

The influence of various gasses and gas mixtures on the bacterial growth on retail packed pork cutlets and in comminuted pork

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### Introduction

The principle of utilizing modified atmospheres as a means of prolonging the shelf life of fresh meats was first demonstrated by Kolbe (1882). Between the two World Wars fresh meat was shipped from South America and Australia to Europe in an atmosphere added 10% CO<sub>2</sub> (Hales, 1963). At that time it was claimed that higher CO<sub>2</sub> concentrations would damage the colour of fresh meat irreversibly. However, retailing of fresh meats in modified atmosphere has only been attempted on a large scale within the past couple of decades.

In Denmark two large supermarket chains are marketing fresh meats packed in a modified atmosphere consisting of a mixture of CO<sub>2</sub> and O<sub>2</sub>, sometimes also added small amounts of N<sub>2</sub>. This gas mixture will protect the colour of the meat and at the same time to some extent retard the growth of spoiling organisms and thus result in a prolonged shelf life. To achieve this the packaging operation must be combined with a high hygienic level, very fresh raw materials with a low level of bacteria, and effective chilling during distribution and retailing, preferably at 2°C.

The purpose of this investigation was to examine, how other gas mixtures than those ordinarily used would affect the growth of the microbial flora of fresh pork, notably *Brochotrix thermosphacta*, H<sub>2</sub>S producing and other psychotrophic bacteria.

In modified atmosphere many of the bacteria usually held responsible for the spoilage of fresh pork are affected. Shaw and Nicol (1969) thus found a doubled generation time of *Pseudomonas* at 5°C in 10% CO<sub>2</sub>. The growth of *Alcaligenes* was retarded in 20% CO<sub>2</sub>, and completely inhibited in 100% CO<sub>2</sub>. Similarly, Gill and Tan (1979 and 1980) found increasing inhibition of *Pseudomonas*, *Alteromonas putrefaciens* and *Yersinia enterocolitica* with increasing concentrations of CO<sub>2</sub>. However, these authors also found that *Lactobacilli* were unaffected by CO<sub>2</sub>, and that growth of *Brochotrix thermosphacta* was only affected in pure CO<sub>2</sub>.

With regard to the effect of N<sub>2</sub> the picture seems more complicated. Weidemann (1965) thus found no effect on *B. thermosphacta* in 100% N<sub>2</sub> at 0°C. Partmann et al (1971) found a retarding effect of bacterial growth on meat in 100% N<sub>2</sub> at 3°C and 7°C, but stressed that as little as 1% O<sub>2</sub> in N<sub>2</sub> was sufficient to change the growth conditions. Enfors et al (1979) found twice as long a generation time of the flora on pork at 4°C, when comparing 100% N<sub>2</sub> with the ordinary atmosphere. However, after 10 days they still found non-lipolytic and non-proteolytic *Pseudomonas* dominating. Huffman (1974), Huffman et al (1975) and Seideman et al (1979) found no retarding effect of 100% N<sub>2</sub>.

### Experimental

**Materials:** In each series was used pork cutlets and comminuted pork supplied by a supermarket chain.

**Packaging:** The cutlets were packed separately in packs and the comminuted pork was packed in ca. 100 g portions. The packaging operation was carried out on a Multivac deep-draw packaging machine. The bottom film consisted of a lamination of cast-polypropylene/poly-acrylonitrile/LD polyethylene and the top film of a lamination of polyester/poly-vinylidene chloride/LD polyethylene.

Head space analyses during storage were kindly performed by Otto Nielsen Emballage A/S

pH measurements were made on each series.

**Bacteriological examinations** were performed as follows: Total counts on Plate Count Agar (PCA), coliforms (37°C) on Violet Bile Agar (VBA), *B. thermosphacta* on Streptomycin-Thallium-Actidione Agar (STAA), *Lactobacilli* on Nitrite-Actidione-Polymyxine Agar (NAP) and H<sub>2</sub>S producing bacteria on Iron Agar (IA).

The investigation was carried out in 4 series: 1: in pure N<sub>2</sub>, 2: in pure CO<sub>2</sub>, 3: in a gas mixture of 20% CO<sub>2</sub> and 80% O<sub>2</sub> and 4: in a gas mixture of 40% CO<sub>2</sub> and 60% O<sub>2</sub>.

