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Effect of growth of selected lactic acid bacteria on storage life of beef stored under vacuum and in air

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Abstract

The effect of growth of different types of lactic acid bacteria (LAB) on the storage life of normal pH beef was determined anaerobically (under vacuum) and aerobically. Four LAB from meat were inoculated separately onto sterile slices of lean beef. Inoculated samples were stored anaerobically at 2° C for 10 weeks or stored aerobically in an oxygen permeable film at 7° C for 10 days, with and without previous storage under vacuum at 2° C. The LAB strains used were *Carnobacterium maltaromicus* (previously *C. piscicola)* LV17 and UAL26, *Leuconostoc gelidum* UAL187-22 and *Lactobacillus sake* Lb706. Storage life was determined by sensory panel evaluation of colour and odour. Under anaerobic conditions *Lb. sake* Lb706, inoculated at log 2 CFU/cm^2, grew rapidly to reach maximum population within three weeks of storage. *L. gelidum* UAL187-22 also grew on the meat but at a slower rate. In contrast, growth of *C. maltaromicus* LV17 and UAL26 was unpredictable, achieving maximum population after 2 to 8 weeks. None of the test strains caused spoilage of the meat within the 10-week storage period under vacuum. When the test organisms were inoculated at an initial level of log 4 CFU/cm^2, *C. maltaromicus* LV17 and UAL26 produced off-odours after 8 weeks of storage under vacuum at 2° C. Under aerobic conditions at 7° C, all four of the strains grew well on the beef samples. *C. maltaromicus* LV17 and UAL26 and *Lb. sake* Lb706 all caused off-odours and discoloration. The rate of aerobic deterioration in meat quality was faster with increased time of storage under vacuum. *L. gelidum* UAL187-22 could be a suitable antagonistic strain with the potential to extend the storage life of beef, stored anaerobically and packaged aerobically for retail sale, without producing undesirable sensory changes.

Keywords: *Carnobacterium*; *Leuconostoc*; *Lactobacillus*; Bacteriocins; Spoilage; Beef; Storage life

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1. Introduction

Lactic acid bacteria (LAB) are the prevailing microorganisms on chill-stored fresh meat packaged under vacuum or in modified atmosphere with increased CO_2 (Egan, 1983; Dainty and Mackey, 1992). Development of a lactic microflora markedly extends the storage life of meats packaged in this way, but actual extension of storage life depends on several factors, including the type(s) of LAB found on the meat (Shay and Egan, 1981; Borch and Agerhem, 1992; Dainty and Mackey, 1992). The genera of LAB most frequently encountered on vacuum or modified atmosphere packaged meat are *Lactobacillus*, *Leuconostoc* and *Carnobacterium* (Shaw and Harding, 1984; Dainty and Mackey, 1992; McMullen and Stiles, 1993). A widely used practice in meat marketing is the vacuum packaging of primal cuts for distribution and extended storage followed by removal of the meat from the package for preparation of retail cuts. The retail cuts are usually marketed in an oxygen permeable film for display in an open, refrigerated cabinet to facilitate consumer selection. The bacteriological implications and the role of LAB in this transition from an anaerobic environment to aerobic storage have been examined for the natural microbial flora growing on pork (Greer et al., 1993) and after the inoculation of beef steaks with *Lactobacillus* cultures (Smith et al., 1980). Both studies showed that LAB may contribute to undesirable sensory changes of meat during aerobic storage.

There is considerable research on antagonistic proteinaceous compounds (bacteriocins) of LAB with the goal of using these strains or their bacteriocins for extension of the storage life of chilled meats (Stiles and Hastings, 1991). Bacteriocin-producing meat isolates of species of LAB have been described (Schillinger and Lücke, 1989; Ahn and Stiles, 1990a; Hastings and Stiles, 1991; Lewus et al., 1991). However, the growth of these organisms and their effects on sensory quality of beef has not previously been compared in a single study. Because LAB can cause spoilage of meats (Egan et al., 1980; Shay and Egan, 1981), it is necessary to screen isolates before inoculation of meat surfaces with bacteriocin-producing isolates to assess their preservative effect in situ.

This paper reports the growth of pure cultures of four bacteriocinogenic strains of LAB and their influence on sensory quality of vacuum packaged beef stored at 2° C, and the growth of the same organisms and their influence on sensory quality of beef stored aerobically at 7° C, with or without previous storage for various periods of time under vacuum at 2° C.

