SHELF-LIFE OF EMULSION SAUSAGE STORED IN VACUUM OR MODIFIED ATMOSPHERES

ELISABETH BORCH & EVA NERBRINK

Swedish Meat Research Institute,
P.O. Box 504, S-244 00 Kävlinge,
Sweden

SUMMARY
The microbiological, physico-chemical (pH, drip) and sensory changes of an emulsion type of sausage stored in vacuum, 100% N_2, 30% CO_2 + 70% N_2, 50% CO_2 + 50% N_2, 70% CO_2 + 30% N_2 and 100% CO_2 atmospheres at 4°C, were studied. The total aerobic count reached 10^6 cfu/g after 17 days in vacuum and after about 27 days in modified atmospheres. The spoilage flora was dominated by homofermentative Lactobacillus spp.. L. alimentarius dominated in vacuum and 100% N_2, while Lactobacillus groups C, D and F dominated in CO_2 concentrations ≥30%. All sausages had an acceptable flavour after 40 days of storage. Before any defects in flavour were noted, the sausages were judged unacceptable due to the formation of slime. The amount of drip varied from 4-5% in 100% CO_2 to 0% in 100% N_2.

INTRODUCTION

Emulsion sausage stored in 100% CO_2 at 4°C may achieve an extremely good shelf-life (Blickstad & Molin, 1983). However, due to the dissolving of CO_2 in the sausage leading to swelling, modified atmospheres with mixtures of CO_2 and N_2 may be preferable. The effect of N_2 on the shelf-life of emulsion sausage varies from no advantage over vacuum (Simard et al., 1983) to an effective reduction in the growth rate of spoilage bacteria as opposed to vacuum (Blickstad & Molin, 1983).

The present study reports on the changes in microflora, pH, drip and flavour scores during 50 days of storage at 4°C in vacuum and modified atmospheres with varying CO_2 and N_2-concentrations.

MATERIALS AND METHODS

Experimental design

The details of the composition and the processing of the emulsion sausage ("falukorv") studied are given by Borch et al., (1988). Sausages with retained Naturin casing (Tripasin, Malmö, Sweden) were packed in gas-impermeable film (Lam-o-Foil A 15/9/75, a polyamid/ alufolie/ polyamid/polyethene film; permeability at 1 atm, 75% R.H., 25°C: O_2, <0.02 ml/m^2/24 h; CO_2, <0.1 ml/m^2/24 h; Otto Nielsen Ltd., Lyngby, Denmark) with one sausage of 550 g in each pack. The pouches were evacuated (4 mb) and then either sealed directly (vacuum-packed) or filled with a precise mixture of CO_2 and N_2 (100% N_2; 30% CO_2 + 70% N_2; 50% CO_2 + 50% N_2; 70% CO_2 + 30% N_2; 100% CO_2). The headspace in the modified atmosphere-packs was about 3 l. During storage at 4°C the sausages were subsequently removed for analysis.

Microbiological analysis

Slices (30 g) were cut through the casing and sausage. The samples were homogenized and analysed for total aerobic count (TGE agar, APT agar), lactic acid bacteria (AcA agar; pH 6.2), Brochothrix thermosphacta (STAA-agar), Enterobacteriaceae (VRBD agar), yeast/moulds (PD agar) and pH as described by Borch et al., (1988). Two sausages were examined on each occasion.

Identification

Isolates were picked at total aerobic counts of 5.3 to 6.9 log cfu/g from the duplicate TGE plates used for the total aerobic count - about 15 isolates were picked from each plate. All isolates were initially tested for Gram reaction, catalase reaction at 22°C, morphology, acid production from glucose in MRS fermentation broth and growth on STAA agar as described by Borch et al., (1988). Isolates which were Gram-positive, catalase negative, producing acid from glucose and not growing on STAA (i.e. lactic acid bacteria) were

further characterized by assimilation, acid production and other tests as described by Borch et al., (1988).

