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"Selective effect of salt and smoking on the bacterial flora of bacon, with special reference to vacuum packing".

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Summary

Experiments with a model system, and then with commercially vacuum packed smoked and unsmoked Wiltshire bacon, showed that both the immediate and long-term effects of smoking favour the growth of lactobacilli and adversely affect the growth of micrococci. In consequence, the balance of the microflora is changed. Increasing salt concentration, such as could result from desiccation during smoking, also affects the balance between bacterial types, but it was found to have the opposite effect to smoking i.e. more salt led to a greater preponderance of micrococci. Higher temperatures of storage of the vacuum packs favoured lactobacilli; as did, apparently, the elevation of temperature during the smoking of the bacon before packing.

Introduction

Various publications (e.g. Alm, Erichsen & Molin, 1961; Shank & Lundquist, 1963) conclude that lactobacilli dominate the flora of vacuum packed meats, but it is clear that this gives only a partial view of the situation for bacon. The importance of the micrococci has been known for several years (Ingram, 1960, Hansen 1962, Cavett, 1962). Casual experience shows that the spoilage is only sometimes souring, but at others cheesy or putrid (Ingram, 1960; Cavett, 1952) or even fruity, while the temperature (Ingram, 1960; Cavett, 1962), the salt content of the bacon (Cavett, 1962; Eddy & Ingram, 1962; Tonge, Baird-Parker & Cavett, 1964) and the smoking of the bacon (Eddy & Ingram, 1962; Kitchell & Ingram, 1963; Handford & Gibbs, 1964) are all known to have a significant

Experimental

Bacon

Two of the experiments involved the use of a model system employing psoas muscles of bacon pigs prepared and matured as described by Eddy, Gatherum & Kitchell, (1960). When these were smoked, they were hung for one hour in a smoking oven with forced air circulation operating at 90°F, as a result of which they lost 5 to 6% of their fresh weight.

Normally, packs containing ½ lb. of Wiltshire bacon, middle cut or back, smoked or unsmoked, were taken directly from the production line in a factory, where possible cut from several different sides of bacon. They were stored at a number of different temperatures and sampled at intervals to determine the changes in the microflora.

In the storage experiments, three packs were examined at the beginning and on each subsequent occasion of sampling. The packs were opened and the first slice removed for chemical analysis. The second and third slices were taken to make up a 50 g. lot for bacteriological examination which was homogenized with 200 ml. sterile distilled water for 2 minutes at 12,000 r.p.m.

Where cured psoas muscles were used, eight were examined at each time. Each muscle was minced twice and a 10 g. sample taken for maceration in 90 ml. sterile diluent (0.1% peptone + 0.85% NaCl). The remainder was used for chemical analysis.

Bacterial counts

The total count was made on nutrient agar containing phenolphthalein phosphate (Barber & Kuper, 1951) incubated at 20°C for 4 days. Lactobacilli were counted on Rogosa agar (Oxoid) incubated under 10% CO_2 in hydrogen at 20°C for 4 days. Lactobacilli growing on the total count medium were excluded from the count by use of the test of Deibel and Evans (1960) in which o-tolidine replaced the more carcinogenic benzidine. In the case of cured psoas muscles, the micrococci were detected by Gram staining and testing for catalase all colonies growing on the highest dilution plated on Plate Count Agar (Oxoid) + 1% NaCl.

Chemical analyses

The top slice from each pack was chopped finely, weighed, and extracted three times with boiling water. The volume of the combined extracts was made

up to 100 ml. The minced psoas muscles were extracted slightly differently but in both cases sodium chloride was determined by Volhard's method.

