INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

UNIVERSITY OF ALBERTA

Ground Beef Quality and Extended Storage Life

by

Rodney Jason Worobo 

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science

IN

Food Microbiology

Department of Agricultural, Food and Nutritional Science

Edmonton, Alberta

Spring 1997

UNIVERSITY OF ALBERTA

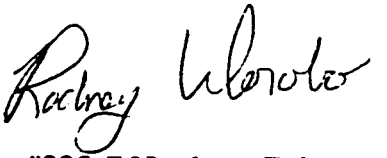
RELEASE FORM

NAME OF AUTHOR: Rodney J. Worobo

TITLE OF THESIS: Ground Beef Quality and Extended Storage Life

DEGREE: Master of Science

YEAR THIS DEGREE GRANTED: 1997



#205, 7 Northstar Drive
Lacombe, Alberta, CANADA
T4L 1C6

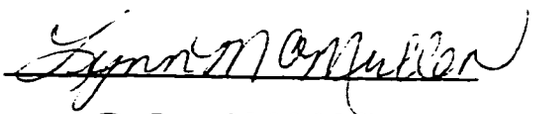
January 28, 1997

UNIVERSITY OF ALBERTA

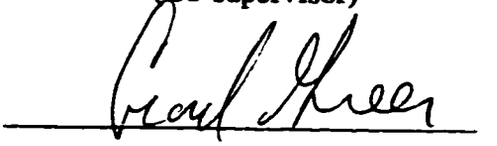
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Ground Beef Quality and Extended Storage Life** submitted by Rodney Jason Worobo in partial fulfillment of the requirements for the degree of **Master of Science in Food Microbiology**.

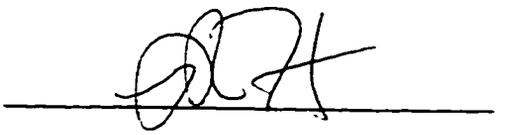
Dr. Michael E. Stiles
(Supervisor)



Dr. Lynn M. McMullen
(Co-supervisor)



Dr. G. Gordon Greer



Dr. Gordon R. Finch

December 19, 1996

DEDICATION

To Mom, Dad and Randy

ABSTRACT

Centralized preparation of ground beef at a large meat packing plant was evaluated to determine if it could produce a high quality product with an improved shelf-life. The results showed ground beef can be stored in vacuum for 20 days with a retail storage life of 2.5 days. Biopreservation by the bacteriocin-producing *Leuconostoc gelidum* UAL187 and nonbacteriocinogenic UAL 187-13 was studied in commercially produced vacuum packaged (VP) ground beef. The adventitious LAB, *Enterobacteriaceae* and *Pseudomonas* spp. were 1 to 2 log lower in the inoculated ground beef than in the uninoculated control after 35 days of storage. The colour stability of retail ground beef after extended storage was improved by the addition of *L. gelidum*. The odour intensity of the inoculated VP ground beef was significantly lower than the uninoculated sample, but the overall flavour intensity and sour off-flavour notes of ground beef inoculated with *L. gelidum* UAL187 and 187-13 deteriorated at the same rate as the uninoculated stored control samples.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisory committee members: Dr. Michael E. Stiles, Dr. Gordon Greer and Dr. Lynn McMullen for all the advice, support and time they dedicated to me during my graduate studies.

A special thanks to the all the staff at the Lacombe Research Centre - Meat Research Section for all the help you gave me and also for making me feel right at home while working there. For all his technical advice I would like to acknowledge Bryan Dilts.

I would like to acknowledge all the panelists at the University of Alberta who took part in my research. A special thanks to Jean Chaw Kant and Susan Gibson for all the guidance and advice they gave me while working with them.

I would like to thank a couple of my friends Ken Fahner and John McCormick for all the good times we had during my graduate studies.