All 4 series were stored at 2°C and 5°C.

### Results and discussion

Figures 1 through 4 show the results of the bacteriological and head space analyses, as well as the results of the pH measurements of the 4 series.

It will be seen that the initial bacterial numbers are higher than usual for gas packed meats except for the series packed in 20% CO<sub>2</sub> and 80% O<sub>2</sub>. However, in all cases the initial bacterial numbers were low enough to observe the influence of the various gasses on the composition of the bacterial flora during storage.

**In pure N<sub>2</sub>,** see figure 1, no inhibition takes place of neither *B. thermosphacta* nor of H<sub>2</sub>S producing bacteria, and lactic acid bacteria seem to be favoured by growth in comminuted meat in comparison to on meat surfaces. There was an evident bloom in all packs when they were opened, even after 7 days, although there was a putrid odour at that time. The gas analyses showed that the production of CO<sub>2</sub> was of the order of 4 to 5% within 7 days at 2°C and 1 to 2% higher at 5°C.

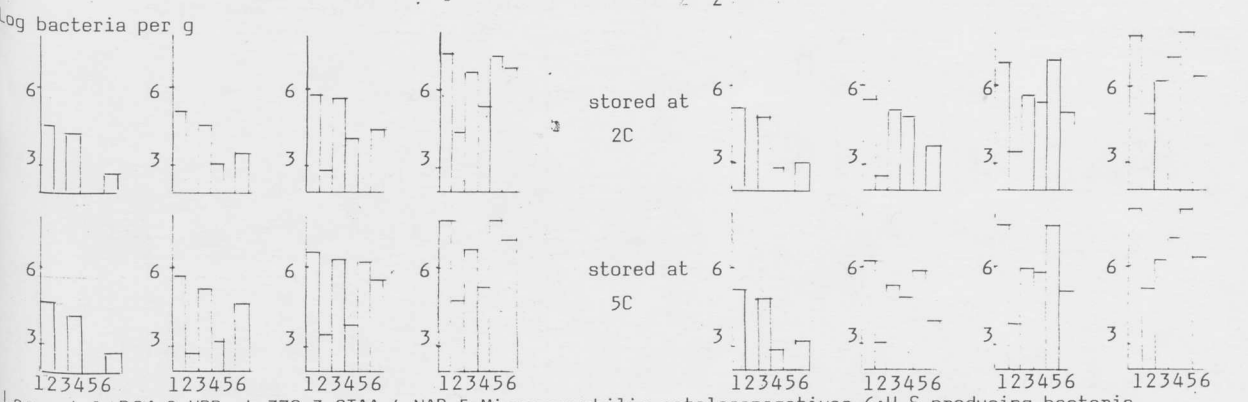
**In pure CO<sub>2</sub>,** see figure 2, the coliform bacteria were retarded in growth. Although the initial numbers of the meat packed in N<sub>2</sub> and in CO<sub>2</sub> were fairly much identical, it appears that the coliforms were suppressed in growth especially at 2°C. With regard to H<sub>2</sub>S producing bacteria, no growth was observed during storage. The low pH of the meat in this series may in itself be of importance, but the fact that the CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> ratio is high in the water phase of the meat at this pH, is probably also a contributing factor. Further, the growth of *B. thermosphacta* is strongly affected on the cutlets, and is only found in moderate numbers. Since *B. thermosphacta* was growing relatively well in pure N<sub>2</sub> it may be concluded that CO<sub>2</sub>, rather than lack of O<sub>2</sub> has an inhibiting effect.

The gas analyses revealed that some of the packs were slightly leaking, but on none of the days where packs were investigated, both packs were leakers. In the cases where packs were leaking, the results of the bacteriological analyses of these packs are omitted, i.e. the reported results are based on single determinations. The colour of the meat was light pink throughout storage, and bloom was observed when the packs were opened. Within the 7 days' storage, a faint off-odour was only observed on the last day in packs stored at 5°C.

