NITRATE REDUCING ORGANISMS IN BACON CURING BRINES

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INTRODUCTION

Curing brines for Wiltshire style bacon have the following composition: 25–27% sodium chloride and 0·5–1% sodium nitrate. The amount of nitrite varies, depending on the bacterial flora: the recommendation for cover brines is about 0·05%. Prior to the introduction of Wiltshire cure, pigs were killed on farms and taken to small factories for dissection, and the cuts were dry-cured. The carcases were not chilled until butchery was completed, usually 24 hours post-mortem. The temperature of the cellar for dry curing ranged from 6·5–9°C. On the introduction of the tank cure, carcases were cooled at atmospheric temperature in a hanging hall for 18-24 hours, butchered, chilled, and placed in tanks at 5°C. The flora of the cover brines was predominantly micrococci, and the nitrite levels were remarkably stable. When chilling took place immediately after slaughter, it was noticed that the proportion of cocci in the brines decreased, with a corresponding increase in rods. The immediate effect was a tendency for the nitrite content of the brine to fluctuate. If the carcase or brine temperature rose, the nitrite level increased rapidly, and measures had to be taken to control the excess amount produced. The changes occurring in a cover brine over a period of twelve months are illustrated in Figure 1. It was decided to investigate the activity of these dominant rod types.

MATERIALS AND METHODS

Halophilic bacteria from Wiltshire curing brines were isolated on the pork juice medium of Jesperson and Riemann (1). The colonies were picked off into nitrate broth (1% peptone, 0·1% KNO_3, 25% NaCl), and strains showing active nitrate reduction at this salt concentration were selected. In all, there were 20 strains from 11 different cover brines.

Salt tolerance was measured in nitrate broth containing various salt concentrations at 22°C.

Temperature range was measured in nitrate broth with 25% NaCl.

Sugar fermentations, starch hydrolysis, phosphatase production, indole and ammonia production were carried out by the methods of Penso, Ortali and Gori (2).

Casein hydrolysis, gelatine liquefaction, urease production, citrate utilization, lecithinase production and acetoin were tested by the usual methods, except that 20% NaCl was added to the media.

Total counts were carried out on a Helber counting chamber using phase contrast illumination.

Mossel and Martin's medium (3), with the salt concentration increased to 20%, was used for testing the breakdown of glucose.

The Gram strain was performed as recommended by Dussault (4).

Nitrite estimations were carried out on a Unicam SP500 spectrophotometer, using the method of Grau and Mirna (5).

EXPERIMENTAL PROCEDURE

Tubes of nitrate broth containing 25% NaCl were seeded with heavy inocula of the strains to be tested. These tubes were incubated at 7°C and 4°C (±0·25°), and samples were removed at intervals for nitrite estimations and total counts.

RESULTS

The results are shown in Tables 1 and 2. It will be seen that, although the initial inocula at 4°C were considerably higher, the production of nitrite was substantially slower than at 7°.

The biochemical reactions of the organisms are shown in Table 3. The organisms were all Gram-positive rods. All grew well at 22°, some at 37° and none at 44°. Almost all the strains required at least 10% NaCl for growth, although some strains showed slight growth in 5% NaCl. Some strains produced brown pigment. It was not possible to demonstrate spores, but this may have been due to straining difficulties. In most cases the final pH on nitrate broth was 8·2–8·4.

DISCUSSION

Various authors have found different types of organisms to be responsible for nitrate reduction in curing brines. Buttiaux (6) described a **Vibrio**, but it should be borne in mind that his brines contained much less salt than Wiltshire brines. This type does not appear to be present in brines in this country. Ingram, Kitchell and Ingram (7) described Gram-negative rods from Wiltshire curing brines, which reduced nitrate and nitrite. Hornsey (8) described a Gram-negative rod which actively reduced nitrate and nitrite in beef curing brines. Jones (9) described a brine containing **Bacillus** types which showed an increasing production of nitrite. Leistner (10) isolated **Bacillus** for cover brines, but in small numbers.

The results given above indicate that reduction of nitrate can be controlled by storage at temperatures below 4°C. Unfortunately this also retards the cure, and a compromise is inevitable. Most factories cure at 4·5°, and experience has shown that even a small rise above this temperature causes a rapid increase in the total numbers of bacteria, followed by an increase in nitrite concentration.

