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Bacterial mediated off-flavours in retail-ready beef after storage in controlled atmospheres

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Abstract

The effects of anoxic storage under CO_2 at 2°C for up to 10 weeks followed by 28 h aerobic display in a retail case on flavour attributes and bacteriology of beef were examined. Pseudomonads reached 2.5 log cfu/cm^2 in one replication only and their numbers decreased with increasing storage time. *Enterobacteriaceae* and *Brochothrix thermosphacta* were not above levels of 1.0 and 2.0 log cfu/cm^2, respectively. Presumptive lactic acid bacteria increased to maximum numbers of 5.5 log cfu/cm^2 after 8 weeks. A subpopulation of lactic acid bacteria, able to grow in the presence of 12 g/l acetate, developed later in storage reaching a maximum population of 4.3 log cfu/cm^2. Flavour amplitude dropped to an inappropriate level after 6 weeks. Between 6 and 8 weeks, the numbers of lactic acid bacteria able to grow in the presence of 12 g/l acetate increased in the absence of an increase in presumptive lactic acid bacteria. This time coincided with the development of "off barny" aromatic, "off barny" aftertaste and unidentified "off" aromatic and a reduction in flavour amplitude suggesting a relationship between the growth of these organisms and changes in organoleptic properties.

Keywords: Beef; Anoxic packaging; Lactic acid bacteria; Flavour

1. Introduction

Changes in meat distribution and merchandising have required extension of product storage life without compromising product quality and safety. Preservative packaging has emerged as a promising method for product storage life extension, without the need for chemical preservatives (Farber, 1991; Gill and Molin, 1991). The most effective packaging system for fresh, chilled meats uses high CO_2 controlled atmosphere (Gill, 1989), which provides both an atmosphere which inhibits the growth of most bacteria (Dainty, Shaw, Harding & Michanie, 1979; Egan, 1983), and maintains desirable meat colour, when oxygen levels and storage temperatures are properly controlled (Gill 1989). In this packaging system the bacterial flora becomes dominated by lactic acid bacteria, which attain maximum numbers of 10^8/cm^2.

When meat is removed from controlled atmosphere storage and is displayed aerobically in a retail display case discolouration is relatively rapid and the product becomes unacceptable to consumers after two to four days (Greer & Jones, 1991; Greer, Dilts & Jeremiah, 1993; Moore & Gill, 1987; Shay & Egan, 1990). Bacterial levels increase upon removal from the CO_2 package. With both beef and pork, lactic acid bacteria predominate and are the only detectable organisms until after about 4 days of retail display, when pseudomonad numbers increase (Greer 1993; Nesom-Fleet, Greer, Jeremiah & Wolfe, 1993). When beef is removed after up to 24 weeks of storage at −1.5°C in CO_2, colour and odour remain acceptable (Nesom-Fleet et al.).

Optimally beef flavour consists only of appropriate flavour notes, or those usually associated with fresh (unstored) beef with normal muscle properties, in the proper balance and blend to provide a favourable overall impression and a high flavour amplitude ($\geqslant$7.50). Beef with these characteristics would be exemplified by high quality, unstored samples, with a relatively undeveloped microflora.

Presently, the limiting factor for storage life extension, early off-flavour development, appears to result from a flora of lactic acid bacteria which reach maximum numbers between 6 and 9 weeks on chilled pork primals stored in high CO_2 controlled atmosphere packaging (CAP) at −1.5°C (Jeremiah & Gibson, 1995; Jeremiah, Gibson & Arganosa, 1995a, 1995b).

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The present study was designed to examine the relationship between bacteria and off-flavour development in retail-ready beef steaks during high CO_2-CAP storage with subsequent aerobic display. The "off barny", "sour/off", and unidentifiable "off" aromatics and aftertastes were examined since they had previously been identified as coinciding with early off-flavour development (Jeremiah & Gibson, 1995). The design simulated actual merchandising of retail-ready beef and consumer handling after purchase. The possibility that a subpopulation of lactic acid bacteria might be responsible for organoleptic deterioration was addressed.

