Effect of Lactic Acid Bacteria on Beef Flavour

Frances M. Nattress, Rodney J. Worobo, G. Gordon Greer and Lester E. Jeremiah

Agriculture & Agri-Food Canada
Lacombe Research Centre
6000 C & E Trail
Lacombe, Alberta, Canada T4L 1W1

Background

Changes in handling of meat require that the shelf life of the product be extended without compromising its quality and safety. Preservative packaging has emerged as a promising method of fulfilling these requirements without the need for the addition of chemical preservatives (Gill and Molin, 1991; Farber, 1991). The controlled atmosphere packaging system for fresh, chilled meats that is most effective is the Captech process (Gill, 1989). It provides an atmosphere that inhibits the growth of most bacteria (Dainty *et al.*, 1979; Egan, 1983), and when oxygen levels and storage temperatures are properly controlled, colour quality can be maintained (Gill, 1989). Bacterial levels increase upon removal from the CO_2 package and, in both beef and pork, lactic acid bacteria (LAB) predominate and are the only detectable organisms until after about 4 days of retail display when pseudomonad numbers increase (Nesom-Fleet *et al.*, 1993, Greer *et al.*, 1993). When beef that has been stored in CO_2 is subsequently frozen, the meat is badly discoloured and spoiled once defrosted (Nesom-Fleet, 1994).

Objectives

The objectives of this study were 1) To determine the types of bacteria that might be responsible for overt spoilage of fresh beef packaged in CO_2, stored up to 10 weeks at low temperature and subsequently displayed for 28 h in a retail case and 2) To establish whether freezing the product resulted in changes in the bacterial population.

Materials and Methods

Fresh beef rib-eyes were obtained from a federally inspected abattoir (Edmonton Meat Packers, Edmonton, AB, Canada), were cut into rib eye steaks and packaged in foil laminate pouches (MET nylon bags, Securefresh Pacific Limited, Auckland, New Zealand) having a gas transmission rate of <0.01 cc/m^2/24 h which were evacuated, filled with bone dry CO_2 (2L of CO_2 / kg of meat, Liquid Air, Red Deer, AB) and heat sealed using the Captron III packaging system (RMF, Grandview, MO). Residual O_2 levels were <300 ppm as measured by a Mocon Oxygen Analyzer (Model FBP-08B12L, Mocon Modern Controls Inc., Minneapolis, MN, U.S.A.). The samples were stored at 2°C. At weekly intervals randomly selected packages were opened and the steaks were repackaged in individual styrofoam trays (Scott National, Calgary, AB), which were overwrapped with an oxygen permeable polyvinyl chloride film (Vitafilm Choice Wrap, Goodyear Canada Inc., Toronto, ON) with an oxygen transmission rate of 8000 cc/m^2/24 h. They were placed in a fan-circulated horizontal-type retail display case (Model LPM12T, Hill Refrigeration of Canada, Ltd., Barrie, ON) (Greer and Jeremiah, 1980). The meat was displayed for 28 h at which time 5 steaks were stored at -20°C for 85 days, 4 were subjected to sensory evaluation by a flavour profile panel (FPP) and were evaluated for bacterial levels using standard methods. The other steaks removed after 85 days, were defrosted and were subjected to sensory evaluation and bacterial analysis. At each storage time, colonies of presumptive LAB picked from MRS (Difco Laboratories Inc., Detroit, Michigan, U.S.A.) and Rogosa agar (acetate agar, pH 5.6, Oxoid Inc, Nepean, ON), were purified and characterized. Four trials were done.