It is paradoxical that an improvement in factory hygiene leads to a predominance of these nitrate reducing rods. It has been noticed that where the total number of rods exceeds 10^8 per ml. (phase contrast count), nitrite increases very rapidly. It may be noted that increasing the salt concentration does not effectively control this increase, and it is necessary to either dilute the brine or pass it through a Seitz filter.

SUMMARY

The predominant bacteria in Wiltshire curing brines appear to be Gram-positive rods, which actively reduce nitrate. The results obtained emphasise the importance of strict control of temperature during curing.

ZUSAMMENFASSUNG

Die vorwiegende Keime in Pokellaken scheinen Gram-positive Stabe zu sein, welche das Nitrat aktiv reduzieren. Die Versuchsergebnisse heben die Wichtigkeit einer strengen Temperaturkontrolle wahrend der Pokelung hervor.

RESUME

Les bacteries principales dans les saumures de salaison semblent etre des batonnets Gram-positifs, qui reduisent activement le nitrate. Les resultats obtenus soulignent l'importance d'un controle strict de la temperature pendant la salaison.

LITERATURE

(1) Tofte Jespersen N. J. & Riemann H. Proc. 2nd Int. Symp. Food Microb. 1957, p.177.

(2) Penso, G., Ortali V. & Gori G. B. — Ibid. p.21.

(3) Mossel & Martin, Ann. Inst. Pasteur Lille 12, 225.

(4) Dussault H. P. (1955), J. Bact. 70, 484-5.

(5) Grau R. & Mirna A. (1957), Z. Anal. Chem. 158, 182.

(6) Buttiaux R. Proc. 2nd Int. Symp. Food Microb. 1957, p.137.

(7) Intram M., Kitchell A. G. & Ingram G. C. Ibid. p.205.

(8) Hornsey H. C. Ibid. p.197.

(9) Jones O. Meat 9c, no.12.

(10) Leistner L. (1959), Die Fleischwirtschaft 11 (9), 726.

Table 1 NITRATE BROTH @ 4°

Phase contrast count (× 10⁶)*
Nitrite p.p.m.†

No.	Day 0	Day 3	Day 7	Day 10	Day 13	Day 17	Day 19
1	*107·5	118·5	72·0		103·5	142·25	238·75
	†			260	260	260	500
2	136·5	109·0	100·5		232·0	216·0	277·0
				340	460	340	
3	153·5	141·0	136·5		144·0	275·0	268·5
				380	515	380	470
4	86·5	65·0	33·5		68·25	102·5	66·0
				T	T	T	T
14	122·5	88·0	77·0		104·5	134·25	189·75
				300	415	300	630
15	150·0	126·5	95·0		73·0	164·5	205·5
				305	400	305	520
29	85·0	45·0	46·0		52·0	75·5	75·25
				130	150	130	315
30	113·0	94·0	82·0		94·0	139·0	160·5
				395	415	395	650
35	113·0	131·0	126·5		152·5	164·5	300·0
				405	525	405	650
37	143·0	129·5	122·0		120·5	230·0	265·5
				375	480	375	685
38	91·0	84·5	77·5		68·5	159·5	218·0
				300	310	300	480
43	70·5	74·0	63·0		39·5	68·5	85·0
				270	280	270	425
51	125·0	128·5	130·5		116·5	183·5	191·5
				530	410	530	630
52	113·0	102·5	110·5		124·5	139·0	159·5
				260	380	260	560
56	109·0	80·5	76·0		87·0	130·0	141·25
				190	240	190	380
59	113·0	93·5	103·0		104·5	145·5	114·0
				220	360	220	430
B1	108·0	110·5	101·5		98·5	138·5	209·0
				305	410	305	585
B5	107·0	88·0	100·0		112·0	199·0	240·0
				250	290	250	450
C7	135·0	115·5	139·5		141·0	205·5	267·5
				380	415	380	680
C19	92·0	89·5	67·5		88·0	109·0	166·5
				235	330	235	450