2. Materials and methods

2.1. Meat samples

Vacuum packaged beef rib eyes were obtained from a federally inspected abattoir (Edmonton Meat Packers, Edmonton, AB, Canada) following a 48 h chill and were transported to the Lacombe Research Centre. Immediately upon arrival the vacuum packages were opened and the rib eyes were cut into boneless, rib-eye steaks. Rib-eye steaks were randomly allocated to 11 storage intervals (0, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days). All cuts were placed on hard plastic trays and put into oxygen impermeable metallized pouches (Securefresh Pacific Ltd., Auckland, New Zealand) with a gas transmission rate of <0.1 cm/m^2/24 h/atm at 25°C and 100% r.h. The pouches were evacuated and filled with 100% CO_2 (2l/kg of product) (Liquid Air Inc., Red Deer, AB, Canada), using a modified Captron III packaging machine (RMF Inc., Grandview, MO, USA). Residual O_2 levels were <300 ppm as measured by a Mocon Oxygen Analyzer (Model FBP-08B12L, Mocon Modern Controls Inc., Minneapolis, MN, USA). The samples were stored at 2°C and sampled weekly for up to 10 weeks.

2.2. Retail display

At weekly intervals, a package was removed and opened. The product was placed individually on to 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) with an oxygen transmission rate of 8000 cc/m^2/24 h. Packaged steaks were placed into a horizontal, retail display case (Model LPM12T, Hill Refrigeration of Canada, Ltd., Barrie, ON, Canada) with fan-circulated air set to operate at a temperature of 4°C. The temperature on the surface of the meat was monitored using a Delphi temperature logger (Delphi Industries Limited, Auckland, New Zealand). A detailed description and evaluation of this retail environment has been published (Greer & Jeremiah, 1980). Steaks were displayed for 28 h and then their sensory, organoleptic and microbiological properties were evaluated.

2.3. Muscle pH, objective colour and sensory evaluation

pH was measured using an Oaktron Digital pH meter (Model Wo-0060500-000, Anachemia Scientific, Calgary, AB, Canada) and colour was objectively measured using a Minolta chromameter II (Minolta Camera Co. Ltd., Japan). Triplicate readings of Commission Internationale de l'Eclairage (CIE, 1978) values (L^*, a^* and b^*) were recorded for each steak.

Sensory evaluation consisted of the assessment of muscle colour (9-point scale: 0 = completely discoloured; 1 = white; 8 = extremely dark red), surface discolouration (7-point scale: 1 = no surface discolouration; 7 = complete discolouration), retail appearance (7-point scale: 1 = extremely undesirable; 7 = extremely desirable), off-odour intensity (5-point scale: 1 = no off-odour; 5 = prevalent off-odour), and odour acceptability (5-point scale: 1 = acceptable; 5 = unacceptable) by an experienced, trained, 5-member sensory panel (Greer et al., 1993).

2.4. Microbiological assessment

A 10 cm^2 core was aseptically excised from each steak at each sampling period. Cores were homogenized for 2 min in 90 ml of 0.1% peptone diluent using a Colworth Stomacher (Baxter Diagnostics Corp., Canlab Division, Edmonton, AB, Canada). Ten-fold dilutions were prepared and aliquots were plated. The lower limit of sensitivity using this procedure was log cfu/cm^2 of 1.0 for *Enterobacteriaceae* and 2.0 for other bacteria. Selective media described by Baird, Corey and Curtis (1987) were used to enumerate pseudomonads, *Brochothrix thermosphacta*, lactic acid bacteria and *Enterobacteriaceae*. Total psychrotrophs were enumerated using plates of Plate Count Agar (Difco Laboratories Inc., Detroit, MI, USA) incubated for 10 days at 5°C. Cephaloridine-fucidin-cetrimide agar (CFC) was used to enumerate pseudomonads with a 2 day incubation at 25°C, and streptomycin sulfate-thallous acetate-actidione agar (STAA) was used to enumerate *Brochothrix thermosphacta* with a 3 day incubation at 25°C. Enumeration of lactic acid bacteria (LAB) and those LAB able to grow in the presence of 12 g/l acetate at pH 5.6 (AcLAB) was done on MRS agar (Difco) and Rogosa agar (pH 5.6, Oxoid Inc., Nepean, ON, Canada), respectively with a 3 day anaerobic incubation at 25°C. Anaerobic conditions were established using a BBL anaerobic system with an atmosphere containing 5 to 10% CO_2 (Becton Dickenson Co., Cockeysville, MD, USA). Pour plates of Violet Red Bile Glucose Agar

(Difco) with an overlay were used to enumerate *Enterobacteriaceae*. Plates were incubated for 18 to 24 h at 35°C.

