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G.A. Gardner & J. Patton

Ulster Curers' Association, 2 Greenwood Avenue, Belfast 4.

Variations in the Composition of the Flora on a Wiltshire Cured Bacon Side.

The microbial association of any food is dependent on numerous physical and chemical influences. These vary between and even within a single product. The physical, chemical and microbiological principles of Wiltshire bacon production have been reviewed by Gibbons (1953). The most numerous bacteria on sides of bacon are micrococci (e.g. Garrard & Lochhead, 1939; Ingram, 1952). Other organisms such as Clostridium, Streptococcus, Enterobacteriaceae, and Alcaligenes are less frequently found (Ingram & Hobbs, 1954).

In the production of bacon the whole sides after immersion in a curing brine are matured either in a stack or on a rail for 7 - 14 days at ca. 4°C. This period of maturation permits the drainage of excess brine from the side and also ensures the even distribution of curing salts.

The work reported in this paper was carried out to determine the numbers and types of bacteria on a bacon side after the maturation period. Three areas of the side were examined: the singed rind, the unsinged rind, and the cut muscle surface. The latter two areas are those recognised

by the factory personnel as the positions where bacterial slime production can be first noted.

Methods and materials

Bacon sides. Bacon sides were obtained from 3 factories (X, Y and Z). The sides were matured in a stack for 9 and 8 days in factories X and Y respectively. Those from factory Z had been suspended from a rail for 7 days in the maturation cellar.

Samples. Samples of 10 sq.cm. from three areas were taken from each side, using a metal template and sterile instruments. The areas sampled were: (i) the skin from the outside of the hind leg (singed rind), (ii) the skin from the fold underneath the foreleg (usinged rind) and (iii) the cut surface of the gracilis muscle (meat).

Enumeration of bacteria. Samples for analysis were transported to the laboratory in bottles containing sterile sand. To each was added 10 ml of sterile 0,1 % (W/v) peptone water and the bottles were shaken vigorously on a mechanical shaker for 3 min. Serial dilutions were prepared in the same diluent. Aliquots of 0,1 ml were spread on the surface of plates by the technique of Davis & Bell (1959). The medium used for samples from factory X was the basal medium of Gardner (1966) with the addition of 4 % (W/v) NaCl; other samples were plated out on the same medium, in which the level of glycerol was reduced to 0,5 % (W/v) and to which 1 % (W/v) glukose was added. Colonies were enumerated after 4 days' incubation at 22°C.

Classification of isolates. After counting, each plate was divided into eight sectors and all the colonies on a number of sectors were picked off to give, where possible, ca. 30 isolates/sample. Purified cultures were classified by the methods shown in Table 1. Yeasts were identified solely on the morphology of Gram-stained preparations.

Table 1.

Classification of bacon bacteria

Gram reaction	Morphology	Catalase	Method of classification
-	Rods	+	Harrigan & McCance (1966)
+	Cocci	+	Anaerobic acid production from glucose: + Staphylococcus, - Micrococcus (Baird-Parker, 1966)
+	Rods or coccobacilli	-	Lactic acid bacteria
+	Pleomorphic rods	+	Coryneform bacteria
+	Rods	+	For Microbacterium thermosphactum (Gardner, 1966)

Results

The numbers and types of bacteria on the bacon sides matured in a stack are shown in Tables 2 and 3 and for those matured in a hanging position in Table 4. Within one treatment there was a great variation in the number of bacteria/sq.cm.; in a few cases there was a difference of a factor of x200. Therefore because of the small number of samples, the figures only give a general indication of the level of contamination. In general the numbers on the unsinged sites were higher than the singed sites, and both were higher than the meat site. The overall degree of contamination was higher in the sides examined from factory Z.

Micrococcus was the predominant genus in the singed rind samples. In the stacked sides Acinetobacter spp. were also isolated. In factory Y they accounted for 10 % of the flora.

Micrococcus remained the predominant genus on the unsinged rind samples from factory X, but Acinetobacter spp. were

more frequently isolated from sides from factories Y and Z. In this site the flora was found to be more diverse in the sides of factory Y.

Micrococcus spp. also predominated on the muscle samples, but to a lesser extent. Acinetobacter spp. were isolated from most sides, and other organisms such as Vibrio spp., coryneform bacteria and lactic acid bacteria were more frequently found.