2. Materials and methods

2.1. Bacterial strains

The LAB used in this study included *C. maltaromicus* LV17 [Collins et al., 1991; *C. piscicola* 2852, National Collection of Food Bacteria (NCFB), Institute of Food Research, Reading, UK], and UAL26 (Ahn and Stiles, 1990a), *L.euconostoc*

gelidum UAL187-22 (Hastings and Stiles, 1991) and *Lb. sake* Lb706 (Schillinger and Lücke, 1989). All of these are bacteriocin-producing strains that were isolated from meat. *L. gelidum* UAL187-22 is a partially cured variant of the meat isolate *L. gelidum* UAL187 that contains two of the three native plasmids of the parent strain (Hastings and Stiles, 1991). *C. divergens* LV13 (NCFB 2855) was used as the indicator strain to monitor bacteriocin production.

2.2. Beef inoculation

Bacteria were grown in APT broth (Difco Laboratories Inc., Detroit, MI) at 25° C for 18 h and diluted in sterile, 0.1% peptone water to give an initial density of bacteria of log 2 CFU/cm^2 of the meat surface. In one experiment the initial density was log 4 CFU/cm^2. Sterile, lean beef slices (surface area 20 cm^2) were aseptically excised from normal pH, *Longissimus dorsi* muscle of beef (surface pH 5.6) as described by Greer and Jones (1991). Beef slices were suspended from sterile wire hooks and immersed in bacterial inocula for 15 s. An equal number of slices immersed in sterile 0.1% peptone water was used as controls. After inoculation, three or six slices from each treatment were placed in sterile Stomacher bags (Seward Medical, UK). These bags were enclosed in gas impermeable foil laminate (Printpac-UEB, Auckland, New Zealand) and vacuum packaged using a Captron III Packaging System (RMF, Grandview, MO, USA). For aerobic storage, six slices from each treatment were placed in a square Petri plate (9 × 9 cm) and wrapped in an oxygen permeable (8000 cc/m^2/24 h) polyvinyl chloride film (Goodyear Canada Ltd., Toronto, Ontario).

2.3. Beef storage

Vacuum packaged beef was stored at 2° C for 10 weeks and samples were removed for analysis at weeks 0, 1, 2, 3, 4, 6, 8 and 10. At certain time intervals, samples were removed from storage under vacuum for storage aerobically at 7° C. Samples inoculated with *C. maltaromicus* LV17, UAL26 and the corresponding sterile controls were either stored aerobically without previous storage under vacuum or transferred from anaerobic to aerobic storage after 3 and 6 weeks. Samples inoculated with *L. gelidum* UAL187-22, *Lb. sake* Lb706 and the corresponding sterile control were treated in the same way, except that samples were transferred from anaerobic to aerobic storage after 1 and 6 weeks. During aerobic storage samples were withdrawn for analysis on day 0, 2, 4, 7 and 10. At each sampling time, three beef slices were used for microbiological analysis and another three slices were used for sensory analysis.

The growth of *C. maltaromicus* LV17 and UAL26 under vacuum at 0, 2 or 4° C was determined following inoculation of beef samples with one of the test strains at log 2 CFU/cm^2. Uninoculated beef samples were used as controls. Three slices of beef were withdrawn for microbiological analysis after storage for 0, 1, 2, 3, 4, 6, 8 and 10 weeks.

2.4. Bacterial sampling and determination of antimicrobial activity

Three beef slices from one package at each sampling time were homogenized separately in a Lab-blender 400 stomacher (Seward, UK) in 90 ml of sterile 0.1% peptone water, diluted and surface plated onto APT agar. Plates were incubated for 3 days at 25° C. Colonies were counted and plates were overlayered with soft APT agar (0.75% agar) containing 1% of an overnight culture of *C. divergens* LV13 to indicate antimicrobial activity by the deferred test (Hastings and Stiles, 1991). The inhibitory activity of *L. gelidum* UAL187-22 was determined after growth of an initial inoculum of log 4 CFU/cm^2 on meat slices stored under vacuum at 2° C for 10 weeks. To assess the inhibitory activity, three meat slices were homogenized in 90 ml of 0.1% peptone water; heated in a boiling water bath for 15 min; cooled rapidly on ice and centrifuged at $8000 \times g$ for 15 min. The supernatant was filtered through Whatman #2 filter paper and stored at −70° C (McMullen and Saucier, unpublished procedure). A 1.5 ml sample was freeze-dried and resuspended in 50 μl peptone water. Twenty μl of undiluted samples and samples diluted 1:1 in peptone water were spotted onto the surface of freshly prepared indicator lawns containing log 7 CFU of *C. divergens* LV13 per ml and incubated anaerobically overnight (Ahn and Stiles, 1990a, Ahn and Stiles, 1990b). Samples were also treated with pronase E (Sigma; St Louis, MO) by adding 10 μl of a concentration of 20 mg/ml to the spotted samples.