Pork cutlets

Figure 1: stored in 100% N<sub>2</sub>

Comminuted pork



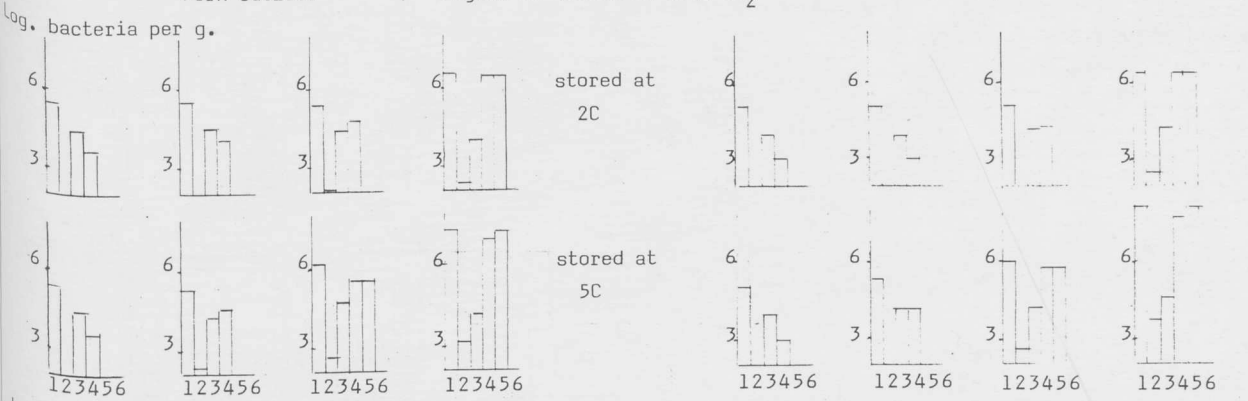
Legend 1:PCA 2:VRB at 37C 3:STAA 4:NAP 5:Microaerophilic catalasenegatives 6:H<sub>2</sub>S producing bacteria

Days	0				1				3				7				HEAD SPACE ANALYSES
	2C		5C		2C		5C		2C		5C		2C		5C		
O <sub>2</sub>	-	-	.09	.30	.24	.23	.09	.07	-	-	.07	.08	.24	.23	.06	.06	
			.10	.12	.24	.25	.08	.07			.07	.07	.23	.22	.07	.06	
N <sub>2</sub>	-	-	97	97	96	93	95	93	-	-	97	96	96	96	94	92	
			96	96	95	97	94	94			96	96	96	96	92	92	
CO <sub>2</sub>	-	-	1.8	1.5	2.9	4.9	3.6	6.0	-	-	1.8	2.5	2.6	2.2	3.5	6.2	
			2.8	2.4	3.7	2.5	4.0	4.8			2.6	2.1	2.2	2.2	5.7	6.2	
H <sub>2</sub> O	-	-	1.1	1.1	.80	1.4	1.4	1.1	-	-	1.5	1.4	1.3	1.4	2.1	1.6	
			1.4	1.3	1.4	1.4	1.7	1.4			1.4	1.3	1.3	1.5	2.1	1.7	
pH	6.48										6.25						
	6.41										6.16						