2.5. Flavour profiles

The four steaks evaluated after each time interval for bacterial numbers and colour were also utilized to obtain complete flavour profiles. They were grilled to 72°C on an electric grill. All samples were cut into cubes (1.9 cm^3) taking care to avoid large pieces of fat and connective tissue. Samples underwent flavour profile analysis, and complete flavour profiles were formulated by a highly trained 6 to 12 member professional flavour/texture profile panel, using methods previously described in detail (Jeremiah, Gibson & Burwash, 1997). Flavour profiles were rated on a 15 point scale. For inappropriate flavour notes a score of 0 indicates that the flavour note is nonexistent and a score of 15 is the highest score these flavour notes normally attain. A score of 15 is usually the highest flavour amplitude score attainable. When the flavour amplitude scores fall below 7.5 the appropriateness, balance and blendedness of the overall flavour becomes inappropriate.

2.6. Data analysis

Data were analyzed using the General Linear Models Analysis of Variance Procedure (ANOVA) and Pearson correlation coefficient analysis of the Statistical Analysis System (SAS Institute, 1995). When ANOVA showed an effect of storage time at $P<0.05$ the least square means and standard error were presented graphically.

3. Results

3.1. Storage and display conditions

The temperature during 10 weeks of storage in CO_2 ranged from 0.3 to 3.6°C with a mean temperature of 2°C. The temperature at the meat surface on display ranged from 2.3 to 23.9°C. The mean temperature during retail display was 8.6°C.

3.2. Muscle pH, objective colour and sensory evaluation

L^* and b^* were not affected by storage time in CO_2 (data not shown) but a^* decreased from 15.8 prior to storage to 13.6 after 10 weeks. pH values ranged from 5.6 to 5.9 and were not affected by duration of storage.

Visual assessment of the steaks during retail display (Fig. 1) demonstrated that colour was not affected by duration of storage. However, discolouration increased and retail appearance deteriorated as storage time increased. Off-odour intensity also increased and odour acceptability deteriorated as storage was extended (Fig. 2).

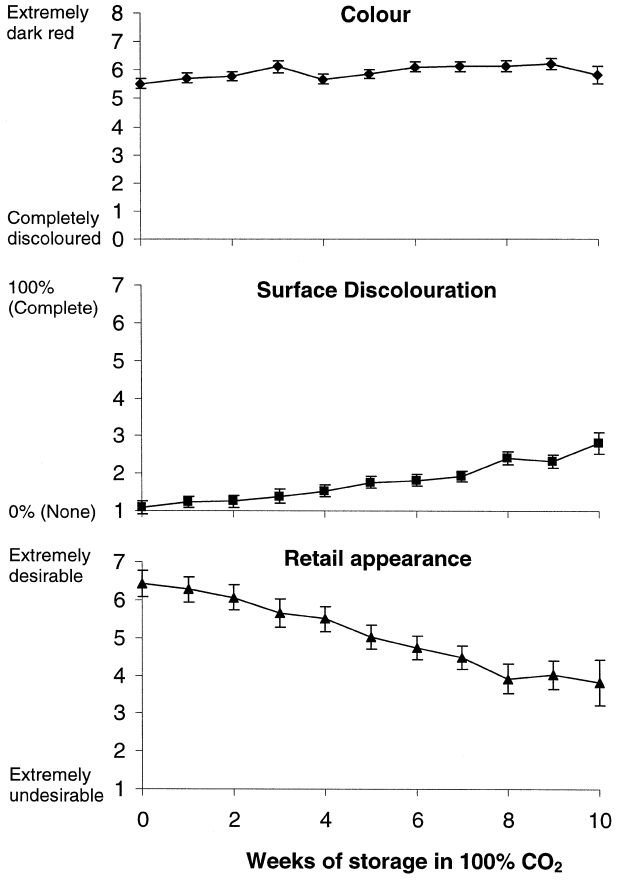

Fig. 1. Least square means and standard errors for subjective measurements of colour, discolouration and retail appearance of steaks stored in 100% CO_2 for 10 weeks followed by 28 h of retail display.

