HEAT RESISTANCE OF THERMODURIC ENTEROCOCCI ISOLATED FROM FRANKFURTERS IN JAMAICA.

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INTRODUCTION

The heat processing operation is critical in the manufacture of shelf stable, microbiologically safe frankfurters. Generally, frankfurters are cooked to an interval temperature of 68-72°C (Ockerman, 1989). At lower cooking temperatures, the shelf life and microbiological safety of the product is reduced because a greater number of organisms survive the process. The enterococci are the major heat-resistant organisms which survive the processing of cured meat products (Deibel, 1964; Duitchaever, 1978; Chyr et al., 1981). However, high numbers of thermoduric enterococci in cured meats indicate inadequate processing (Bell and De Lacy, 1983). Both S. faecalis and S. faecium have been implicated in the spoilage of these products (Bell and De Lacy, 1984; Magnus et al., 1986). In addition, the enterococci may be etiological agents of food poisoning (Bryan, 1979). Hence proper care must be taken to avoid excessive numbers of enterococci in processed meat products.

The heat resistance of enterococci and the mechanisms by which they suffer heat injury and subsequently recover have been investigated by several researchers (Clarke et al., 1968; Duitchaever and Jordan, 1974; White, 1953). The heat resistance of microorganisms is usually expressed as Decimal Reduction Times (D-values). This is the time (in minutes) required to reduce the microbial population by 90 percent. Others have concentrated on the heat resistance of the enterococci in different menstrua or as affected by environmental factors (Sanz Perez et al., 1982; Magnus et al., 1988). Several of these studies used isolates whose heat resistance may have been affected by mutations during storage and transfer. It is more desirable to use newly isolated strains from the product under investigation if the results are to be directly applicable to industry (Magnus et al., 1986). In addition, there are few reports on the thermal resistance of enterococci isolated from frankfurters and, to our knowledge, no such report originating from a developing country. Hence, the objective of this study was to evaluate the heat resistance of freshly isolated enterococci from frankfurters in broth and meat-based menstrua and relate this to their occurrence on, and spoilage of frankfurters.

MATERIALS AND METHODS

Organisms and Culture Conditions

Nine representative strains of enterococci from cooked frankfurters were used in this study. These isolates were identified as S. faecium according to Deibel (1964), and Gross et al. (1975). The isolates were maintained on Brain Heart Infusion (BHI) agar slants at 4°C and revived in BHI broth at 37°C for 24 hours prior to use. Broth cultures were plated onto BHI agar, incubated a further 24 hours to check for purity and single colonies subcultured unto BHI agar slopes. Cultures for heat resistance determinations were grown at 37°C for 18 hours in BHI broth overnight.

Determination of Heat Resistance In Broth Culture

A 10 ml aliquot of each exponential-phase culture was transferred to a 250

Erlenmeyer flask containing sterile lass beads and diluted with 0.1% ptone water to give approximately 10^7 ills/ml. The culture was thoroughly nd-shaken for 1 min to break up clumps arrigan and McCance, 1976), 5 ml liquots aseptically dispensed into terile screw-capped tubes (125 x 12mm) d heated in a thermostatically ntrolled water bath at 63°C (± 0.5°C) r 60 min and 68°C (± 0.5°C) for 30 min. required 1 min for equilibration of mperatures inside and outside of the bes, as determined by a thermocouple serted into a control tube. At this me, a zero time sample was taken (Bell d De Lacy, 1984) with another sample thdrawn at the end of the respective ating periods for each incubation mperature. The samples were cooled to 5°C and pre-poured BHI agar plates oculated with suitable dilutions. hree plates per sample were used and hese were incubated at 37°C for 48 ours before the viable counts were etermined. This method was used to xamine the heat resistance of all nine trains of S. faecium at 63°C and 68°C.

A more detailed study was done on ne of the strains of S. faecium, DP2181 hich were randomly chosen. The heat esistance of S. faecium DP2181 was xamined at 55°C, 63°C, and 68°C for 90, 0 and 30 min intervals respectively. he sampling intervals used were 15, 10 and 5 min respectively and samples were ooled to 20°C and serial dilutions lated on BHI agar. The plates were ncubated at 37°C for 48 hours before acterial counts were estimated.

In Frankfurter Emulsion

Commercially prepared raw rankfurter emulsion was placed into 50 ml beakers which were covered and utoclaved at 121°C for 15 min to eliminate the natural flora. Expo- nential phase broth cultures of S. faecium DP2181 (2 ml) was pipetted into each of 12 beakers, mixed thoroughly for 2 min with a sterile spatula and placed in the water bath. The time required for equilibration of temperatures between the emulsion and the water bath was 4 min. At this point, a zero time sample was taken. Further samples were taken at 20, 40 and 60 min for the determination of heat resistance at 63°C, and 10, 20 and 30 min for samples heated at 68°C. For each treatment three beakers were used. The heated emulsion was cooled at 25°C and blended with 180 ml of 0.1% peptone water in a Waring blender for 2 min. Appropriate dilutions were plated on BHI agar. The experiment was repeated three times.