Pork cutlets

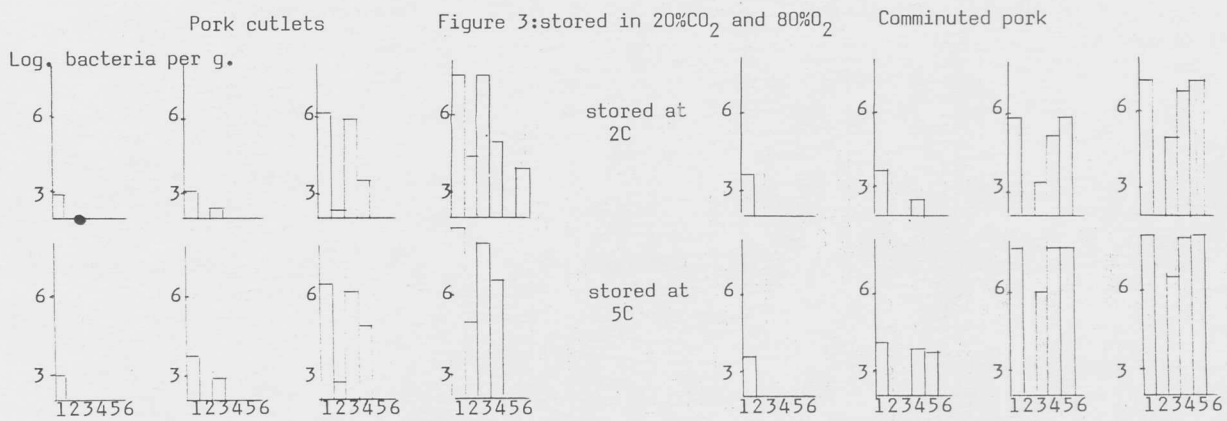
Figure 2: stored in 100% CO<sub>2</sub>

Comminuted pork



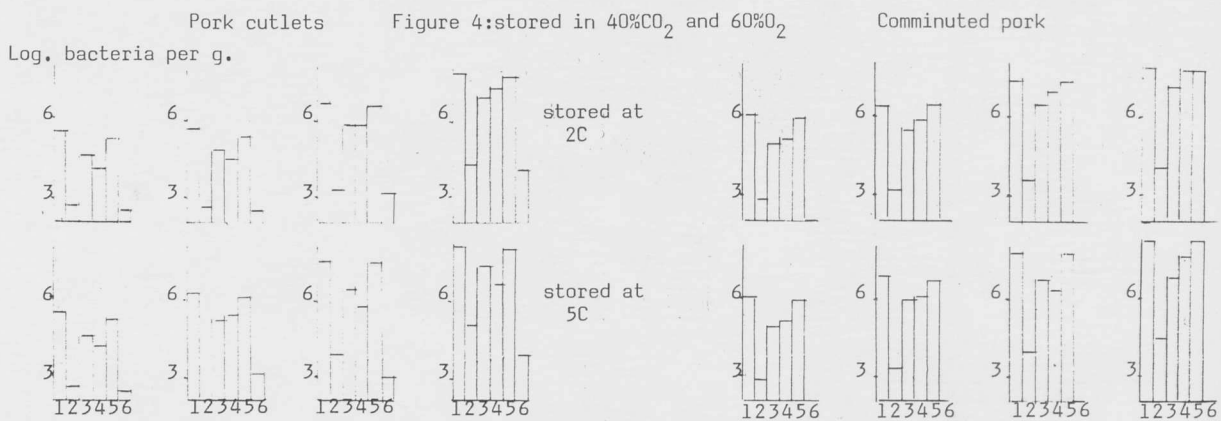
Legend 1:PCA 2:VRB at 37C 3:STAA 4:NAP 5:Microaerophilic catalasenegatives 6:H<sub>2</sub>S producing bacteria

Days	0		1		3		7		HEAD SPACE ANALYSES							
	2C	5C	2C	5C	2C	5C	2C	5C								
O <sub>2</sub>	-	-	.92	.04	.04	.68	.05	.04	-	-	.02	.03	.08	2.8	.05	.08
			.04	.05	.03	.03	.72	.05			.03	.03	.03	.04	.64	.82
N <sub>2</sub>	-	-	4.1	.56	.60	4.5	1.1	.56	-	-	.32	.57	1.5	15	.78	3.4
			.60	.52	.73	.41	7.7	1.2			.39	.61	.71	.66	6.4	12
CO <sub>2</sub>	-	-	94	98	99	94	97	98	-	-	98	98	98	82	98	95
			98	98	99	99	90	97			98	98	99	99	92	86
H <sub>2</sub> O	-	-	.84	1.0	.43	.56	1.4	1.5			1.5	1.2	.72	.42	1.0	1.5
			1.2	1.5	.67	.60	1.8	1.6			1.1	1.3	.67	.48	1.4	1.2
pH	5.88										5.81					
	5.71										5.89					



Legend 1:PCA 2:VRB at 37C 3:STAA 4:NAP 5:Microaerophilic catalasenegatives 6:H<sub>2</sub>S producing bacteria

Days	Pork cutlets								HEAD SPACE ANALYSES	Comminuted pork							
	0		2		7		10			0		2		7		10	
Gas	2C	5C	2C	5C	2C	5C	2C	5C		2C	5C	2C	5C	2C	5C	2C	5C
O <sub>2</sub>	-	-	82	81	81	81	80	66		-	-	79	76	75	73	75	66
N <sub>2</sub>	-	-	.73	.40	.83	.50	.62	1.9		-	-	2.2	.63	.79	.64	.94	.72
			.38	.46	-	-	-	-				.51	.52	-	-	-	-
CO <sub>2</sub>	-	-	16	17	16	18	18	31		-	-	19	23	23	25	22	32
			17	18	-	-	-	-				20	23	-	-	-	-
H <sub>2</sub> O	-	-	1.2	1.2	1.6	.97	1.2	.81		-	-	.56	1.1	1.1	.87	1.6	.76
			1.1	1.0	-	-	-	-				-	1.2	-	-	-	-
pH	5.59									5.72							
	5.60									5.69							



