EFFECTS OF THREE BACTERIA ISOLATED FROM DANISH CURING BRINES IN A STERILE MEAT MODEL SYSTEM.

Hinrichsen, L.L. & Andersen, H.A.
Dept. of Dairy and Food Sciences, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg, DENMARK.

ABSTRACT

The effects of three bacteria strains, *Vibrio proteolyticus* (168), *Halomonas elongata* (16) and *Staphylococcus carnosus* (65) have been examined in a sterile meat model system.
Whole sterile meat cuts were injected with brine containing bacteria, brine containing disrupted bacteria or sterile brine as control. The meat cuts were subsequently vacuum packed and stored at 20°C for 14 days. Sampling was made 2, 6, 8, 10 and 14 days after production, and nitrite, nitrate and pH were either analysed or measured. Volatile compounds were analysed by headspace gas chromatography (HSGC), and smell analyses were carried out on day 14. Bacteria were monitored by plate count technique.
Strain 168 produced nitrite and reduced nitrate, while no effects were obtained with strains 16 and 65. None of the bacteria had any effect on pH in the meat. HSGC analyses showed that strain 168 produced two characteristic peaks after 6 days, whereas there was no effects of strains 16 and 65. The smell analyses showed that strain 168 had a characterictic smell designated cheesy. No difference was observed between strains 16 and 65 and their respective controls. Plate counts showed that strains 16 and 65 did not grow in the meat, but remained at approx. 10^5 cfu/g. In contrast strain 168 died out in 8 days, a result which coincided with changes in nitrite, nitrate and volatile compounds. Injection of lysate from strain 168 did not cause any effect, which may indicate that strain 168 enter a state of dormancy with coincident change in metabolism in 8 days.

INTRODUCTION

The flavour of bacon produced by the modern "cured in bag" (CIB) process is by consumers claimed to be inferior to bacon produced by traditional tank curing. In the CIB process the meat is injected with brine, equilibrated for 5 min. and subsequently vacuum packed, while in the traditional process the meat is initially injected with brine, salted in cover brine for at least three days and then stored aerobically on pallets before distribution. The difference in flavour might be due to the highly selected microflora found in cover brines. These are generated through decades by repeated back-sloppings (Leistner, 1958). It is well known that micro-organisms in brines are beneficial for development and stability of meat colour (Ingram, 1960), but it still remains unknown whether this microflora has any effect on the overall flavour development in traditional produced bacon.
The present study was undertaken to elucidate the bacterial effects, particularly in respect of volatile compounds, of three strains and their respective lysates in a sterile meat model system, simulating conditions in the CIB process. The three bacteria, *Vibrio proteolyticus* (168), *Halomonas elongata* (16) and *Staphylococcus carnosus* (65), were isolated from Danish cover brines as described by Andersen and Hinrichsen (1991). They are selected on their ability to grow at high NaCl-concentrations, pH=5.5, chill temperatures, anaerobic conditions, catalase-production and nitrate-reduction, respectively. The purpose of studying the effect of cell lysates was to examine the necessity of bacterial metabolism in order to introduce the desired effects in a model system during incubation.
Several investigations have already demonstrated the possibility of improving flavour of salted, raw meat by addition of micro-organisms (Leistner, 1958; Petäjä et al., 1973; Bartholomew and Blumer, 1977). However, these investigations have been carried out in aerobic systems and therefore are not representative for the modern CIB process, which is expected to dominate bacon production in the future.

MATERIALS AND METHODS

Sterile meat: The lower part of *m. Longissimus dorsi* from 24 h old pork carcasses was boiled for 60 sec. Heat treated part of the meat was subsequently cut off under sterile conditions. Each meat cut was divided into 5 chops (approx. 250 g). The chops was numbered from the lower part (1) to the upper part (5).

Cultures: Pure cultures of the strains 168, 16 and 65 were grown in 250 ml BHI-bouillon (Brain-Heart-Infusion, Difco, added 4 % NaCl) and harvested after 4 days of incubation at 20°C. Half of the cell material was diluted with sterile brine (21.02 % NaCl, 0.17 % KNO_3 and 0.09 % $NaNO_2$) to an optical density of 0.2 (670 nm). The other half of the cell material was washed 3 times with sterile water, resuspended in equal weight sterile water and added equal weight sterile glass beads (0.2 mm). This suspension was stirred for 30 min. on a Whirli-mixer. After 5 minutes without stirring, the supernatant was pippetted off and diluted with the same amount of brine as used for the first half of cell material.