Estimation of D and z values

Survivor curves for S. faecium DP2181 in BHI broth and frankfurter emulsion at both heating temperatures were obtained by plotting the logarithm of the number of survivors against the sampling time. The D-values (Decimal Reduction Times) were obtained by linear regression analysis of the logarithmic portion of each curve. The D-values were plotted on a logarithmic scale against temperature to produce the thermal death time (TDT) curves. The z-value was derived from the absolute value of the inverse slope of the line obtained and is defined as the temperature interval (°C) required to reduce the D-value by a magnitude of ten.

RESULTS AND DISCUSSION

The effects of both heat treatments on the nine strains of enterococci were similar in terms of number of survivors at the end of the heating period (Table 1). All of the strains were relatively thermoduric and most survived both temperatures in sufficient numbers (>10^3 CFU/ml) to pose a threat to the shelf stability of a product if it was subjected to temperature abuse. The S. faecium strain CF18 was the least heat tolerant while PF40 was the most heat resistant at both cooking temperatures (Table 1). The variation in heat tolerance between strains of S. faecium is not uncommon and Magnus et al. (1986) have also shown wide variations between S. faecium isolates.

S. faecium strain DP2181, a moderately heat resistance isolate , was

chosen for further, more detailed studies of its thermal susceptibility in broth and frankfurter emulsions. Figure 1 shows the survivor curves at 55°C, 63°C and 68°C for DP2181. The 55°C survivor curve was irregular in shape, deviating from the expected logarithmic order of death (Jay, 1986). There were initial and terminal slow periods of death with the latter being very pronounced. There was no substantial reduction in the bacterial population and the D-value of the logarithmic portion of the curve which was 105.63 mm (Table 2).

Duitchaever and Jordan (1974) reported a 2-log reduction for a S. faecium strain after being heated at 55°C for 30 min. However, Magnus et al. (1986) found that very little cell death occurred at 55°C for three strains of S. faecium which agrees with the results reported here. They further observed that S. faecium E-20, a strain which had been shown to be highly heat resistant in earlier studies, had lost much of its heat tolerance. Several other factors besides storage of the isolates may contribute to differences in heat tolerance between S. faecium strains. These include the inherent heat resistance of the enterococcal strain, cultivation prior to and after heating and the method of determining heat tolerance.

In contrast to heating at 55°C, a sharp decline in the number of survivors was observed for DP2181 in the BHI broth at 63°C (Fig. 1). Again, the survivor curve was non-logarithmic and a resistant "tail" of organisms with a D_{63} value of 24.39 min was observed. The D_{63} value for the initial (log) portion of the curve was 9.36 (Table 2). A 4-log reduction in total bacterial numbers was obtained at this time/temperature combination. The survivor curve for DP2181 in BHI broth at 68°C showed a 3-log fall within the first 10 min after which a resistant tail was again evident (Fig. 1). The D_{68} value was 3.34 min for the primary logarithmic portion and 47.62 min for the tail (Table 2). It therefore appears that the thermal resistance of the organisms forming the tail increased with increasing thermal stress.

The shape of the survival curves provides insightful information about heat resistance of the bacterial population being examined. Non-logarithmic curves were obtained under all three heating conditions in this study. These deviations are indicative of the heterogeneity of the test organism with respect to thermal susceptibility (White, 1953). Hansen and Reiman (1963) suggested that the initial lag in the death rate (shoulders) may indicate bacterial clumping or chain formation resulting in a temporary increase in heat stability, since only when the last organism in the clump or chain is destroyed will reductions in viable cell count be observed. They also suggested that the initial lags in death rate might be related to the extent of cell damage which cells can endure before losing their ability to recover. While this latter suggestion may be relevant to our results, care was taken to destroy any chains or clumps formed prior to evaluating the thermotolerance of DP2181 (see materials and methods). This reduces the probability that the formation of aggregates or chains may have resulted in abnormalities in survivor curves.

Deviations from the logarithmic order of death is a relatively frequent occurrence among microorganisms (Blankenship and Craven, 1982; Verrips and van Rhee, 1983; Jay, 1986), and has been previously reported for the enterococci (White, 1953; Sanz Perez et al., 1982; Bell and DeLacy, 1984; Magnus et al., 1986). Magnus et al. (1986) reported tailing of the survivor curves but only at temperatures between 56°C and 58°C. However, other studies have found the consistent appearance of a resistant tail for S. faecium strains at temperatures ranging from 62°C to 70°C (Sanz Perez et al., 1982; Bell and De Lacy, 1984). This tailing phenomena may be due to variations in the thermal resistance of the cells at different stages of cell division (Bell and De Lacy, 1984) or the emergence of cells

FIGURE 1. SURVIVOR CURVES FOR S. FAECIUM DP2181 AT THREE TEMPERATURES.

