G4.

NITRITES AND NITROSAMINES IN PROCESSED MEATS

BACTERIAL STABILITY OF VACUUM PACKED WILTSHIRE BACON CURED WITH AND WITHOUT NITRATE

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Bacon has been made by a factory Wiltshire process incorporating hand pumping and immersion using brines with and without nitrate and with diminishing concentrations of nitrite. The bacterial stability of the bacon during storage in vacuum packs has been compared.

The inclusion of nitrate in a brine containing 26% NaCl and 1000 ppm nitrite was not essential to give stability to back bacon. Brines without nitrate and containing 500 ppm or less nitrite gave back bacon which was prone to souring due to increased growth of lactic acid bacteria.

Collar bacon produced in a brine containing 26% NaCl and 1000 ppm or 2000 ppm nitrite was less stable than that produced in a brine containing 1000 ppm nitrite with 5000 ppm nitrate. High counts of Gram negative bacteria were obtained from collar bacon produced with no added nitrate and may explain its poor shelf-life.

LA STABILITE BACTERIENNE DU BACON WILTSHIRE MIS EN PAQUETS SOUS VIDE ET CONSERVE AVEC OU SANS NITRATE

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Du bacon fut produit selon un procédé industriel du type Wiltshire, comprenant le pompage à la main et l'immersion dans des saumures avec et sans nitrate et avec des concentrations décroissantes de nitrite. La stabilité bactérienne du bacon lors du stockage dans des paquets sous vide fut comparée.

L'inclusion du nitrate dans une saumure contenant 26% NaCl et 1 000 ppm de nitrite n'était pas essentielle pour donner de la stabilité au bacon du dos. Des saumures sans nitrate et contenant 500 ppm de nitrite ou moins donnèrent du bacon du dos qui était susceptible à l'acidification à cause d'une augmentation de la croissance des bactéries d'acide lactique.

Du bacon de collier produit dans une saumure contenant 26% NaCl et 1 000 ppm ou 2 000 ppm de nitrite était moins stable que celui produit dans une saumure contenant 1 000 ppm de nitrite avec 5 000 ppm de nitrate. De grandes quantités de bactéries gram-négatives furent décelées dans du bacon de collier produit sans l'addition de nitrate et cela pourrait expliquer sa courte durée de conservation.

BAKTERIELLE STABILITÄT VON VAKUUMVERPACKTEM WIL.SHIRE-SPECK, DER MIT ODER OHNE NITRAT GEPÖKELT WURDE

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Speck wurde hergestellt mit einem Wiltshire Fabrikverfahren mittels Handpumpen und Eintauchen unter Verwendung von Pökellaugen mit und ohne Nitrat und bei abnehmenden Nitritkonzentrationen. Die bakterielle Stabilität des Specks während der Lagerung in Vakuumpackungen wurde verglichen.

Das Beifügen von Nitrat zu einer Pökellauge aus 26% NaCl und 1 000 ppm Nitrit war nicht wesentlich, um dem Rückenspeck Stabilität zu geben. Pökellaugen ohne Nitrat, die 500 ppm oder weniger Nitrit enthielten, erzeugten Rückenspeck, der leicht versauerte und zwar durch das schnellere Wachstum von Milchsäurebakterien.

Halsspeck, der in einer Pökellauge aus 26% NaCl und 1 000 ppm oder 2 000 ppm Nitrit erzeugt wurde, war weniger stabil als derjenige, der in einer Lauge aus 1 000 ppm Nitrit mit 5 000 ppm Nitrat hergestellt wurde. Ein großes Vorkommen von Gram-negativen Bakterien bestand beim Halsspeck, der ohne Nitratzusatz hergestellt wurde, und das könnte seine geringe Lagerfähigkeit erklären.

БАКТЕРИАЛЬНАЯ СТАБИЛЬНОСТЬ ВАКУУМ-УПАКОВАННОГО УИЛТШАЙРСКОГО БЕКОНА СОЛЕННОГО С НИТРАТОМ И БЕЗ НЕГО

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Бекон был приготовлен на заводе посредством уилтшайрского процесса с ручным накачиванием и погружением в рассолы с ни-тратом и без него и с уменьшаемыми концентрациями нитрита. Затем были сделаны сравнения бактериальной стабильности этого бекона в время хранения в вакуумных упаковках.