Finally, I would like to acknowledge my family. My brother Randy, who has always been there when I needed him. Most of all I would like to thank my parents, Donna and Raymond Worobo. Without all their love, support and understanding this accomplishment would have not been possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. Literature Review

1.1. INTRODUCTION

The bacteriological condition of ground beef has remained unchanged for the past 80 years (Foster et al., 1977; Gill and McGinnis, 1993) and microbial counts of 10^6 to 10^7 colony forming units (CFU)/g are still routinely reported. With these high counts it is not surprising that ground beef deteriorates within a day at the retail level. The reason for this problem is that the process of ground beef production has not changed over the years. Traditionally, beef trimmings from in-store preparation of retail cuts and commercially-produced vacuum packaged (VP) beef trim are blended, ground and displayed for retail sale. The problems with ground beef produced in this manner are the poor microbial quality of the trimmings and retail temperature abuse that results in large numbers of spoilage bacteria and unacceptable levels of coliform bacteria including *Escherichia coli* (Gill and McGinnis, 1993).

An alternative method to the traditional production of ground beef is preparation at a centralized facility where it has been shown that ground beef of superior microbiological quality can be produced (Shoup and Oblinger, 1976). The centralized production of ground beef entails all of the preparation operations of slaughter, dressing, grinding and packaging in the same plant followed by shipment to the retail outlet. Centralized prepackaging systems allow the potential for increased efficiency in the fresh meat industry because it permits improved control of inventory, reduces processing waste, allows for product uniformity, reduces labour and facilitates savings in materials and equipment. In addition centralized preparation minimizes product handling at the retail level which further prevents cross-contamination with potentially pathogenic and spoilage microorganisms. Overall the potential exists for more effective control of processing hygiene with a concomitant improvement in product safety and storage stability.

Centralized operations are based on the technology of vacuum packaging which revolutionized the fresh meat industry with a significant increase in storage life of the meat. The meat normally deteriorates rapidly under aerobic conditions due to the prevalence of gram-negative putrefactive bacteria. Under vacuum, the anaerobic conditions allow for the prevalence of gram-positive, catalase-negative coccus and rod-shaped bacteria that are collectively referred to as lactic acid bacteria (LAB). This group of bacteria generally delays spoilage until some time after maximum population is reached. LAB that dominate on fresh meats are a mixed population of bacteria that are composed primarily of three genera of *Carnobacterium, Leuconostoc* and *Lactobacillus*. As a result of the mixed population of LAB the storage life of vacuum packaged chill stored fresh meats is difficult to predict. A strain of *Lactobacillus sake* that produces sulphur compounds causes overt spoilage within three weeks (Shay and Egan, 1981). The dominance of LAB under these conditions is not only attributed to their ability to grow in an anaerobic environment but it may also be due to their ability to produce a number of inhibitory compounds that are believed to contribute to their dominance in foods, including: organic acids, hydrogen peroxide, CO_2, diacetyl and bacteriocins.

As a novel and "natural" means of reducing the unwanted bacteria in raw meat the use of competitive LAB strains that produce and exert their antagonistic properties during storage and distribution has been suggested (Stiles and Hastings, 1991), and it has been suggested that bacteriocins and bacteriocin-producing LAB could be used as possible biopreservatives to replace chemical preservatives in foods. Bacteriocins are defined as "proteins or protein complexes with bactericidal activity directed against species that are closely related to the producing bacterium" (Klaenhammer, 1988). The genetics of bacteriocin production have been studied but now the focus of bacteriocin research is shifting to the selection of bacteriocin-producing strains that cause minimal spoilage, that are capable of growing in meats under refrigerated conditions and will destroy the meatborne pathogens and spoilage organisms.

This study was designed to determine the hygienic efficiency of VP coarse ground beef produced at a large centralized facility transported to and stored at retail level and to establish the bacteriological profile of the ground beef transported and stored under commercial conditions and simulated retail display; and to determine whether the storage life of the ground beef could be extended by the novel approach of adding a bacteriocin-producing LAB to control or prevent spoilage without affecting the sensory properties.

1.2. MEAT MICROBIOLOGY DURING ANAEROBIC STORAGE

Muscle tissue of a red meat carcass after slaughter typically carries between 10^2 and 10^4 bacteria/cm^2 (Dainty and Mackey, 1992). Contaminants on the carcass are derived from a number of sources, including: the hide, faeces and gut contents and from the hands of workers and the instruments that they use to dress the carcass. The bacteria that contaminate the surface of the carcass are largely mesophilic and include a diverse group of both gram-positive and gram-negative organisms. The environment used for the storage of the meat provides a selective pressure on the microflora and dictates the microflora that develops on the surface of the fresh meat.