Results

Wiltshire bacon which is to be sliced and vacuum packed is first matured for 7-14 days, and may or may not be smoked. The immediate effect of smoking on the bacterial flora is of some relevance to subsequent events within the packs. An experiment was made with psoas muscles in which 4 groups, each of 8 muscles, were first matured for 14 days at 5°; at this time one group was examined, and another group transferred unsmoked to 15°; while the last 2 groups were smoked, one for immediate examination and the other for storage at 15°. Storage at 15° continued for 7 days before the smoked and unsmoked muscles were examined. The results are given in Table 1; in terms of the changes resulting from smoking, immediately and after 7 days at 15°, in the percentage occurrence of the main types of microorganisms. Though smoking reduced the numbers of micrococci, it increased their relative abundance because of the death of most of the Gram-negative bacteria. Also, considerable increases occurred in the proportion of lactobacilli (and incidentally to a much lesser extent in their absolute numbers) though they still formed only a small fraction of the total count. Yeast likewise increased on a relative basis. These effects of smoking were maintained during normal aerobic storage of the cured muscles at 15°, for lower total numbers of bacteria developed, and the proportions of Gram-negative bacteria were lower; /on the smoked muscles/ whilst the proportion of micrococci increased slightly and those of lactobacilli and yeasts markedly. The significance of this in relation to prepacking is obvious, since conditions under vacuum favour the growth of the lactobacilli and yeasts. The greater their initial numbers and relative abundance the sooner are they likely to outgrow the micrococci.

Next the results of storage experiments with over 150 commercial vacuum

of −1, a 10:1 preponderance of lactobacilli. In Table 2a this ratio is given for each period of testing at each of 3 temperatures; and the sums of these values show that the overall effect of smoking is to produce a lower total i.e. to decrease the relative abundance of micrococci. For the sake of brevity, therefore, these totals only are given in Tables 2b and 2c.

In every case except that of Danish bacon stored at 20° (Table 2b), the totals, as in Table 2a, are less for smoked than for unsmoked bacon, though there are obviously distinct differences between bacons of different origin. Thus smoking generally diminishes the preponderance of micrococci. On the whole, in the packs of Danish smoked bacon (Table 2a) there was a tendency for the later stages of storage to be dominated by lactobacilli (ratios negative). In the English bacon this was observed in both unsmoked and smoked bacon, though in the latter they were even more preponderant (cf. large negative ratios in Table 2c).

The figures given in Tables 2a, 2b and 2c for the initial samples, examined at each temperature of storage, cannot be used to derive information about the immediate effect of smoking. Though unsmoked and smoked material came from the same factory their initial counts were different, probably because it is common practice to mature bacon for smoking for a different period from that which is not to be smoked. Nevertheless, the data amply demonstrate a marked effect of smoking expressed during the subsequent period of vacuum packed storage.

Of the conceivable causes of this influence, desiccation is one of the most obvious. Since smoking involves heating there is, inevitably, drying out of the bacon with a resultant increase in salt concentration. Hence, the effect of salt content should also be considered here.

Fifty packs of smoked bacon (English) in 2 lots, were analysed for NaCl, moisture content, and composition of the bacterial flora after storage at 20° for 7 and 14 days. The ratio of micrococci to lactobacilli ($\log_{10}$ M/L) was plotted against the salt content (g. salt/100 g. bacon). The relationship is shown in Figures 1a and 1b. As the salt concentration increases so does the proportion of micrococci.

Though fewer data are available, a group of 8 cured psoas muscles analysed in the same way (Fig. 1c) suggest that the same relationship holds for

actually observed with smoking.

There is little difference between Figs. 1a and 1b, which indicates that there was no pronounced change in the M/L ratio over the period 7-14 days storage, at 20°, in this smoked bacon. There are contrary indications that this is not usually so, from data in Table 2a, which apply to bacon from a different factory, whether smoked or not.

The data in Fig. 1c appear inconsistent with those in Figs. 1a and 1b, in that they display similar M/L ratios at much higher salt contents. A large part of this difference arises, however, from the presentation of salt contents as per cent of fresh weight. When the figures are recalculated on the more fundamental basis of salt/water ratio, Fig. 2 is obtained. The M/L ratios in Fig. 1c are now seen to be, in effect, higher than those of Figs. 1a and 1b; and this corresponds to the difference that the bacon of Fig. 1a and 1b was smoked while that of Fig. 1c was not. Also, the unsmoked bacon was stored at a lower temperature. This suggests that the combined effect of smoking and storage at 20° was to reduce the M/L ratio by about 1 log unit i.e. a factor of 10. The effect of temperature can also be seen in Tables 2a, 2b and 2c; higher temperatures tend to give lower positive ratios or even negative ones, i.e. to encourage lactobacilli.

