SPOILAGE POTENTIAL OF LACTIC ACID BACTERIA ON VACUUM PACKED BEEF

Ylva Blixt and Elisabeth Borch
Swedish Meat Research Institute, P.O. Box 504, S-244 24 Kävlinge, SWEDEN.

Keywords: Spoilage, lactic acid bacteria, *H. alvei*, interaction.

BACKGROUND

Evaluation of the shelf life of meat is today based on microbiological standards. In Sweden, the maximum acceptable level of bacteria on vacuum packed beef is 7 log cfu/g, according to national guidelines. However, the correlation between total bacterial numbers and sensorial spoilage is imprecise (1). If instead specific spoilage bacteria are identified, the growth and activity of these bacteria may be used as spoilage indicators. The dominating bacteria on vacuum packed cold-stored beef are lactic acid bacteria such as *Carnobacterium* spp., *Lactobacillus* spp., *Leuconostoc* spp. and *Weissella* spp. Different bacteria belonging to the group of *Enterobacteriaceae* may also occur at levels of 5 log cfu/g.

OBJECTIVE

To determine the ability of single and mixed cultures of lactic acid bacteria and *Enterobacteriaceae* to produce off odours on vacuum packed cold-stored beef.

METHODS

Beef strip loins (*M. longissimus dorsi*) were sterilised by thoroughly burning the meat surface. The burnt surface was removed with sterile knives and pincers, working from the centre to the periphery.

Pure strain cultures of lactic acid bacteria and *Enterobacteriaceae* were grown in All Purpose Tween (APT) at 25°C and in Tryptone Glucose Extract (TGE) at 30°C, respectively, for 1 day and diluted in 0.85% NaCl before being added to the sterile meat.

Twelve different strains (Table 1) were tested for their ability to produce off odours on vacuum packed normal pH-meat and DFD-meat. The inoculation level (N_0) was 4 log cfu/cm² and the inoculated meat was stored at 4°C in vacuum packs (O_2-permeability= 4ml/(m² x 24h)). The odours that developed were described by a sensory panel.

In addition, pieces of sterile meat were inoculated with mixtures of bacterial strains (N_0=1.7 - 2.5 log cfu/cm²) isolated from spoiled vacuum packed beef strip loin: Series A=10 different isolates of lactic acid bacteria, series B=10 different isolates of *Enterobacteriaceae* and series C= a mixture of the lactic acid bacteria and *Enterobacteriaceae* strains used i series A and B. The lactic acid bacteria were characterised as heterofermentative *Lactobacillus* spp., *Leuconostoc/Weissella* spp., *Leuconostoc pseudomesenteroides*, *Lactobacillus sake* and *Lactobacillus pentosus*, while the Gram negative isolates were all identified as *Hafnia alvei*. The meat was stored at 4°C for 6 weeks in vacuum packs as above. Pieces of meat were analysed for the total number of bacteria and the number of *Enterobacteriaceae* during the storage period. A sensory panel assessed the meat for acidic, vacuum pack spoilage and sulphurous odours. The odours were scored from 1-3, where 1=no odour and 3= a lot of odour.

RESULTS AND DISCUSSION

In general, the bacteria caused more unpleasant odours when grown on DFD-meat than on normal pH meat (Table 1). After 44 days of storage odours on normal pH-meat were characterised as, e.g., sulphurous/H_2S, sweet, acid, fresh and bitter. Similar odours were found on the DFD-meat, though the smell of H_2S was more intense and appeared more frequently, than on normal pH-meat. However, the typical vacuum pack spoilage odour (dense, sour and slightly putrid) was not obtained, neither on normal pH- nor DFD-meat. This may indicate that a bacteria-bacteria interaction is important for this typical off odour.

The total number of bacteria reached a plateau of about 7 log cfu/cm² after 2 weeks of storage on meat inoculated with a mixture of lactic acid bacteria and on meat with a mixture of lactic acid bacteria and *H. alvei* (Figure 1). The presence of lactic acid bacteria clearly inhibited the growth of *H. alvei* since, on meat solely inoculated with *H. alvei*, this bacteria reached a concentration of about 6 log cfu/cm², while in the presence of lactic acid bacteria, this concentration was one log unit lower. The total number of bacteria showed the same growth pattern on meat solely inoculated with lactic acid bacteria and on meat inoculated with both lactic acid bacteria and *H. alvei*. Thus, the presence of *H. alvei* did not influence the growth pattern of the total number of bacteria.

On meat inoculated solely with lactic acid bacteria, the development of the vacuum pack spoilage odour and that of the sulphurous odour were similar (Figure 2). However, if *H. alvei* was also present, it was rather the acidic odour that co-developed with the vacuum pack spoilage odour, while the sulphurous odour only showed a modest increase. The intensities of the acidic, sulphurous and vacuum pack spoilage odours were low and fairly constant on sterile meat and meat inoculated solely with *H. alvei* throughout the storage period (data not shown).

The lactic acid bacteria at the end of the storage period were characterised as *Leuconostoc/Weissella* spp. and *L. sake* on meat that was solely inoculated with lactic acid bacteria at the outset, while, if *H. alvei* was present, the only recovered lactic acid bacteria were *Leuconostoc/Weissella* spp. Thus, without affecting the total number of bacteria, the presence of *H. alvei* influenced the selection of lactic acid bacteria on the stored meat, by specifically repressing the growth of *L. sake*. The difference in the lactic acid bacteria

composition was also mirrored in the different odour patterns. The presence of *L. sake*, a bacterium previously shown to produce H_2S (2) on meat, led to a more sulphurous odour.

