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Survival of *Salmonella eastbourne* and *Salmonella typhimurium* in milk chocolate prepared with artificially contaminated milk powder

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SUMMARY

Milk chocolate mass containing salmonellas was prepared by mixing artificially contaminated milk powder with the other ingredients at a temperature of about 40 °C. From this mass bars were made. Two series were prepared, with *S. eastbourne* and *S. typhimurium* respectively. The number of surviving salmonellas was counted after various periods of storage, up to 19 months. *S. eastbourne* was reduced in numbers during 19 months from an initial count of *ca.* 3×10^4 to *ca.* 3×10^2 per 100 g of chocolate. *S. typhimurium* died off more rapidly, and was not detectable in about 55 g after 15 months, in spite of an initial count of *ca.* 10^5 per 100 g.

In these experiments the salmonellas in the milk powder had had to survive the spraying procedure and the adverse conditions in the dried powder. This may be the reason why *S. eastbourne* showed a distinctly better survival on storage than the same serotype showed in previous experiments in which the organism was added as a broth culture to the chocolate mix. With *S. typhimurium*, however, such a difference was hardly detectable.

Possible explanations of these results are discussed.

INTRODUCTION

In a previous paper (Tamminga, Beumer, Kampelmacher & van Leusden, 1976) experiments were described carried out after we learnt about cases of food poisoning in Canada and the U.S.A. which could be traced back to consumption of chocolate, contaminated with *S. eastbourne* (Craven *et al.* 1975). During these experiments nutrient broth cultures of two strains of Salmonella were mixed with chocolate mass at a temperature of *ca.* 40 °C. From this mass bars were prepared. The bars were kept at room temperature and the number of salmonellas was determined after different periods of storage. In the paper mention was made of positive results, obtained even after 9 months. Especially in milk chocolate

with the *S. eastbourne* strain from the Canadian chocolate the reduction in numbers took place very slowly.

The question arose, whether the above mentioned way of contamination might not show a still too favourable picture of the situation. A well grown broth culture can be expected to contain both sensitive and resistant germs. Salmonellas causing contamination in the chocolate industry are, however, likely to be carried into the product together with dry ingredients such as cocoa beans or milk powder, and hence may be more resistant against the very conditions of low water activity found in chocolate. It appeared desirable to do some more experiments taking these considerations into account.

Artificial contamination of the milk powder used for the preparation of the chocolate appeared to be a useful start of the procedure to obtain bars, contaminated in a more 'natural' way.

In the experiments described here, the same serotypes as in the first experiments were used, i.e. *S. eastbourne* and *S. typhimurium*.

MATERIALS AND METHODS

As in our first experiments, the *S. eastbourne* strain used was the one which had caused the above mentioned food poisoning outbreak. Similarly, the same *S. typhimurium* strain was used, of phage-fermentative type II 505. Both strains were grown in nutrient broth, centrifuged after 24 h at 37 °C and resuspended in physiological saline. Each of these suspensions was added to about 30 l of concentrated milk, after which the milk was spray-dried.

Both portions of milk powder thus obtained were used a few weeks later to prepare chocolate mass by mixing about 1 kg of powder with about 3 kg of a mixture of the other ingredients at a temperature of about 40 °C. From the mass bars were made. For details of the procedure reference may be made to the section on 'Materials and Methods' in the previous paper (Tamminga *et al.* 1976).

In this way two series of chocolate bars were obtained, each infected with one of the two salmonella serotypes.

The salmonellas in the milk powder were counted by the MPN method (Edel & Kampelmacher, 1969, 1973) on a few days preceding the preparation of the chocolate and also, using surplus contaminated milk powder, on 2 days immediately after preparation of the chocolate. In this way two regression lines could be constructed which showed a gradual logarithmic reduction of the numbers of salmonellas, in accordance with the results of LiCari & Potter (1970). *S. typhimurium*, which had the higher initial concentration, showed a higher reduction rate than *S. eastbourne*. The numbers of live salmonellas in the milk powder on the day on which the bars were prepared, calculated from the regression lines and expressed as $\log_{10}$ organisms/100 g of milk powder, were 5·91 for *S. eastbourne* and 6·48 for *S. typhimurium*.

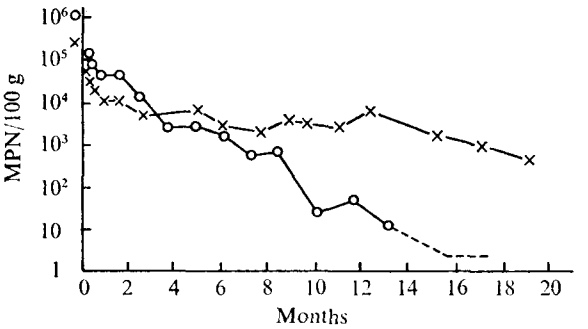

Fig. 1. Reduction of two Salmonella strains in chocolate, contaminated by milk powder, ×, *S. eastbourne*; ○, *S. typhimurium*.