Numerical analysis

In order to group and identify the isolates, a dendrogram was constructed, based on the simple

Table 1. Reference strains included in the numerical analysis.

Strain/Cluster[1)]	Name and origin[2)]
R2/Cluster 4	Lactobacillus sp. SMRICC 247, homofermentative
R3/Cluster 1	Carnobacterium piscicola SMRICC 185
R9/Cluster 2	Carnobacterium divergens SMRICC 198
R12/Cluster 11	Lactobacillus sp. SMRICC 248, homofermentative
R14/Cluster 9	Leuconostoc sp. SMRICC 188
R16/Cluster 9	Leuconostoc sp. SMRICC 206
R20/Cluster 14	Lactobacillus sp. SMRICC 222, homofermentative
R21/Cluster 11	Lactobacillus sp. SMRICC 231, homofermentative
R25/Cluster 14	Lactobacillus sp. SMRICC 223, homofermentative
R29/Cluster 1	Carnobacterium piscicola SMRICC 197
R34/Cluster 12	Lactobacillus sp. SMRICC 236, homofermentative
R42/Cluster 10	Lactobacillus sp. SMRICC 249, homofermentative
R48/Cluster 12	Lactobacillus sp. SMRICC 238, homofermentative
R59/Cluster 9	Leuconostoc sp. SMRICC 215
R60/Cluster 12	Lactobacillus sp. SMRICC 194; homofermentative
R69/Cluster 2	Carnobacterium divergens SMRICC 186
R80/Cluster 12	Lactobacillus sp. SMRICC 250, homofermentative
R82/Cluster 4	Lactobacillus sp. SMRICC 251, homofermentative
R93/Cluster 11	Lactobacillus sp. SMRICC 235, homofermentative
R101/Cluster 8	Lactobacillus halotolerans DSM 20190^T
R102/Cluster 8	Lactobacillus minor DSM 20014^T
R103/ Cluster 8	Lactobacillus viridescens DSM 20248
R104	Lactobacillus confusus DSM 20196^T
R123	Lactobacillus plantarum DSM 20174^T
R128/Cluster 18	Leuconostoc mesenteroides ssp. cremoris CCM 2078^T
R148/Cluster 19	Lactobacillus viridescens CCM 56^T
R162	Lactobacillus sake DSM 20017^T
R163	Lactobacillus alimentarius DSM 20249^T

1) The strain and cluster numbers refer to the major clusters of Borch and Molin (1988). In the present study a prefix R is used to denote these reference strains.

2) SMRICC, Swedish Meat Research Institute Culture Collection
DSM, Deutsche Sammlung von Mikroorganismen

matching coefficient (Sneath, 1978) and clustered together with the unweighted pair group method using arithmetic averages (Romersburg, 1984). In the numerical study, 28 reference strains (Table 1) were included. In total, 196 strains were clustered using 23 characters.

Drip
The drip, i.e. free meat-juice in the pack, was weighted and calculated as a percentage of the sample weight.

Sensoric analysis
Thirteen experienced tasters scored samples for flavour, rating them on a 9-point structured scale (1 - very bad to 9 - very good). The samples were not heat treated before being presented to the tasting panel for evaluation.

The point of slime formation was recorded by a laboratory technician.

RESULTS AND DISCUSSION
Microbial analysis
The bacterial growth of emulsion sausage ("falukorv") stored in different gas atmospheres at 4°C is shown in Figure 1. The total aerobic count reached 10^6 cfu/g after 17 days in vacuum and after about 27 days in the modified atmospheres. The microbiological shelf-life was thus increased by 10 days in modified N_2+CO_2 atmospheres, as opposed to vacuum. For another type of emulsion sausage ("prinskorv"), Blickstad and Molin (1983) reported an extremely good microbiological shelf-life in 100% CO_2; no increase in total aerobic count was detected during 7 months of storage at 4°C. For yet another, but similar type of sausage ("wienerkorv", identical to "prinskorv" although longer) the total aerobic count reached 3.5 log cfu/g after 60 days of storage in a 50% CO_2 + 50% N_2 atmosphere while 6.0 log cfu/g was reached after 22 days in a vacuum-pack, at 8°C (Benny Landén, unpublished results). No significant difference in the microbial shelf-life of frankfurters