Discussion

It now seems well established that smoking favours lactobacilli, for the present work confirms in detail the earlier observations of ourselves (Eddy & Ingram, 1962) and of Handford & Gibbs (1964). Our earlier observations demonstrated a preponderance of lactobacilli in the late stages when the nature of the spoilage is determined. Handford & Gibbs (1964), found, in addition, that the dominance of lactobacilli is established earlier if the bacon is smoked; and (though their observations were not many and were made with slices smoked individually, a practice few manufacturers yet adopt) this indication is fully supported here. Indeed our present observations carry the argument further, by indicating an important swing in favour of the lactobacilli even during the period of smoking; earlier observations on the effect simply of smoking on the flora of bacon, by Gibbons, Rose & Hopkins (1954), did not record the lactobacilli.

Broadly speaking, the cause of this appears to lie in the greater resistance to smoke of lactic acid bacteria as compared with micrococci, which was well demonstrated by Handford & Gibbs (1964).

The reasons for this difference are not known. But it seems to us that Handford & Gibbs dismiss too lightly the possible effects at the surface of bacon of reduced pH, since micrococci are known to be very sensitive to changes below pH 6.0 (Ingram, 1958). It is at least clear that increased salt concentrations, as a result of desiccation during smoking, are not responsible since such conditions have been shown by us to lead to a greater, not a reduced preponderance of micrococci.

A third possibility, which has not been investigated experimentally, is suggested by events reported to occur in the manufacture of smoked, dry sausage. Nitrite production, largely by micrococci (Coretti, 1956), precedes acid production by the lactobacilli (Deibel, Niven & Wilson, 1961). This suggests (Kitchell, 1962) that inhibition of micrococci by nitrite under acid conditions (Tarr, 1941) might account for the fact that lactobacilli dominate the microflora of the smoked and dried product.

On sides of Wiltshire bacon matured before smoking at least 40-50% of the microflora is made up of micrococci (Kitchell, 1958). At this time, the pH of the skin is about 7.0 and the nitrite content as high as 700 p.p.m. (Gatherum & Kitchell, unpublished). It seems likely that at least some commercial smoking processes could result in a reduction of the pH at the surface, due directly to the acidic components of smoke and/or to the growth and metabolic activity of lactobacilli. At pH 6.0, in the presence of only 200 p.p.m. NO_2, micrococci are inhibited (Tarr, 1941). Such inhibition is likely to be more pronounced at the elevated temperatures of the smoke stove.

Finally, of course, the greatest commercial advantage of smoking may not be in the changes reported here, but in the fact that smoke can mask

Résumé

Par comparaison de préparations controlées, fumées ou pas, et avec des paquets de Wiltshire bacon (emballé par pellicule dans le vide, et de fabrication industrielle) fumé ou pas, on a trouvé que le fumage augmente la proportion des lactobacilles et gène celle des micrococques, immédiatement, et pendant le stockage. Enfin, leurs proportions peuvent être beaucoup changées. Une concentration plus elevée de sel, ce qui suivrait la desiccation pendant le fumage, produit un effet d'elle-même, mais contraire à celui du fumage - c.a.d. elle augmente la prepondérance de micrococques. Des températures plus elevées du stockage des paquets, et la chaleur pendant le fumage, sont, au contraire, favorables aux lactobacilles.

Zusammenfassung

Untersuchungen an einem Versuchsmodell, sowie an handelsfertigem vakuumverpackten geräucherten und ungeräucherten Wiltshire-Speck zeigten, dasz die Räucherung sogleich das Wachstum von Lactobacillen fördert und Micrococcen unterdrückt. Diese Wirkung zeigt sich auch bei der Lagerung. Dies kann zu einer grossen Veränderung der normalen Mikroflora führen. Eine Erhöhung des Salzkonzentration, die infolge der Aus-trocknung während der Räucherung auftreten kann, verändert ebenfalls das normale Keimgleichgewicht; aber, in Gegensatz zu der Wirkung der Räucherung, führt eine Erhöhung der Salzkonzentration zu einem Überwiegen der Micrococcen. Lagerung des vakuum-verpackten Specks bei höheren Temperaturen begunstigte, ebenso wie die Temperaturerhöhung wahrend der Räucherung, die Vermehrung von Lactobacillen.

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Table 1. Changes in the microflora of cured psoas muscle as a result of smoking.

