NITRITES AND NITROSAMINES IN PROCESSED MEATS

SOME CHEMICAL STUDIES ON THE NITROSAMINE PROBLEM WITH RESPECT TO BACON PRODUCTION

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Because of the possibility of nitrosamine formation during bacon manufacture, the concentrations of volatile amines and nitrosamines in 10 pork *M. longissimus dorsi* were determined at the following stages of Wiltshire-type curing: immediately after slaughter, after hanging but before curing, after maturation, and after vacuum-packed storage of the bacon. One tertiary, two secondary and five primary amines were detected in concentrations ranging from 10 to 1900 µg/kg. Of the secondary amines detected, only dimethylamine (DMA) was present in significant quantity, and the mean concentration doubled from slaughter to the end of storage. No nitrosamines were found above the detection limit (1 µg/kg) in any of the uncooked pork or bacon.

In laboratory experiments, simulating normal bacon curing, the conversion of added DMA to N-nitrosodimethylamine (NDMA) at pH 5.6 was 0.1%. Addition of sodium ascorbate to the brine suppressed nitrosation in the brine and in the meat. Heating treated meat by canning or frying resulted in increased concentrations of NDMA, particularly in the fried fat. The lowest nitrosamine levels were found in meat cured in the presence of ascorbate, and the minimum molar ratio of ascorbate to nitrite necessary in the starting brine to obtain maximum suppression of NDMA formation was concluded to be 0.5.

QUELQUES ETUDES CHIMIQUES DU PROBLEME DE LA NITROSAMINE DANS LA PRODUCTION DU BACON

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A cause de la possibilité de la formation de nitrosamines au cours de la production du bacon, les concentrations d'amines volatiles et de nitrosamines dans 10 porcs *M. Longissimus dorsi* furent mesurées aux stades suivants de la conservation selon le procédé Wiltshire: immédiatement après l'abattage, après la pendaison mais avant la conservation, après la maturation et après le stockage du bacon dans des paquets sous vide. Une amine tertiaire, deux secondaires et cinq primaires furent décelées dans des concentrations allant de 10 à 1 900 µg/kg. Des amines secondaires décelées, seulement la diméthylamine (DMA) était présente en quantité significative, et la concentration moyenne doubla entre l'abattage et la fin du stockage. Aucunes nitrosamines ne furent décelées au-dessus du seuil de décelage (1 µg/kg) ni dans le porc ni dans le bacon crus.

Dans des expériences de laboratoire, où la conservation normale du bacon fut simulée, la conversion de la DMA ajoutée à N-nitrosodiméthylamine (NDMA) à pH 5,6 était de 0,1%. L'addition du sodium ascorbique à la saumure supprima la nitrosation dans la saumure et dans la viande. Le chauffage de la viande traitée au moyen de la mise en boîtes ou de la friture aboutit à une augmentation des concentrations de NDMA, surtout dans la graisse frite. Les niveaux les plus bas de nitrosamines furent décelés dans la viande conservée en présence d'acide ascorbique, et la proportion moléculaire minimum d'acide ascorbique à nitrite nécessaire dans la saumure initiale pour réaliser la suppression maximum de la formation de NDMA se révéla de 0,5.

EINIGE CHEMISCHE UNTERSUCHUNGEN ZUM NITROSAMINPROBLEM BEI DER SPECKERZEUGUNG

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Wegen einer möglichen Nitrosaminbildung bei der Speckerzeugung wurden die Konzentrationen von flüchtigen Aminen und Nitrosaminen in 10 *M. longissimus dorsi* (Schwein) bei den folgenden Stufen des Pökelns auf die Wiltshire-Art bestimmt: sofort nach der Schlachtung, nach dem Aufhängen aber vor dem Pökeln, nach der Reifung und nach der vakuum-verpackten Lagerung des Specks. Ein tertiäres, zwei sekundäre und fünf primäre Amine wurden in Konzentrationen von 10 - 1 900 µg/kg entdeckt. Von den festgestellten Sekundäraminen war nur Dimethylamin (DMA) in nennenswerten Mengen vorhanden, und der Konzentrationsmittelwert verdoppelte sich von der Schlachtung bis zum Ende der Lagerung. Im ungekochten Schweinefleisch oder Speck konnten keine Nitrosamine über der Bestimmungsgrenze von 1 µg/kg gefunden werden.