Добавление нитрата в рассол, содержащий 26% NaCl и 1000 миллионных долей нитрита не играло существенной роли в прид[...] стабильности шпиговому бекону. Рассолы без нитрата и содер[...]щие 500 или меньше миллионных долей нитрита давали шпиговый бекон с тенденцией к закисанию в результате разрастания мол[...]чно-кислых бактерий.

Шейный бекон, изготовленный в рассоле, содержащем 26% Na[...] и 1000 или 2000 миллионных долей нитрита, оказался менее ус[...]тойчивым, чем бекон, изготовленный в рассоле с 1000 миллион[...]ных долей нитрита и 5000 миллионных долей нитрата. В случа[...] шейного бекона, изготовленного без добавления нитрата, были обнаружены большие количества грамотрицательных бактерий, что может объяснить его плохое выдерживание хранения.

BACTERIAL STABILITY OF VACUUM PACKED WILTSHIRE BACON CURED WITH AND WITHOUT NITRATE

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INTRODUCTION

Wiltshire bacon is typically produced by curing with brines containing salt, nitrate and nitrite. The concentrations of nitrate and nitrite in cured and preserved meats are limited by law in the United Kingdom to 500 ppm and 200 ppm respectively (Statutory Instrument No.882, 1971). Concern with the safety of nitrite may cause these limits to be reduced and it is essential to understand the consequences of this on the properties of Wiltshire bacon. Nitrite may contribute to the stability of bacon since it inhibits bacterial growth (Tarr, 1941). The effect has been determined of the omission of nitrate and the reduction of nitrite level in curing brines on the storage stability of vacuum packed collar and back bacon. A detailed microbiological examination is not described, but the approach has been to monitor, during storage, total bacterial numbers and numbers of selected groups of organisms with the aim of explaining alterations in storage stability.

EXPERIMENTAL

Bacon manufacture

A series of comparisons were made of bacon produced by different curing treatments (Table 1). The bacon was processed at a local factory and in each comparison paired sides of Grade 1 bacon pigs were taken after chilling from the normal factory throughput. In each comparison left sides were given one curing treatment (Cure 1) and right sides the other (Cure 2). In each curing treatment pumping and immersion brines had the same concentrations of salt, nitrite and nitrate. All sides were stitch pumped by hand to gain 8% of their trimmed weights and 6 gallons brine per side were used for immersion in plastic tanks. Immersion was for 5 days, and sides were then withdrawn and stacked for 7 days at 5°C.

Slicing, packing and storage

In each comparison matched pairs of sides were taken under refrigeration to the Laboratory for examination. In Comparisons A, B and C 10 pairs of sides were used and 60 slices (3mm) cut from each back to be vacuum packed consecutively, 6 to a pack, in Metathene X (Metal Box Co., London) pouches. The packs from the 10 sides from each curing treatment were grouped according to a 10 x 10 Latin Square design so that at each examination time when 10 packs were sampled, each side and position along a sliced back were represented. Comparison between curing treatments was thus made by examining the corresponding slices from opposite sides of the same pig. In Comparison D 6 pairs of sides were sampled and 36 slices cut from each back to give 6 packs. These were grouped according to a 6 x 6 Latin Square design for sampling. In Comparisons E and F 6 pairs of sides were sampled and 18 slices cut from each collar section. The slices were numbered consecutively and the numerically equivalent slices from each collar were packed together to give 18 packs each containing 6 slices. At each sampling time 2 corresponding packs were taken from each curing treatment.

One set of packs was examined initially and the remaining sets examined periodically during storage at 15°C and 5°C.

Microbiological examination

Back bacon (Comparisons A, B, C and D). Packs were opened aseptically and slices 1 and 3 removed. The eye muscle was cut from these slices to form the sample which was minced first through a 10 mm screen and then through a 5 mm screen. Twenty five grams were then homogenized for 2 min at 6,000 r.p.m. in an Atomix Blender (MSE, London) in 100 ml ¼ strength Ringers + 0.1% peptone as diluent. Drops (0.017 ml) of suitable decimal dilutions were transferred in duplicate to the surface of plates of Plate Count Agar (PCA, Oxoid) + 4% NaCl by means of calibrated dropping pipettes (Astell Cat. No.851 and 852). The drops were each spread separately over the area of a quarter of a plate. After incubation at 25°C for 5 days the colonies were counted to give the total viable count. Cavett's (1963) modification of acetate agar (AA; Rogosa, Mitchell & Wiseman, 1951) incubated at 25°C for 5 days under 95% H_2 and 5% CO_2 was used to enumerate lactic acid bacteria. Yeasts were enumerated on Yeast Salt Medium (Davis, 1959) and Gram negative bacteria on PCA + 2 ppm crystal violet (Holding, 1960), both media being incubated at 25°C for 5 days.