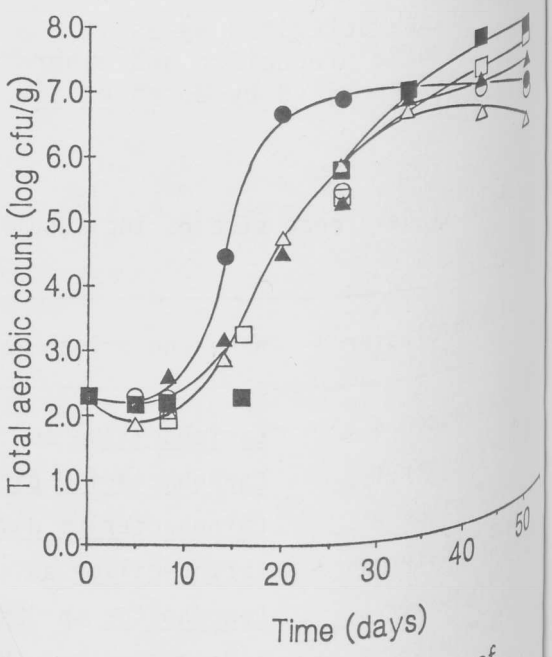

Figure 1. Total aerobic count of emulsion sausage stored at 4°C in ●, vacuum-pack; ▲, 100% N_2; △, 30% CO_2 + 70% N_2; ○, 50% CO_2 + 50% N_2; □, 70% CO_2 + 30% N_2; ■, 100% CO_2.

was found when comparing vacuum and 100% N_2 (Simard et al., 1983). Thus, the effect of modified N_2/CO_2 atmospheres, as opposed to vacuum, ranges from null to a significantly increased shelf-life prolonging effect. It was speculated by Blickstad and Molin (1983) that water activity/drip may be a controlling parameter. However, in the present study no difference in bacterial growth was found when comparing atmospheres without drip (100% N_2) with those having 1-5% drip (Table 2). Differences between other attributes of prinskorv/wienerkorv and falukorv such as film permeability, NaCl + $NaNO_2$ concentrations and amount of headspace/g of product do not explain the differences in the shelf-lives obtained. However, one factor that needs to be further evaluated is the type of casing used; naturin casing was used for falukorv, while natural casing was used for prinskorv/wienerkorv.

Table 2. Microbial counts, pH, drip and flavour scores of emulsion sausage stored in vacuum or modified atmospheres at 4°C.