Legend 1:PCA 2:VRB at 37C 3:STAA 4:NAP 5:Microaerophilic catalasenegatives 6:H<sub>2</sub>S producing bacteria

Days	Pork cutlets								HEAD SPACE ANALYSES	Comminuted pork							
	0		1		3		7			0		1		3		7	
Gas	2C	5C	2C	5C	2C	5C	2C	5C		2C	5C	2C	5C	2C	5C	2C	5C
O <sub>2</sub>	-	-	68	67	70	65	64	58		-	-	75	67	72	63	56	44
			69	66	69	65	64	58				69	65	73	63	56	43
N <sub>2</sub>	-	-	.80	.85	1.7	1.9	1.5	.82		-	-	2.0	.66	1.9	.74	1.6	1.2
			.98	.67	1.6	.84	2.6	.81				.78	.51	1.7	.87	.98	1.0
CO <sub>2</sub>	-	-	30	31	27	32	33	39		-	-	22	31	24	35	41	53
			29	33	28	33	32	40				29	33	24	35	42	54
H <sub>2</sub> O	-	-	1.8	.71	.95	.73	1.3	1.6		-	-	.98	1.4	1.6	1.2	1.7	1.8
			1.6	.75	1.0	1.2	1.8	1.6				1.3	1.2	.94	1.2	1.4	1.8
pH	6.02									5.89							
	6.07									5.87							

The results of the series packed in a mixture of 80% O<sub>2</sub> and 20% CO<sub>2</sub> are shown in figure 3. Since the initial bacterial numbers were very low, it was decided to extend the storage to 10 days. The results after 7 and 10 days' storage are therefore based on single determinations. The results of the gas analyses show a considerable increase in the CO<sub>2</sub> concentration after 10 days at 5C, whereas the concentration at 2C remained fairly constant. On the cutlets, *B. thermosphacta* increased considerably in numbers during storage, especially at 5C. In this series, gramnegative, catalasepositive rods were found in large numbers in several cases which indicate that 20% CO<sub>2</sub> is too low to inhibit growth at high O<sub>2</sub> concentrations.

In the comminuted meat, *Lactobacilli* dominated the flora after a few days' storage, both at 2C and 5C. The colour was found poor after 7 days in all cases, and unacceptable after 10 days. Moderate off-odour was detected after 7 days, but except for the comminuted meat stored at 2C all other combinations were completely unacceptable after 10 days' storage.

The results of the series packed in 60% O<sub>2</sub> and 40% CO<sub>2</sub> are shown in figure 4. The gas analyses showed a large decrease in CO<sub>2</sub> concentration already after 24 hours, the largest decrease taking place in the packs stored at 2C. Presumably the gas has been dissolved in the water phase of the meat. During storage there was an increase of CO<sub>2</sub> though, especially in the comminuted meat. It is noticeable that the O<sub>2</sub> concentration remained high in the packs with cutlets, even after 7 days, and at both storage temperatures.

Unfortunately, the initial bacterial numbers were high in this series, but on the whole the development of the flora composition showed the same trend as shown in the packs with a gas mixture of 80% O<sub>2</sub> and 20% CO<sub>2</sub>, i.e. in the cutlets the dominating flora after 7 days' storage comprised lactic acid bacteria and *B. thermosphacta*. There was some growth of H<sub>2</sub>S producing bacteria and of coliforms.

In the comminuted meat lactic acid bacteria and *B. thermosphacta* also dominated, whereas no H<sub>2</sub>S producing bacteria were demonstrated, although the initial pH was above 6, so they would be expected to be able to grow. It therefore appears that the increased CO<sub>2</sub> concentration does not retard neither the growth of *B. thermosphacta* nor of coliforms in the presence of O<sub>2</sub>.

Because of the high initial numbers the storage stability was short in this series. Weak off-odour was detected already after 3 days at 5C and after 7 days at 2C. Both the cutlets and the comminuted meat had faded and showed no bloom on opening after 7 days' storage.

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