THE MICROBIOLOGY OF 'BACK BACON' MANUFACTURED IN 'NITRATE-FREE' BRINES AFTER STORAGE AT 5°C

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INTRODUCTION

In Ireland, Wiltshire bacon is manufactured in brines containing potassium nitrate and sodium nitrite. However, several factories have cured successfully without the addition of nitrate for more than 40 years and previous results have confirmed this (Evans et al 1971).

The current controversy about nitrite has led investigators to study the stability of bacon manufactured with and without nitrate (Shaw 1974) and with lower levels of nitrite than are normally employed (Taylor and Shaw 1975).

Although the microbiology of Wiltshire-style bacon has been extensively studied (Ingram 1960; Cavett, 1962; Gardner and Patton (1969a); Gardner 1971; Dempster 1973), few reports have been published in which the microbial changes in 'nitrate-free' cured bacon have been related to the levels of input nitrite. The work described here refers to these changes.

EXPERIMENTAL

Three Grade A Wiltshire bacon carcasses were selected in a local factory after overnight chilling. The sides were injected with brines of the following composition after routine cutting and dressing;

Brine A: 2,000 ug/g sodium nitrite, no added nitrate (2 sides).
Brine B: 500 ug/g sodium nitrite, no added nitrate (2 sides).
Brine C: 1,000 ug/g sodium nitrite + 1,000 ug/g sodium nitrate (2 sides).

The brines were constituted to contain 18% (w/v) sodium chloride. The sides were immersed in 'cover' brines of the same composition for 4 days at 5°C. After maturation for a further 4 days, they were deboned, sliced (backs only) and vacuum packaged.

Source of isolates

At weekly intervals, 12 packs were examined representing bacon from both left-hand and right-hand sides, and from each curing treatment. After the total aerobic count was made on plates of Oxoid Plate Count Agar with 4% (w/v) of added salt (4 PCA), all the colonies in two segments of the same dilution showing different plate morphology were picked off into 4% saline and incubated at 25°C/2 days. A loopful was then streaked on to fresh 4 PCA plates for a purity check. This was repeated until pure cultures were obtained. The cultures were transferred to Bijou bottles and stored at 5°C.

Classification scheme

The following schemes were used to classify the isolates into broad groups:-

1. Gram positive, catalase positive cocci
   - Baird Parker (1966)
2. Gram positive, catalase positive rods/coccoid rods
   - Gardner (1966)
   - Harrigan and McCance (1966)
   - Gardner (1969)
   - Bergey (1975)
3. Gram negative cocci/rods/coccobacilli
   - Harrigan and McCance (1966)
   - Gardner (1973)

Statistical analysis

The percentage of each of the main colony types was analysed using a logistic model with week of storage and nitrite levels as two factors. A linear effect of nitrite concentration was tested, using equally spaced codes (1, 2 and 3) for the three levels. Tests of significance were based on the deviance, which has, approximately, a chi-square distribution.

RESULTS AND DISCUSSION

One hundred and forty of the isolates were identified as Gram positive, catalase positive cocci of which 14% were coagulase positive staphylococci, Table 1. These tended to die out as storage progressed and none were recovered after 3 weeks.

The majority of the micrococci (55%) were phosphatase positive strains (Group 6 of Baird-Parker 1966) and they persisted throughout. The proportions of micrococci have been reported to be 94% of the flora on sides of pork and 89 - 100% of the flora on matured sides of bacon by Kitchell (1962). They play a significant part in the curing process because of their ability to reduce nitrate, (Shaw et al 1951; Kitchell 1958,).
The majority of the strains studied by Patterson (1966) were tolerant of nitrite and this was confirmed in the present study; at 2,000 ug/g nitrite, nearly half of the isolates were micrococci, whereas at 500 ug/g only

20% of the colonies were micrococci ($P < 0.01$), Table 2.

They predominated in the middle storage period and died out towards the end. This might be significant since Taylor and Shaw (1975) stated that bacon made with 1,000 ug/g nitrite was still acceptable after 5 weeks at 5°C, whereas bacon made in 500 ug/g and less gave a product which was more prone to souring due to increased growth of lactic acid bacteria. This was confirmed by Shaw and Harding (1978) who showed that lactic acid bacteria were commonest on bacon containing the lowest initial nitrite concentration (34 parts/10^6) and might also explain why lactic acid bacteria were not found in the present study. Another explaination may be that none of the isolates here were taken from a medium selective for lactics.