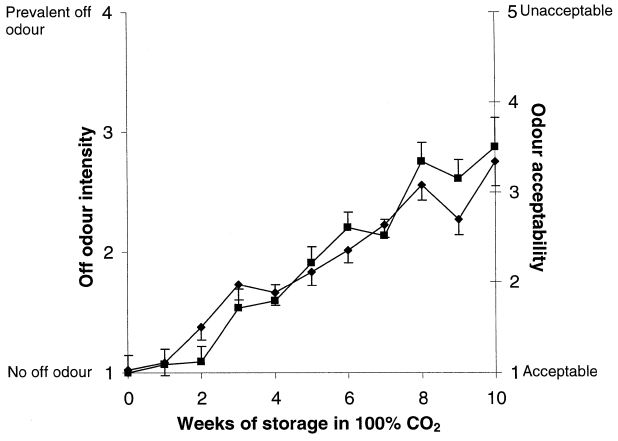

Fig. 2. Least square means and standard errors for subjective measurements of off-odour intensity (◆) and odour acceptability (■) of steaks stored in 100% CO_2 for 10 weeks followed by 28 h of retail display.

3.3. Microbiological properties

No *Brochothrix thermosphacta* or *Enterobacteriaceae* were detected at limits of 2.0 and 1.0 log cfu/cm^2, respectively, during storage in CO_2. Pseudomonads were detected only in replication 2 (Fig. 3) and their

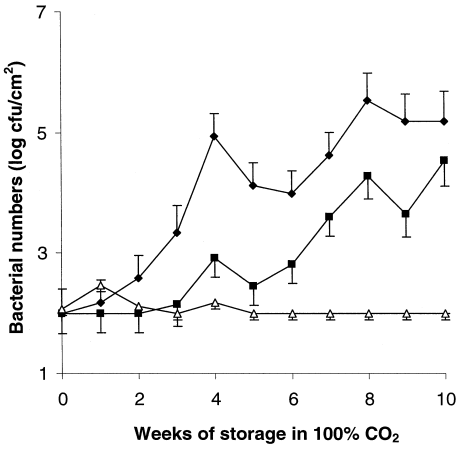

Fig. 3. Least square means and standard errors for numbers of presumptive lactic acid bacteria (LAB) (◆), aciduric lactic acid bacteria (AcLAB)(■) and pseudomonads (replication 2 only)(△) on steaks stored in 100% CO_2 for 10 weeks followed by 28 h of retail display.

numbers decreased with increased storage time in CO_2. Numbers of psychrotrophs had Pearson correlation coefficients of 0.98 and 0.97 ($P=0.0001$) with LAB enumerated on MRS (Fig. 3). LAB were divided into two groups, those capable of growing on MRS (the total presumptive LAB population) and a subgroup capable of growing in the presence of 12 g/l acetate at a pH of 5.6 (acetate resistant lactic acid bacteria, AcLAB; Fig. 3). LAB were enumerated earlier than AcLAB.

3.4. Palatability attributes

The development of the inappropriate "off barny" aromatic and aftertaste and unidentified "off" aromatic and aftertaste with increasing storage time is shown in Fig. 4. These inappropriate notes were significantly affected by storage time; but there was no relationship between the development of a "sour/off" aromatic and aftertaste during storage in CO_2. Flavour amplitude (appropriateness, balance and blend of the character notes) decreased with increasing storage time and fell below 7.5 after 4 weeks of storage (Fig. 4).

3.5. Bacterial numbers and sensory and palatability attributes

In three of the four replications, an increase in AcLAB numbers in the absence of an increase in LAB numbers coincided with the development of "off barny" aftertaste and unidentified "off" aromatic and aftertaste, and a decrease in flavour amplitude. This observation is depicted in Figs. 5 and 6 for replications 2 and 3. In replication 2 the changes occurred between 7 and 8 weeks of storage in CO_2 and in replication 3 they occurred between 6 and 7 weeks.