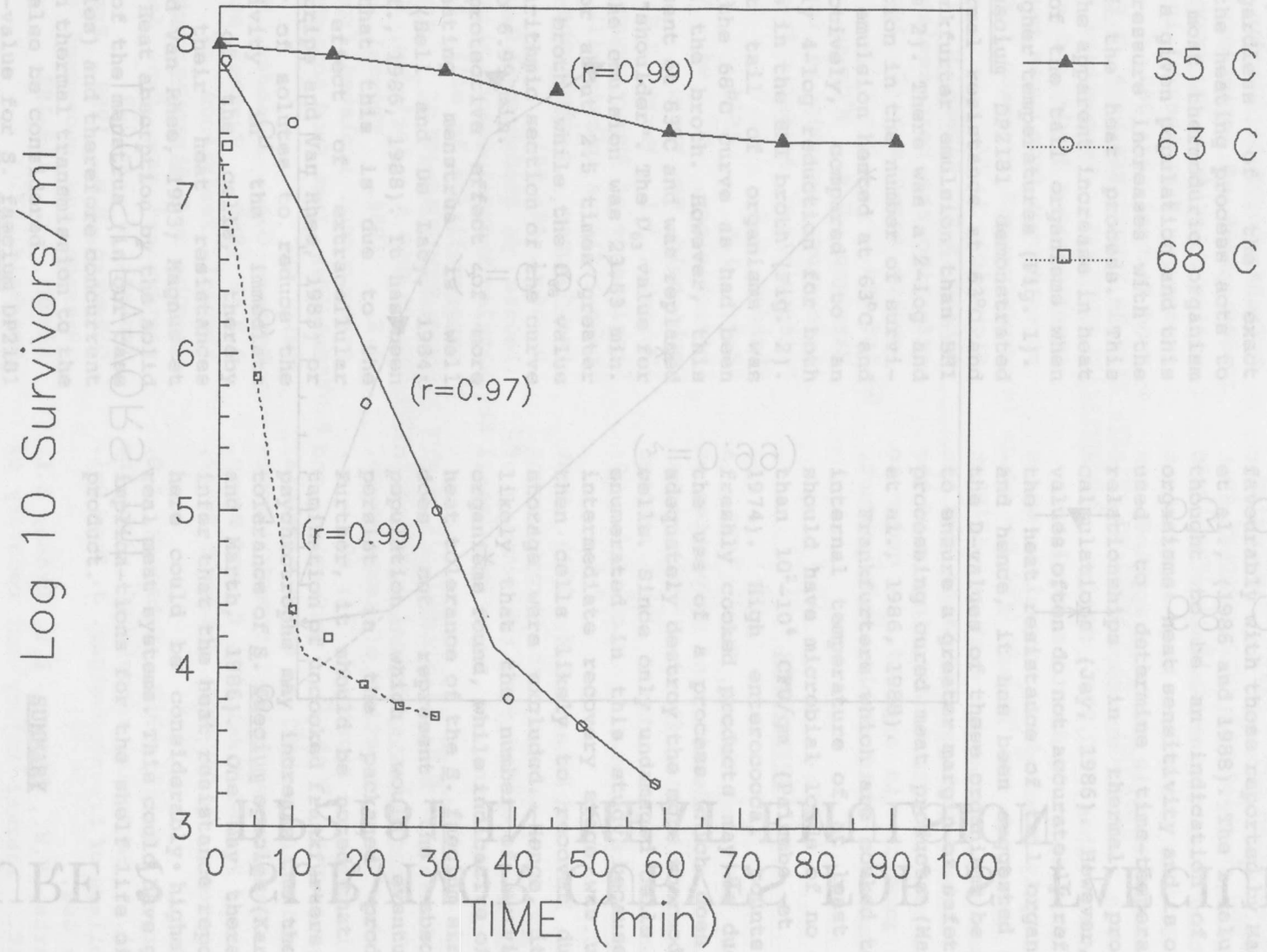

FIGURE 2. SURVIVOR CURVES FOR S. FAECIUM DP2181 IN MEAT EMULSION

within the population with membrane structures which enhance their thermotolerance (Duitchaever and Jordan, 1974). Regardless of the exact mechanism, the heating process acts to select the most thermoduric organism from within a given population and this selective pressure increases with the severity of the heat process. This results in the apparent increase in heat resistance of the tail organisms when heated at higher temperatures (Fig. 1).