Gas atmosphere	Storage time (d)	Total aerobic count (log cfu/g)	Lactic acid bacteria (log cfu/g)	pH	Drip (w/w, %)	Flavour score
Vacuum	0	2.3	0.4	6.0	-	5.8
	8	2.2	<1.0	-	1.2	6.2
	14	4.5	4.5	-	-	-
	20	6.7	6.9	6.0	1.0	5.6
	26	6.9	7.1	-	1.1	5.9
	35	-	-	-	2.2	5.5
	44	-	-	-	1.5	4.4
	50	6.4	6.4	-	0.9	4.2
100% N_2	8	2.5	<1.2	-	0	5.9
	14	3.1	3.0	-	-	-
	20	4.5	4.7	6.0	0	5.0
	26	5.3	5.3	-	0	5.9
	33	6.8	6.9	-	0	5.4
	42	6.8	6.9	6.0	0	5.6
	50	6.7	6.9	-	0	6.0
30% CO_2 + 70% N_2	8	2.1	<1.0	-	0	6.5
	14	2.9	3.3	-	-	-
	20	4.7	4.8	6.0	0	5.4
	26	5.9	5.9	-	0	6.4
	33	6.5	6.6	-	0.3	6.1
	42	6.3	7.0	5.8	0.1	6.2
	50	5.8	5.9	-	0.1	5.9
50% CO_2 + 50% N_2	8	2.3	<1.0	-	0	5.6
	16	2.7	2.7	-	-	-
	26	5.4	5.5	5.8	0	5.5
	33	7.0	7.1	-	0.1	5.5
	42	6.8	7.0	5.8	0	5.7
	50	6.3	6.4	-	0	5.5
70% CO_2 + 30% N_2	8	2.0	<1.0	-	0.2	6.1
	16	3.3	3.3	-	-	-
	26	5.4	5.6	5.8	0	5.6
	33	6.9	6.9	-	0.2	5.7
	42	7.0	6.8	5.7	0	5.1
	50	7.2	7.2	-	0	5.8
100% CO_2	8	2.2	<1.0	-	3.2	5.6
	16	2.3	<2.7	-	-	-
	26	5.8	6.0	5.6	5.1	5.8
	33	6.9	7.0	-	5.6	5.1
	42	7.5	7.6	5.4	4.0	6.0
	50	7.3	7.3	-	4.1	4.8

No *Brochothrix thermosphacta*, *Enterobacteriaceae* or yeasts and moulds were dectected (detection level 10 cfu/g) during storage. The count of lactic acid bacteria equalized to the total aerobic count after two weeks of storage (Table 2).

Identification

The spoilage flora consisted of *Lactobacillus* spp.. The relationship between the lactobacilli isolated from the different gas atmospheres is shown in Figure 2. Among the type reference strains included only *Lactobacillus alimentarius* DSM 20249^T and *Lactobacillus confusus* DSM 20196^T could be grouped together with the sausage isolates. The *L. alimentarius* group comprised 48 strains, among them *L. alimentarius* DSM 20249^T. Group A consisted of four homofermentative *Lactobacillus* strains. Group B consisted of 14 homofermentative *Lactobacillus* strains including R60 and R80 which belong to the unidentified cluster 12 of Borch and Molin (1988). In groups C, D and E 44, 22, and 23, respectively homofermentative *Lactobacillus* strains were included but no reference strains. Group F consisted of four homofermentative strains including R2 and R82 from cluster 4 (unidentified) of Borch and Molin (1988).