Discussion

The average levels of contamination of the three sites examined in the present study ranged from ca. 10^4-10^6/sq. cm. Using an excision method of sampling, Gibbons (1940) reported mean counts on the pleural membrane of sides on receipt from the factory of $10^{4.2}$-$10^{4.5}$/sq.cm. After 10 - 12 days storage at 1,1°C these mean counts had rised to $10^{5.18}$-$10^{6.94}$/sq.cm. Gibbons found large variations in counts of particular sites at any one time, e.g. the difference between the maximum and minimum loads on sides as received from the factory were log 3,0 (i.e. 1000x) per sq.cm., whereas after 10 - 12 days at 1,1°C this had increased to ca. log 5,0 (100,000x) per sq.cm.

These variations may result from differences in the flora, different types of Growth of the same organism, or variability in the method of detection (Gibbons, 1940). Garrard & Lochhead (1939) found that sides which were highly contaminated before going into cure remained contaminated after maturation. Therefore variation in levels of contamination brought about by slaughter and butchery operations would be reflected in the bacon. Different practices at each factory would contribute to this. In most maturation cellars temperature is controlled, but variations between factories do exist. The relative humidity can vary within and between different factories. The results from factory Z suggest that the overall level of contamination of bacon

sides is higher. This is not due to a difference in the method of maturation, as some of our unpublished data has shown that statistically there is no significant difference between the rates of growth of bacteria on bacon sides matured in a stack or in a hanging position over a period of 7 days at 4°C. In addition, the types of bacteria isolated from sides from factory Y were more diverse than the two other factories examined.

Tofte Jespersen & Riemann (1958) demonstrated that the bacterial load on the rind was greater than that from the meat of a bacon side. After 7 days' maturation at 5°C count on nutrient agar containing 4 % NaCl were ca. 10^5/sq.cm. for the rind and ca. 10^4/sq.cm. for the meat. Ingram (1960) reported that bacon immediately out of cure carried a surface load of 10^4-10^5/sq.cm., which increased more than tenfold during a week of maturation at 4°. Counts on the skin were on average higher than counts from the longissimus dorsi at both times.

Counts on the bacon sides in the present study, although variable, are of the same order as those reported by other workers. The bacterial contamination of the meat site tended to be lower than either the singed or unsinged rind, thus confirming the observations of Tofte Jespersen & Riemann (1958) and Ingram (1960). Of all the sites this would probably have the least opportunity for outside contamination. The unsinged rind had on average the highest levels of contamination. This is probably due to the fact that most of the organisms on the skin of the pig are killed in the singeing process. The area of unsinged rind, underneath the foreleg, tends to be less well scraped during the slaughter operation.

It is now well established that micrococci are the predominant bacteria on matured Wiltshire bacon (Garrard & Lochhead, 1939; Brooks, Haines, Moran & Pace, 1940; Ingram, 1952, 1960). The present work has shown this to be valid

for both the rind and meat surfaces of a bacon side. However, there does appear to be an increase on the meat site of a proportion of Gram-negative organisms, such as Acinetobacter spp. and Vibrio spp.

Tofte Jespersen & Riemann (1958) found that, on a nutrient agar with 4 % NaCl, cocci predominated on the rind side at most stages during a maturation period of 14 days at 5°. However, on the meat side rods became predominant after 7 days at this temperature. Kitchell (1958) using a 10 % NaCl medium found 42 % Gram-positive bacteria on the skin and 63 % Gram-positive bacteria on the muscle of bacon after 14 days' maturation. Tofte Jespersen & Riemann (1958) obtained higher counts from bacon using a brine agar (20 % NaCl) on both the rind and meat of bacon after curing. Rod shaped bacteria predominated in the meat, whereas both cocci and rods were of the same order on the rind except after 7 days when rods appeared to out number the cocci. Gibbons (1940) found that the highest counts of bacon samples were obtained on media with 3 - 6 % NaCl. Occasionally counts equal to or higher than those on 4 % NaCl were obtained on 10 % NaCl. It is difficult to assess these results accurately because of the different media used and also the isolates were not classified.

Ingram (1960) found that the flora of matured bacon consisted of micrococci plus a roughly similar number of lactobacilli and a smaller proportion of Gram-negative rods. The latter accounted for 4 - 79 % of the flora of the l. dorsi samples and 0,1 - 20 % of the skin samples of bacon sides after 7 days' maturation.

Slime on the muscle of refrigerated bacon can contain 50 % lactobacilli, 33 % Micrococcus, 10 % Enterobacteriaceae and 7 % M. thermosphactum (Gardner, unpublished data).

