

GASCHROMATOGRAPHIC HEADSPACE PROFILES OF CURED IN BAG BACON AND TANK CURED BACON.

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SUMMARY.

Four different gaschromatographic (GC) headspace methods have been evaluated for their suitability to analyse meat relevant volatiles in aqueous solutions, and a "purge and trap" method with a detection limit of 10 ppb was found to be most advantageous. This method was subsequently used to evaluate influence of two processing techniques used in bacon production ("cured in bag" versus "tank cured") on development of different volatiles during storage of the two products at 5°C. There was not found systematic differences in the GC-profiles of the two products during the first two weeks after slaughtering, corresponding to 13 days after vacuum packaging of the "cured in bag" product and 8 days after draining of the "tank cured" product. This was confirmed by an 8 membered trained sensory panel by sniffing. 25 days after slaughtering there was found a significant difference in the GC-profiles between the two products, which might be due to different microbial activity. This was confirmed by different nitrate-levels in the two products at this stage of storage.

INTRODUCTION.

It is well known that there is a highly selected microflora in cover brine used for production of tank cured bacon (Petäjä and Niinivaara, 1973; Gardner, 1980-81; Andersen and Hinrichsen, 1991). However, the influence of these organisms are unknown, although colour and flavour have been showed to improve, when some of these bacteria are injected together with brine in the production of ham (Meisel, 1989). "Tank cured" bacon types are claimed to be preferred by consumers to the more cost effective "cured in bag" type of bacon due to a better overall flavour production. The aim of present work has been to examine any difference in the overall production of volatiles by GC headspace techniques and sensory evaluation between "tank cured" and "cured in bag" bacon, as this may indicate differences between the two processing techniques regarding overall flavourproduction.

In the examination of a possible difference in developed volatiles in the two bacon types during storage, it was necessary to find and optimize a suitable and gentle gaschromatographic headspace method.

MATERIALS AND METHODS.

Optimization of gaschromatographic method. Several headspace methods (tabel I) were tested with a standard solution of components identified from porc-meat (van Straten and Maarse, 1983). The composition of the standard solution was acetaldehyde, dimethylsulfide, acetone, dimethyldisulfide, ethylbutanoate and acetoin, representing boilingpoints from 21 - 143°C. Internal standard was n-chloro-heptane, and resolution, recovery and reproducibility were observed.

Gaschromatographic analysis. Products were sliced (2 mm) and cut into small pieces. Approx. 50 g was placed in a Dreschelbottle and equilibrated for 30 min. at 50°C. Subsequently, headspace was purged through a tube containing 250 mg Tenax TA (mesh. 60-80) with a purgeflow at 60 ml/min for 10 min. using high quality N₂ as purge-gas. The loaded Tenax-tube were then thermally desorbed in a Spantech TD4 two stage thermal desorber, and chromatographed on a Perkin Elmer Capillary Gas Chromatograph 8420 equipped with PE Nelson data collection interface and a flame

ionisation detector. All analyses without MS were carried out with 5 replicates. Meat-samples for gaschromatographic/mass-spectrometric (GC/MS) analyses were placed in 20 ml headspace vessels, which were flushed with N₂ to avoid oxidation, and subsequently sealed with a teflon membrane. Vessels equilibrated for 30 min. at 70°C, and 10 ml headspace gas was withdrawn with a gastight syringe, injected into a coldtrap (modified Chrompack-unit) and finally transferred to a Hewlett-Packard Gas Chromatograph 5890 interfaced with a VG Analytical mass spectrometer using EI with a ionisation-potential at 70 eV. Data were compared with a data library and tentatively identified.

Tabel I. Headspace techniques tested for suitability for analysis of volatiles in non-heated meat.

I	Static headspace	- direct
		- direct - addition of KCl
		- direct - splitless
		- direct - splitless - addition of KCl
		- direct - splitless - coldtrapping
		- direct - splitless - coldtrapping - addition of KCl
II	Static headspace	- direct - cryofocusing
III	Vacuumdestillation	- direct
		- extraction
IV	Purge and trap	- onto Tenax TA

All GC-analyses were carried out with a DB-1701 capillary column (J & W Scientific) initially maintained at 40°C for 10 min, and programmed from 40 to 250°C at a rate of 6°C/min, with a final holding time of 5 min.

