EFFECT OF THE HEATING RATE ON THE THERMAL INACTIVATION OF ENTEROCOCCUS FAECIUM IN CURED PORK MEAT

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INTRODUCTION: Many factors may influence the heat resistance of a nonsporeforming microorganism. Some workers (THOMPSON et al.,1979; MACKEY and DERRICK, 1987; QUINTAVALLA and CAMPANINI,1991) have proved that the heating rate may induce a greater thermal tolerance in some microorganisms, and may have a great importance especially when low processing temperatures are employed. Enterococcus faecium and Enterococcus faecalis are common contaminants in cured ham prior to processing and have been identified as spoilage organisms in the pasteurized products (HOUBEN,1980). The purpose of this work was to demonstrate and measure the effect of the heating rate, simulating an industrial process, on the thermal inactivation of Enterococcus faecium RR1 strain in function also of two different meat substrates.

MATERIALS AND METHODS: The Enterococcus faecium RR1 strain used in this study was isolated from a cooked ham. The culture was maintained at −18°C in a frozen medium; after thawing, the cells were grown in broth for 18–20 hours at 37°C to obtain the inoculum suspension. The two cured pork meats used differed both for the curing mixture composition and the percentage of brine injection: for C22 meat the % of brine injection was 22% (w/w) and brine contained 14.8% NaCl, 4.32% dextrose, 1.5% sodium glutammate, 0.48% phosphates, 0.25% sodium ascorbate, 0.09% $NaNO_2$, 0.04% $NaNO_3$; for C50 meat the % was 50% (w/w) and the brine contained 7.55% NaCl, 5.31% dextrose, 4.00% caseinate, 0.30% sodium ascorbate, 0.13% phosphates, 0.08% $NaNO_2$ and 0.03% $NaNO_3$.
Cured raw ham was chopped and each of a number of 100 g portions obtained was placed in a sterile container and was frozen at −18°C until required. After thawing, a 1 ml aliquot of E. faecium suspension was added to the cured meat to give a final concentration of about 1×10^7 cfu/g. The ham and the culture were blended in a Stomacher for 2 minutes to obtain an homogenously inoculated mixture. About 2.5 g of the mixture were aseptically packed in 7 x 10 cm polythene pouches, forming a thin layer, then heat sealed excluding most of the air. To determine the heat resistance at constant temperature, tests were conducted as described previously (QUINTAVALLA et al.,1987). For the tests at rising temperature, bags containing the inoculated cured meat were completely submerged in water at 40°C, allowed to equilibrate, then heated at constant rates of 0.1 and 0.07°C/min up to 62, 65, 68 and 70°C. Temperature was monitored using a type−T thermocouple (Ellab) connected to a CMC821 Temperature Microprocessor Ellab. After reaching the final temperature, the bags were maintained at the same, but constant temperature and samples were removed at specific intervals. Viable counts were determined by direct plating on TSAP (MACKEY and DERRICK,1987) after incubation at 37°C for 3 days. D−values were calculated from the slopes of the linear regression lines of the survivors.

RESULTS AND DISCUSSION: The thermal resistance characteristics of E.faecium RR1 in cured pork meat C22 at 62, 65, 68 and 70°C after different heating procedures are shown in table 1.

Temperature (°C)	D-values (min)		
	instant. heating	0.1°C/min	0.07°C/min
62	69.8	n.d.	104.6
65	45.0	95.1	57.6
68	25.1	37.9	21.0
70	13.6	24.5	12.8

n.d.: not determined

Table 1. Heat resistance of Enterococcus faecium RR1 in cured pork meat C22

The strain of E. faecium showed a high heat resistance, with $D_{70} = 13.6$ min and a z−value of 11.8°C. The microorganism proved to be more resistant when the cells were heated up at 0.1°C/min to constant temperature; D−values were approximately 1.5−2 times higher than those calculated with a near instantaneous heating. It was not possible to calculate the D−value at 62°C after heating at 0.1°C/min because the inactivation did not follow a first−order kinetic. The heat resistance did not further increase after heating at the slowest rate (0.07°C/min); on the contrary, when the data were analysed by the t−test, no significant differences (α=0.01) in D−values were observed between the cells heated at linearly rising temperature and those heated instantaneously, except for the tests at 62°C. While heating to lower temperatures than 68°C did not cause a decrease in the initial number, heating up to 68° and 70°C resulted in a 1 log− and in a 4 log−cycle decrease, for both the heating rates tested.
An example of the time−temperature profile obtained with a heating rate of 0.07°C/min and the corresponding thermal inactivation of E. faecium RR1 in cured pork meat C22 are illustrated in figure 1 for the tests conducted at 68°C as the set temperature.