CONCLUSIONS

* It was indicated that a bacteria-bacteria interaction is needed for the development of spoilage odours on vacuum packed beef.
* *H. alvei* specifically repressed the growth of *L. sake* and thereby affected the spoilage on vacuum packed beef by decreasing the production of sulphurous odours.

LITERATURE

1. Borch, E. and Agerhem, H., 1992, Chemical, microbial and sensory changes during the anaerobic cold storage of beef inoculated with a homofermentative *Lactobacillus* sp. or a *Leuconostoc* sp., Int. J. Food Microbiol. 15:99-108.
2. Egan, A F. Shay, B J. Rogers, P J., 1989, Factors affecting the production of hydrogen sulphide by *Lactobacillus sake* L13 growing on vacuum-packaged beef. J Appl. Bact. 67:255-262.

TABLES AND FIGURES

Table 1. Odour development of single bacterial strains on normal pH-and DFD-beef stored in vacuum packs at 4°C for 44 days.

Strain	Odours, normal pH	Odours, DFD meat	Origin[1)]
Brochothrix thermosphacta	Butter	Fresh, yeast	ATCC 11509^T
Carnobacterium divergens	Acid, putrid, pungent	Fresh, aromatic, bitter	NCFB 2763^T
Lactococcus raffinolactis	Sweet, sour milk, bitter, fresh herring	Sweet, sour milk, bitter, fresh herring	NCFB 617^T
Lactobacillus sake	Acid, fresh, H_2S, slightly sweet, heavy	H_2S	100% CO_2 packed pork
Homofermentative *Lactobacillus* sp.	Butter, H_2S	H_2S	SMRICC 235, vacuum packed beef
Homofermentative *Lactobacillus* sp.	Fresh, sour milk, slightly H_2S	Slightly H_2S	Vacuum packed pork
Homofermentative *Lactobacillus* sp.	H_2S	H_2S	Vacuum packed beef
Homofermentative *Lactobacillus* sp.	Slightly H_2S	H_2S, fresh herring	Vacuum packed pork
Leuconostoc sp.	Acid, fresh	H_2S	SMRICC 219, vacuum packed beef
Leuconostoc sp.	Acid, sour milk	H_2S	Vacuum packed pork
Serratia liquefaciens	Fresh, bitter, sweet	Very heavily sweet, acid, aromatic	CCM 2717
Serratia liquefaciens	Acid, bitter	Fresh, slightly H_2S, aromatic	Vacuum packed pork

1) ATCC=American Type Culture collection, NCFB=National Collection of Food Bacteria, SMRICC=Swedish Meat Research Institute Culture Collection, CCM=Czechoslovak Collection of Microorganisms, T=Type strain.

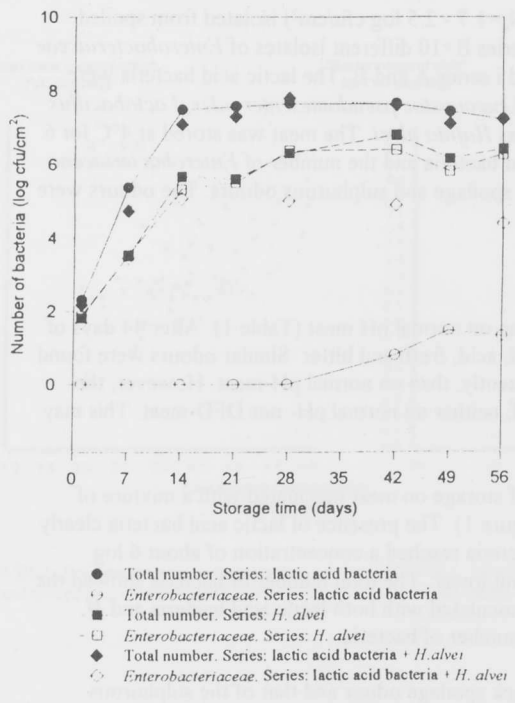

Figure 1. Total number of bacteria and number of *Enterobacteriaceae* on beef stored at 4°C in vacuum packs. Sterile beef was inoculated with lactic acid bacteria, *H. alvei* or a mixture of lactic acid bacteria and *H. alvei*.

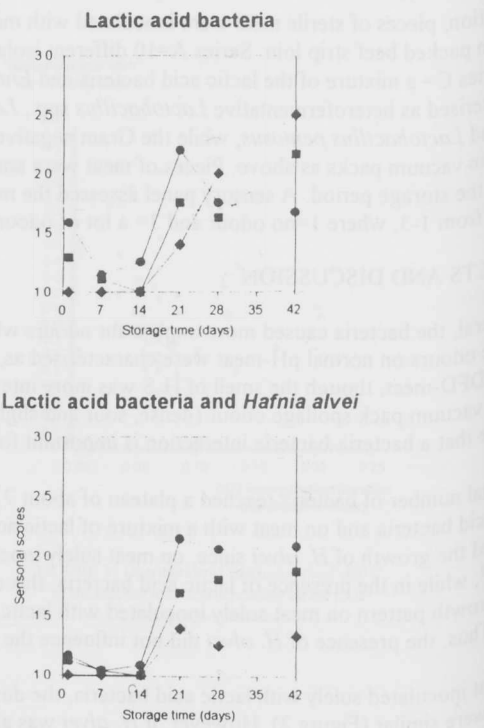

Figure 2. Evaluation of vacuum pack spoilage (■), sulphurous (◆) and acidic (●) odours on vacuum packed beef stored at 4°C. Sterile beef was inoculated with lactic acid bacteria or a mixture of lactic acid bacteria and *H. alvei*.