Samplepreparation. In order to avoid variation among individuals backs from left and right side, respectively, of a first class pig were processed in a slaughterhouse. Rinds were left on the backs, which subsequently were injected with brine. One back was cut into 5 pieces, and each piece was vacuumpacked and stored at 5°C. The other back was placed in brine for 4 days at 5°C. After tank-curing this back was stored aerobic at 5°C. Samples were taken out just before injection of brine (day 0) and then at day 7, 13, 20 and 25. Immediately after breakage of vacuumpackage the samples were analysed in Dreschel-bottles as described above to avoid loss of very volatile components. To avoid discrepancies in pH and fat along backs, they were sampled, so that the same place on the left respective the right back were sampled at the same time. The remaining part of the samples were kept frozen until analysed for NaCl, NO₃⁻, NO₂⁻ and total fat-content.

Sensory evaluation. At day 13 sensory evaluation was carried out. One slice of bacon was put into petri-dishes, sealed and equilibrated at 20°C. An eight membered trained sensory panel broke the sealed petri-dishes represented in a triangle-test, and examined samples by sniffing.

RESULTS AND DISCUSSION.

Based on resolution, recovery and reproducibility of tested standard solution, the GC headspace method deploying "purge and trap" was found most suitable for present application, and results after further optimalization of the "purge and trap"-method are listed in tabel II. Acetaldehyde, acetone and acetoin were not recovered at all, why 10 ppb was the lowest limit for detection of selected standards. The 95-% confidence intervals are low compared to the low concentration level, which means, that this method had a good reproducibility, which is also indicated by low values of coefficient of variance. At higher concentration-levels it was observed, that all standards were very well resolved on the chromatograms, despite the fact that most of them had little retention.

In evaluation of the gaschromatograms from the bacon-analyses the total amount of peak area were compared using a t-test and a general linear model. An examination of total production of volatiles during storage showed, significant difference between day 0 - 7 and 13 - 20 for the "tank cured" type. Contrary, no significant difference was found for the "cured in bag" between day 0 - 7, day 13 - 20 being significant. Not untill day 25 was there significant differences between the two types of bacon. Using principal component analysis on GC-data, chromatograms from day 0 were very similar. At day 25 chromatograms fell clearly into two parts in accordance with the two bacon-types as analyzed by principal component analysis, which indicate a qualitative difference in development of volatiles between "cured in bag" and "tank cured" bacon at this stage of storage. At least 6 peaks had a significant role in grouping of chromatograms. There was no systematic grouping of chromatograms from day 7 to 20.

Table II shows the results from optimalization of "purge and trap"-method evaluated with 1 ml 10 ppb standard solution.

Component	Mean ¹ (ppb)	Conf. ² (ppb)	CV ³	Recovery (%)
Acetaldehyde	0	-	-	0
Dimethylsulfide	3.04	0.61	0.196	30.4
Acetone	0	-	-	0
Dimethyldisulfide	1.24	0.07	0.055	12.4
Ethylbutanoat	1.84	0.11	0.057	18.4
Acetoin	0	-	-	0

¹Mean of 5 replicates. ²95-% confidence interval. ³Coefficient of variance.

Based on the fact, that "tank cured" bacon is exposed to oxygen during storage, and also have a higher NaCl content (Table III) compared to vacuumpacked "cured in bag" bacon, a higher production of volatiles in the "tank cured" bacon could be expected due to enhanced lipid oxidation, but as seen in fig. 1 the total production of volatiles during the storage period did not differ before very late in the storage period, and this difference is therefore likely to be due to enhanced microbial activity in the "tank cured" product. This is supported by a dramatic decrease (457%) in NO₃⁻-content of the product and a simultaneous increase in NO₂⁻-content (61%) from day 20 to 25 indicating high nitrate-reductase activity, compared to only a small decrease in NO₃⁻-content (62%) and no production of NO₂⁻ in the "cured in bag" product during the same period (table III). Observed late appearance

Table III. Results from chemical analyses. All results are mean of two analyses except pH, which is a mean of six analyses. Sampling at day 0 was just before injection of brine.

