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SECTION A 7

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Heat resistance and significance of enterococci

The enterococci have long been accepted as heat resistant bacteria. As early as in 1916 Houston & McCloy (1) reported the high heat resistance of streptococci, and since that time numerous data were published on this topic. Though it is generally agreed, that enterococci exhibit high heat resistance, the D values measured by various authors are extremely diverse. While Richards & White (2), White (3), Ott et al. (4) measured a D value of 2 - 13 min at 60°; Hansen & Riemann (5), Greenberg & Silliker (6), Möller-Madsen et al. (7), Kelch & Stehle (8) found much higher heat resistance (D values), sometimes reaching even hours at the same temperature. In our experiments we have come across with all these data, depending on the strain and environmental conditions, and it is evident, that although the majority of enterococci do not have a D value higher than 6 - 8 min at 65°C, (2, 3, 4, 9) nevertheless there do exist quite a few strains which have much higher resistance. On the other hand the D values measured in vitro do not seem to be effective in the practice, i.e. in meat cooking, in other words the time necessary for total killing of bacteria in ham is longer than the calculated time. This well-known fact on the one hand, and the sometimes extremely high resistance of enterococci on the other is the cause of and explanation for their survival

in canned ham.

Experiments

In our investigations we tested the heat resistance of identified enterococci (Strep. faecalis, Strep. faecalis v. liquefaciens, Strep. faecalis v. zymogenes) at 60° and 65°C, and the effect of 2 mg% nitrite and/or 2 % NaCl on heat resistance of Strep. faecalis. Further we tested the heat resistance of a highly resistant enterococcus, which proved to be Strep. faecium and the difference in heat resistance values of the same strain when examined in bouillon or in phosphate buffer. The cultures used in the experiments were 18 hrs old. The experiments were carried out in ultrathermostate, the suspensions to be heat treated were added to a medium the temperature of which was previously adjusted to the desired value. The heat resistance of the highly resistant Streptococcus was tested at 60° 70° and 75°C in bouillon containing 2 % NaCl and in M/15 phosphate buffer at 60°C.

Results and Discussion

The identified enterococci exhibited about similar heat resistance as the other "regular" enterococci, i.e. D_{60}=10-12 min. D_{65}=6 - 8 min. (Fig. 1,2,3) Neither NaCl alone nor with nitrite in the used concentration did affect the heat resistance of Strep. faecalis (Fig. 4). It is generally accepted that nitrite has bacterostatic action, but in the concentration it was used - and the Hungarian meat products contain not more than the above-mentioned 2 mg% NO_2 - showed no inhibiting effect on heat resistance (this amount of nitrite was added to the recovery medium, too).

The D values of the resistant Streptococcus (Strep. faecium) were as follows: D_{60}= 50 min; D_{70}= 5 min; D_{75}= 2,8 min. When heated in phosphate buffer, the D values were lower:

D_{60}= 15 min. These data are calculated mechanically from the decrease in number of survivors during a certain period of time. The fact is that all curves consist of two parts: a steeper initial slope and a gentle slope thereafter (Fig. 5 - 6). This phenomenon is known from other publications too, let me quote just the result of Hansen & Rieman (5) who measured an initial D value of 8 min. and 100 min. thereafter at 62°C with streptococci. With this in mind our above-mentioned D values of resistant Streptococcus would be changed to: 15 min. initial and 80 min. there-after at 60°C; 1 min. initial and 8 min. thereafter at 70°C; 0,13 min. initial and 40 min. thereafter at 75°C. In phosphate buffer 7 min. initial and 24 min. thereafter.

No matter how we calculate the D value, the fact is that there do exist enterococcus strains, which exhibit extreme heat resistance and the regular heat treatment of meat products do not kill all of them. And here arises the rather delicate question: how we should judge the presence of enterococci in meat products. This question is to be examined from 3 viewpoints:

a) their role in technological faults
b) their role in food poisoning
c) their role as indicators of fecal contamination

Ad a) It is commonly said: the enterococci are responsible for the sour odour-flavour of ham, for the liquefaction of ham and sometimes for colour changes of ham (10, 11, 12, 13). As for the sour flavour, we think that it has importance only in those countries where the ham contains carbohydrate additive, otherwise there is no source for acid formation. In our country no carbohydrate additive is in use.

It is self-evident that Strep. faecalis v. liquefaciens causes liquefaction of meat protein, more precisely of the intramuscular and intrafibrillar connective tissue (13). Nevertheless -out of enterococci- only Strep.

faecalis v. liquefaciens is capable of liquefaction and the occurence of this species in heat treated products is far less frequent, than that of the other types, mainly of Strep. faecium because of the latter's higher heat resistance (8, 13, 14, 15, 16, 17).

Ad b) The other accusation against enterococci is that they cause food poisoning (18). This fact has not been exactly proven yet; the experiments to make human volunterers sick by feeding them either enterococcus cultures or enterococcus- containing foods - failed up to now (19). On the contrary, ten Cate considers - and Niven too - the enterococci necessary organisms in sausage ripening (20).

Keeping in mind how often the enterococci occur in nature, it seems strange that the poisoning caused by enterococci are not more numerous, as Deibel put it: "if enterococci are at all capable of producing food poisoning, then this ability is peculiar to a truly rare strain or else to an extremely unusual set of environmental conditions" (14).