Figure 1. Time-temperature profile at 0.07°C/min and thermal inactivation of E.faecium RR1 in cured pork meat C22 at 68°C
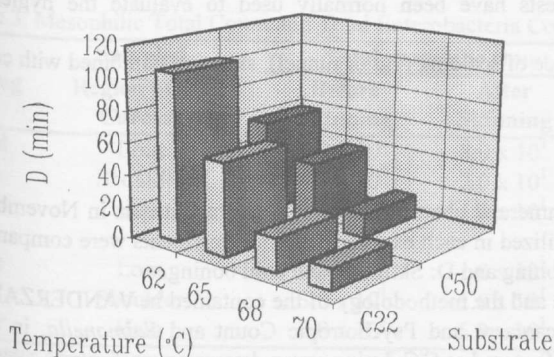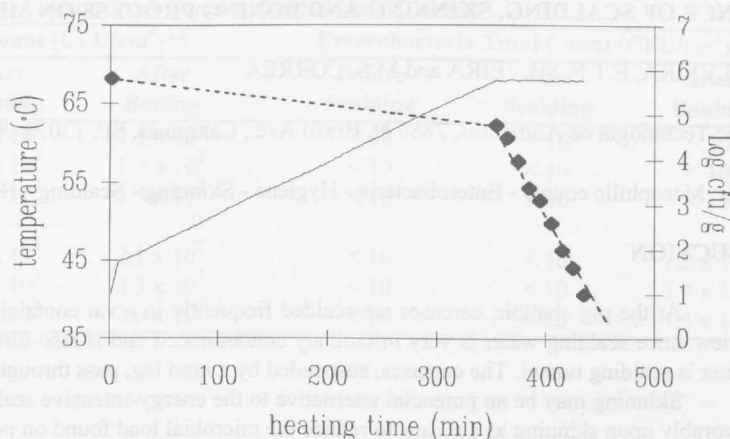
Figure 2. Changes of heat resistance of E.faecium RR1 as a function of the medium where heat treatment takes place (0.07°C/min)

Resistance to constant temperatures of 62, 65, 68 and 70°C, after heating up at 0.07°C/min, was also determined in cured pork meat C50. The results, compared to those obtained in the same conditions in cured pork meat C22 are shown in Fig.2. No data are reported at 70°C for C50 meat beacause no viable cell remained after the heating up phase (> 7 decimal reductions). Unexpectedly, the D-values were approximately the same than those obtained after the virtual instantaneous heating in C22: D_{68} was 21.0 min and D_{70} was 12.8 min.

When the heating up phase is very slow, and when the final temperatures are high (>68°C), it is possible that, in a population of cells, the increase in thermotolerance and the cell inactivation take place simultaneously: the prevalence of the one or the other effect could be much more a function of the type of substrate.

CONCLUSIONS: The strain of Enterococcus faecium used in this work, as expected, demonstrated a great thermoresistance in meat substrate. Heating cells at rising temperatures of 0.1°C/min increased the heat resistance of the organism when subsequently exposed to constant temperatures, but at rate of heating of 0.07°C/min there was no further increase in thermotolerance inducted. It is evident that there is a combination of different factors affecting the heat resistance. The thermal history of a population of cells acts as a fundamental factor influencing the heat sensitivity of that population. Also the type of substrate and the presence in it of substances such as the curing additives are responsible for the increase, or the decrease, in the heat tolerance of the cells. All these phenomena could have implications in the survival of E. faecium, as well as other microrganisms, in those foods, like cooked ham, which are heated slowly up and do not always reach a final high internal temperature.

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