44

14.50 – 15.05.

152

BACTERIOLOGICAL AND ASSOCIATED CHEMICAL CHANGES IN BACON STORED IN VACUUM PACKS

by B. P. Eddy and D. P. Gatherum
Low Temperature Research Station, Cambridge.

During storage experiments on commercial packs of bacon it was noticed that, generally speaking, deterioration of quality was governed more by the source of the bacon than by the temperature or duration of storage. It was also noticed that to a certain extent the same was true of the accumulation and disappearance of nitrite in the bacon. We have observed parallels between these changes and the development of lactobacilli and micrococci on the bacon. In this paper a comparison is made of the behaviour of green bacon from one factory and smoked bacon from another stored at 10° and 20°C.

Methods

Six storage experiments were carried out altogether. Packs ($\frac{1}{2}$ lb.) of middle cut or back bacon were taken directly from the production line in the factory, where possible from several bacon sides. Three factories were used, from one of which both green and smoked bacon were obtained. Storage was at 5°, 10°, 15°, 20° and 25°: one individual experiment consisted of the storage of one type of bacon either at 5°. 10° and 20°, or at 15°, 20° and 25°. In this way one temperature was duplicated for comparison. Packs were stored for up to 73 days, depending upon temperature, and were sampled at intervals during the storage period.

Sampling. Three packs were used at each sampling time. The packs were opened aseptically and the top rasher removed for chemical analysis. From the remainder, 50 g. (usually a little over 2 rashers) were taken, lean and fat together, and homogenized with 200 ml. sterile distilled water. A sample of the homogenate was removed and put into a 1 oz. screw capped bottle for bacterial counts.

Bacterial counts. Dilutions of the homogenate were made in $\frac{1}{4}$ strength Ringers solution. General counts were made on phenolphthalein phosphate agar plates incubated at 44°/1 day, 37°/2 days and 20°/4 days. Lactobacilli were counted on Rogosa agar (Oxoid) incubated under 10% CO_2 in H_2 at 20° for 4 days.

Phosphatase positive micrococci were detected on the phenolphthalein agar plates by exposing them to ammonia vapour and lactobacteria were excluded from the general counts by using the cytochrome reagent of Deibel & Evans (1960) with the benzidine replaced by the less carcinogenic o-tolidine. Cytochrome negative counts never exceeded, and were usually lower, than those in Rogosa agar.

Chemical analyses. The eye muscle was removed from the selected rasher, chopped finely, weighed and extracted three times with boiling water. The volume of the combined extracts was made up to 100 ml. Sodium chloride was determined by Volhard's method (Callow, 1929) and sodium nitrite and potassium nitrate as described by Eddy, Gatherum & Kitchell (1960).

It was not possible to determine the initial potassium nitrate content of the bacon in each pack. However, most of the nitrate and chloride in the bacon come from the pump pickle and hence should be present in a roughly constant ratio. This ratio was determined in the three packs sampled at the beginning of each experiment and the nitrate content of subsequent packs calculated by applying it to the chloride content found. Analyses on several packs of bacon indicated an accuracy of $\pm$ 15%.

In the results the chloride contents are expressed as % NaCl and the calculated initial nitrate, the nitrate lost and the nitrite found as p.p.m. N, all based on the fresh weight of the eye muscle.

Results

The experiments whose results are described in this paper are those in which green bacon from one factory and smoked bacon from another were stored at 20° and 10°. The results obtained with the green bacon are shown in Figs 1 and 2 and those with the smoked bacon in Figs. 3 and 4. In all cases the value plotted is the mean (geometrical mean in the case of the bacterial counts) of the results obtained from three packs.

In the packs of green bacon stored at 20°, (Fig. 1; NaCl % = 6.56 $\pm$ 1.13) the micrococci maintained higher numbers than the lactobacilli until some point between the 3rd and 7th days when the lactobacilli overtook the micrococci and may thereafter be presumed to have predominated. The total counts at 20°, joined by the broken lines, probably comprised mainly lactobacilli: the o-tolidine test was not yet known. In the packs at 10° (Fig. 2; NaCl % = 5.80 $\pm$ 0.86), however, the micrococci maintained their predominance

throughout the duration of the experiment, suggesting that the lactobacilli were the more sensitive organisms to the lowered temperature. These differences are reflected in the results for nitrite accumulation and nitrate loss. At 20° the nitrite only reached about 40% of the theoretical, although all the nitrate was lost, whereas at 10° the nitrite reached about 80% of theoretical. Clearly, therefore, nitrite was being destroyed as it was produced and was destroyed relatively more rapidly at 20° than at 10°.