Fresh meats that are stored aerobically at chill temperatures allow growth of a putrefactive aerobic microflora that consists largely of the *Pseudomonas* sp. (Ingram, 1962; Gill and Newton, 1977; Dainty and Mackey, 1992). Fresh meats that are stored in the absence of oxygen under vacuum or in a modified atmosphere (MAP) with elevated levels of CO_2, inhibits growth of the putrefactive aerobes and allows a population of gram-positive LAB to dominate (Enfors et al., 1979). LAB are a group of gram-positive, usually non-motile, nonsporulating, microaerophilic bacteria that produce lactic acid as a major or sole product of their fermentative metabolism (Kandler, 1983). LAB grow anaerobically, but they can grow in the presence of oxygen making them facultative anaerobes. LAB can be further differentiated on the basis of the end-products formed during sugar fermentation. LAB that produce lactic

acid as a single end-product of fermentation are called homofermenters; whereas those that produce a variety of end-products including lactic acid, acetic acid, and/or ethanol and CO_2 are known as the heterofermenters. The homofermentative LAB ferment hexose sugars via the glycolytic pathway when there is an unlimited carbohydrate supply, and the heterofermentative LAB ferment hexose sugars via the 6-phosphogluconate pathway. The production of acetic acid or ethanol depends on the oxidation potential of the system. Under anaerobic conditions the production of ethanol is favoured (Blickstad and Molin, 1984).

LAB are considered to be preservative agents in foods including meats because in oxygen depleted environments the spoilage process is slowed. However, LAB eventually spoil meat as a result of the end-products of fermentation. Spoilage is a result of the acidity or souring of the meat (Sutherland et al., 1976; Dainty et al., 1983). Odours that spoil the meat do not become noticeable until sometime after maximum numbers have been reached. Some LAB that dominate the meat microflora are potent spoilage agents and their growth drastically reduces the storage life of meats during anaerobic storage (See Section 1.5).

Early studies classified the gram-positive LAB of meats as "atypical streptobacteria, lactobacilli and or leuconostocs". The LAB are comprised of heterogeneous genera that include: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus* and *Vagococcus*. The genera *Aerococcus* and *Vagococcus* are not associated with foods. *Lactococcus* spp. are not commonly associated with meats (Holzapfel, 1992), but they have been identified in some instances on meats (Schillinger and Lücke, 1987a, b). Carnobacteria, lactobacilli and leuconostocs are the usual prevailing LAB on anaerobically packaged, chill stored meats (Hitchener et al., 1982; Shaw and Harding; 1984; Schillinger and Lücke, 1987 a, b; Borch and Molin, 1988; McMullen and Stiles, 1993). In a taxonomic study of LAB isolated from VP beef, pork and lamb, Shaw and

Harding (1984) found that the lactobacilli could be divided into aciduric and nonaciduric strains. The aciduric strains were initially identified as *Lactobacillus sake* or *Lactobacillus bavaricus* (Shaw and Harding, 1984). *Lactobacillus divergens* (Holzapfel and Gerber, 1983) and *Lactobacillus carnis* (Shaw and Harding, 1984) were identified as the nonaciduric strains. These strains were reclassified as *Carnobacterium divergens* and *Carnobacterium piscicola*, respectively (Collins et al., 1987). The nonaciduric strains were differentiated from the *Lactobacillus* spp. isolated from MAP and VP meats by their failure to grow on acetate agar and other distinguishing phenotypic characteristics (Shaw and Harding, 1985; Collins et al., 1987). The specific LAB that prevail on fresh meat stored for extended periods of time plays a significant role in storage life of the meat (Section 1.5)