2.5. Determination of storage life, beef colour and pH

Three muscle slices from each package were evaluated for acceptability of both appearance and odour using an experienced five-member panel based on established criteria for determination of storage life (Greer and Jones, 1991; Greer and Murray, 1991). After removal of the barrier film, beef slices stored under vacuum were exposed to air for 15 min prior to evaluation for acceptance of meat colour. In addition, meat colour (CIE, 1976) reflectance coordinates (L*, a*, b*) were measured using a Minolta Chroma Meter II (Minolta Camera Co., Ramsey, NJ). Lightness or paleness is expressed by the L*-coordinate with higher values indicating lighter colours; the red-green spectrum is expressed by the a*-coordinate with higher values indicating a more red colour; and the yellow-blue spectrum is expressed by the b*-coordinate with higher values indicating a more yellow colour. Surface pH was determined using an Oakton microprocessor WD-00605-00 (Anachemia Scientific, Calgary, Alberta) equipped with a flat surface electrode.

2.6. Statistical analyses

For each trial a randomized experimental protocol was followed. Sensory assessment data, beef colour coordinates (L*, a*, b*) and surface pH were subjected tc analysis of variance according to the General Linear Models procedure of the Statistical Analysis System (SAS Institute, 1985)

3. Results

3.1. Bacterial growth and changes in pH

C. maltaromicus LV17 and UAL26 exhibited variable rates of growth on beef slices when inoculated at initial densities of log 2 CFU/cm^2 and stored under vacuum at 2° C (Fig. 1a,b). Standard deviations up to log 1.6 CFU/cm^2 were observed at some sampling times for these two strains, whereas standard deviations for *L. gelidum* UAL187-22 and *Lb. sake* Lb706 at any sampling time were less than log 0.5 CFU/cm^2. Increase of storage temperatures from 0 or 2 to 4° C resulted in a predictably faster growth of *C. maltaromicus* LV17, but the growth of *C. maltaromicus* UAL26 on beef was still unpredictable.

L. gelidum UAL187-22 grew slowly (Fig. 2a), but *Lb. sake* Lb706 grew rapidly at 2° C (Fig. 2b). Samples inoculated at higher densities (log 4 CFU/cm^2) with *C. maltaromicus* LV17 and UAL26 increased less than one log cycle during 10 weeks of storage at 2° C. *Lb. sake* Lb706 and *L. gelidum* UAL187-22 exhibited similar types of growth to that observed when the beef was inoculated at a density of log 2 CFU/cm^2.

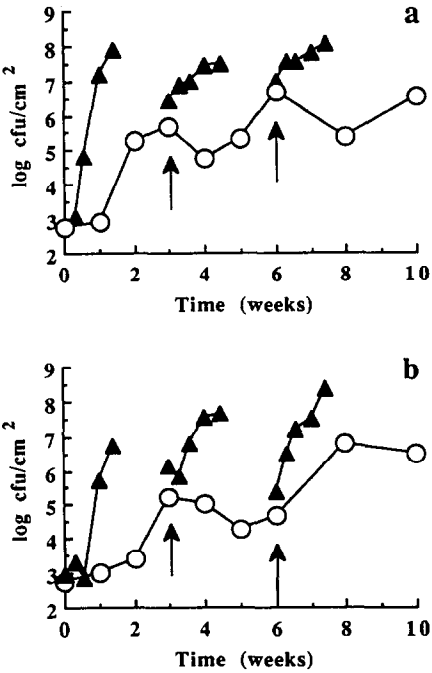

Fig. 1. Growth of (a) *C. maltaromicus* LV17 and (b) *C. maltaromicus* UAL26 under anaerobic (○) or aerobic (▲) conditions after inoculation at low (log 2 CFU/cm^2) initial level. Arrows indicate times of transition from anaerobic (vacuum) to aerobic storage.

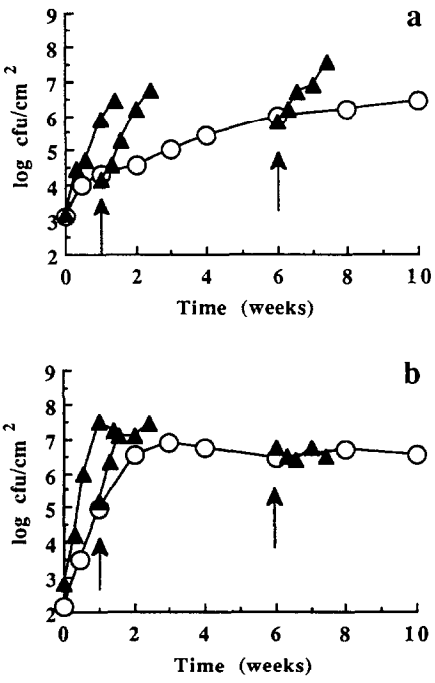

Fig. 2. Growth of (a) *L. gelidum* UAL187-22 and (b) *Lb. sake* Lb706 under anaerobic (○) or aerobic (▲) conditions after inoculation at low (log 2 CFU/cm^2) initial level. Arrows indicate times of transition from anaerobic (vacuum) to aerobic storage.