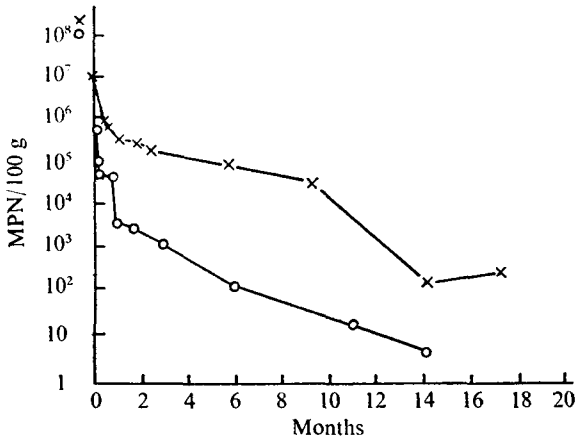

Fig. 2. Reduction of two Salmonella strains in chocolate, contaminated by broth culture. ×, *S. eastbourne*; ○, *S. typhimurium*.

As a consequence of the mode of inoculation, in contrast with previous experiments no bitter chocolate could be prepared.

From the bars most probable numbers (MPN) were determined in two laboratories (Edel & Kampelmacher, 1969, 1973), on the day of preparation and after several intervals, up to 19 months. The bars were stored at 20 °C. The results of the two laboratories were averaged. More details of the determination are given in our previous paper.

RESULTS AND DISCUSSION

Results of the salmonella determinations in the chocolate bars after different periods are given in Fig. 1. For comparison, in Fig. 2 data are shown, obtained with the chocolate contaminated directly with broth cultures, which has already been reported in our previous paper. A few data, however, obtained with remaining bars after that paper had been accepted for publication, are added. For this comparison only milk chocolate with high inoculation has been used.

In both graphs the points to the left of the vertical axis represent the theoretical (calculated) values in the chocolate if no adverse effects had occurred as a

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consequence of the inoculation of the chocolate mass and during the short time between inoculation and first MPN determination.

In Fig. 1 the line for *S. typhimurium* has been dotted as soon as all MPN portions gave negative results (which means absence in about 55 g).

Our former conclusion, that salmonellas can survive in chocolate for long periods is confirmed by the experiments using milk powder contamination. Especially striking is the longevity of the *S. eastbourne* strain. The reduction rate is distinctly lower than that obtained with the same strain if nutrient broth contaminated bars were examined (Fig. 2).

There is a clear difference between the two strains. In the first place the number of *S. eastbourne* declines considerably slower than that of *S. typhimurium* in the milk powder contamination experiments. There were already indications of such a difference during the experiments with broth culture contamination, but then the difference manifested itself especially in the beginning of the storage period. Later on a stabilization of the difference was observed without much further divergence of the lines.

Secondly, the behaviour of *S. typhimurium*, as distinct from that already mentioned for *S. eastbourne*, does not differ greatly whether contamination was carried out by means of broth or by means of milk powder (apart from initial rapid reduction effects shortly after the start of the experiments).

Taking into consideration the smaller initial numbers of *S. eastbourne* in the milk powder a possible explanation for the greater resistance of this strain in the milk powder contamination experiment might be that preparation of the milk powder containing *S. eastbourne* may have been carried out under somewhat more unfavourable circumstances as compared with those of the milk powder containing *S. typhimurium*, resulting in the relatively high percentage of resistant germs in the former. Yet this explanation appears less probable, considering that, even when in the chocolate after some months both strains are present in about equal numbers, the relatively sharper decline of *S. typhimurium* continues, so that after 15 months this strain is no longer detectable, whereas after a longer period – i.e. 19 months – *S. eastbourne* is still about 2 log cycles removed from the non-detectability level. This gives rise to the presumption that the larger numbers of resistant germs of the *S. eastbourne* strain in the milk powder contaminated chocolate, as compared with those of *S. typhimurium*, is the result of an intrinsically better resistance of the former.

This better resistance of *S. eastbourne* might have also caused the clear difference between the curves for broth contaminated chocolate and milk powder contaminated chocolate (by way of a concentration of resistant germs from the broth); for *S. typhimurium* apparently the number of germs having a comparable resistance in the broth culture is too low to cause such a difference.

It may be wondered whether a difference in water activity resulting from the two ways of contamination has exerted an influence on the difference between the series. During the experiments mentioned in the Introduction it has been shown that the water activity is somewhat increased by the water from the broth culture. Investigations (albeit with other commodities) by Christian &

Stewart (1973) have demonstrated that comparable differences in this water activity range may influence the reduction rate of bacteria, including salmonellas, in such a way that, by a lower water activity the reduction rate will be decreased. Yet in our view this need not take away from our conclusions concerning the existence in itself of a difference between *S. eastbourne* and *S. typhimurium*. One might say that possibly an intrinsic difference between the strains is exposed more clearly under conditions of slightly lower water activity.

For the sake of completeness attention is drawn to the differences, for both ways of contamination, between the values, calculated on the assumption that no losses will occur during and shortly after preparation of the bars (as represented in the graphs by the figures to the left of the vertical axis), and the corresponding first values actually found. Indeed a considerable 'shock effect' is noted. Such an effect might, of course, appear during the actual commercial preparation in the factory.

CONCLUSIONS

These results reinforce our previous conclusions that, in view of the small number of organisms which proved sufficient to cause food poisoning, strict hygienic control of raw materials and processing is absolutely necessary in the manufacture of chocolate. If salmonellas find their way into the chocolate they will remain in the product, albeit in gradually diminishing numbers, for quite a long time.

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