Treatment	Ratio: % occurrence in smoked psoas / % occurrence in unsmoked psoas				Ratio of total counts
	Lactobacilli	Micrococci	Yeasts	Gram negative rods.	
Smoking	45	1.6	7	1/7	1/13
Smoking and storage at 15° for 7 days	5	1.4	117	1/2	1/29

Table 2. The proportions of micrococci and lactobacilli developing on vacuum packed smoked and unsmoked bacon at different temperatures.

Origin	Temperature of storage	Duration of storage (days)	Log_{10} ratio micrococci/lactobacilli	
			Unsmoked	Smoked
(a) Danish	20°C	0	0.61	1.10
		2	2.12	1.60
		6	1.77	1.09
		10	-0.09	-0.48
		16	0.06	-1.15
		23	-0.22	-0.64
		28	0.05	41.88
		35	-0.78	-0.98
			3.52	-1.34
	10°C	0	0.61	1.10
		3	1.91	1.36
		10	0.87	1.30
		21	1.60	-0.04
		28	1.93	-0.31
		37	1.92	-0.36
		43	1.95	-0.19
			10.79	2.86
	5°C	0	0.61	1.10
		3	1.10	1.45
		13	2.67	1.08
		21	0.35	-0.73
		30	0.85	-0.60
		43	1.32	-0.16
		57	2.55	-1.60
			9.45	0.54
(b) Danish	25°C	0 - 16	5.27	1.51
	20°C	0 - 23	4.42	4.56
	15°C	0 - 34	10.99	5.47
(c) English	20°C	0 - 17	0.13	-3.58
	5°C	0 - 24	2.94	-2.02