4. Discussion

When CO_2 packaging is properly applied, a population of lactic acid bacteria (LAB) dominates the microflora which develops on muscle with extended storage at low temperatures (Blickstad & Molin, 1983; Enfors, Molin & Ternström, 1979; Gill, 1989). An apparent residual effect of CO_2 storage existed after transfer to an aerobic environment, as demonstrated by the lack of growth of pseudomonads, other aerobic bacteria and facultative anaerobes. Continued dominance of lactic acid bacteria on pork during 3 to 4 days in retail display has been demonstrated (Enfors et al., 1979; Greer et al., 1993).

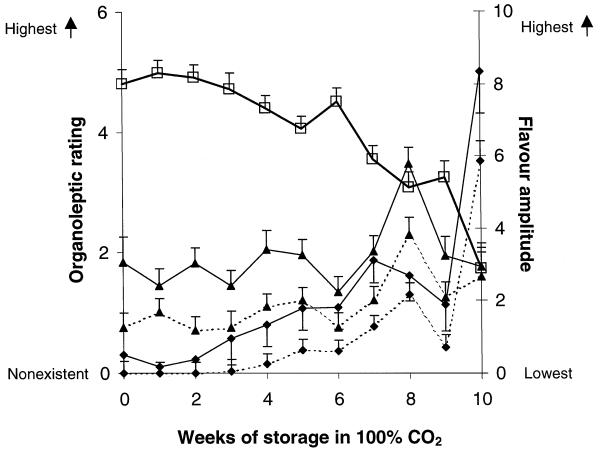

Fig. 4. Least square means and standard errors for intensity of "off barny" aromatic (◆, solid line), "off barny" aftertaste (◆, dashed line), unidentifed "off" aromatic (▲, solid line) and unidentified "off" aftertaste (▲, dashed line) and the flavour amplitude (□) for steaks stored in 100% CO_2 for 10 weeks followed by 28 h of retail display.

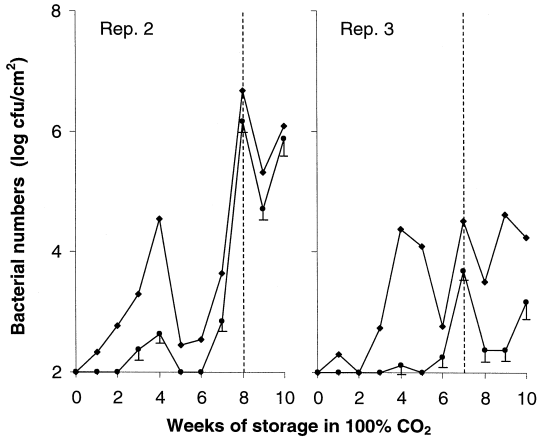

Fig. 5. Least square means for growth of acetate resistant lactic acid bacteria (●) and presumptive lactic acid bacteria (◆) during 10 weeks of storage in CO_2 followed by 28 h of retail display in replications 2 and 3. The dashed lines show the time at which the increase in LAB numbers was entirely due to an increase in AcLAB numbers.

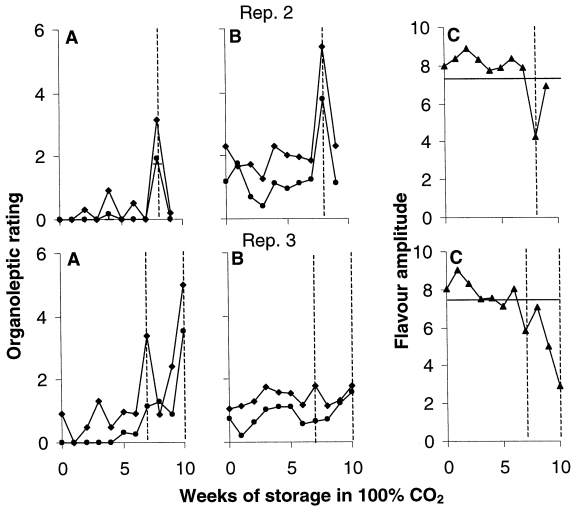

Fig. 6. Least square means for A: intensity of "off barny" aromatic (◆) and "off barny" aftertaste (●); B: intensity of unidentifed "off" aromatic (◆) and unidentified "off" aftertaste (◆) and C: the flavour amplitude (▲) for steaks in replications 2 and 3 stored in 100% CO_2 for 10 weeks followed by 28 h of retail display. The dashed lines show the time at which the increase in numbers of presumptive lactic acid bacteria numbers was entirely due to an increase in the number of acetate resistant lactic acid bacteria.