Inoculation of meat: Brine containing bacteria, disrupted bacteria or sterile brine as control were injected into the meat to obtain a 10 % increase in weight. The chops were subsequently vacuum packed in polyethylene coated alu-bags and stored at 20°C. 5 replicates (same place on different muscles) were made, and sampling was made after 2, 6, 8, 10 and 14 days (numbered from 1 to 5 corresponding to place on muscles). Comparison of treatments were always within the same position on the muscles. This was done to minimize variation in lipid, pH and myofibrillar composition within treatments.

Analyses: NaCl, nitrate and nitrite were analysed according to the NMKL standard. pH was measured with injection electrode (Knick). Volatile compounds were analysed by the headspace gas chromatographic method described by Hinrichsen and Andersen (1991). Bacteria were cultivated on PCA (Plate Count Agar, Oxoid, added 4 % NaCl). All plates were incubated aerobically and anaerobically at 20°C for 5 days.

Sensory analyses: A laboratory panel evaluated the blind coded meat samples by smelling after 14 days.

RESULTS AND DISCUSSION.

Results of chemical analyses of sterile meat cuts injected with either brine containing bacteria strains 168, 16 and 65 or cell lysates from the same respective bacteria strains or brine (control) during the 14 days storage period are shown in tables 1A, B and C.

As the chemical results show, no significant differences in NaCl-concentrations between treatments were found, indicating that all meat samples had the same initial concentration of nitrate and nitrite after injection of the meat cuts.

Table 1A. Results from chemical analyses of cured meat inoculated with strain 168, lysate of strain 168 or sterile brine as control. Results with different letters are significantly different from each other (numbers in parenthesis are standard deviations).

	Days	2	6	8	10	14
Nitrite (ppm)	168	20.4 (9.15)^a	7.8 (3.03)^b	9.6 (1.1)^b	8.2 (2.77)^b	7.4 (2.30)^b
	Control	19.2 (3.03)^a	5.3 (1.53)^b	4.3 (1.15)^c	3.5 (1.00)^c	1.6 (0.55)^c
	Lysate	21.0 (3.65)^a	5.0 (0.00)^b	4.8 (0.96)^c	3.0 (0.82)^c	2.3 (0.50)^c
Nitrate (ppm)	168	87.0 (12.50)^a	71.7 (8.78)^b	74.4 (14.14)^b	77.8 (13.26)^b	52.8 (11.76)^c
	Control	105.2 (6.18)^a	91.3 (4.51)^a	89.6 (4.04)^a	115.0 (5.35)^a	108.4 (6.23)^a
	Lysate	98.8 (10.21)^a	97.8 (5.00)^a	108.3 (12.04)^a	111.5 (5.57)^a	101.3 (7.27)^a
NaCl (%)	168	1.7 (0.16)^a	1.6 (0.10)^a	1.6 (0.10)^a	1.6 (0.05)^a	1.5 (0.00)^a
	Control	1.6 (0.07)^a	1.5 (0.06)^a	1.6 (0.15)^a	1.7 (0.07)^a	1.7 (0.04)^a
	Lysate	1.6 (0.08)^a	1.6 (0.12)^a	1.6 (0.08)^a	1.6 (0.06)^a	1.6 (0.06)^a
pH	168	5.4 (0.09)^a	5.6 (0.04)^a	5.8 (0.04)^b	5.6 (0.04)^b	5.7 (0.03)^b
	Control	5.6 (0.17)^a	5.5 (0.00)^a	5.7 (0.06)^b	5.5 (0.05)^a	5.8 (0.03)^b
	Lysate	5.4 (0.05)^a	5.6 (0.05)^a	5.7 (0.05)^b	5.6 (0.05)^b	5.8 (0.05)^b

Table 1B. Results from chemical analyses of cured meat inoculated with strain 16, lysate of strain 16 or sterile brine as control. Results with different letters are significantly different from each other (numbers in parenthesis are standard deviations).

	Days	2	6	8	10	14
Nitrite (ppm)	16	19.4 (2.51)^a	7.4 (0.55)^c	4.2 (0.44)^d	2.2 (0.44)^d	3.0 (1.22)^d
	Control	18.8 (1.30)^b	9.0 (0.00)^c	3.3 (1.26)^d	2.8 (0.84)^d	1.6 (0.89)^d
	Lysate	16.6 (2.30)^b	5.8 (1.30)^c	3.2 (0.45)^d	2.0 (0.00)^d	2.6 (1.34)^d
Nitrate (ppm)	16	86.6 (12.81)^a	89.6 (3.65)^a	94.6 (1.52)^a	91.6 (5.11)^a	92.6 (11.37)^a
	Control	95.6 (5.08)^a	99.0 (0.00)^a	98.0 (6.16)^a	97.0 (4.64)^a	91.6 (13.07)^a
	Lysate	90.2 (14.54)^a	85.4 (7.92)^a	94.6 (2.04)^a	93.2 (7.05)^a	91.2 (1.34)^a
NaCl (%)	16	1.2 (0.18)^a	1.4 (0.05)^a	1.5 (0.09)^a	1.5 (0.06)^a	1.5 (0.10)^a
	Control	1.4 (0.10)^a	1.5 (0.00)^a	1.6 (0.10)^a	1.4 (0.09)^a	1.3 (0.19)^a
	Lysate	1.3 (0.25)^a	1.4 (0.11)^a	1.5 (0.06)^a	1.4 (0.09)^a	1.6 (0.06)^a
pH	16	5.5 (0.01)^a	5.6 (0.04)^a	5.6 (0.02)^a	5.7 (0.03)^b	5.8 (0.01)^b
	Control	5.5 (0.02)^a	5.6 (0.00)^a	5.5 (0.06)^a	5.7 (0.03)^b	5.8 (0.04)^b
	Lysate	5.5 (0.05)^a	5.6 (0.08)^a	5.6 (0.04)^a	5.6 (0.05)^b	5.7 (0.04)^b