Brochothrix thermosphacta, previously known as *Microbacterium thermosphactum*, is a gram-positive, facultative anaerobe that utilizes glucose as its major substrate (Newton and Gill, 1978; Dainty and Hibbard, 1980). Under aerobic conditions the organism metabolizes glucose to produce acetic acid and acetoin which are linked to the "dairy" odours associated with spoilage by *B. thermosphacta*. Under anaerobic conditions the organism produces lactic acid from glucose (Gardner, 1980). *B. thermosphacta* has been reported in high numbers on chill stored, aerobically packaged meats and it has been reported to reach numbers of $10^6/cm^2$ under anaerobic conditions (Sutherland, 1975b; Dainty et al., 1979; Erichsen and Molin, 1981). The reports that *B. thermosphacta* prevail under anaerobic conditions are inconsistent with earlier studies in which the growth of *B. thermosphacta* was not detected in the abscence of oxygen (Pierson et al., 1970; Roth and Clark; 1972). It has been shown that *B. thermosphacta* will not grow on anaerobically packaged meats with a pH lower than 5.8 and therefore growth of the organism on meat is largely dependent upon pH and packaging environment.

Enterobacteriaceae can also grow on VP and MAP fresh, chill stored meats. These bacteria have not been enumerated in all studies but results have shown that they can constitute up to 10% of the microflora after 4 weeks of anaerobic storage (Beebe et al., 1976; Seideman et al., 1976) and numbers as high as $10^6/cm^2$ have been reported (Dainty et al., 1979). The key to control of growth of *Enterobacteriaceae* on fresh meats is temperature. Storage temperatures greater than 10°C allow the *Enterobacteriaceae* to flourish, but on normal pH meat at temperatures below 7°C these organisms do not grow well. However, at a storage temperature of 5°C *Enterobacteriaceae* have been isolated from MAP and VP meats (Hanna et al., 1976; Patterson and Gibbs, 1977; Vanderzant et al., 1982; Manu-Tawiah et al., 1993). The *Enterobacteriaceae* isolated from these meats at 5°C include: *Enterobacter, Hafnia, Serratia* spp. and *Yersinia enterocolitica.* Psychrotrophic *Enterobacteriaceae* can dominate the microflora of MAP meats stored below 2°C after periods of extended storage (Newton et al., 1977; Blickstad and Molin, 1983; Gill and Harrison, 1989).

Growth and large numbers of *Enterobacteriaceae* on anaerobically packaged meats have been attributed to high initial levels of contamination on the meat surface (Gill and Penney, 1986). The *Enterobacteriaceae* can be associated with undesirable off-odours on meats and *Serratia liquefaciens* and *Yersinia enterocolitica* have been identified with production of these odours (Gill and Newton, 1978; Dainty et al., 1979). On meat with a relatively high pH value (pH>6.0) *Shewanella* (formerly *Alteromonas*) *putrefaciens*, in combination with an increase in total numbers of cold tolerant *Enterobacteriaceae*, can cause rapid production of a particularly obnoxious form of spoilage associated with hydrogen sulphide (Dainty and Mackey, 1992). Growth of psychrotrophic *Enterobacteriaceae* that dominate under anaerobic conditions are inhibited in most circumstances by the combination of CO_2 and lactic acid (Grau, 1981), but the inhibitory action of these products is reduced and growth is enhanced when temperature, pH and oxygen permeability of the packaging material is increased.

It is imperative to control the growth of the *Enterobacteriaceae* in anaerobically packaged meats because they drastically reduce the storage life.

An unusual type of spoilage of anaerobically packaged, normal pH meat is characterized by blown packages and "sulphurous", "fruity", "solvent-like" and "strong cheese" odours have been reported, and the organism responsible is a *Clostridium* sp. (Dainty et al., 1989; Kalchayanand et al., 1989; 1993). *Clostridium* spp. are not normally associated with spoilage of fresh meat, because they do not grow well in the competitive environment found on meat tissue (Hauschild, 1989); however, results of Dainty et al. (1989) showed that clostridia can be isolated with prevalent LAB microflora. Kalchayanand et al. (1989) also reported spoilage of VP beef by *Clostridium* spp. with LAB levels of 10^6/ml in the liquid purge from the meat. The organism responsible for the spoilage was classified as *Clostridium laramie* sp. nov. (Kalchayanand et al., 1993). They confirmed that *C. laramie* was responsible for spoilage by inoculation studies in which the purge was used to inoculate aseptically prepared beef, and the same spoilage pattern occurred in 1 to 2 weeks. *Clostridium botulinum* can grow on meat under anaerobic conditions at very abusive temperatures (Hauschild et al., 1985); but the potential for it to be hazardous at chill temperatures in fresh meat is small because spoilage precedes toxigenesis (Hauschild, 1989).