Collar bacon (Comparisons E and F). The rind and surface fat was cut from slices and the remaining portion of the slices used for microbiological analysis. Treatment of samples was the same as that described for back bacon except that PCA + 1% NaCl was used for the total viable count.

Chemical analysis

Back bacon. The eye muscle was cut from slices 2 and 6 from each pack to form the sample. Each of the bulk samples was chopped and minced. One gram of sample was homogenized with 10 ml distilled water and the pH measured. Nitrite and nitrate were estimated by the method of Follet & Ratcliffe (1963). Sodium chloride was estimated in the same extract by precipitating the chloride with an excess of silver nitrate and titrating the excess with potassium thiocyanate.

Collar bacon. Analyses were carried out as described for back bacon on a minced sample taken from the bulk sample used for microbiological analysis.

Assessment of shelf life

When the packs were opened the odour of the bacon was noted. A slight sour or slight putrid odour was considered to be the limit of shelf life. When a strong sour or putrid odour was noted then the shelf life was considered to have been exceeded.

RESULTS

Analysis of bacon

Table 1 shows the pH and concentration of nitrite, nitrate and salt at the beginning of storage for the different bacon samples. Several of the bacons, particularly the collar samples, contained nitrate levels in excess of those permitted by law but these bacons were still thought suitable for comparative experiments.

Shelf life

Back bacon. None of the bacon produced in Comparisons A, B and C exhibited off-odour after storage and on this basis shelf life was in excess of 5 weeks at 5°C and 2 weeks at 15°C. In Comparison D the bacon containing 250 ppm nitrite (D2) had a strong sour odour after 19 days at 15°C and 36 days at 5°C at which time the D1 bacon had no off-odour. A precise shelf life cannot be given for the D2 bacon because of the infrequency of sampling but it was between 1 and 3 weeks at 15°C and 2 and 5 weeks at 5°C.

Collar bacon. The E1, F1 and F2 bacons exhibited no off-odour after storage at 5°C for 20 days. The E2 bacon had a shelf life of up to 20 days at 5°C since a slight putrid odour was noted at this time. Shelf life of E1 and F1 bacon was up to 14 days at 15°C (slight putrid) of E2 up to 9 days at 15°C (slight putrid) and of F2 less than 14 days but more than 9 days at 15°C (no off odour at 9 days, putrid odour at 14 days).

Microbiology

Back bacon. The total viable counts and numbers of lactic acid bacteria during storage of bacon in Comparisons A, B, C and D are shown in Table 2. In Comparison A the addition of 5,000 ppm nitrate to the brine containing 1,000 ppm nitrite had no effect on the total viable count at the time of packing. During storage the total viable count was higher and lactic acid bacteria in general more common in the bacon cured with added nitrate. Reducing the level of nitrite in the brines from 2,000 ppm to 1,000 ppm and 500 ppm (Comparisons B and C) did not affect the total viable count at the time of packing. In general, total viable counts were higher in the bacons cured in 1,000 ppm and 500 ppm nitrite during storage than in that cured in 2,000 ppm. During storage similar numbers of lactic acid bacteria were detected on the bacon cured with 2,000 ppm and 1,000 ppm nitrite. Decreasing the nitrite concentration in the brine to 500 ppm (Comparison C) resulted in increased numbers of lactic acid bacteria during storage at 5°C and 15°C. The total viable count and numbers of lactic acid bacteria were higher both initially and during storage on the bacon cured in 250 ppm nitrite (Comparison D) compared with that cured in 2,000 ppm nitrite.

Yeasts were not detected on C2 bacon on packing and on all other bacons they were present in low numbers on packing (25 to 4,300/g). During storage at 5°C a slight increase in yeast numbers (less than tenfold) was detected on most bacons but at 15°C numbers generally declined. There was no marked difference between yeast numbers on compared bacons.