The microbiological effect of salt in a food probably depends on osmotic withdrawal of water. This will be reflected in the water activity of the food. A brine

concentration in bacon exceeding about 5 % will exclude the normal pork spoilage flora, Pseudomonas, but permit the growth of the Micrococcus (Ingram & Kitchell, 1967). On the basis of a_w it could be postulated that the musculature of bacon would have a higher value than the skin and thus explain the apparent increasing importance of Acinetobacter spp. and Vibrio spp. in the flora.

Gibbons & Rose (1950) found with cured psoas muscle that at pH of 5,7 the flora was almost entirely composed of yeasts, whereas at pH 6,3 the flora was predominantly micrococci. Kitchell & Ingram (1965) demonstrated that the ante-mortem feeding of sugar to pigs resulted in a lowering of the pH of the musculature. There were fewer bacteria on the muscles of bacon from pigs treated in this manner, but there was no difference in the loads from the skin. These authors also concluded that there may be factors other than pH which affect the numbers and types of microorganisms on bacon sides.

However, factors such as level of NaCl (a_w), amount of NO_2^-, pH (i.e. the physical and chemical characteristics of the environment) would have to be measured in order to obtain a better understanding of the microbial ecology of Wiltshire cured bacon.

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Table 2.

The number and types of bacteria found on wiltshire cured bacon sides which were matured in a stack (factory X)

Site	Total no. of sides examined	Count/sq.cm. x 10^4			Total no. isolates examined	Incidence (%) of				
		Min.	Max.	Mean		Micrococcus	Staphylococcus	Acinetobacter	Vibrio	Miscellaneous
Singed rind	5	0,78	83,5	20,2	101	88,3 (5) **	5,7 (2)	2,8 (1)	0	3,2 +
Unsinged rind	5	1,2	16,5	7,98	99	94,4 (5)	1,6 (1)	4,0 (1)	0	0
Meat	5	0,03	8,5	2,72	104	64,2 (5)	-	5,9 (4)	19,0 (2)	10,9 ++

** The figures in parentheses are the number of bacon sides on which the organism was found.

\+ Includes M. thermosphactum and yeasts.

++ Includes M. thermosphactum, yeasts, Alcaligenes, Aeromonas and flavobacteria.

Table 3.

The number and types of bacteria found on wiltshire cured bacon sides which were matured in a stack (factory Y)

Site	Total no. of sides examined	Count/sq.cm. x 10^4			Total no. isolates examined	Incidence (%) of						
		Min.	Max.	Mean		Micrococcus	Staphylococcus	Acinetobacter	Coryneform bacteria	Vibrio	Lactic acid bacteria	Miscellaneous
Singed rind	5	0,13	7,7	2,4	107	80,5 (5) **	0	9,2 (4)	1,6 (1)	3,3 (2)	0,5 (1)	4,6 +
unsinged rind	5	0,15	50	15,3	168	64,9 (5)	10,4 (5)	10,4 (5)	3,1 (3)	1,2 (2)	1,2 (2)	8,8 ++
Meat	5	0,08	4,35	1,7	141	59,8 (5)	1,8 (1)	13,0 (5)	6,9 (3)	2,7 (3)	3,7 (2)	12,1 +++

** The figures in parentheses are the number of bacon sides on which the organism was found.

\+ Includes Achromabacter, Alcaligenes and Enterobacteriaceae.

++ Includes Achromabacter, yeasts and M. thermosphactum.

+++ Includes Achromabacter, yeasts, M. thermosphactum adn Enterobacteriaceae.

Table 4.

The number and types of bacteria found on wiltshire cured bacon sides which were matured in a hanging position (Factory Z)

Site	Total no. of sides examined	Count/sq.cm. $\times 10^3$			Total no. of isolates examined	Incidence (%) of		
		Min.	Max.	Mean		Micrococcus	Acinetobacter	Miscellaneous
Singed rind	4	0,38	11,4	4,5	100	95,2 (4)**	0	4,8 +
Unsinged rind	5	0,66	30,4	13,8	119	90,5 (5)	5,8 (3)	3,7 ++
Meat	5	1,4	23,5	9,1	110	86,0 (5)	9,8 (3)	4,1 +++

** The figures in parentheses are the number of bacon sides on which the organism was found.

\+ Includes Aeromonas, yeasts and unclassified Gram positive, catalase positive rods.

++ Includes Aeromonas, Enterobacteriaceae and unclassified Gram positive, catalase positive rods.

+++ Includes yeasts, lactic acid bacteria and unclassified Gram positive, catalase positive rods.