With the exception of *Lb. sake* Lb706, values for surface pH of beef samples inoculated at a density of log 2 CFU/cm^2 showed little change and was not significantly different ($p > 0.05$) from sterile control values during storage under vacuum at 2° C. At the start of the experiment the mean value for inoculated and sterile samples was 5.60 ± 0.05. Surface pH of beef slices inoculated with *Lb. sake* Lb706 decreased from an initial value of 5.60 ± 0.07 to 5.31 ± 0.06 during 10 weeks of storage. The drop in pH occurred after 6 weeks of storage at 2° C. After 8 and 10 weeks of storage at 2° C, samples inoculated with *L. gelidum* UAL187-22 or *Lb. sake* Lb706 at log 4 CFU/cm^2 had a significantly lower surface pH ($p < 0.05$) than the sterile control samples. In contrast, for samples inoculated with *C. maltaromicus* LV17 and UAL 26 there was no decrease in surface pH.

C. maltaromicus LV17 and UAL26 grew rapidly on beef stored aerobically at 7° C (Fig. 1a,b). On samples that had not been stored under vacuum, an initial lag phase of 2 to 4 days was observed. *Lb. sake* Lb706 and *L. gelidum* UAL187-22 also grew rapidly under aerobic conditions (Fig. 2a,b). *Lb. sake* Lb706 reached the stationary phase of growth before the end of 10 days of aerobic storage (Fig. 2b). In contrast, the other three test organisms did not reach the stationary phase during 10 days of aerobic storage (Fig. 1a,b and 2a). With the exception of *Lb.*

sake Lb706, all of the test organisms reached higher counts on the beef slices when stored aerobically than under vacuum.

Transfer of vacuum-packaged beef to storage in air resulted in significantly lower pH values ($p < 0.05$) of samples inoculated with LAB compared with sterile control samples, especially for samples inoculated with *L. gelidum* UAL187-22 or *Lb. sake* Lb706 toward the end of aerobic storage. The most marked differences were observed after 10 days of aerobic storage of samples previously stored under vacuum for 6 weeks. Surface pH values of 5.22 ± 0.13 and 4.99 ± 0.08 were recorded for beef inoculated with *L. gelidum* UAL187-22 and *Lb. sake* Lb706, respectively; whereas the pH values for beef inoculated with *C. maltaromicus* LV17, UAL 26 and the sterile control samples were 5.56 ± 0.03, 5.56 ± 0.08 and 5.52 ± 0.16, respectively. No growth of bacteria was observed on uninoculated control samples stored under vacuum or aerobically throughout storage ($\leq$ log 2 CFU/cm^2).

3.2. Changes in beef colour

Beef colour coordinates L* (lightness), a* (red-green), b* (yellow-blue) were measured in all trials. All of the beef samples that were stored anaerobically 'bloomed' when exposed to air. In comparison to uninoculated beef slices the only significant ($p < 0.05$) and consistent variation in the L*, a*, b* coordinates that could be attributed to bacterial growth was in the a* values and these changes were only significant during aerobic storage. The effects of storage time upon a* colour coordinates are illustrated in Fig. 3. The data show a faster decrease in a* values with time of storage in air for beef inoculated with *C. maltaromicus* LV17, UAL26 or *Lb. sake* Lb706 than was observed for sterile control samples. This difference increased with the extension of the period of storage under vacuum. Compared with the sterile control samples, significantly lower a* values ($p < 0.05$) were observed for beef inoculated with *C. maltaromicus* LV17 and UAL 26 after 10, 4 or 2 days of aerobic storage of samples previously stored under vacuum for 0, 3 or 6 weeks, respectively. Similarly, significantly lower a* values ($p < 0.05$) were observed for *Lb. sake* Lb706 after 10 or 2 days of aerobic storage of samples previously stored under vacuum for 0 or 6 weeks, respectively. In contrast, a* values of samples inoculated with *L. gelidum* UAL 187-22 were, with one exception (Fig. 3e, day 7), significantly higher ($p < 0.05$) or not significantly different from the sterile control samples.