To determine whether a subpopulation of LAB was implicated in reductions in palatability, several differentiation regimes (Cavett, 1963; Hitchener, Egan & Rogers, 1982; Leisner, Milan, Huss & Larsen, 1994; Shaw & Harding, 1984; Wilkinson & Jones, 1977) were evaluated in an attempt to divide the population of LAB into groups. Use of Rogosa agar to separate LAB capable of growing in the presence of 12 g/l acetate demonstrated one subgroup of LAB that had different growth characteristics than the LAB grown on MRS (2.2 g/l CH_3COO^-). The numbers of this subgroup did not start to increase until after 3 weeks of chilled storage in 100% CO_2. Their maximum population was 1.25 log cfu/cm^2 less than LAB enumerated on MRS. Between 6 and 8 weeks of chilled storage an increase in numbers of AcLAB (1.46 log cfu/cm^2) was almost entirely responsible for an increase in LAB (1.52 log cfu/cm^2). It was during this time period that an increase in intensity of inappropriate flavour character notes ("off barny" aftertaste, unidentified "off" aromatic and unidentified "off" aftertaste) and a decrease in flavour amplitude occurred. When individual replications were examined separately these relationships became more defined in three of the four replications. In replication 2 there was an increase of 3.3 log cfu/cm^2 in AcLAB and 3.0 log cfu/cm^2 between 7 and 8 weeks of storage, while in replication 3 (and in replication 1, data not shown) this increase occurred between 6 and 7 weeks and the increase was 1.4 log cfu/cm^2 for AcLAB and 1.8 cfu/cm^2 for LAB. Changes in intensity of the inappropriate flavour notes, "off barny" aromatic, "off barny" aftertaste and unidentified "off" aromatic and the decrease in flavour amplitude coincided with the increase in AcLAB. The intensity of "off barny" aromatic was similar in both replications while the unidentified "off" aromatic was lower in Replication 3. It is possible, therefore, when the AcLAB population begins to develop, there is a concomitant deterioration in some organoleptic properties of beef steaks stored in 100% CO_2 and subsequently displayed for 28 h in a retail display case. The extent of deterioration may be related to the numbers of AcLAB when they are present and when storage conditions permit them to develop.

No relationship was observed between numbers of the LAB and AcLAB and the "sour/off" aromatic and aftertaste, which had previously been identified as coinciding with LAB approaching maximum numbers (Jeremiah & Gibson, 1995). Since the LAB in this study reached a maximum of only 5.5 log cfu/cm^2, their expected maximum numbers of approximately 8 log cfu/cm^2 were not reached within the allowed storage time.

5. Conclusions

Storage of steaks in CO_2 followed by 28 h of aerobic display in a retail display case resulted in a gradual deterioration of retail appearance, odour acceptability and flavour and a gradual increase in "off" odour and flavour development. None of the olfactory or visual characteristics decreased to unacceptable levels, but flavour eventually became inappropriate (undesirable). Therefore the product became unpalatable before visual or olfactory observation resulted in rejection under these experimental conditions, confirming previous findings (Jeremiah and Gibson, 1995; Jeremiah et al., 1995a,1995b). A subpopulation of lactic acid bacteria capable of growth in the presence of 12 g/l acetate developed rapidly after 6 to 7 weeks of storage in most samples. This increase was responsible for the entire increase in presumptive lactic acid bacteria during this time and coincided with the development of inappropriate "off barny" aromatic, "off barny" aftertaste and unidentified "off" aromatic and a decrease in flavour amplitude to inappropriate levels.

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