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THE BACTERIOLOGY AND CASE-LIFE OF RETAIL-READY BEEF AFTER EXTENDED STORAGE IN CARBON DIOXIDE

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Please refer to Folio 71.
[Ed. note: Folio 71 incorrectly labelled S8P14.WP]

INTRODUCTION

Preservative packaging has been used to prolong storage life, maintain meat quality, control spoilage and assure safety (Gill and Molin, 1991; Greer *et al.*, 1992). One of the most effective controlled atmosphere packaging systems for fresh, chilled meats is the Captech process (Gill, 1989).

Although this technology will facilitate the transport of fresh meat to domestic and export markets, the efficiency can be improved upon by incorporating centralized packaging of retail ready cuts. The advantages of retail ready packs include decreased spoilage, improved hygiene, increased profit, improved inventory control, improved quality control and decreased labour at the retail level (Scholtz *et al.*, 1992a).

Unfortunately, there is limited information on the bacteriological, sensory and colour changes that occur during prolonged CO_2 storage of retail-ready beef steaks and subsequent retail display. Thus, some research has suggested extended anoxic storage of chilled meats caused retail acceptability to decrease under aerobic conditions (Moore and Gill, 1987; Shay and Egan, 1990). Contrary studies, however, conclude CO_2 inhibition continues to be expressed once pork chops were transferred to the aerobic conditions of retail display (Spahl *et al.*, 1981; Scholtz *et al.*, 1992b).

The objectives of the study were to provide quantitative data on the bacteriology and case life of retail-ready rib-eye steaks following prolonged CO_2 storage.

MATERIALS AND METHODS

Longissimus dorsi muscles were obtained from a federally inspected commercial abattoir and shipped under commercial conditions. The muscles were trimmed of subcutaneous fat and cut into 2.5cm thick rib-eye steaks.

Steaks were randomly placed on plastic (polypropylene) cafeteria trays (Russell Food Equipment Ltd., Edmonton, AB, Canada), six steaks per tray. Each tray was placed inside an oxygen impermeable EVOH bag (60 gauge nylon, 60 gauge ethyl vinyl alcohol co-polymer, 3mil polyethylene; Packaging Industries, Inc., San Leandro, CA, United States), filled with 2.5l CO_2/kg of meat and packaged using a Captron III packaging system (RMF, Grandview, MO, United States). After packaging, residual oxygen levels were <300ppm.

Following sealing, steak packages were placed inside cardboard boxes and randomly allocated to storage intervals (0, 6, 12, 18 or 24 weeks at one of two temperature -- -1.5°C or 2.0°C). One box of five packages was assigned to each storage interval and each temperature.

After each storage interval, steak were removed from the CO_2 master-pack, placed on individual styrofoam trays (Scott

National, Calgary, AB, Canada) and overwrapped with an oxygen permeable polyvinyl chloride film (Vitafilm Choice Wrap, Goodyear, Canada Inc., Toronto, ON, Canada) having an oxygen transmission rate of 8000cc/m2/24 hours. Retail steaks were randomly placed in a fan-circulated, horizontal-type retail case (Hill Refrigeration of Canada, Ltd., Barrie, ON, Canada). The mea surface temperature of the steaks was 6°C.

Ten steaks were evaluated by an experienced 5-member sensory panel on days 0, 2, 4, 6, 8 and 10 of retail display at each storage interval (0, 6, 12, 18 and 24 weeks). Retail appearance was evaluated using a 7-point hedonic scale (1=extremely undesirable; 7=extremely desirable) and odour was evaluated using a 5-point acceptability scale (1=acceptable; 5=unacceptable).

Steaks were sampled by the aseptic removal of 10cm² of tissue from the surface at one location only. The sample was combined with 90ml of 0.1% peptone-water and homogenized for two minutes using a Colworth Stomacher (Baxter Diagnostics Corp., Canlab Division, Edmonton, AB, Canada). Ten steaks were sampled at each display time.