Table 1C. Results from chemical analyses of cured meat inoculated with strain 65, lysate of strain 65 or sterile brine as control. Results with different letters are significantly different from each other (numbers in parenthesis are standard deviations).

	Days	2	6	8	10	14
Nitrite (ppm)	65	20.2 (1.64)^a	6.8 (0.84)^c	4.8 (1.30)^c	3.6 (1.52)^d	4.0 (1.22)^d
	Control	15.7 (3.06)^b	9.0 (1.41)^c	2.5 (0.71)^d	8.7 (10.69)^d	2.5 (0.71)^d
	Lysate	22.2 (1.48)^a	8.0 (2.12)^c	5.4 (0.89)^c	3.2 (0.84)^d	2.8 (1.48)^d
Nitrate (ppm)	65	106.6 (7.52)^a	110.0 (7.28)^a	101.0 (4.12)^a	108.2 (4.92)^a	90.4 (27.98)^a
	Control	91.3 (4.51)^a	104.0 (11.31)^a	91.5 (3.53)^a	99.7 (11.84)^a	94.0 (9.90)^a
	Lysate	106.2 (7.79)^a	105.0 (3.67)^a	105.0 (7.07)^a	97.8 (8.14)^a	88.4 (21.34)^a
NaCl (%)	65	1.5 (0.06)^a	1.5 (0.08)^a	1.5 (0.05)^a	1.6 (0.02)^a	1.7 (0.18)^a
	Control	1.4 (0.07)^a	1.5 (0.11)^a	1.4 (0.08)^a	1.5 (0.16)^a	1.5 (0.10)^a
	Lysate	1.5 (0.05)^a	1.5 (0.11)^a	1.5 (0.10)^a	1.5 (0.13)^a	1.6 (0.22)^a
pH	65	5.6 (0.06)^a	5.7 (0.06)^a	5.6 (0.03)^a	5.7 (0.03)^a	5.6 (0.05)^a
	Control	5.6 (0.07)^a	5.7 (0.08)^a	5.6 (0.06)^a	5.6 (0.03)^a	5.7 (0.01)^a
	Lysate	5.6 (0.04)^a	5.8 (0.05)^a	5.6 (0.02)^a	5.7 (0.04)^a	5.7 (0.06)^a

Strain 168 was able to produce nitrite and reduce nitrate as seen in Table 1A. These effects occurred after 6 days of storage at 20°C. Strains 16 and 65 had only a slight effect on nitrite formation most pronounced initially in the storage period (see Table 1B and C).

None of the tested bacteria had any effect on pH. There was, however, a small increase in pH during the storage period, which might be due to autolytic processes in the meat or to intramuscular variations.

HSGC analyses showed that strain 168 produced two characteristic volatile compounds (A and B) during the storage period. Figures 1A and B show gas chromatograms of separated constituents in the headspace of cured meat inoculated with strain 168 and the respective control after 14 days of storage.

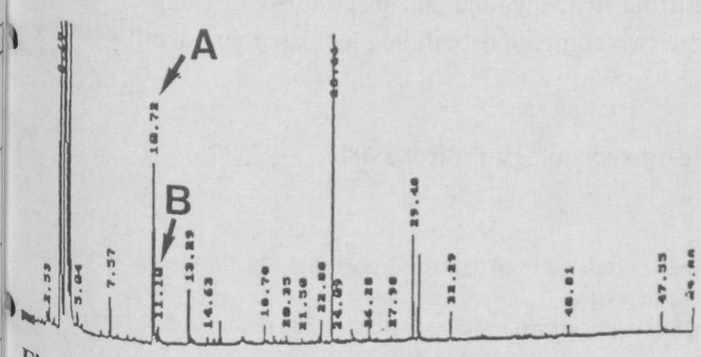

Figure 1A. Gas chromatogram of cured meat inoculated with strain 168 after 14 days of storage. A and B points out the volatile compounds produced by strain 168.