In the smoked bacon the situation was quite different. At both 20° (in two separate experiments: Fig. 3) and at 10° (Fig. 4) the lactobacilli predominated over the micrococci almost from the beginning of storage. In the first set of experiments with this smoked bacon, in which the packs were stored at 25°, 20° and 15°, no nitrite developed in the packs stored at 25° but nitrite did develop in the packs stored at 20° (Fig. 3 - ringed symbols; NaCl % = 4.03 $\pm$ 0.75), and at 15°. In the second set of experiments (20°, 10° and 5°) no nitrite developed in the packs stored at 20° (Fig. 3 - unringed symbols; NaCl % = 4.16 $\pm$ 0.69) but nitrite did develop in the packs stored at 10° (Fig. 4; NaCl % = 4.24 $\pm$ 0.70). At 5° no nitrite developed during storage for 73 days. The nitrate analyses show that at 20° nitrate was lost during the first experiment but not during the second. At 10° there was no significant loss of nitrate until after 20 days storage.

The bacteriological differences between the packs at these storage temperatures in the two experiments consist mainly of a higher count of micrococci in the first experiment at 20° than in the second and of a consistently higher count of micrococci at 10° than at 20°.

Discussion

The interpretation of the results presented is made easier by the fact that as far as we know only two groups of bacteria are significantly involved viz. micrococci and lactobacilli. The bacteriological differences between the green and smoked bacon suggest that smoking favours the lactobacilli or inhibits the micrococci or both. Eddy & Ingram (in preparation) have shown that the growth rate of a strain of Staphylococcus aureus is the same on green and smoked bacon pre-sterilized by irradiation and the present results (Figs. 3 & 4)

suggest that the growth of micrococci may be reduced in the presence of large populations of lactobacilli. It seems likely that nitrite would inhibit the growth of micrococci more than that of lactobacilli (Castellani & Niven, 1955) but nitrite was clearly not the cause of the poor development of micrococci in the second experiment with smoked bacon at 20° since none was produced. It is therefore suggested that, in some way, smoking favours the lactobacilli and that the rapid growth of these is in turn inimical to the growth of micrococci. The ability of lactobacilli to produce conditions unfavourable to staphylococci has been reported by other workers (Grossowics, Kaplan & Schneerson, 1947; Wheater, Hirsch & Mattick, 1951).

These factors influence the metabolism of nitrate and nitrite in the bacon. Lactobacilli are generally believed to be unable to reduce nitrate and this belief is confirmed by these experiments (Fig. 3). Micrococci, however, are able to reduce nitrate (Eddy, 1958) but are only able to do so measurably when the lactobacilli permit them to attain a high enough population density (cf. Figs. 1 & 3).

There was a variable discrepancy between the nitrite found and the nitrate lost (= theoretical nitrate present initially less that found) in the various experiments due to the destruction of nitrite simultaneously with its production. Eddy & Kitchell (1961) found that in a bacon brine, where metabolism of nitrate and nitrite was probably due mainly to micrococci, nitrite was not attacked until nearly all the nitrate had been reduced and, in the present experiments, large losses of nitrite appear to be associated with higher counts of lactobacilli (cf. Figs. 1 & 2). It seems possible therefore that the discrepancies observed are due to the action of lactobacilli on the nitrite, and preliminary experiments with buffered cultures suggest that lactobacilli have the ability to destroy nitrite. Similar losses of nitrite were observed by Eddy, Gatherum & Kitchell (1960) in bacon undergoing prolonged maturation.

These interpretations of the experimental data, and particularly those involving the lactobacilli about whose behaviour in bacon and towards nitrite little is known, clearly require confirmation by experiments with pure cultures and work along these lines is at present in progress.

References

Callow, E. H. (1929). The determination of chloride in animal tissues. Biochem. J., 23, 648.

Castellani, A. G. & Niven, C. F., Jr. (1955). Factors affecting the bacteriostatic action of sodium nitrite. Appl. Microbiol., 3, 154.

Deibel, R. H. & Evans, J. B. (1960). Modified benzidine test for the detection of cytochrome-containing respiratory systems in micro-organisms. J. Bact., 79, 356.

Eddy, B. P. (1958). The effects of salt concentration and temperature on nitrite production and metabolism. Proc. 2nd Int. Symp. Fd Microbiol., 1957, p. 87, London, H.M.S.O.

Eddy, B. P., Gatherum, D. P. & Kitchell, A. G. (1960). The metabolism of nitrate and nitrite in maturing bacon. J. Sci. Fd Agric., 11, 727.

Eddy, B. P. & Kitchell, A. G. (1961). Nitrate and nitrite metabolism in a bacon-curing brine and their relation to the bacterial population. J. Sci. Fd Agric., 12, 146.

Grossowics, N., Kaplan, D. & Schneerson, S. (1947). Production of an antibiotic substance by a Lactobacillus. Abstr. 4th Int. Cong. Microbiol., p.22.

Wheater, D. M., Hirsch, A. & Mattick, A. T. R. (1951). 'Lactobacillin', an antibiotic from lactobacilli. Nature, Lond., 168, 659,

Pakattu bacon on mikrobiologisesti säilyttävämpi kylmässä kuin ei-pakattu.

Captions

Fig. 1. The growth of micrococci and lactobacilli, together with the accumulation of nitrite and the loss of nitrate, in vacuum packed green bacon from factory A stored at 20°C.