Results and Discussion

The average temperature in the centre of the retail case during the display was 7.59°C. This temperature would permit the growth of psychrotrophic bacteria and some pathogens if they were present (Gill *et al.*, 1997 a,b). The pH of the steaks was consistently between 5.6 and 5.9 with no changes attributed to storage time or freezing. The pH of all the steaks could be considered "normal" so that when properly packaged in CO_2 LAB would be expected to dominate the microbial flora (Gill, 1989). Colour measurement showed that time of storage had little effect on L*, a* and b* (data not shown) but freezing did have an effect on L* and a*. L* had a net change with freezing of -1.17 indicating a slightly darker product after frozen storage and the change in a* was -4.72 indicating a duller product after freezing. No *Enterobacteriaceae*, *Brochothrix thermosphacta* or pseudomonads were detectable. As expected the predominant flora during CO_2 storage was made up of LAB. The numbers of LAB increased from an undetectable level to an average maximum of log CFU/cm^2 of 5.84 after 8 weeks in CO_2 storage (Fig. 1A). Those LAB that were able to grow on acetate agar also peaked as the total LAB population peaked (Fig. 1A) although their lag phase was longer. Rejection of the product by the flavour profile panel coincided with the growth of acetate tolerant LAB (Fig. 1A, Fig. 2A). Frozen storage accelerated spoilage by approximately 2 weeks (Fig. 1B) with flavour profile panel rejection coinciding with growth of acetate resistant LAB (Fig. 1B, 2B).

In this study overt spoilage was associated with the growth of a population of LAB able to grow on acetate agar. Unlike Nissen *et al.* (1996) in which after 5 weeks of storage of beef in CO_2 at 2°C *Leuconostoc* spp. was the dominant organism, few heterofermentative organisms were observed. With increasing time however Nissen *et al.* (1996) suggested *Lactobacillus* spp. numbers increased, an observation consistent with those of the present study. LAB isolated from MRS and Rogosa agars at spoilage and immediately prior to spoilage are being characterized (Leisner *et al.*, 1994). Results indicate four groups of organisms that are all homofermentative. One quarter of the isolates cannot be subcultured more than once. For the other groups the ability to grow on acetate agar is lost upon subculturing. One group consistently appears mixed despite repeated purification steps. Isolates that have been characterized have been tentatively classified as *L. curvatus* and *L. sake.*

Conclusions

The results of this study suggest that it is the development of a population of LAB that are able to grow, at least transiently, on acetate agar that are responsible for development of off/undesirable flavours and product rejection in retail ready steaks stored in controlled atmospheres. These are unusual organisms some of which quickly lose the ability to grow on defined media. One group of isolates appears mixed despite several attempts to purify them.

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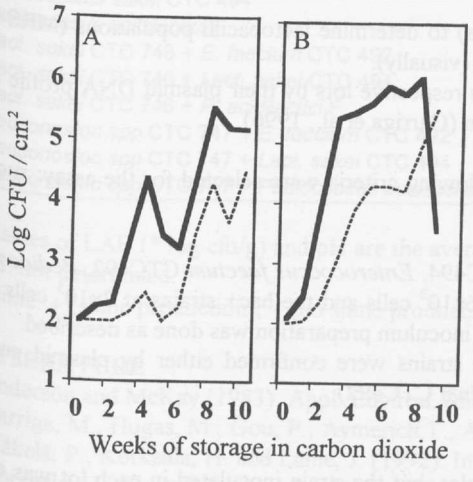

Figure 1. Growth of total (——) and acetate resistant (·····) LAB on steaks which had been stored in carbon dioxide at 2°C, subsequently displayed in a retail case for 28 h (A) and after frozen storage (B).

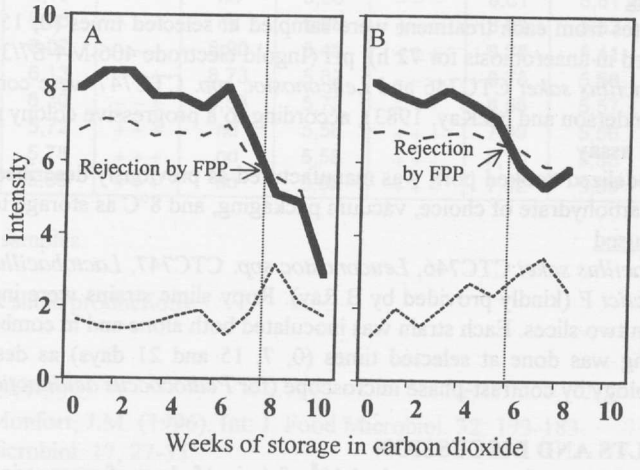

Figure 2. Intensity of selected flavour notes and overall blendedness of flavour notes (amplitude ——) for steaks which had been stored in carbon dioxide at 2°C, subsequently displayed in a retail case for 28 h (A) and after frozen storage (B). 7.5 is an average score. Beefy flavour (– –) and off/undesirable flavour (·····).