzeresistenz-Daten der Organismen und der Erhitzungseigenschaften einer bestimmten Lebensmittelprobe ermittelt. Unsere Ergebnisse zeigen, dass auf Grund dieser Annahme das Schicksal von Salmonellen während eines Bratvorganges im voraus beurteilt werden kann. Wir glauben, dass unsere Ergebnisse auch als Grundlage zur Beurteilung der Wirkung einer Hitzebehandlung auf Salmonellen bei anderen Zubereitungsmethoden und in anderen Lebensmitteln dienen können. Die beschriebenen Annahmen und Versuchsmethoden sollten auch auf Probleme verbunden mit dem Schicksal anderer sporenfreier, pathogener Organismen anwendbar sein.

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FIG. 1

THERMAL DEATH TIME CURVES FOR SALMONELLAE SUSPENDED IN PORK HOMOGENATE (cell load 2×10^7 per tube)

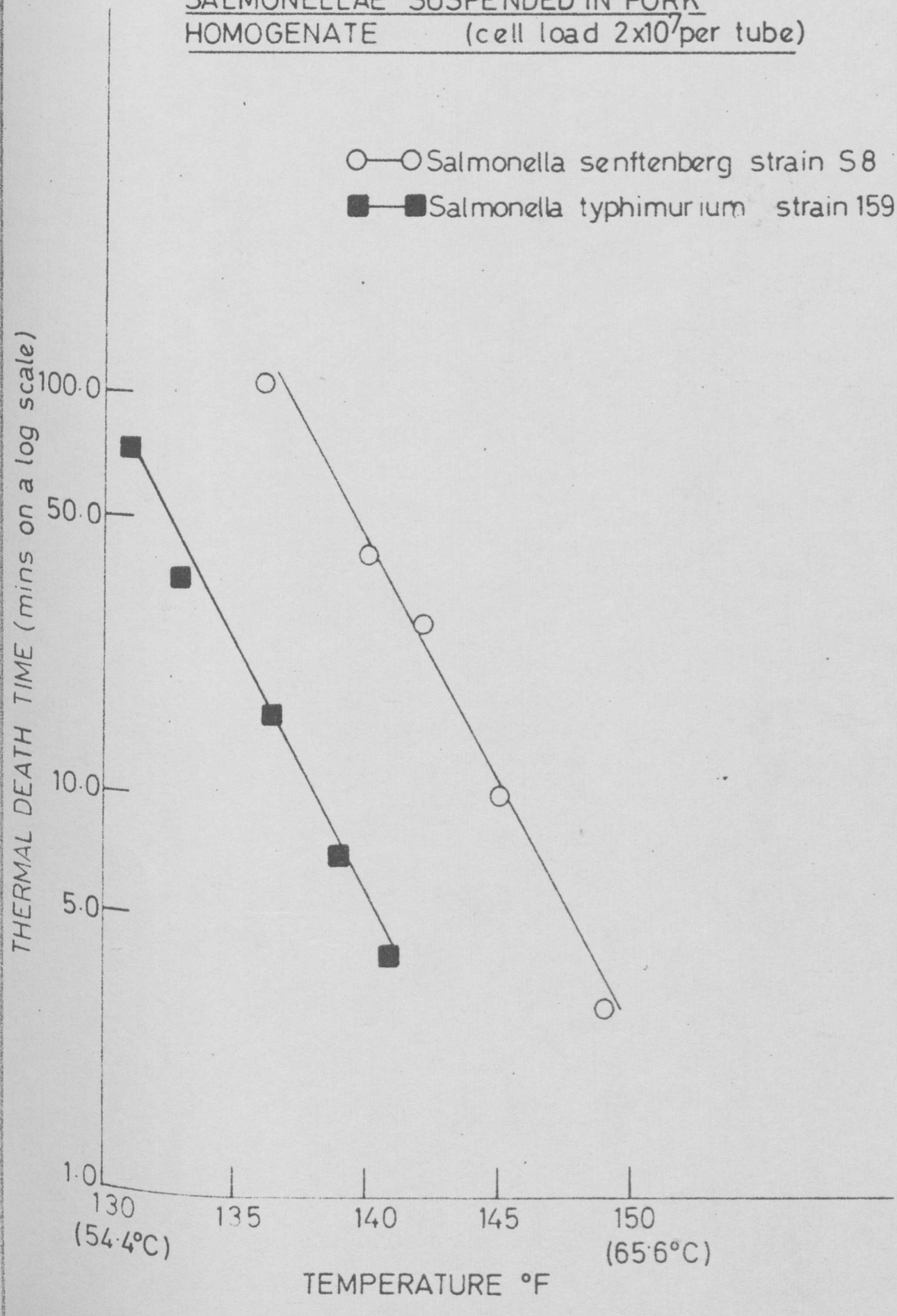

Fig 2 HEATING CURVES FOR A COMMINUTED MEAT SAMPLE DURING "SLOW FRY AT MODERATE TEMPERATURE"
(Broken and full lines indicate recorder tracings from 2 separate thermocouples in one sample)

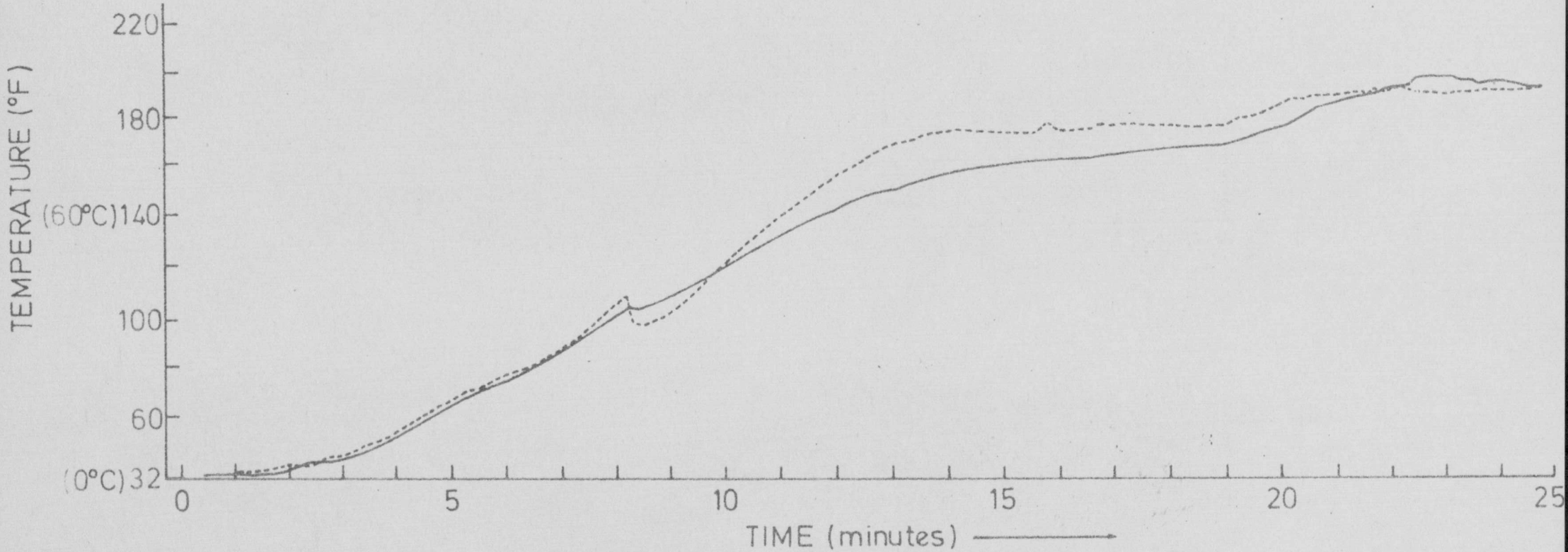

Fig 3 HEATING CURVES FOR A COMMINUTED MEAT SAMPLE DURING "SHORT HOT FRY" (Broken and full lines indicate recorder tracings from 2 separate thermocouples in one sample)