1.3. CENTRALIZED AND TRADITIONAL PREPARATION OF GROUND BEEF

A large proportion of beef is sold as ground beef (Foster et al., 1977). With the advancement in technology and knowledge of this area of food production it is surprising that the bacteriological condition of ground beef has not changed over the past 80 years (Foster et al., 1977; Gill and McGinnis, 1993). It is not uncommon for the bacterial load of retail displayed ground beef to exceed 10^7 CFU/g (Duitschaever et al., 1977, Hudson et al., 1986; Nychas et al., 1992). The ultimate control of ground beef quality relies on three main factors: initial bacteriological load of the meat used for

grinding, sanitation of the equipment and time-temperature of the storage. The ground beef that is produced today is still done primarily by "traditional preparation

1.3.1. Traditional Preparation of Ground Beef

The traditional preparation of ground beef is defined by Shoup and Oblinger (1976) as the "cutting, grinding, packaging and other preparatory operations on the premises of a retail store." The bacteriological quality of the ground beef produced in this manner relies mainly on the retailer. In most instances the retailer does not have the knowledge of the factors that are required to maintain ground beef quality. In the past, all of the operations of the ground beef production operations were carried out at the retail outlet, but today the retailer utilizes trim that accumulates at the retail and supplements it with trim from a centralized producer. The trim that is obtained from the centralized producer requires preparation prior to grinding for retail display.

A study by Gill and McGinnis (1993) showed that the bacterial load of ground beef still exceeds 10^7 CFU/g at the retail level. They evaluated ground beef produced from trim that was fabricated at a large centralized plant from the time of fabrication through to retail display. At the point of fabrication prior to vacuum packaging, the trim had a bacterial load ranging from 10^3 to 10^5 CFU/g with an average of 10^4 CFU/g. After refrigerated transportation and grinding at the retail outlet, the load had increased to 10^4 to 10^7 CFU/g with 75% of the samples exceeding 10^6 CFU/g. Gill and McGinnis (1993) concluded that there are two factors that affect the bacteriological condition of ground beef produced in this manner: (1) wholesalers and retailers maintain stocks of meat that allow them to deal with the fluctuations in supply and demand and it is inevitable that some of stocks will be stored for extended times that will result in inadequate stability when displayed; (2) temperature control is lost in the retail case (James and Bailey, 1990; Greer et al., 1994). In the retail display case it is not unusual to find meat at temperatures greater than 10°C (Bogh-Sorensen and Olsson, 1990; Greer et al., 1994). This temperature is affected by the lighting, display design,

inadequate temperature setting, placement of meat in the retail case and also temperature of the meat prior to grinding (Dempster and Cody, 1978).

1.3.2. Centralized Production

An alternative method to traditional preparation of ground beef is centralized production. Centralized preparation can be defined as production in an "autonomous facility in which all preparatory operations are completed before the packaged product is transported to retail outlets" (Shoup and Oblinger, 1976). Ground beef that is centrally produced is shipped either coarsely ground, which requires a fine grind at the retail outlet, or retail ready. A study by Shoup and Oblinger (1976) showed the centralized preparation of ground beef resulted in lower microbiological load than ground beef produced in the traditional manner. Another study showed that 1.5 kg VP "chub" packs of ground beef that were fabricated commercially and supplied unfrozen to a retailer had bacterial loads less than 10^5 CFU/g (Emswiler et al., 1976). Centralized preparation of ground beef allows for more effective control of process hygiene and this allows for improved product stability and safety. Centralized preparation allows for these improvements mainly by minimizing the handling operations at the retail level.