Fig. 2. As Fig. 1 but stored at 10°C.

Fig. 3 The growth of micrococci and lactobacilli, together with the accumulation of nitrite and the loss of nitrate, in vacuum packs of smoked bacon from factory B stored at 20°C (squares and ringed symbols, 1st expt: circles and unringed symbols, 2nd expt.).

Fig. 4. As Fig. 3 but stored at 10°C (One expt. only).

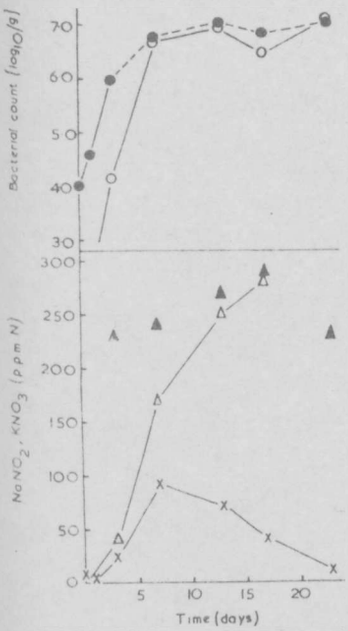

Fig. 1

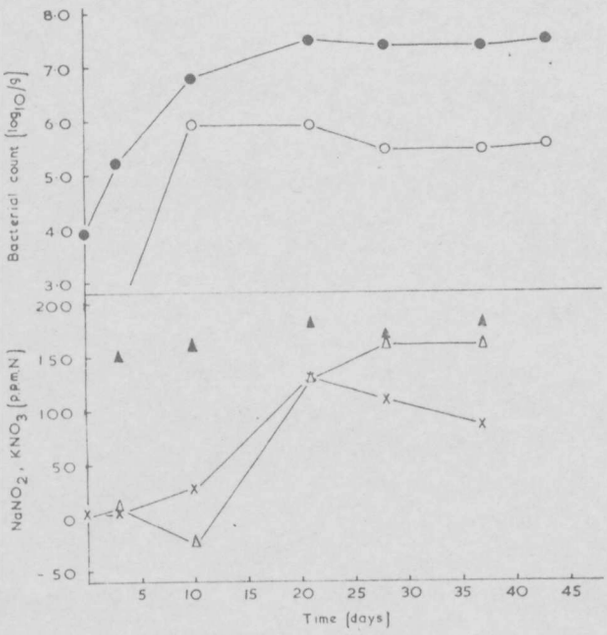

Fig. 2

□,O Lactobacilli
■,● Micrococci
⊗,▲ Theoretical KNO_3
⊗,Δ Lost KNO_3
⊗,X $NaNO_2$

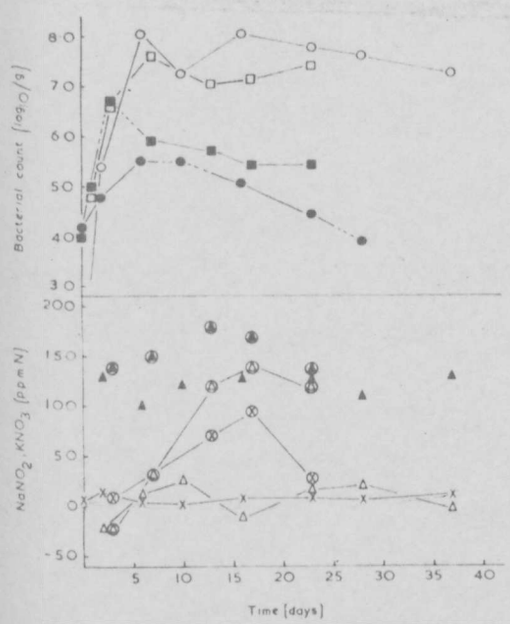

Fig. 3

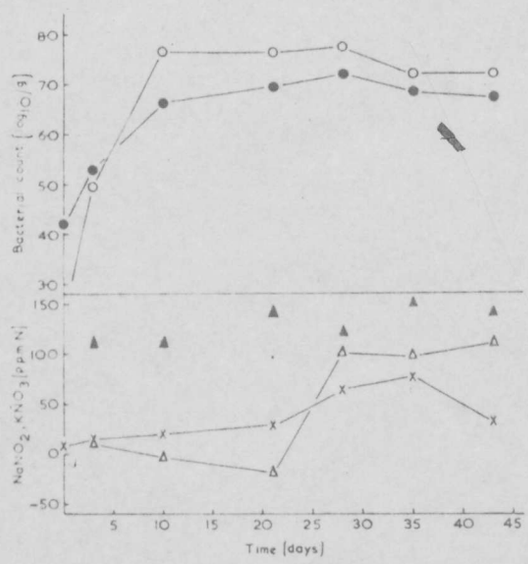

Fig. 4

□,O Lactobacilli
■,● Micrococci
⊗,▲ Theoretical KNO_3
⊗,Δ Lost KNO_3
⊗,X $NaNO_2$