Following serial, ten-fold dilutions in 0.1% pepton-water, lactic acid bacteria *Brochothrix thermosphacta* and pseudomonads were determined by the spread plate technique and *Enterobacteriaceae* (enterics) by the pour plate technique.

Enterics were determined after incubation for 18 to 24 hours at 35°C using overlaid plates of violet red bil glucose agar (VRBGA). Pseudomonads were determined using cephaloridine-fucidin-cetrimide agar (CFC) after two days incubation at 25°C. *B.thermosphacta* were enumerated on streptomycin sulfate-thallous acetate-actidione agar (STAA) after incubation for three days at 25°C. Lactic acid bacteria were determined on MRS agar after anaerobic incubation for three days at 25°C using a BBL anaerobic system containing five to 10% CO2 (Becton and Dickenson Co., Cockeysville, MD).

A completely randomized design was used to analyze bacterial growth and retail case-life. Data were analyzed by analysis of variance according to the General Linear Model Procedure of the SAS Institute (1985).

RESULTS AND DISCUSSION

Data in Figure 1 show the effect of temperature on bacterial growth on beef steaks during storage in CO_2. Throughout the 24-week storage interval, at both storage temperatures, *B.thermosphacta*, pseudomonads and enterics were undetectable. Lactic acid bacteria were the only bacteria detected during storage in CO_2 for up to 24 weeks. These bacteria reached maximum cell numbers (log bacteria/cm² = 6 to 7) within six weeks at 2.0°C and 12 weeks at -1.5°C. Bacteria grew faster at 2.0°C and overall numbers of lactics were slightly higher on steaks stored at 2.0°C compared to -1.5°C but this difference was not significant ($P>0.05$).

Data in Figure 2 shows the effect of CO_2 storage temperature and retail display time on bacterial populations. To allow for a simplified comparison of bacterial growth on steaks during retail display, data in Figure 2 were pooled over CO_2 storage interval.

Lactic acid bacteria were the dominant group of organisms throughout the ten day display interval at both CO_2-storage temperatures, bacteria grew from initial numbers of log bacteria/cm²=5.00 to reach maximum densities of log bacteria/cm≤=7 within ten days of retail display ($P<0.05$). Bacteria growth was not significantly effected by temperature ($P>0.05$).

The only other detectable bacterial population during retail display were the pseudomonads. They grew slowly after a lag period of four days to reach populations of 10^3 at 2.0°C and 10^4 at -1.5°C ($P<0.05$). CO_2 storage at 2.0°C resulted in lower populations of pseudomonads during subsequent retail display compared to those developing after storage at -1.5°C ($P<0.05$). CO_2 storage temperature had no significant effect upon the numbers of lactics found during retail display ($P>0.05$). In the absence of CO_2 storage (storage time=0weeks), pseudomonads dominated the spoilage flora throughout retail display (data not shown).

The effects of CO_2 storage temperature and time on the number of pseudomonads during retail display are shown in Figure 3. Data were pooled over retail display time. As CO_2 storage time increased, there was a significant decrease ($P<0.05$) in the numbers of detectable pseudomonads and this decrease was greater after CO_2 storage at 2.0°C than at -1.5°C ($P<0.05$). At -1.5°C, pseudomonads were reduced from log bacteria/cm²=3.81 at 0 weeks of storage to undetectable levels (log bacteria/cm²<2.00) at 18 weeks. At 2.0°C, pseudomonads were reduced from log bacteria/cm²=2.46 at 0 weeks of storage to undetectable levels at 12 weeks. Thereafter, no pseudomonads could be recovered from retail steaks at any retail display time.

The effects of CO_2 storage time and temperature upon retail case-life are shown in Table 1.

The retail case-life of rib-eye steaks decreased, for both storage temperature, as storage time in CO_2 increased ($P<0.05$). Appearance deteriorated more quickly than odour. During the first storage interval (0-6weeks), case-life of rib-eye steaks was reduced by as much as 60% at -1.5°C and 75% at 2.0°C.