1.4. STORAGE OF FRESH MEAT UNDER VACUUM

VP chill stored meat has been used in the meat industry for more than 35 years. The simplicity of the technology makes it very attractive. VP involves placing the product in a film of low oxygen permeability (30 to 50 cc/m ^{2}/day), the air is evacuated and the package is sealed. VP is currently used for transportation of fresh meat and retail-ready products from centralized producers to retail outlets. VP retail ready fresh meats have not been widely used at the supermarket level because of the negative perceptions that consumers have of the purplish red colour of myoglobin in the absence of oxygen. The accumulation of liquid purge from the meat also makes the product unacceptable. Despite these disadvantages, chill stored VP fresh meats offer many advantages, including reduced weight loss of product due to dehydration, preserved

meat colour, aging of meat in the package, elimination of further contamination, and most importantly, extended shelf-life (Seideman and Durland, 1983).

Despite these advantages, spoilage of meat products is inevitable but there are means of extending the shelf-life. To achieve maximum shelf-life of VP meat there are a number of interrelated factors, including intrinsic properties of the meat and extrinsic properties that are described in detail below.

1.4.1. Intrinsic Factors

Meat is highly susceptible to spoilage because the favourable conditions that it provides for microbial growth, including: nutrients, growth environment (Aw 0.96 to 0.97, pH 5.6 to 5.8) and minerals (Hammes et al., 1990). Meat components fall into one of three groups of potential nutrients for microbial growth (Lawrie, 1974). The first group includes the major meat constituents of protein and fat. These components must be degraded before they can be utilized by the bacteria. They are insoluble and are not readily available for microbial growth. The second group include the low molecular weight soluble nitrogenous compounds including: creatine and nucleotides, that are derived during rigor mortis from creatine phosphate and adenosine triphosphate, amino acids and peptides. The peptides and amino acids become more available with time because of proteolysis. The third group of meat constituents is derived from muscle glycogen. Muscle glycogen is degraded by glycolysis during onset of rigor (Bendall, 1973) and lactic acid is the most abundant end-product that is formed.

Newton and Gill (1978) showed that spoilage bacteria utilize the following substrates during anaerobic growth: *B. thermosphacta* utilizes glucose; *Enterobacteriaceae* utilize glucose and glucose-6-phosphate; and *Lactobacillus* spp. utilize glucose and arginine. In contrast to reports by Newton and Gill (1978), there have been reports of the formation of hydrogen sulphide by LAB in anaerobically packaged meats which suggest that LAB may utilize amino acids other than arginine.

Lactobacillus and *Carnobacterium* spp. are capable of producing hydrogen sulphide (Schillinger and Lücke, 1987b; Egan et al., 1989).

Meat that has a high pH is referred to as "dark, firm, dry" (DFD). In normal muscle tissue the pH ranges from 5.5 to 5.8, but if circumstances permit, the pH can remain above 6.0 and this is referred to as DFD. The spoilage of DFD meat packaged under vacuum differs from that of normal pH meat. DFD meat spoils rapidly (3 to 6 weeks) due to production of putrid odours and undesirable greening of the exudate (Bem et al., 1976; Taylor and Shaw, 1977). Spoilage of vacuum packaged DFD meat is attributed to hydrogen sulphide odours and hydrogen sulphide binding with the myoglobin to form sulphomyoglobin which gives the meat exudate an undesirable green appearance (Nicol et al., 1970). *Alteromonas (Shewanella) putrefaciens* can be of critical importance on meat of pH 6.0 and above because, under anaerobic conditions, the organism produces large amounts of hydrogen sulphide (Gill and Newton, 1979; Nicol et al., 1970). Although the LAB are the dominant bacteria on vacuum packaged DFD meat (Dainty et al, 1979; Patterson and Gibbs, 1977; Erichsen and Molin, 1981), the *Enterobacteriaceae* can reach numbers high enough to cause spoilage.