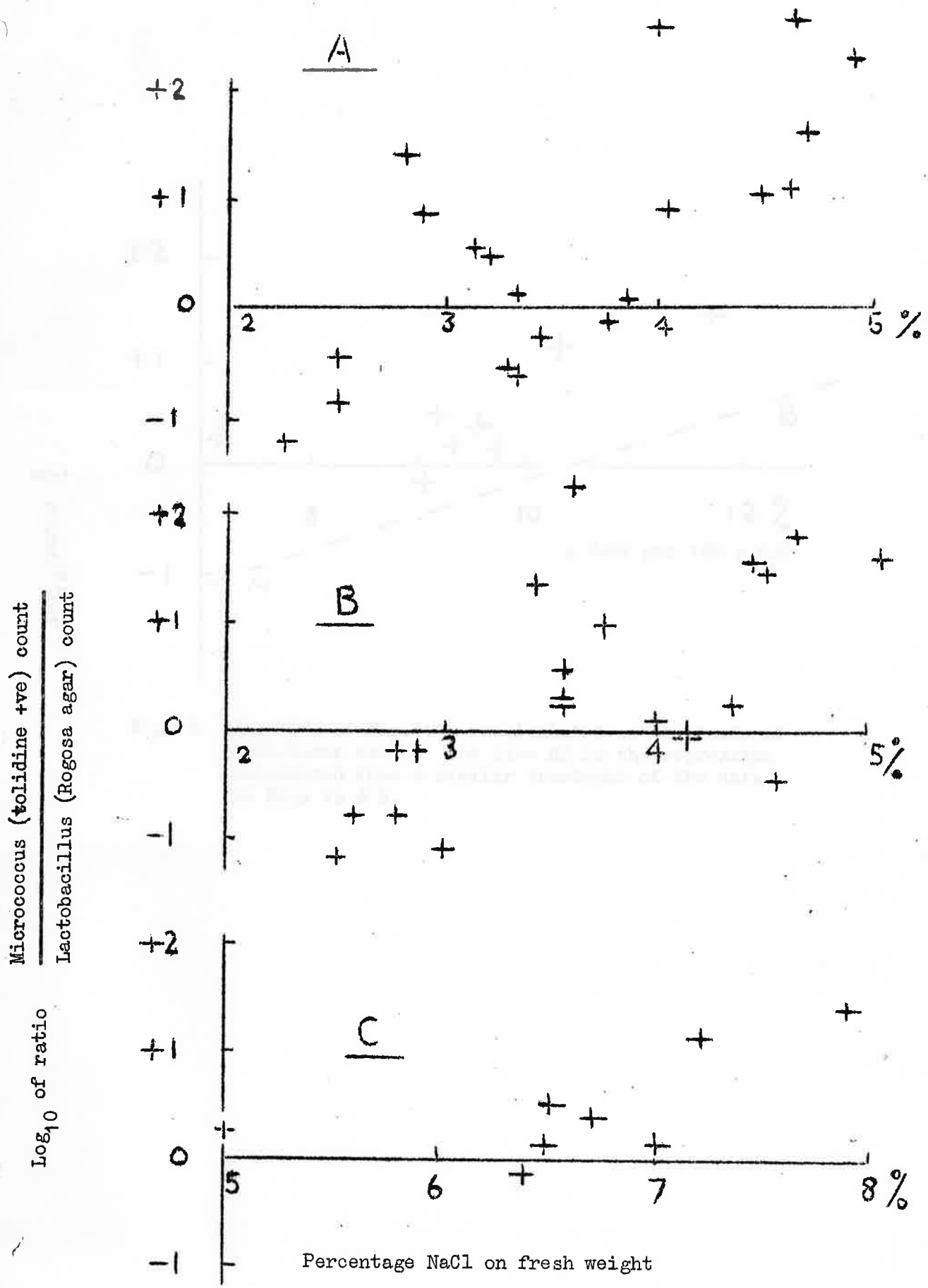

Fig. 1. Effect of salt content on composition of flora of vacuum packed Wiltshire bacon.

A. Smoked, stored at 20°C for 7 days

B. the same, _____ 14 days

C. Not smoked, ——— 15°C for 7 days.

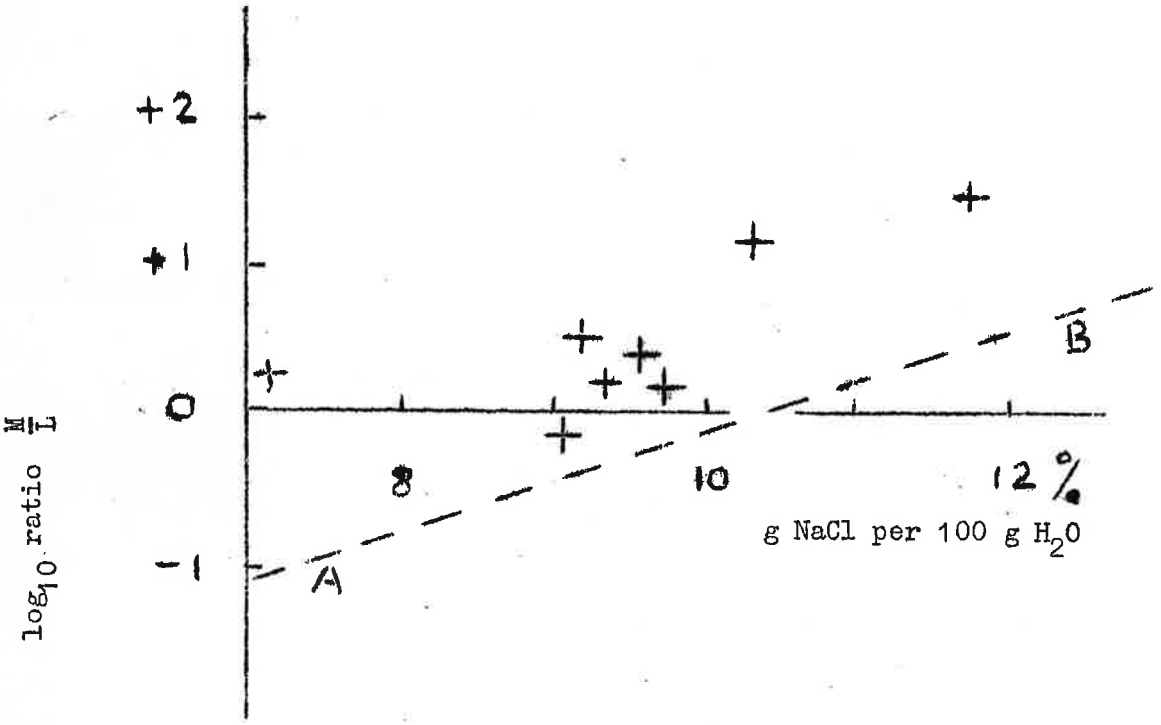

Fig. 2. The data of Fig. 1C, recalculated on the basis of NaCl/water ratio. The line AB is the regression calculated from a similar treatment of the data in Figs 1A & B.