Present results show the only detectable bacterial population throughout 24 weeks of storage (-1.5°C and 2.0°C) in CO_2 was the lactic acid bacteria. Thus, the growth of B.thermosphacta, pseudomonads and Enterobacteriaceae were completely inhibited. Similar results have been shown with CO_2-packaged pork (Egan and Roberts, 1987) and beef (Rousset and Renerre, 1991).

Present data support previous conclusions that high concentrations of CO_2 were very effective in inhibiting the growth of potent spoilage organisms (Gill and Harrison, 1989; Shay and Egan, 1987), allowing the CO_2-resistant lactic bacteria to thrive.

More importantly, changes occurred when beef steaks were removed from anoxic storage in CO_2 and subjected to the aerobic conditions of retail display. Lactic acid bacteria remained dominant during retail display, which supports studies with CO_2-packaged pork (Greer *et al.*, 1992). Lactic acid bacteria reached maximum levels of 10^7 log bacteria/cm² and remained the dominant group of organisms during ten days of retail display. Pseudomonads emerged as the only other group of bacteria detectable at the retail level. However, their numbers were always lowers than the lactic acid bacteria.

As CO_2 storage time increased (-1.5°C and 2.0°C), the number of pseudomonads recovered from steaks during retail display decreased significantly until they were no longer detectable. The inhibition of meat spoilage bacteria, including pseudomonads, by CO_2 supports previous findings (Gill ad Tan, 1980). However, this is the first observation of extended storage of beef in CO_2 reducing the numbers of pseudomonads recovered from steaks during subsequent retail display under aerobic conditions. Furthermore, this effect was more pronounced at the high CO_2 storage temperatures. This is contrary to reports of inhibitory effects of CO_2 increasing with decreasing temperatures (Egan and Molin, 1981). Apart from CO_2, it is conceivable increasing numbers of lactic acid bacteria during ÇO storage antagonize the pseudomonads and mediate a reduction in their numbers.

Present findings may have important implications in regard to the aerobic shelf-life of retail beef after storage in CO_2. Through the elimination of pseudomonads and the dominance of lactic acid bacteria, the usual putrid odours may be replaced by a delayed souring (Egan and Shay, 1982).

There has been limited work on the aerobic bacteriology and case-life of beef steaks after storage for extended periods of time in CO_2. Previous research found retail-ready steaks packaged and stored in CO_2 had significantly more surface discoloration, decrease appearance ratings and higher bacterial numbers in comparison to steaks derived from vacuum-packaged primals (Seideman *et al.*, 1980; Christopher *et al.*, 1980). In contrast to these earlier reports, the current study would complement recent data of Rousset and Renerre (1991) who reported CO_2 could maintain quality and improve case-life of retail steaks. That is, retail-ready beef steaks could be stored for up to 12 weeks in CO_2 at -1.5°C or 2.0°C and still have a retail case-life of two days.

CONCLUSION

The prolonged storage of retail-ready beef in CO_2 produced a substantial reduction in retail case-life following removal from the master-pack. However, retail keeping quality, even after 12 weeks of CO_2 storage is comparable to that presently achieved under retail conditions. Therefore, the described packaging system should allow more than sufficient time for the distribution, storage and display of retail-ready beef cuts in both the domestic and export market place.

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Table 1. Effects of CO_2 storage time and temperature upon the retail case-life of beef steaks[1].

Time in CO2 (weeks)	Case-life (days) Appearance -1.5°C	2.0°C	Odour -1.5°C	2.0°C
0	5.8	6.9	8.8	9.6
6	2.2	1.6	3.5	2.9
12	2.3	2.0	4.1	2.8
18	2.2	1.4	4.7	1.4
24	1.9	1.1	5.2	1.4

[1] Data are least squares means of five steaks. The standard error for appearance was 0.44 and for odour it was 0.34.