1.4.2. Storage Atmosphere

The success of extending the storage life of chill stored VP meats has been attributed to the inhibition of growth of the putrefactive aerobic spoilage organisms under anaerobic conditions. VP fresh meats with a good vacuum contain <1.0% oxygen, which is theoretically sufficient to allow pseudomonads to grow to appreciable numbers (Dainty and Mackey, 1992). Pseudomonads can grow under conditions that are considered to be "good vacuum" to levels of 10^3 to 10^6 CFU/cm^2, but reports of this are not consistent (Dainty et al., 1983). Growth of pseudomonads on meat that is packaged commercially is probably related to the oxygen permeability of the packaging film (Gill, 1985).

Failure of the pseudomonads and other gram-negative bacteria to grow on VP meat is linked to the relatively low oxygen content, combined with the presence of carbon dioxide. Carbon dioxide is bacteriostatic and inhibits the growth of the putrefactive gram-negative bacteria, but gram-positive LAB are tolerant to carbon dioxide (Sutherland et al., 1977; Enfors et al., 1979). The *Pseudomonas* spp. and *A. putrefaciens* are inhibited by levels of 10 to 20% carbon dioxide (Gill and Tan, 1980) while *B. thermosphacta*, *Lactobacillus* spp. and psychrotrophic *Enterobacteriaceae* are not affected by carbon dioxide in the presence of low levels of oxygen (Newton et al., 1977). *B. thermosphacta* withstands up to 50% carbon dioxide (Gardner, 1980), while lactobacilli tolerate and grow at 100% carbon dioxide (Blickstad et al., 1981). The inhibitory effect of carbon dioxide results in an increased lag phase and reduced growth rate of the organisms (Sutherland et al., 1977; Blickstad and Molin, 1984). There are many theories of how carbon dioxide inhibits bacterial growth. This was reviewed in detail by Daniels et al. (1985).

Controlling the residual oxygen content of fresh meat is important for controlling microbial growth and maintaining the colour stability of the meat. If there is too much residual oxygen in the VP meat, oxymyoglobin will be oxidized to the undesirable brown form of metmyoglobin (Rennere, 1990). Oxygen concentration below 0.1% residual oxygen does not oxidize myoglobin to metmyoglobin (Penney and Bell, 1993; Gill and Molin, 1991). VP wholesale meat has been successful because of acceptance at the distributor level. Exposure of wholesale cuts to air allows the myoglobin to "bloom" to its desirable oxymyoglobin state (cherry red colour). The extent to which the meat will "bloom" depends on the amount of oxygen present in the vacuum package, and that depends on the oxygen permeability of the packaging film. Meat that has had minimal contact with oxygen prior to its preservative packaging will "bloom" much more quickly than meat that has been exposed to low levels of oxygen

for a period of time prior to VP. Red meat colour is the most important attribute indicating a fresh product to the consumer (Renerre, 1990).

1.4.3. Storage Temperature

Storage temperature of meats in any environment is the single most important environmental factor that influences the growth of bacteria. As storage temperature increases, the growth rate of the bacteria increases which reduces the time for spoilage to occur. At normal storage temperatures for VP fresh meats the psychrotrophic lactobacilli will dominate (Newton and Gill, 1978) and *Enterobacteriaceae* will be present and grow, even at a storage temperature of 5°C. An increase in temperature reduces the inhibitory action of carbon dioxide and lactic acid (Grau, 1981). Under extreme circumstances where the anaerobically packaged meats have been exposed to gross temperature abuse, growth of clostridia becomes important. If *Clostridium perfringens* is present as part of the initial population they can become dominant (Ingram and Dainty, 1971; Gill and Newton, 1979). Hauschild et al. (1985) reported that meats stored at ambient temperatures will support the growth of *Clostridium* spp. that cause rapid spoilage.

1.5. SPOILAGE BY LAB IN VACUUM PACKAGED MEATS

With time, LAB cause spoilage of VP meats. Meat is considered spoiled when metabolic end-products of bacterial growth make the meat offensive to the senses. This is usually detected by appearance or odour of the meat. LAB on VP fresh meat generally do not cause overt spoilage until some time after they have reached their maximum population of 10^7 to 10^8 CFU/cm^2 (Egan and Shay, 1982; Borch and Nerbrink, 1989). The end-products produced during anaerobic fermentation eventually reach levels that cause an off-odour. Oxygen, pH and glucose concentration are the main factors that affect end-products of fermentation, with pH and glucose being the two most important determining factors (Thomas et al., 1979; Rhee and Pack, 1980; Borch and Molin, 1989).