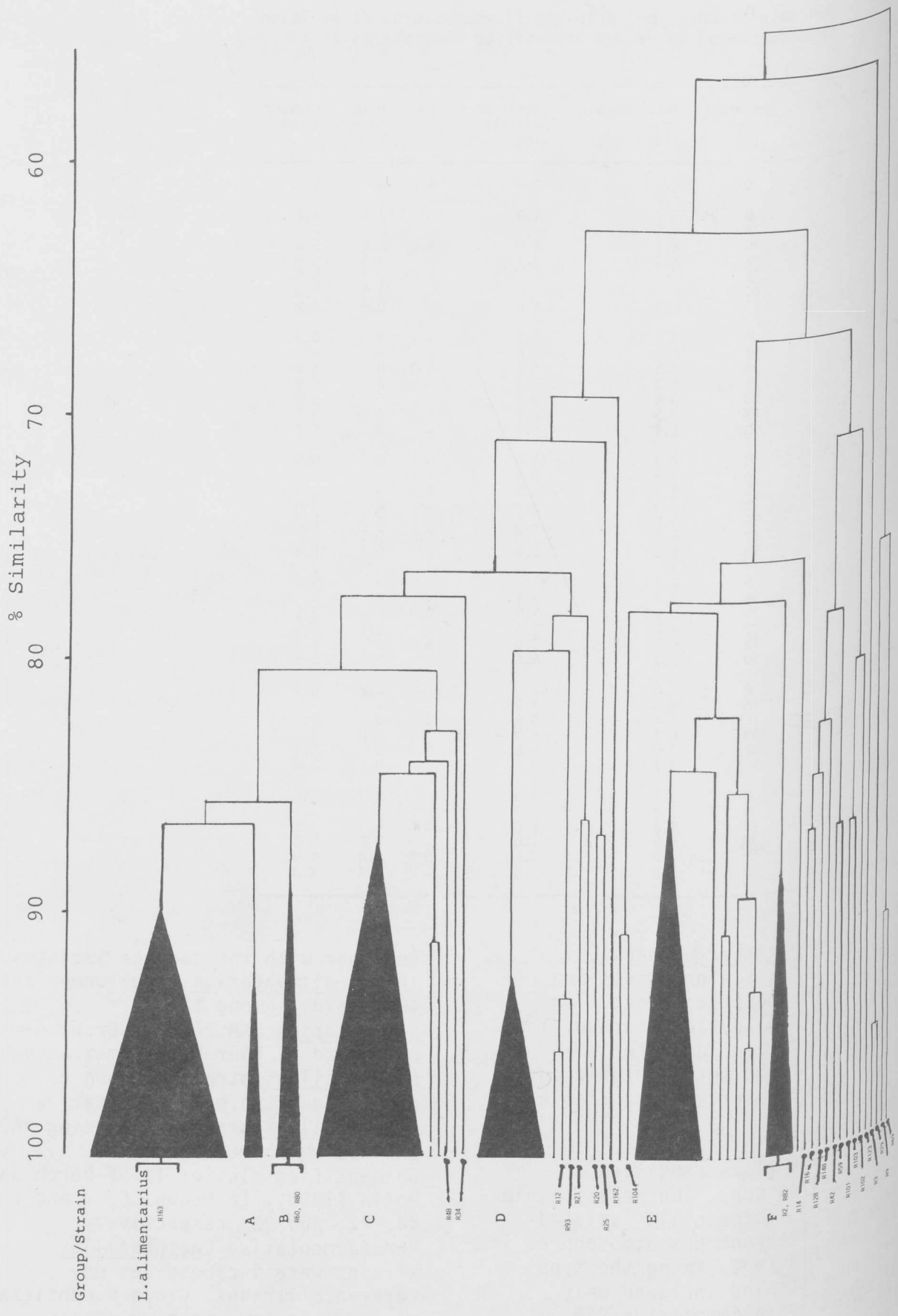

Figure 2. Simplified dendrogram showing the relationship between lactic acid bacteria of emulsion sausage.

In total, 8% of the strains could be assigned to two of the clusters (nos. 4 and 12) in the paper by Borch and Molin (1988). A major part of the strains (63%) was not identifiable with the reference strains included. The problem of identifying lactic acid bacteria from meat and meat products is well-known (Reuter 1975; Egan, 1983; Morishita & Shiromizu, 1986). Numerical taxonomic studies (Shaw & Harding, 1984; Borch & Molin, 1988) indicate that the reason for this is the occurrence of hitherto undiscovered/validly described Lactobacillus, spp. on meat and meat products.

L. alimentarius dominated the flora in vacuum and 100% N_2 atmospheres (Table 3). Groups C, D and E dominated in modified atmospheres with a CO_2 concentration $\geq$ 30%.

Sensoric evaluation

The flavour score during storage in vacuum is shown together with the increase in total aerobic count in Figure 3. The flavour score had significantly decreased after 50 days of storage and was at this point considered unacceptable (flavour score < 5). In contrast, the flavour of sausages stored in modified atmospheres was unaffected during 50 days of storage, with the exception of 100% CO_2 where a significant decrease was found at 50 days of storage (Table 2). The flavour remained acceptable until $\geq$20 days after the total aerobic count had reached the maximum level. This lack of correlation between total aerobic count and flavour defects is also demonstrated by Hill et al. (1976), Egan et al. (1980) and Korkeala et al. (1985). However, in the present study, before any defects in flavour were noted, the sausages were judged unacceptable due to the formation of slime. Slime formation was detected on sausages sampled after 20 d (vacuum), 33 d (100% CO_2) 33 d (70% CO_2), 42 d (30% CO_2) and 50 d (50% CO_2) of storage. No slime was formed in 100% N_2.