Bei Laborversuchen, die das normale Speckräuchern simulierte, betrug die Umsetzung von beigefügtem DMA in N-Nitrosodimethylamin (NDMA) 0,1% bei einem pH-Wert von 5,6. Der Zusatz von Sodiumaskorbat zum Salzwasser unterdrückte die Nitrosierung im Salzwasser und im Fleisch. Ein Aufwärmen des behandelten Fleisches durch Kochen oder Braten ergab erhöhte Konzentrationen von NDMA, besonders im gebratenen Fett. Die geringsten Nitrosaminmengen fanden sich in Fleisch, das mit Zusatz von Askorbat gepökelt wurde, und das geringste Molverhältnis von Askorbat zu Nitrit der anfänglichen Pökellauge muß 0,5 betragen, um die NDMA-Bildung optimal zu verhindern.

НЕКОТОРЫЕ ХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ ПРОБЛЕМЫ НИТРОЗАМИНА ПО ОТНОШЕНИЮ К ПРОИЗВОДСТВУ БЕКОНА

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Ввиду возможности образования нитрозамина в процессе изготовления бекона были установлены концентрации летучих аминов и нитрозаминов в 10 свинины *M. longissimus dorsi* на следующих этапах изготовления бекона методом Уилтшайра: непосредственно после убоя, после подвески но перед посолом, после вызревания и после хранения в вакуумных фасовках. Были обнаружены один третичный, два вторичных и пять первичных аминов в концентрациях от 10 до 1900 мкг/кг. Из обнаруженных вторичных аминов только диметиламин (DMA) присутствовал в значительных количествах и средняя концентрация его увеличилась вдвое с убоя до конца хранения. Ни в одной из исследованных проб сырой свинины или бекона не было обнаружено нитрозаминов в количествах, превышающих порог детектирования (1 мкг/кг).

В лабораторных опытах, моделирующих принятые процессы изготовления бекона, конверсия добавленного DMA в N-нитрозодиметиламин (NDMA) при pH 5,6 была равна 0,1%. Добавление к рассолу натриевого аскорбата подавляло нитризацию как в рассоле, так и в мясе. Нагревание обработанного мяса консервированием или жарением вызывало повышенную концентрацию NDMA, в особенности в жареном жиру. Низшие уровни нитрозамина были обнаружены в мясе соленом в присутствии аскорбата и было установлено, что минимальное мольное отношение аскорбата и нитрита в исходном рассоле, необходимое для максимального подавления образования NDMA должно быть порядка 0,5.

SOME CHEMICAL STUDIES ON THE NITROSAMINE PROBLEM WITH RESPECT TO BACON PRODUCTION

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Introduction

During recent years, there has been increasing concern at the possible occurrence of carcinogenic compounds in food ; N-nitrosodimethylamine (NDMA) has been found in various cured meat products [1-6] at the µg/kg (ppb) level and N-nitrosopyrrolidine has been detected in fried bacon [4-7]. Many nitrosamines can be formed by the reaction of the corresponding secondary amines with nitrite. Amines occur widely in nature as intermediate products in the protein metabolism of plants, animals and microorganisms and therefore can be expected to occur in most foods including meat and meat products. Thus the use of nitrite in the preparation of cured meats makes these food particularly suspect.

At present nitrite is essential for bacon manufacture. It imparts cured flavour, colour and microbiological stability, in particular inhibiting the growth of Clostridium botulinum, and, unless banned in future by law, its continued use seems certain for some considerable time, since no single alternative has been found which meets all these requirements.

In the first part of this paper, qualitative and quantitative data are presented on the volatile amines found in pork carcasses at four stages during bacon production. In the second part, results are reported of studies on the conversion of the secondary amine, dimethylamine (DMA) to NDMA under curing conditions, both in model chemical systems and in pilot-scale Wiltshire-type curing, and include the effect on nitrosamine formation of added ascorbate and of canning or frying the cured meat.

Part I Volatile amines in pork meat during bacon manufacture

Little information is available on the occurrence of individual amines, particularly secondary dialkylamines in red meats. Trace quantities of methylamine (MA) and trimethylamine (TMA), together with considerably higher concentrations of ammonia [8-10], have been reported in studies on the irradiation and flavour chemistry of beef. Low concentrations of TMA (2.5 mg/kg (ppm)) and DMA (0.4 mg/kg) were found [11] in minced meat (pork : beef, 1 : 1), and Japanese workers reported similar concentrations of secondary amines (calculated as DMA) in separate analyses of beef and pork (uncooked, < 0.2 mg/kg; cooked, < 0.5 mg/kg) [12]. Thirteen amines were identified in fresh beef during a study of packed meats [13], and included the C_1 - C_6 primary aliphatic amines, DMA and some diamines. None of these reports indicated whether the amines detected in the various meat samples were endogenous; nor did they deal with pork carcass meat intended for bacon production where the presence of secondary amines could lead to the formation of nitrosamines.