Gram negative bacteria were detected on all bacons on packing (25 to 1,000/g). Numbers of Gram negative bacteria remained constant or declined during storage of bacons in the A and B comparisons. In C1 and D2 bacons certain stored samples had higher counts of Gram negative bacteria than on packing but numbers were always low (<4,000/g) and no relationship emerged between their numbers and the different curing treatments.

Collar bacon. Total viable counts, numbers of Gram negative bacteria and numbers of lactic acid bacteria on collar bacon during storage at 5°C and 15°C are shown in Table 3. The inclusion of 5,000 ppm nitrate in the cure (Comparison E) reduced the total viable count on packing. During storage at 5°C and 15°C the total viable count was always highest on the bacon cured without nitrate. The same observations were made in Comparison F although differences between cures were smaller.

Growth of lactic acid bacteria was more rapid on collar bacon cured with no added nitrate (Comparison E) but in that cured with 2,000 ppm nitrite numbers of lactic acid bacteria were similar to those on bacon cured with 1,000 ppm nitrite + 5,000 ppm nitrate (Comparison F).

In Comparison E Gram negative bacteria were more common on packing on the bacon cured without added nitrate. During storage numbers of Gram negative bacteria increased slightly on the bacon cured without nitrate and decreased slightly on that cured with nitrate. Doubling the nitrite level without adding nitrate (F2) had little effect on Gram negative bacteria and numbers were still higher than on bacon cured with a mixture of nitrate and nitrite (F1).

In Comparisons E and F bacons, yeast numbers were in the range 6.0×10^3 to 4.0×10^4/g on packing and declined during storage. There was no marked effect in curing without nitrate on yeast numbers in collar bacon.

DISCUSSION

Back bacon (pH 5.5) with 50 ppm residual nitrite and 3.5% salt made from a nitrate-free brine gave a vacuum packed product which was as stable during storage as bacon made with a brine containing nitrate. Further, curing in a brine with no added nitrate and containing 1,000 ppm nitrite to produce bacon containing 80 ppm nitrite and 4.5% salt gave a vacuum packed product which was as stable as that cured to contain the maximum permissible levels of nitrite. Storage stability was also acceptable in back bacon cured with 500 ppm nitrite to contain 34 ppm nitrite and 4.5% salt, but higher numbers of lactic acid bacteria on this bacon indicated an increased risk of souring with storage. Storage stability was unsatisfactory in back bacon cured with 250 ppm nitrite to contain 17 ppm nitrite. At this low nitrite level souring occurred, possibly the result of increased growth of lactic acid bacteria (see also Wood, Evans & Razvi, 1972). No spoilage problems relating to yeasts or Gram negative bacteria were detected in vacuum packed back bacon cured without nitrate even when very low nitrite levels were used.

It is concluded that curing with a brine containing 1,000 ppm nitrite to produce back bacon with 60-100 ppm nitrite and 4% salt will allow, with a margin of safety, vacuum packed storage for at least 5 weeks at 5°C and between 1 and 2 weeks at 15°C.

Collar bacon differed in several respects from back bacon. The stability of collar bacon was improved by the inclusion of nitrate in the brine and shelf life was limited by the development of putrid off-odours. The interpretation of microbiological data in relation to shelf life is difficult since techniques may not detect the critically important bacteria. Nevertheless Gram negative bacteria commonly produce a putrid type of spoilage and it may be significant that the growth of these bacteria is reduced both before and after packing in the bacon cured with nitrate, in which putridity developed less rapidly. Further, nitrate contributed to the inhibition of lactic acid bacteria in collar bacon and may therefore delay souring. Although increasing the nitrite concentration in the cure (F2) resulted in similar growth of lactic acid bacteria in a bacon cured with nitrate, it did not bring about a similar stability with regard to shelf life, total viable counts and Gram negative bacteria. It is concluded that nitrate makes an important contribution to the stability of vacuum packed collar bacon with a specific effect on Gram negative bacteria and that the omission of nitrate from the Wiltshire cure may result in a reduced shelf life for this product.

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TABLE 1. Concentrations of salt, nitrite and nitrate added to brines and analysis of bacon at beginning of storage.