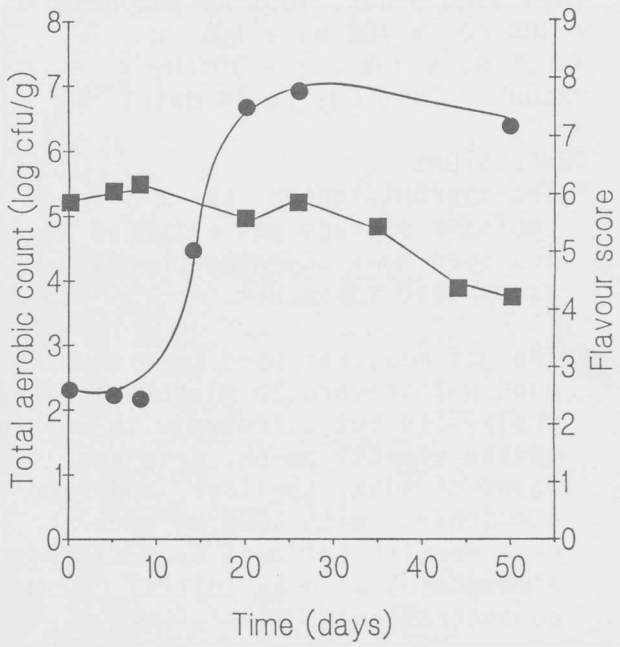

Figure 3. ●, Total aerobic count and ■, flavour score of emulsion sausage stored at 4°C in vacuum-pack.

Lactic acid bacteria may form slime from sucrose (Sharpe, 1962; Borch & Molin, 1988). In the present study, 65% of the isolated lactobacilli (homofermentative strains) were able to do so. However, strains being able to form slime from sucrose could be isolated from sausage which wasn't slimy (Table 3). Moreover, the sausage type studied does not contain sucrose. Consequently, in the present study, the source of the bacterial production of slime was not sucrose. These observations are in accordance with Korkeala et al., (1988) who reported that slime may be formed by homofermentative lactobacilli from some other carbon source than sucrose.

Physico-chemical changes

The initial pH of the sausages was 6.0 (Table 2). During storage, the pH decreased by 0.6 pH units in 100% CO_2, 0.2-0.3 pH units in atmospheres with pH 30-70% CO_2 and not at all in 100% N_2.

The drip, i.e. free meat-juice in the package, was affected by the atmosphere used and increased in the

following order; 100% N_2 (no drip) < 30% CO_2 + 70% N_2 ≈ 50% CO_2 + 50% N_2 ≈ 70% CO_2 + 30% N_2 < vacuum < 100% CO_2 (4-5% drip).

CONCLUSIONS

* The microbiological shelf-life of emulsion sausage was extended in modified N_2 + CO_2 atmospheres, as opposed to vacuum.

* The gas mixtures used were equally good with regard to microbiological shelf-life but differed with regard to the effects on pH, drip and point of slimy spoilage. Modified atmospheres with 100% N_2 gave the best result, followed by modified atmospheres with an initial CO_2 concentration ≤50% .

REFERENCES

Blickstad, E. & Molin, G. (1983): The microbial flora of smoked pork loin and frankfurter sausage stored in different gas atmospheres at 4°C. Journal of Applied Bacteriology 54:45-56.

Borch, E. & Molin, G. (1988): Numerical taxonomy of psychrotrophic lactic acid bacteria from prepacked meat and meat products. Antonie van Leeuwenhoek 54:301-323.