In the present study, the volatile amines in pork M.longissimus dorsi were extracted with trichloroacetic acid and also identified by gas chromatography and mass spectrometry; and the muscles were also analysed for the presence of volatile nitrosamines. Samples were taken successively from the right-hand side of each of 10 bacon-weight carcasses, selected at random after slaughter in a commercial abattoir, at the following stages of bacon manufacture by a commercial Wiltshire process : a) within 1 hour of slaughter, b) after 76 h. in commercial chill prior to injection and curing, c) at the end of curing and maturation (15 days after slaughter) and d) after vacuum-packed storage for either 28 days at 5°C (2 samples) or 43 days at 1°C (4 samples).

Results

MA, DMA, TMA, diethylamine (DEA), n-propylamine (n-PA) and iso-propylamine (iso-PA) were detected in the fresh pork, MA being present in greatest concentration. The mean concentrations of each of the other amines were less than one fifth that of the MA value and decreased in the order TMA, DMA, iso-PA and DEA (Table I).

Table I Changes in the concentrations of volatile amines in pork M. longissimus dorsi at various stages of curing and storage.

Means and ranges of 10 carcasses except where indicated; units µg/kg wet tissue.

Amine	Samples examined: immed. after slaughter	3 days post-slaughter, before curing	after curing and maturation	after storage (vacuum packed) 28 days/5°C	after storage (vacuum packed) 43 days/1°C
Methylamine	1490 (910-1940)	1064 (540-1520)	730 (520-920)	830^{+} (650-1010)	673^{++} (590-760)
Dimethylamine	135 (80-200)	175 (110-250)	191 (110-250)	205^{+} (160-250)	348^{++} (250-520)
Trimethylamine	286 (60-750)	365 (210-730)	477 (330-840)	635^{+} (510-760)	568^{++} (460-720)
Ethylamine	0	15 (0-20)	0	0^{+}	0^{++}
Diethylamine	10	10	30	10^{+}	10^{++}
n-Propylamine	41 (20-90)	58 (20-100)	116 (50-240)	90^{+} (90-90)	138^{++} (80-270)
iso-Propylamine	55 (20-110)	88 (50-120)	142 (60-430)	70^{+} (60-80)	70^{++} (50-110)

$^{+}$ 2 carcasses only; $^{++}$ 4 carcasses only.

During hanging at 4-5°C for 76h. between slaughter and curing, the mean concentration of MA decreased by 30%, whilst those of DMA and TMA each increased by approximately 30%, n-PA by 40% and iso-PA by 60%. DEA remained unchanged at less than 10 µg/kg, and in addition EA was detected in two samples at concentrations of less than 20 µg/kg.

After curing and maturation, the MA concentration decreased by a further 30%; TMA and iso-PA increased as previously by 30 and 60% respectively, and n-PA doubled. DMA increased by 8% and DEA marginally. No EA was detected.

Comparing freshly matured bacon with the same material after vacuum-packed storage, statistically significant increases (paired t-test, $p < 0.05$) were found in the concentrations of DMA and TMA; the concentrations of MA, DEA, n-PA and iso-PA were unchanged.

Thus, during the period of bacon manufacture, the concentrations of DMA, TMA, n-PA and iso-PA increased continuously up to the end of maturation, despite the presence of nitrite both in the cure and the bacon; thereafter, in storage, only DMA, TMA and n-PA contained to increase. The mean value for the concentration of DMA was below 200 µg/kg at the end of maturation but this increased to 350 µg/kg during storage; however, some individual figures were considerably higher, the maximum recorded being 520 µg/kg in a long-stored sample. The concentration of the other secondary amine, DEA, remained constant at a very low level throughout the entire period. As demonstrated in Part II of this paper, even the highest concentration of DMA recorded (520 µg/kg) is unlikely to yield, under normal conditions of curing, a concentration of NDMA in excess of the current detection limit of 1 µg/kg; at pH 5.6 and in the presence of sodium chloride, the conversion of DMA to NDMA is about 0.1%. This conclusion was supported by the results of analyses for steam volatile nitrosamines which showed no positives above the detection limit in any sample up to the end of maturation. Similarly, no nitrosamines were detected in three vacuum-packed samples stored at 1°C for 43 days; however, in one of two samples stored at 5°C for 28 days, there was an indication of NDMA and N-nitrosopyrrolidine at the detection limit, but identification could not be confirmed. Samples of commercial brines, containing initially 0.1% (1000 ppm) and 0.2% nitrite were also analysed for nitrosamines and none was found.