3.3. Storage life of beef

Storage life of vacuum packaged samples inoculated at the lower inoculum level (log 2 CFU/cm^2), assessed by acceptability of odour or appearance, was in excess of 10 weeks irrespective of the test strain inoculated onto the beef (data not shown). Samples inoculated at the higher level (log 4 CFU/cm^2) and stored under vacuum at 2° C were assessed by acceptability of odour. Storage life of samples inoculated at this level with *L. gelidum* UAL187-22 or *Lb. sake* Lb706 was in excess of 10 weeks; while samples inoculated with *C. maltaromicus* LV17 or UAL

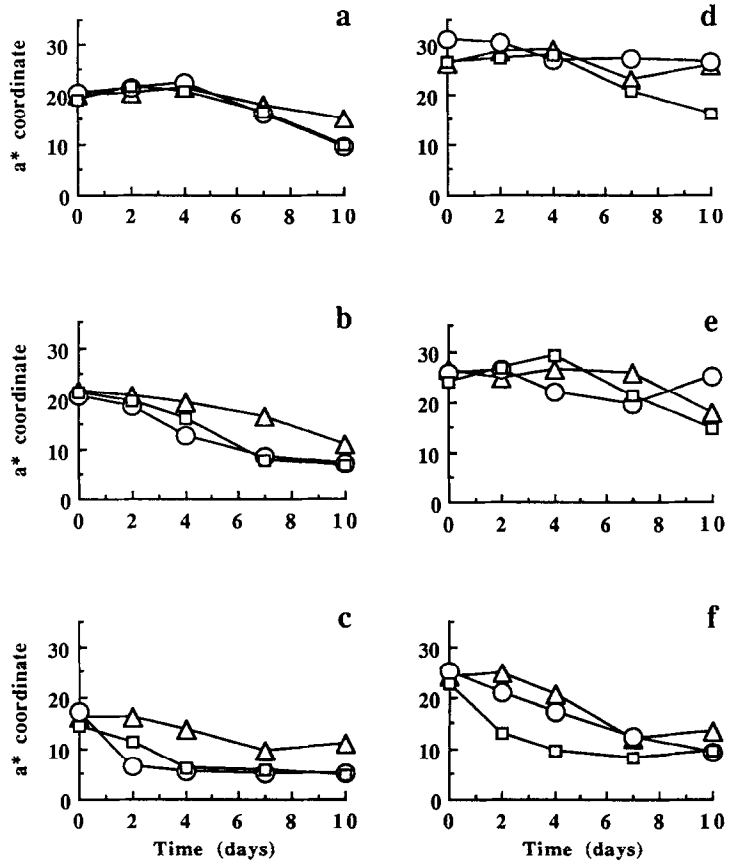

Fig. 3. Values of the a* meat colour coordinate observed for beef slices inoculated with *C. maltaromicus* LV17 (○), UAL26 (□) or sterile control samples (△) and stored aerobically at 7° C after 0 (a), 3 (b) or 6 (c) weeks of previous storage under vacuum at 2° C or beef slices inoculated with *L. gelidum* UAL187-22 (○), *Lb. sake* Lb706 (□) or sterile control samples (△) and stored aerobically at 7° C after 0 (d), 1 (e) or 6 (f) weeks of previous storage under vacuum at 2° C.

26 spoiled after 8 weeks of storage. The data in Fig. 4 (appearance) and Fig. 5 (odour) compare the effects of bacterial strain on the aerobic storage life of beef before and after various periods of storage under vacuum. Beef inoculated with *C. maltaromicus* LV17, UAL26 or *Lb. sake* Lb706 and stored aerobically, had a storage life that was significantly reduced compared with sterile control samples based on both appearance and odour of the samples. Hence, aerobic storage life of samples previously stored for 6 weeks under vacuum was 4 to 8 days shorter than samples that had not previously been stored under vacuum. In comparison to control samples, the aerobic storage life of beef was not affected by the growth of *L. gelidum* UAL187-22 when determined by acceptability of appearance (Fig. 4) or odour (Fig. 5). Aerobic storage trials after six weeks of previous storage of samples under vacuum were repeated with *L. gelidum* UAL187-22 and the sterile controls.