Borch, E., Nerbrink, E. & Svensson, P. (1988): Identification of major contamination sources during processing of emulsion sausage. International Journal of Food Microbiology 7:317-330.

Table 3. The microbial flora of emulsion sausage and slime formation after storage in vacuum or modified atmospheres at 4°C.

Organism	Distribution (%) in different gas atmospheres					
	Vacuum	100% N_2	30% CO_2 + 70% N_2	50% CO_2 + 50% N_2	70% CO_2 + 30% N_2	100% CO_2
Lactobacillus alimentarius	57	77	-	23	-	-
Lactobacillus confusus	3	-	-	-	-	-
Lactobacillus group A	3	10	-	-	-	-
Lactobacillus group B	27	7	-	7	-	4
Lactobacillus group C	-	3	30	47	63	64
Lactobacillus group D	-	-	17	10	-	9
Lactobacillus group E	7	3	37	-	27	-
Lactobacillus group F	3	-	-	-	-	14
Lactobacillus spp.[a]	-	-	13	13	7	9
Non-culturable	-	-	3	-	3	
Total no. of isolates	30	30	30	30	30	22
Log cfu/g	6.9	5.3	6.5	5.4	6.9	6.9
Storage time (d)	26	26	33	26	33	33
Slimy spoilage	yes	no	no	no	yes	yes
% isolates forming slime from sucrose	53	33	80	87	100	27

a Non-groupable according to the dendrogram structure (Fig. 2)

Egan, A.F. (1983):
Lactic acid bacteria of meat and meat products. Antonie van Leeuwenhoek 49:327-336.

Egan, A.F. Ford, A.L. & Shay, B.J. (1980):
A comparison of Microbacterium thermosphactum and lactobacilli as spoilage organisms of vacuum-packaged sliced luncheon meats. Journal of Food Science 45:1745-1748.

Hill, W.M., Reaume, J. & Wilcox, J.C. (1976):
Total plate count and sensory evaluation as measures of luncheon meat shelf life. Journal of Milk Food Technology 11:759-762.

Korkeala, H., Lindroth, S., Suihko, M., Kuhmonen, A. & Penttilä, P.-L. (1985):
Microbiological and sensory quality changes in blood pancakes and cooked ring sausage during storage. International Journal of Food Microbiology 2:279-292.

Korkeala, H., Suortti, T. & Mäkelä, P. (1988):
Ropy slime formation in vacuum-packed cooked meat products caused by homofermentative lactobacilli and Leuconostoc species. International Journal of Food Microbiology 7:339-347.

Morishita, Y. & Shiromizu, K. (1986):
Characterization of lactobacilli isolated from meats and meat products. International Journal of Food Microbiology 3:19-29.

Reuter, G. (1975):
Classification problems, ecology and some biochemical activities of lactobacilli of meat products. In: Lactic acid bacteria in beverages and food. Eds. Carr, J.G., Cutting, C.V. and Whiting, G.C. pp. 221-229, Academic press, London.

Romesburg, H.C. (1984):
Cluster analysis for researchers. Lifetime Learning Publications, Belmont, California.

Sharpe, M.E. (1962):
Lactobacilli in meat products. Food Manufacture 37:582-589.

Shaw, B.G. & Harding, C.D. (1984)
A numerical taxonomic study of lactic acid bacteria from vacuum-packed beef, pork, lamb and bacon. Journal of Applied Bacteriology 56:25-40.

Simard, R.E., Lee, B.H., Laleye, C.L. & Holley, R.A. (1983):
Effects of temperature, light and storage time on the microflora of vacuum- or nitrogen-packed frankfurters. Journal of Food Protection 46:199-205.

Sneath, P.H.A. (1978):
Classification of microorganisms In: Essays in Microbiology. Eds. Norris, J.R. and Richmond, M.H. pp. 9/1-9/31. Wiley, London.