The results suggest that MA in pork was not of microbiological origin because of the significant concentrations present in the muscle immediately after slaughter, and the marked decrease which occurred during hanging for 3 days. Although the concentration of most of the other amines increased slowly throughout the 14 days of bacon manufacture, levels of secondary amines did not develop which could give rise to the formation of detectable quantities of nitrosamines. The results do not indicate whether these amines are of microbiological origin but the introduction of salt and nitrite, which would be expected to cause a change in microflora, did not influence their formation.

Part II Conversion of DMA to NDMA under conditions simulating Wiltshire-type curing

Ascorbic acid readily reduces nitrite and it has been shown to compete effectively with amines for available nitrite [14]. Ascorbate was therefore added to curing systems to determine its effect in reducing the formation of NDMA.

In order to ensure a measurable reaction, known concentrations of amine were introduced into pork meat before it was cured in one of the following systems: a) in vitro curing of pork slices in the laboratory, or b) experimental pilot-scale curing of pork middles.

In a) 1 cm thick slices of M.longissimus dorsi were suspended in physiological saline solution containing 150 mg/kg DMA (hydrochloride) held in a small chromatography tank. At the end of 24h., the meat contained about 100 mg/kg DMA. After draining, the slices were then suspended in freshly prepared curing brine for 72h. The brines, buffered with trisodium citrate and citric acid to pH values of 4.5, 5.0 and 5.6, contained 10% NaCl, 14.5 mM (1000 ppm) $NaNO_2$ with or without ascorbic acid (29.0 mM, 5100 ppm). Three additional concentrations of ascorbic acid (14.5, 7.25 and 3.62 mM) were also examined in brine buffered to pH 5.6. During this period, the concentration of amine in the meat fell to approximately one-third of its original concentration, the balance being leached into the brine, whilst the concentration of NaCl in the meat rose to about 5%. After curing the slices were allowed to mature at 2°C for 7 days before being vacuum packed and heated at 90°C for 0.5h.

b) Pork middles (weights: approx. 10kg) were pumped by repeated single needle injection until a 7 to 8% increase in weight was achieved. Four brines were used, each containing 26% NaCl and 14.5 mM (1000 ppm) $NaNO_2$, to which a solution of DMA (hydrochloride) was added immediately prior to use to give an ultimate concentration of 2.2 mM (100 ppm) DMA. At the same time, sodium ascorbate solution was added to give a final concentration of either 1.42, 7.1 or 14.2 mM ascorbate (equivalent to 250, 1250 or 2500 ppm ascorbic acid); the fourth brine was ascorbate-free. After pumping, the middles were soaked in a cover brine of the same composition for 4 days, followed by maturation at 4°C for 6 days.

Rashers from each middle were fried in a thermostatically controlled electric frying pan for 10 minutes after their temperature, recorded by thermocouple, had risen to 95-100°C; experience had shown that a final temperature of 140 ± 5°C was obtained in the rashers. The fat and lean were separated, and the procedure replicated until sufficient fried material was accumulated for analysis of NDMA in both the fat and the lean.

Table 2. NDMA formation in laboratory curing systems using pork slices containing added DMA

pH	Molar ratio of ascorbate to nitrite	NDMA (μg/kg) in Meat	NDMA (μg/kg) in Brine	Residual nitrite (ppm) Meat	Residual nitrite (ppm) Brine
5.6	2.0	20	2	45	380
"	1.0	7	3	90	490
"	0.5	14	9	140	510
"	0.25	45	50	240	600
"	0	100	80	245	650
5.0	2.0	ND	ND	5	-
"	0	60	190	135	685
4.5	2.0	1	ND	2	-
"	0	50	400	70	680

ND = not detected; - = not analysed.

Table 3. NDMA formation in pork middles cured in brines containing added DMA

Molar ratio of ascorbate to nitrite	NDMA (μg/kg) in cured meat (uncooked) lean	NDMA (μg/kg) in cured meat (uncooked) fat	NDMA (μg/kg) in meat after canning	NDMA (μg/kg) in meat after drying lean	NDMA (μg/kg) in meat after drying fat	Residual nitrite (ppm) in meat
1.0	ND	1	3	9	32	9
0.5	ND	5	6	7	88	12
0.1	10	4	18	26	144	87
0	7	3	10	29	84	84

Lean from each middle was also canned, cooked in an autoclave at 110° for 130 min (exceeding a commercial Fo = 3.0 process) and analysed for NDMA.