Sample	Day for sampling	NaCl (%) ⁵	NO ₃ ⁻ (ppm)	NO ₂ ⁻ (ppm)	total-fat (%)	pH
TC ¹						
TC	0	0	0	0	28.1	5.66
TC	7	3.2	224	28	19.2	5.82
TC	13	2.1	156	31	25.2	5.63
TC	20	1.8	128	28	28.9	5.86
TC	25	2.2	23	45	29.5	6.00
CIB ²						
CIB	0	0	0	0	27.6	5.49
CIB	7	1.3	102	17	22.9	5.85
CIB	13	1.3	104	16	25.2	5.86
CIB	20	1.4	112	12	28.6	5.58
CIB	25	0.8	68	4	26.3	5.91
TB ³						
TB	0	20.4	1700	1100		
IB ⁴	4	14.5	300	1200		
	0	17.1	1600	800		

¹TC: "Tank cured" bacontype. ²CIB: "Cured in bag"-type. ³TB: Tank brine and ⁴IB: Injection brine. ⁵Total NaCl-content.

in production of microbial volatiles in the "tank cured" product could be due to the fact, that strictly halphilic micro-organisms from the cover brine, where they showed a high nitrate-reductase activity (a decrease of 467% in

the cover brine during the curing proces, table III) enter an elongated lag-phase when changing micro-environment (from 20% NaCl in the cover brine to 3.2% in the product).

Tentatively identification of volatiles from the "tank cured" bacon product using GC-MS showed i.a. 3-methyl butanal, 3-methyl butanol, ethylbutanoate and pentylformate among a wide spectrum of hydrocarbons, carbonyls, esters and alcohols, indicating activity from a mixed flora of gram negative and positive organisms, respectively (Dainty, 1991).

Sensory evaluation of the two product types at day 13 after slaughtering showed no significant difference. This indicate, that it is doubtfull, whether micro-organisms from the cover brine produced volatiles at this stage of storage, which could result in any organoleptic significant contribution with respect to the overall flavour of the "tank cured" product compared to the "cured in bag" product. As

these kinds of green bacon usually are consumed 7 to 14 days after slaughtering the enhanced production of microbial produced volatiles found by the GC-analyses at day 25 in the "tank cured" product may not have any practical relevance for the overall flavour of this product. However, further investigations are nessecary to establish, whether this is the case or not for this particular product.

CONCLUSION.

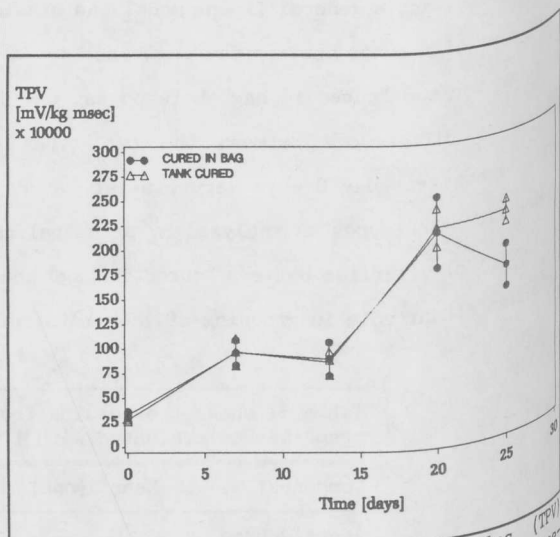
Students t-test, a general linear model and principal component analysis showed a difference in the fraction of volatiles between "cured in bag" and "tank cured" bacon after 25 days of storage by GC-analyses. This coincided with a dramatic drop in NO_3^- -content and a simultaneous increase in NO_2^- for "tank cured" bacon compared to "cured in bag" bacon, indicating microbial nitratereductase-activity in the "tank cured" product. Observed difference in total production of volatiles between the two products arose very late during storage, which make it unlikely, that observed difference has any effect on the overall organoleptic quality, as green bacon normally are consumed approx. 13 days after production, at which stage there was not found any difference in development of volatiles between the two products as confirmed by sensory evaluation.

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Figur 1. Total production of volatiles against storage time. On the plot 95%-confidence intervals are stated.