Comparison	Cure	Brines (Added Conc.) NaCl (%)	$NaNO_2$ (ppm)	$NaNO_3$ (ppm)	Bacon pH	NaCl (%)	$NaNO_2$ (ppm)	$NaNO_3$ (ppm)
BACK BACON								
A	1	26	1,000	5,000	5.5	3.2	52	524
	2	26	1,000	-	5.5	3.5	57	27
B	1	26	2,000	-	5.8	4.5	176	39
	2	26	1,000	-	5.7	4.5	81	25
C	1	26	2,000	-	5.7	4.5	150	26
	2	26	500	-	5.6	4.5	34	18
D	1	26	2,000	-	5.8	4.2	144	42
	2	26	250	-	5.7	3.5	17	7
COLLAR BACON								
E	1	26	1,000	5,000	6.0	5.4	126	746
	2	26	1,000	-	5.9	5.0	105	186
F	1	26	1,000	5,000	5.9	5.2	110	756
	2	26	2,000	-	6.0	5.3	225	97

TABLE 2. Total viable count and numbers of lactic acid bacteria on back bacon during vacuum packed storage at 5°C and 15°C.

Storage Period (Days)	LOG_{10} TOTAL VIABLE COUNT/g 5° CURE 1	5° 2	15° 1	15° 2	LOG_{10} COUNT OF LACTIC ACID BACTERIA/g 5° 1	5° 2	15° 2	15° 2
Comparison A								
0	5.0	5.1	5.0	5.1	<1.7	2.2	<1.7	2.2
2			4.9	4.8			<1.7	<1.7
6	4.8	4.4	5.0	4.6	<1.7	<1.7	5.0	4.3
13	5.2	4.4	5.8	5.3	4.4	3.3	6.3	5.8
20			5.4	5.2			5.3	6.0
22	5.2	4.2			5.2	4.5		
36	5.3	5.2			6.0	6.0		
Comparison B								
0	4.3	4.4	4.3	4.4	<1.7	<1.7	<1.7	<1.7
3							2.6	1.7
7	4.1	4.8	5.1	5.4	<1.7	<1.7	4.6	4.6
15	4.5	5.0	5.5	5.7	4.4	4.2	5.6	5.2
22	5.5	5.3			4.9	5.1		
34	4.8	5.1			5.5	5.6		
Comparison C								
0	5.3	5.2	5.3	5.2	<1.7	<1.7	<1.7	<1.7
3			5.0	5.5			3.9	6.2
7	5.0	5.1	5.1	5.1	2.5	2.8	5.7	6.5
13	5.0	5.3	5.6	5.8	4.5	6.1	6.9	6.9
21	5.2	5.6			5.6	6.4		
35	5.4	5.9			6.6	7.4		
Comparison D								
0	4.9	6.0	4.9	6.0	2.2	3.0	2.2	3.0
8			6.3	7.5			6.4	7.4
14	5.3	5.5			4.2	6.1		
19			6.8	7.7			6.9	7.3
35	6.8	7.5			5.4	7.2		

TABLE 3 Total viable count and numbers of Gram negative and lactic acid bacteria on collar bacon during vacuum packed storage at 5°C and 15°C

Storage period (Days)	Log_{10} total viable count/g 5° CURE 1	5° 2	15° 1	15° 2	Log_{10} count of Gram negative bacteria/g 5° 1	5° 2	15° 1	15° 2	Log_{10} count of lactic acid bacteria/g 5° 1	5° 2	15° 1	15° 2
Comparison E												
0	5.0	7.2	5.0	7.2	3.3	4.9	3.3	4.9	2.2	<1.7	2.2	<1.7
5			5.6	6.9			3.9	4.5			4.4	4.4
9	5.1	7.0	6.4	7.0	2.7	5.0	3.1	6.0	2.1	4.7	5.8	6.3
14	5.3	6.9	6.7	7.0	3.0	5.8	2.9	5.7	4.2	5.9	6.7	7.3
20	6.3	6.8			2.6	5.3			5.0	5.9		
Comparison F												
0	5.8	6.7	5.8	6.7	4.2	4.9	4.2	4.9	<1.7	<1.7	<1.7	<1.7
5			6.1	6.7			2.2	5.0			4.3	5.0
9	5.8	6.4	6.5	6.5	3.3	5.4	3.0	5.1	3.5	4.3	6.3	6.0
14	5.4	6.8	7.0	7.0	3.0	5.7	3.5	5.6	4.9	4.4	6.8	6.8
20	6.1	7.3			2.9	5.4			5.5	4.9		