One hundred and eighteen of the isolates were either Gram positive rods (regular or pleomorphic) or Gram negative rods, Table 3. The majority (59%) were classified as heat sensitive corynebacteria. They did not survive 63°C/30 min. There was also a time effect on the corynebacteria; they only constituted 13% of the flora in week 1, but increased in weeks 5 and 6 to between 50 - 60% and their proportion of the total flora increased with decreasing nitrite concentration, Table 2. Only 3% of the isolates were Brochothrix thermosphacta (Microbacterium thermosphactum)and were recovered in the first two weeks only, Table 3. It is unusual to find this organism in cured meats because of its sensitivity to curing salts (Brownlee 1966). However, Gardner and Patton (1969b) found that is constituted 4, 6 and 14% of the flora in three samples of Wiltshire bacon (fresh and spoiled). It was also found in vacuum packed bacon in proportions ranging from 21% (fresh) to 10% (spoiled), (Dempster 1973).

Six cultures were classified as Acinetobacter spp. They were found at all storage intervals except weeks 1 and 3 and particularly in the 2,000 ug/g nitrite bacon.

Twelve (10%) Gram negative, pigmented rods were described simply as 'flavobacteria' without further identification, Table 3. They formed cream/yellow/orange pigments on the total count medium (4 PCA) on standing at room temperature. Aeromonas spp. constituted 13% of the flora of Gram negative rods and were found in the different bacons up to 4 weeks. Only two isolates were identified as Vibrio spp., one of which did not grow in 10% salt (NaCl) broth, was Arginine negative and therefore resembled the bacon strains studied by Gardner (1973). However, both were motile and one of them did not reduce nitrate. In these respects, they differed from Gardner's strains.

Four isolates were yeasts and were identified only by microscopic examination. Five cultures could not be identified to genus or group level. They were isolated in the 6th week from the control (Wiltshire) and the 500 ug/g NO_2 bacon. They were pigmented, Gram negative, catalase negative rods. All except one were motile and all but one did not utilise glucose in Hugh and Leifson's medium (1953).

CONCLUSIONS

Several broad indications are evident from the results presented.

1. The main bacterial groups isolated from vacuum packed bacon manufactured in the brines described here were staphylococci, micrococci and coryneform bacteria.
2. In weeks 1 and 2, there was a higher percentage of Staphylococci than in the other weeks ($P < 0.001$) and they increased as the nitrite concentration decreased ($P < 0.05$).
3. Micrococci increased up to week 4 and decreased thereafter ($P < 0.001$). They increased with increasing nitrite concentration ($P < 0.01$).
4. Coryneform bacteria increased with time ($P < 0.001$) and as nitrite concentration decreased ($P < 0.05$).

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TABLE I: Distribution of Gram + vs, catalase + cocci in vacuum packed bacon during storage at 5°C

Week No	Input Nitrite ug/g	GENUS/GROUP: Group I Staphylococcus I	II	III	IV	V	VI	Group II Micrococcus 1	2	3	4	5	6	7	8
1	2,000	4	1	3						1		1	3		1
	500		2	2		2	1						1		
	C*		4	3			2						2		
2	2,000	2	1	1									5		1
	500	5	2	2											
	C	4		4									2		
3	2,000		1										11		
	500	2											3		
	C	2											9		
4	2,000												11		
	500			1									3		
	C												10		
5	2,000		1	1									6		
	500												4		
	C		1										4		
6	2,000									2					
	500									3					
	C												3		
Total		19	13	17	-	2	3	-	-	6	-	1	77	1	1
%		13.6	6.9	12.1		1.4	2.1			4.3		0.7	55.0	0.7	0.7

* Control (Wiltshire cure: 1,000 ug/g NO_2 + 1,000 ug/g NO_3)

TABLE 2: Percentages of the main bacterial groups fitted by the logistic model

Week No	COLONY TYPE Staphylococci	Micrococci	Coryne form
1	52	17	13
2	51	17	5
3	14	58	16
4	3	62	25
5	4	29	51
6	-	19	60
2,000 ug/g NO_2	10	46	15
1,000 ug/g NO_2	16	31	23
500 ug/g NO_2	24	20	33
Mean	15	31	23
Deviance Week	50.7 (4 df)	37.1 (5 df)	46.3 (5 df)
NO_2	5.4 (1 df)	10.1 (1 df)	5.7 (1 df)
Interaction	8.5 (8 df)	8.8 (10 df)	16.0 (10 df)

TABLE 3: Distribution of Gram + ve and Gram - ve rods in vacuum packed bacon during storage at 5°C

Week No	Input Nitrite ug/g	GENUS/GROUP Coryne-bacterium	B. thermos-phacta	Acineto-bacter	Flavo-bacterium	Aero-monas	Vibrio	Yeasts	Unclassified
1	2,000	2	2			1			
	500	2				3			
	C	2	1			2			
2	2,000					3	2	1	
	500			1		1			
	C	2	1		2				
3	2,000	3						1	
	500	2				2			
	C	1				1			
4	2,000			2					
	500	4							
	C	5				2			
5	2,000	5		1				2	
	500	8		1	1				
	C	10			2				
6	2,000	9		1	2				
	500	13			1				2
	C	2			4				3
Total		70	4	6	12	15	2	4	5
%		59.3	3.4	5.1	10.2	12.7	1.7	3.4	4.2