T = trace present

Table 2 NITRATE BROTH @ 7°

Phase contrast count (× 10⁶)*
Nitrite p.p.m.†

No.	Day 0	Day 3	Day 5	Day 7	Day 11
1	*70·0	91·0	117·0	173·5	194·0
	†	135	250	420	770
2	34·5	65·0	66·0	111·5	171·5
		110	230	400	635
3	72·5	76·0	83·0	41·0	100·0
		125	115	T	350
4	58·5	73·5	97·0	85·5	129·5
		105	115	T	410
14	60·0	81·5	105·0	146·0	192·5
		145	280	460	620
15	84·5	120·5	157·5	167·5	193·5
		102	200	375	650
29	50·5	67·5	99·5	146·5	193·5
		110	140	285	450
30	53·5	148·25	111·0	162·5	220·0
		160	295	460	710
35	58·5	78·25	105·0	148·5	217·0
		125	180	340	615
37	57·0	100·0	128·5	156·0	230·5
		95	160	265	570
38	73·0	170·75	160·0	165·5	205·5
		100	150	235	570
43	63·0	106·0	127·5	119·0	215·0
		160	260	385	730
51	62·5	146·0	167·0	152·0	215·0
		105	210	235	560
52	72·5	143·75	175·5	200·0	290·5
		160	295	485	680
56	86·0		144·5	158·0	262·5
		130	250	365	680
59	88·5		108·75	190·5	232·5
		110	200	285	590
B1	49·5		69·5	126·0	194·5
		100	180	215	460
B5	65·5	68·0	77·25	83·0	133·5
		T	T	125	235
C7	87·5	112·5	191·0	172·5	267·5
		140	230	365	535
C19	68·0	92·0	87·0	139·5	215·0
		T	T	90	360

T = trace present

Table 3 BIOCHEMISTRY OF HALOPHILIC BACTERIA

No.	Temp. range			Salt tolerance (% NaCl)							Acetoin	Glucose	Arabinose	Xylose	Starch
	30°	37°	44°	0·5%	5%	10%	15%	20%	25%	30%					
1	+	±	−	−	−	+	+	+	+	+	−	−	−	−	−
2	+	±	−	−	−	+	+	+	+	+	−	−	−	−	−
3	+	+	−	−	−	±	+	+	+	+	−	−	−	−	−
4	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−
14	+	±	−	−	−	+	+	+	+	+		−	−	−	−
15	+	±	−	−	−	+	+	+	+	+	−	−	−	−	−
29	+	±	−	−	−	+	+	+	+	+	−	−	−	−	−
30	+	±	−	−	−	+	+	+	+	+	−	−	−	−	−
35	+	−	−	−	−	+	+	+	+	+	−	−	−	−	−
37	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−
38	+	+	−	−	−	+	+	+	+	+	−	−	−	−	−
43	+	−	−	−	−	+	+	+	+	+	+	−	−	−	−
51	+	−	−	−	−	+	+	+	+	+	−	−	−	−	−
52	+	−	−	−	−	+	+	+	+	+	−	−	−	−	−
56	±	−	−	−	−	+	+	+	+	+	+	−	−	−	−
59	+	−	−	−	±	+	+	+	+	+	−	−	−	−	−
B1	+	+	−	−	±	+	+	+	+	+	−	−	−	−	−
B5	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−
C7	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−
C19	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−

*By the method of Mossel and Martin: Ox, oxidative; NC, no change.

Casein	Gelatine	Lecith-inase	Urea	Citrate	Breakdown* of Glucose	Catalase	Motility	Phospha-tase	Indole	Pigment	Ammonia
—	—	—	—	—	NC	+	—	+	—	White	+
—	—	—	—	—	NC	+	—	+	—	Yellow	+
—	—	—	—	—	NC	+	—	—	—	White	+
—	—	—	—	—	NC	+	—	+	—	Brown	+
—	—	—	—	—	NC	+	—	—	—	White	+
—	—	—	—	—	NC	+	—	—	—	White	+
—	—	—	—	—	OX	+	+	—	—	White	+
—	—	—	—	—	NC	+	—	—	—	White	+
—	—	—	—	—	NC	+	+	—	—	Brown	+
—	—	—	—	—	NC	+	—	—	—	Yellow	+
—	—	—	—	—	NC	+	+	+	—	Yellow	+
—	—	—	—	—	NC	+	—	+	—	White	+
—	—	—	—	—	NC	+	—	—	—	White	+
—	—	—	—	—	NC	+	—	+	—	Yellow	+
—	—	—	—	—	NC	+	—	—	—	Brown	+
—	—	—	—	—	NC	+	—	—	—	Yellow	+
—	—	—	—	—	NC	+	—	—	—	Yellow	+
—	—	—	—	—	NC	+	—	—	—	White	+
—	—	—	—	—	NC	+	—	+	—	White	+
—	—	—	—	—	NC	+	—	—	—	Yellow	+