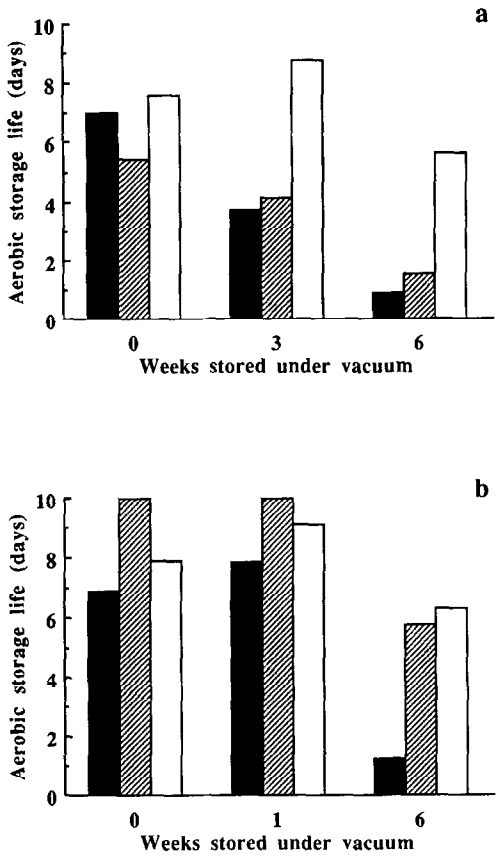

Fig. 4. Storage life assessed by meat appearance for beef slices inoculated with (a) *C. maltaromicus* LV17 (black bars), *C. maltaromicus* UAL26 (striped bars) or sterile control samples (open bars), and (b) *Lb. sake* Lb706 (black bars), *L. gelidum* UAL187-22 (striped bars) or sterile control samples (open bars), and stored aerobically at 7° C after periods of previous storage at 2° C under vacuum, as indicated.

Even with an inoculum level of log 4 CFU/cm^2, growth of this strain on the meat did not affect appearance or odour of the inoculated samples.

3.4. Antimicrobial activity

Bacteria isolated from beef at all storage intervals retained their bacteriocinogenic potential when tested by the deferred test for antagonistic activity (data not shown). The inhibitory activity of *L. gelidum* UAL187-22 inoculated initially at log 4 CFU/cm^2 on sterile beef slices was demonstrated after 10 weeks of storage under vacuum at 2° C. Inhibitory activity of 2.5 arbitrary units/cm^2 was recovered from beef slices inoculated with *L. gelidum* UAL187-22, while no inhibitory activity was observed for sterile samples. This inhibitory substance was pronase

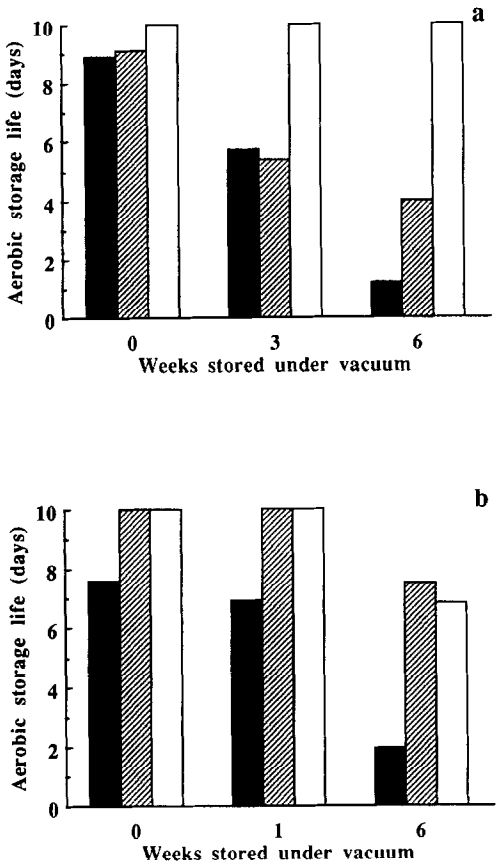

Fig. 5. Storage life assessed by presence of off-odours from beef slices inoculated with (a) *C. maltaromicus* LV17 (black bars), *C. maltaromicus* UAL26 (striped bars) or sterile control samples (open bars), and (b) *Lb. sake* Lb706 (black bars), *L. gelidum* UAL187-22 (striped bars) or sterile control samples (open bars), and stored aerobically at 7° C after periods of previous storage at 2° C under vacuum, as indicated.

sensitive, and therefore it was assumed to be leucocin A produced by this strain of *L. gelidum*.

4. Discussion

Two important criteria must be considered for the use of bacteriocin-producing LAB to extend storage life of beef. They must be able to grow and inhibit unwanted microorganisms, and they should not cause spoilage of the product. All four strains in the present work grew on beef stored under vacuum at 2° C although the extent of growth of *C. maltaromicus* LV17 and UAL26 was variable. None of the strains caused spoilage (colour or odour) of beef during 10 weeks of

storage under these conditions, except *C. maltaromicus* LV17 and UAL26 inoculated at log 4 CFU/cm^2. Sensory evaluation did not allow for detection of so-called confinement odours. These odours are evident immediately after opening the barrier bag but they are not associated with the meat and dissipate rapidly on exposure to air (Edwards et al., 1985). Because the consumer is not exposed to confinement odours as a result of current beef handling practice, the detection of such odours was not relevant to the objectives of this study.