Results and Discussion

Table 2 shows the effect of pH and ascorbate on NDMA formation in the meat slices and associated brines. Table 3 shows the effect of using different molar ratios of ascorbate to nitrite in the curing of pork middles in the presence of added DMA, and the effect on nitrosamine formation of subsequent canning or frying.

At pH 5.6, in the absence of ascorbate, 100 μg/kg (100 ppb) NDMA were found in the meat, representing 0.1% conversion of the DMA in the meat at the start of curing i.e. under laboratory conditions simulating normal bacon curing, only 0.1% of the secondary amine was converted to detectable nitrosamine. As shown in Part I of this paper, the concentrations of DMA found in pork carcasses in a commercial bacon factory ranged from 80 to 250 μg/kg between slaughter and the start of curing; hence it may be calculated that the maximum concentration of NDMA which might be expected in normal uncooked bacon would be approximately 0.2 μg/kg, a concentration below the limit of detection by current methods of nitrosamine analysis.

The addition of ascorbate (2 mol/mol nitrite) to curing brines at pH 4.5 and 5.0 suppressed the formation of NDMA to 1 μg/kg or less in the meat and brine, while at pH 5.6, approximately 80% suppression of DMA nitrosation was found. In the five brines containing different molar ratios of ascorbate to nitrite at pH 5.6, a minimum ratio of 0.5 was required to give maximum suppression of nitrosation at pH 5.6.

The addition of ascorbate at a concentration of 0.5 mol/mol nitrite or greater to the brines spiked with DMA used to cure the pork middles, inhibited NDMA formation in the uncooked cured lean but did not appear to affect the small concentration of NDMA found in the uncooked fat (Table 3). Concentrations of NDMA were always increased by cooking; the highest concentrations were found in meat which had been injected with less than 0.5 mol ascorbate per mol nitrite, irrespective of the type of heat treatment. Nitrosation of DMA during curing and canning was suppressed approximately 80% when 1 mol ascorbate per mol nitrite was present in the brine.

High concentrations of NDMA were found in all the fried bacon. Quantities in the fried lean did not differ greatly from those found in the canned lean, but concentrations in the fatty tissue were variable and greater by a factor of 10 in some cases. The weight lost from the fat and lean parts of the rashers during frying averaged 50 and 58% respectively; thus drying out or rendering did not account for the ten-fold difference in the concentrations of NDMA. One possible explanation is that NDMA is fat soluble and would be expected both to accumulate in the fat and to remain dissolved rather than be lost by volatilisation.

Summary (Parts I and II)

1) One tertiary, two secondary and five primary amines were detected in pork before and after curing in concentrations ranging from 10 to 1900 μg/kg.

2) Up to 250 μg/kg DMA was detected in pork carcass meat before curing.

3) Introduction of sodium chloride and sodium nitrite did not influence the formation of amines in the cured meat.

4) No nitrosamines were found above the detection limit (1 μg/kg, 1 ppb) in any of the uncooked pork or bacon.

5) Conversion of DMA to NDMA at pH 5.6 in laboratory curing of pork slices was 0.1%, suggesting from 2) that the concentration of NDMA in Wiltshire cured bacon could be about 0.2 μg/kg (below detection limits).

6) Addition of ascorbate at a concentration in excess of 0.5 mol per mol nitrite would suppress nitrosation of DMA by 80%. The minimum molar ratio of ascorbate to nitrite necessary in the starting brine to obtain maximum suppression of NDMA formation was found to be 0.5.

7) Heating of the treated meat by canning or frying resulted in increased concentrations of NDMA, particularly in the fried fat.

Experimental details

Analysis of amines. Extraction was based on the method of Keay and Hardy /15/ using trichloroacetic acid, followed by steam distillation of the extract at alkaline pH. Identification and analysis was by gas chromatography and combined GC-MS; the most useful GC columns were Chromosorb 103 coated with 15% KOH and 20% Ucon fluid LB550X + 20% KOH on Chromosorb P (60-80 mesh). Recoveries from spiked meat samples were of the order:- MA, 40%; DMA and higher MW amines, 75-85%.

Analysis of Nitrosamines

All analyses for nitrosamines were carried out by the Laboratory of the Government Chemist using gas chromatography-mass spectrometry /4, 16, 17/ or by reduction and conversion of NDMA to the polyfluorinated amide derivative prior to estimation by electron capture gas chromatography /5/. The detection limit for both methods was 1 μg/kg.

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