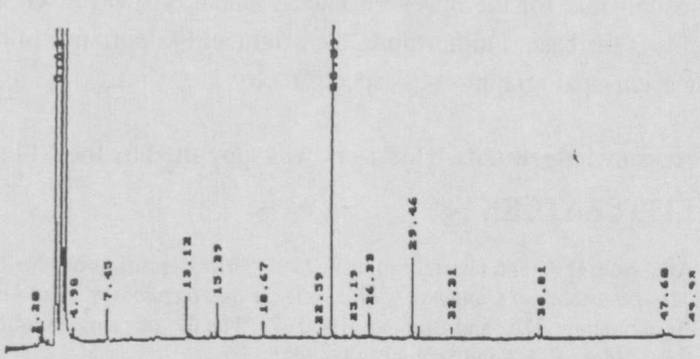

Figure 1B. Gas chromatogram of sterile cured meat after 14 days of storage.

Table 2 shows that the characteristic compounds, A and B, detected after 6 and 8 days of storage, respectively, in cured meat incubated with strain 168. No volatile compounds could be detected from incubation of cured meat with strains 16

and 65, and no volatile compounds were demonstrated in meat cuts injected with cell lysate of strains 168, 16 and 65 when compared to their respective controls.

Table 2. Quantitative estimation of compounds produced by 168. '+' designates a total peak area 0<+<500, 500<++<2000 and 2000<+++<5000.

Compounds\Days	2	6	8	10	14
A	-	+	+	++	+++
B	-	-	+	+	+

None of the three strains were found to be able to grow in the cured meat, although strains 16 and 65 remained at their initial inoculation level approx. 10^5 CFU/g throughout the storage period. Strain 168 could not be found after 8 days of storage as shown in Figures 2 and 3. The inability to grow coincided with observed changes in nitrite, nitrate and production of volatile compounds.

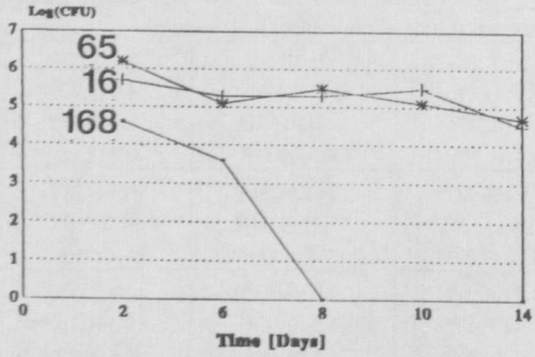

Figure 2. Changes in CFU (aerobic incubation) during the storage of meat inoculated with *Vibrio proteolyticus* (168), *Halomonas elongata* (16) or *Staphylococcus carnosus* (65).

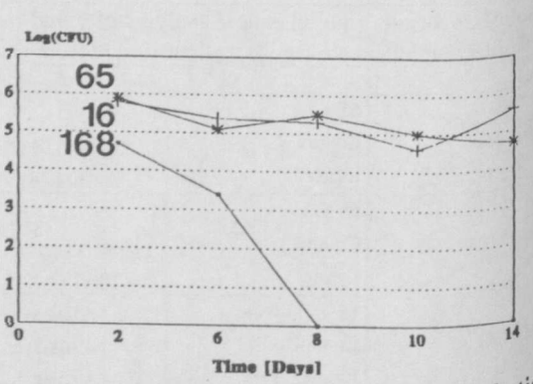

Figure 3. Changes in CFU (anaerobic incubation) during the storage of meat inoculated with *Vibrio proteolyticus* (168), *Halomonas elongata* (16) or *Staphylococcus carnosus* (65).

The formation of characteristic volatile compounds observed by HSGC in cured meat injected with strain 168 could be due to the liberation of endocellular enzymes by cell lysis. Apparently, this is not the case, as the same effect could not be obtained by injection of disrupted cells of strain 168. Another explanation could be that strain 168 becomes stressed under the conditions in question. As a consequence the bacteria turn into a state of dormancy and hereby a change in metabolism of the cells occur. This would also explain that the lysed cells did not show any effects.

Sensory analyses showed that only meat inoculated with strain 168 gave rise to different odour when compared to controls. Odour was described as cheesy by the sensory panel.

It is not known whether the two characteristic compounds found by HSGC in meat inoculated with strain 168 are responsible for the observed cheesy odour. However, GC-sniffing investigations are in progress to examine whether this is the case. Furthermore, the origin and importance of these two compounds will be elucidated via identification by gas chromatography-mass spectrometry.

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