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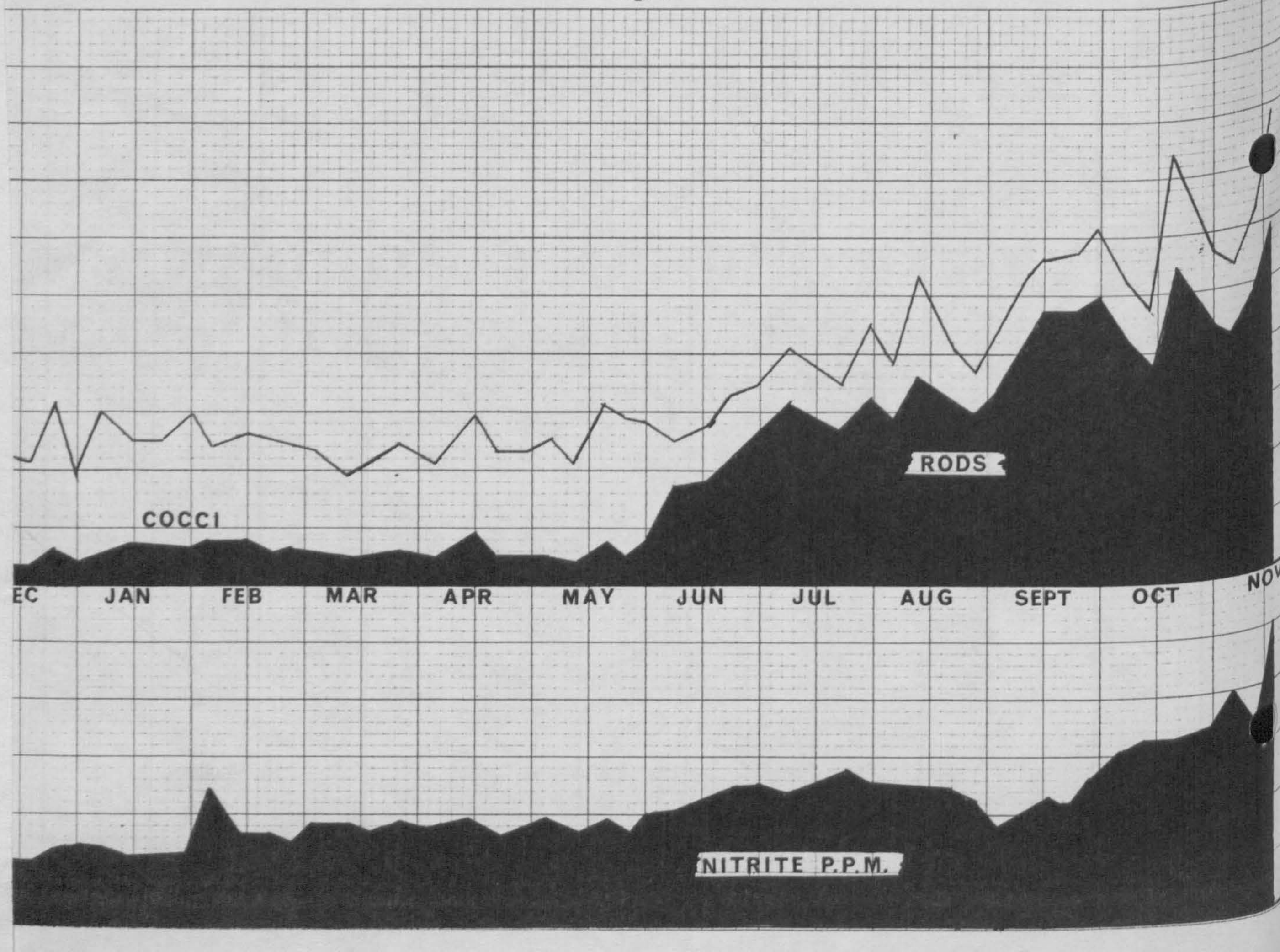
Fig. 1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