S. faecium DP2181 demonstrated greater thermal resistance at 63°C and 68°C in frankfurter emulsion than BHI broth (Table 2). There was a 2-log and 3-log reduction in the number of survivors in the emulsion heated at 63°C and 68°C respectively, compared to an approximately 4-log reduction for both temperatures in the BHI broth (Fig. 2). A resistant tail of organisms was evident in the 68°C curve as had been observed in the broth. However, this tail was absent at 63°C and was replaced by a slight "shoulder". The D_{63} value for DP2181 in the emulsion was 23.53 min. (Table 2) or about 2.5 times greater than in the broth, while the D_{68} value for the logarithmic section of the curve increased to 6.99 min.

The protective effect of more complex heating menstrua is well documented (Bell and De Lacy, 1984; Magnus et al., 1986, 1988). It has been suggested that this is due to the protective effect of extracellular protein (Verrips and Van Rhee, 1983) or the ability of solutes to reduce the water activity in the immediate environment of the cells, thereby increasing their heat resistances (Verrips and Van Rhee, 1983; Magnus et al., 1986). Heat absorption by the solid components of the menstrua (in our case meat particles) and therefore concurrent reduction in thermal transmission to the cells must also be considered.

The z-value for S. faecium DP2181 was calculated from a plot of the $\log_{10}$ of the D-values (obtained at each temperature) against temperature. The z-value obtained was 8.56 which exceeds the value of 5.56 which is typical of vegetative cells (Lovett et al., 1982) and therefore indicates the thermoduric nature of the cells. It compared favourably with those reported by Magnus et al., (1986 and 1988). The z-value is thought to be an indication of the organisms' heat sensitivity and is often used to determine time-temperature relationships in thermal process calculations (Jay, 1986). However, z-values often do not accurate-ly reflect the heat resistance of tail organisms and hence, it has been suggested that the D-values of these organisms be used to ensure a greater margin of safety in processing cured meat products (Magnus et al., 1986, 1988).

Frankfurters which are cooked to an internal temperature of at least 68°C should have microbial loads of no more than 10^2-10^4 CFU/gm (Palumbo et al., 1974). High enterococcal counts on freshly cooked products may be due to the use of a process which does not adequately destroy the more thermoduric cells. Since only undamaged cells were enumerated in this study because no intermediate recovery stage was used, then cells likely to recover during storage were excluded. Hence, it is likely that the number of surviving organisms found, while indicative of the heat tolerance of the S. faecium strain, does not represent the absolute population which would eventually persist in the packaged product. Further, it should be noted that contamination of uncooked frankfurters with psychrotrophs may increase the thermal tolerance of S. faecium species (Karneke and Marth, 1986). One may therefore infer that the heat resistance reported here could be considerably higher in real meat systems. This could have grave implica-tions for the shelf life of the product.

SUMMARY

Both treatments resulted in a 3-4 log decline in bacterial numbers for the nine S. faecium strains tested. The survivor curves for S. faecium DP2181 deviated from the logarithmic order of

death at all three heating temperatures. A resistant tail of organisms was observed at 63 and 68°C. The survival of DP2181 was enhanced in frankfurter emulsion. Our results indicate that heating frankfurter emulsions at 68°C for 30 min, which is a more severe heat treatment than is normally applied, can leave a residual enterococcal flora of greater than 10,000 CFU/gm. This residual flora is due to a population of highly thermoduric organisms which are part of the normal flora. Consequently, processors must ensure that an adequate heat process based upon the most heat resistant contaminants of the raw products is applied. in order to guarantee a shelf stable, safe product.

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TABLE 1. HEAT RESISTANCE OF NINE STRAINS OF ENTEROCOCCI ISOLATED FROM FRANKFURTERS

Strains[a]	Log_{10} Counts/ml at 63 and 68°C at Time T (min)			
	63°C		68°C	
	T = 0	T = 60	T = 0	T = 30
CF10	7.62	2.74	7.82	2.43
CF22	7.31	4.19	7.19	4.03
CF34	7.29	4.09	7.42	3.92
CF35	7.36	3.99	7.38	3.97
PF7	7.48	2.85	7.21	2.81
PF10	7.20	3.87	7.01	3.72
PF19	7.33	3.74	7.23	3.64
PF40	7.45	4.52	7.31	4.43
DP2181	7.49	3.78	7.61	3.77

a - strain designation eg DP2181; CF - isolated from freshly cooked frankfurters; PF - isolated from packaged frankfurters.

TABLE 2. DECIMAL REDUCTION TIMES FOR S. FAECIUM DP2181 IN BROTH AND FRANKFURTER EMULSION

HEATING MEDIUM	TEMPERATURE (°C)	D-value (min)
Broth	55	105.63
Broth	63	9.36 (24.39)[a]
Broth	68	3.34 (47.62)
Frankfurter Emulsion	63	23.53
Frankfurter Emulsion	68	6.99

a - D-value of "tail" phase organisms.