Several studies have shown that LAB can cause spoilage of beef stored anaerobically due to development of objectionable odours. Odours such as sulphur (Shay and Egan, 1981; Hanna et al., 1983) and buttermilk (Hanna et al., 1983) have been described for pure culture meat systems; in addition to putrid/painted (Borch and Agerhern, 1992) for meats in which pure cultures were added to the indigenous microflora. The development of such defects are contradicted by the results of this study with pure cultures, and results reported for inoculated meats that contain their indigenous microflora by Schillinger and Lücke (1987) in which no objectionable odours were detected during growth of various LAB on beef stored under vacuum. However, the many differences in experimental methodology (test organisms; inoculum levels; packaging techniques; storage temperatures; sensory methodology; sample size) and the fact that pure cultures were used in this study may account for the differences in results. A final evaluation of the spoilage potential of the four test organisms in this study must also take into account possible flavour changes. The indigenous flora of LAB have been reported to spoil vacuum-packaged beef by the development of a sour off-flavour (Egan, 1983).

The unpredictable growth patterns observed for *C. maltaromicus* LV17 and UAL26 were not studied in detail, but it has been observed that *C. maltaromicus* LV17 grows slower in normal pH (pH = 5.6) meat extract than in high pH (pH = 6.1) meat extract (Leisner, unpublished results). It is possible that meat-related variations in parameters such as pH and concentration of lactic acid could affect growth of nonaciduric *C. maltaromicus*. Further research is needed to resolve this question.

Few studies have focused on the effect of an aerobic environment after previous storage under vacuum, on the growth of the indigenous flora of LAB and their potential for promoting spoilage of beef (Smith et al., 1980; Greer et al., 1993). The results obtained in this study revealed that all four of the test strains grew well in an aerobic environment, but *L. gelidum* UAL187-22 did not decrease storage life caused by changes in acceptability of beef appearance or odour. In contrast, *C. maltaromicus* LV17 and UAL26 and *Lb. sake* Lb706 all decreased storage life as a result of changes in beef appearance and odour compared with the sterile control samples. Smith et al. (1980) showed that inoculation of various meat isolates of LAB on beef steaks stored up to 35 days at 1 to 3° C and displayed under retail conditions for 3 days had a higher incidence of off-odours, but had an improved overall appearance, compared with uninoculated control samples. Because the beef cuts used by these researchers were not sterile, spoilage changes could be attributed to the indigenous flora rather than the inoculated strain of LAB.

It was evident from the present study that the rate of deterioration of beef

quality is related to the length of time of previous storage under vacuum. This supports observations that an increased rate of deterioration of retail pork meat was related to the time of previous storage of refrigerated primal cuts in controlled atmosphere (Greer et al., 1993). LAB dominate the microbial flora of retail meat cuts after extended storage of commercial primal cuts in controlled atmosphere and these organisms are therefore the most probable cause of spoilage (Greer et al., 1993). The effects observed in our study could be due to the transition from anaerobic to aerobic environment, the transition from 2 to 7° C, or both of these factors. This indicates the need for careful evaluation of changes in the metabolic activities of LAB after transition from anaerobic to aerobic environments and from lower to higher storage temperatures.

The change in appearance observed for three of the four test organisms was caused by 'greening' of the meat. The subjective assessment was supported by a decrease in the Minolta a* values which measure colour reflectance changes on the red-green axis. In contrast, Renerre and Montel (1986) did not show such differences in a* values between samples inoculated with lactobacilli or sterile control samples of beef stored under vacuum followed by a period of aerobic storage. The relatively constant b* values in our study indicate that no major changes occurred in the yellow-blue spectrum.

The metabolites that caused the reduction of storage life assessed by odour for three of the four test organisms was not determined in this study. However, the result was not caused by acidification because there was no correlation between the surface pH of inoculated beef slices and aerobic storage life. For instance, *C. maltaromicus* LV17 and UAL26 did not consistently change pH during aerobic storage, yet they reduced storage life by production of off-odours.

The results obtained in this study indicate that *L. gelidum* UAL187-22 is the strain with the best potential to be utilized as an antagonist of spoilage and pathogenic bacteria in beef stored under vacuum and subsequently transferred to aerobic, retail conditions. This is due to its reliable growth, activity of the bacteriocin in beef and absence of spoilage under anaerobic and aerobic conditions. It remains to be shown whether the relatively slow growth rate of *L. gelidum* UAL187-22 will allow it to compete efficiently with spoilage and pathogenic bacteria on meat.