Ad c) Some years ago microbiologists - looking for a better indicator, than E. coli - suggested enterococci as indicator of fecal contaminations (21, 22, 23, 24), since these latter organisms tolerate the heat and cold, and survive the antibiotic treatment more easily than E. coli, and they belong to the normal microflora of human and animal intestines.

This opinion was unfortunately generalized, and therefore it is not correct. Although enterococci may be good as indicators e.g. in water, and everywhere where they are not able to multiply, they are useless for the same role in foods which support their growth. This is the case with meat too; in other words enterococci grow in environments far removed from the original source of fecal contamination.

Concluding all this, we consider the presence of enterococci in meat products not so serious and objectionable as it is usually done, and if the strain in question does

not cause organoleptic change in the product, we have no objection at all.

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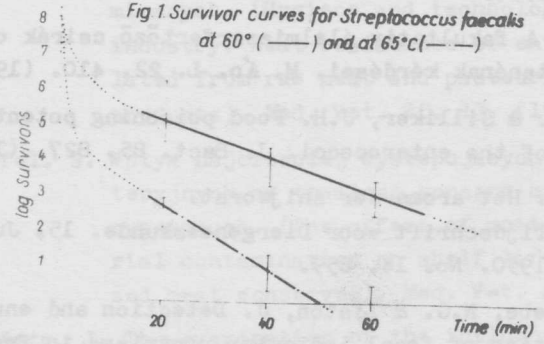


Fig 1 Survivor curves for Streptococcus faecalis at 60° (——) and at 65°C(— —)

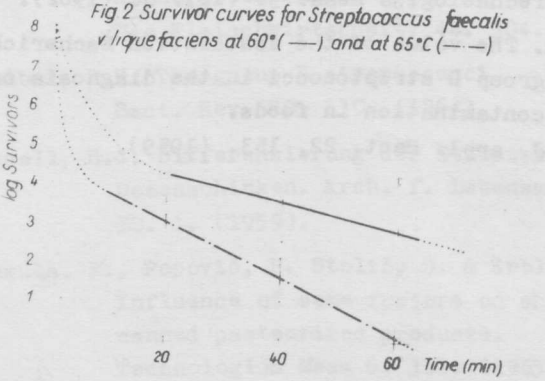


Fig 2 Survivor curves for Streptococcus faecalis v liquefaciens at 60°(——) and at 65°C (— —)

Fig 3 Survivor curves for Streptococcus faecalis v zymogenes at 60° (——) and at 65°C (——·——)

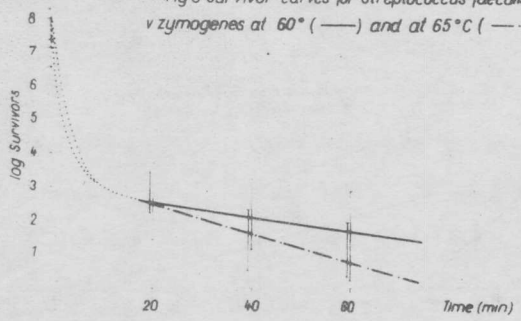
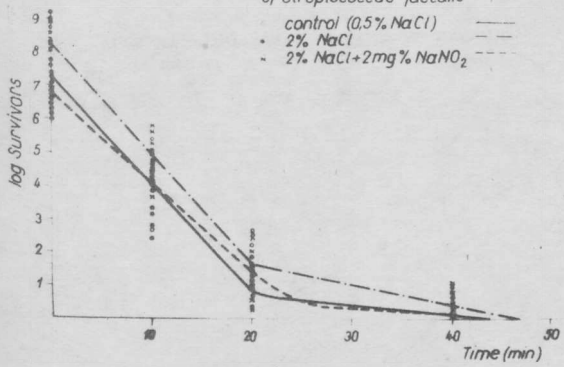
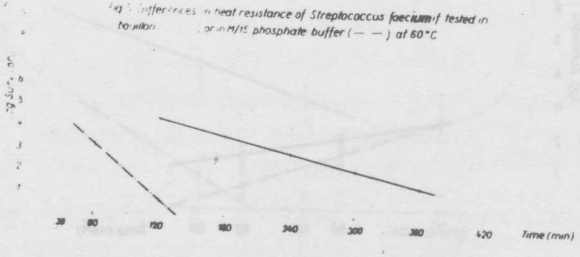


Fig 4 The effect of nitrite and salt on heat resistance of Streptococcus faecalis




heat resistance of Streptococcus faecium if tested in
phosphate buffer (— —) at 60°C
180
240
300
360
420
Time (min)

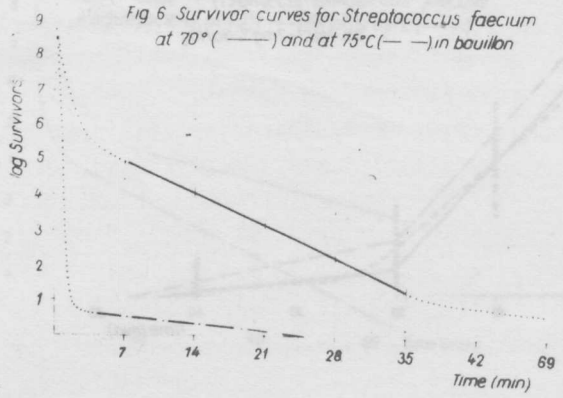


Fig 6 Survivor curves for Streptococcus faecium
at 70°(———) and at 75°C(— —)in bouillon
log survivors
9
8
7
6
5
4
3
2
1
7
14
21
28
35
42
69
Time (min)