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References

Ahn, C. and Stiles, M.E. (1990a) Antibacterial activity of lactic acid bacteria isolated from vacuum-packaged meats. J. Appl. Bacteriol. 60, 302–310.

Ahn, C. and Stiles, M.E. (1990b) Plasmid-associated bacteriocin production by a strain of *Carnobacterium piscicola* from meat. Appl. Environ. Microbiol. 56, 2503–2510.

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.

CIE (1976) Commission Internationale de l'Eclairage. 18th Session, London, England. Sept. 1975. CIE Publication 36. Bureau Central de la CIE, 4, Avenue du Recteur Poincaré, 75782 Paris Cedex 16, France.

Collins, M.D., Rodrigues, U., Ash, C., Aguirre, M., Farrow, J.A.E., Martinez-Murcia, A., Phillips, B.A., Williams, A.M. and Wallbanks, S. (1991) Phylogenetic analysis of the genus *Lactobacillus* and related lactic acid bacteria as determined by reverse transcriptase sequencing of 16S rRNA. FEMS Microbiol. Lett. 77, 5–12.

Dainty, R.H. and Mackey, B.M. (1992) The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. J. Appl. Bacteriol. Symp. Suppl. 73, 103S–114S.

Edwards, R.A., Dainty, R.H. and Hibbard, C.M. (1985) Putrescine and cadaverine formation in vacuum packed beef. J. Appl. Bacteriol. 58, 13–19.

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

Egan, A.F., Ford, A.L. and Shay, B.J. (1980) A comparison of *Microbacterium thermosphacta* and lactobacilli as spoilage organisms of vacuum-packaged sliced luncheon meats. J. Food Sci. 45, 1745–1748.

Greer, G.G. and Jones, S.D.M. (1991) Effects of lactic acid and vacuum packaging on beef processed in a research abattoir. Can. Inst. Food Sci. Technol. J. 24, 161–168.

Greer, G.G. and Murray, A.C. (1991) Freezing effects on quality, bacteriology and retail case life of pork. J. Food Sci. 56, 891–894.

Greer, G.G., Dilts, B.D. and Jeremiah, L.E. (1993) Bacteriology and retail case life of pork after storage in carbon dioxide. J. Food Prot. 56, 689–693.

Hanna, M.O., Savell, J.W., Smith, G.C., Purser, D.E., Gardner, F.A. and Vanderzant, C. (1983) Effect of growth of individual meat bacteria on pH, color and odor of aseptically prepared vacuum-packaged round steaks. J. Food Prot. 42, 216–221.

Hastings, J.W. and Stiles, M.E. (1991) Antibiosis of *Leuconostoc gelidum* isolated from meat. J. Appl. Bacteriol. 70, 127–134.

Lewus, C.B., Kaiser, A. and Montville, T. (1991) Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. Environ. Microbiol. 57, 1683–1688.

McMullen, L. and Stiles, M.E. (1993) Microbial ecology of fresh pork stored under modified atmosphere at −1, 4.4 and 10° C. Int. J. Food Microbiol. 18, 1–14.

Renerre, M. and Montel, M.-C. (1986) Inoculation of steaks with *Lactobacillus* species and effect on colour and microbial counts. Proceedings of the 32nd meeting of European Meat Research Workers, pp. 213–216.

SAS Institute (1985) SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC.

Schillinger, U. and Lücke, F.-K. (1987) Lactic acid bacteria on vacuum-packed meat and their influence on storage life. Fleischwirtsch. 67, 1244–1248.

Schillinger, U. and Lücke, F.-K. (1989) Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol. 55, 1901–1906.

Shaw, B.G. and Harding, C.D. (1984) A numerical taxonomic study of lactic acid bacteria from vacuum-packed beef, pork, lamb and bacon. J. Appl. Bacteriol. 56, 25–40.

Shay, B.J. and Egan, A.F. (1981) Hydrogen sulphide production and spoilage of vacuum-packed beef by a *Lactobacillus*. In: T.A. Roberts, G. Hobbs, J.H.B. Christian and N. Skovgaard (editors), Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Academic Press, New York, pp. 241–251.

Smith, G.C., Hall, L.C. and Vanderzant, C. (1980) Inoculation of beef steaks with *Lactobacillus* species before vacuum packaging. II. Effect on meat quality characteristics. J. Food Prot. 43, 842–849.

Stiles, M.E. and Hastings, J.W. (1991) Bacteriocin production by lactic acid bacteria: potential for use in meat preservation. Trends Food Sci. Technol. 2, 247–251.