"Sour" or "acid" are the most common descriptors for off-odours of meat stored anaerobically at chill temperatures. These odours are usually a result of accumulation of short chain organic acids (Sutherland et al., 1976; Dainty, 1981). Dainty (1981) reported that for meat of normal pH inoculated with LAB and stored under vacuum, the level of acetic acid increased from 0.7 to 1.6 mmol/kg. Another study demonstrated that heterofermentative lactobacilli after 4 weeks of storage produced levels of 6 mmol acetic acid per kg of meat (Borch and Agerhem, 1992). Kandler (1983) reported that acetate was not an expected end-product of homofermentative lactobacilli but others believe that the acetate may be induced at semi-starvation conditions where glucose is limited (Borch and Agerhem, 1992; Hjörleifsdottir et al., 1990).

The accumulation of D-lactate has been suggested as a parameter to determine the extent of spoilage of VP meats (Sinell and Lücke, 1979; de Pablo et al., 1989). Sinell and Lücke (1979) reported that D-lactate levels of less than 5.5 mmol/kg were sensorial acceptable, at 11 mmol/kg detectable spoilage begins and levels of 33 mmol/kg the meat is completely spoiled. However, the level of D-lactate depends on the type of LAB used in the study. In another study it was reported that meat samples spoiled at 40 mmol/kg and 10 mmol/kg of D-lactate in the presence of lactobacilli and leuconostoc, respectively (Borch and Agerhem, 1992). This indicates that the level of D-lactate that results in spoilage of meat depends on the genus of LAB, and that there may be many more factors than D-lactate that contribute to spoilage. When Borch and Agerhem (1992) inoculated meat with a *Leuconostoc* sp. they detected increased levels of ethanol but they did not determine whether it was ethanol that negatively affected the sensory aspects of the meat or whether it was some other metabolite. Edwards and Dainty (1987) suggested that methanethiol and dimethyl sulphide may be responsible for the sour odour of meat after extended vacuum storage. They believe these compounds are partially responsible because when the two compounds are present in

an aqueous solution with levels similar to those in VP meat the odours were described as sour (Shewan, 1974).

Lactobacillus spp. that produce hydrogen sulphide spoil VP beef and greatly reduce its shelf-life (Shay and Egan, 1981). Hanna et al. (1983) inoculated sterile beef steaks with several species of *Lactobacillus* and found that a majority of the 41 strains tested produced sulphurous odours (Lee and Simard, 1984). Hydrogen sulphide causes greening of meat resulting in visual rejection of VP meats. Greening is usually associated with high pH meats (Nicol et al., 1970; Taylor and Shaw, 1977; Gill and Newton, 1979), which allows gram-negative bacteria to grow and produce hydrogen sulphide that causes greening and off-odours (Nicol et al., 1970).

The hydrogen sulphide producing strain of *Lactobacillus* (L13) was identified as a strain of *Lactobacillus sake* (Kandler et al., 1988). Hydrogen sulphide production associated with the lactobacilli was found to be a plasmid-mediated trait (Shay et al., 1988). Hydrogen sulphide production in VP meat is dependent on oxygen and glucose levels (Egan et al., 1989). The formation of sulphomyoglobin occurs when packaging films of low oxygen permeability are used (Egan et al., 1989). Formation of hydrogen sulphide is affected by availability of glucose and Shay et al. (1988) showed that *L. sake* utilizes cysteine as a result of low levels of glucose in the meat (Shay et al., 1988; Egan et al., 1989). Glucose content of meat varies from 200 μg/g at pH 5.40 to 5.49 to less than 10 μg/g in meat of high pH (Newton and Gill, 1978). Therefore in meat with high pH, the glucose is rapidly utilized, and certain LAB metabolize cysteine resulting in the production of hydrogen sulphide. Hydrogen sulphide producing lactobacilli are readily isolated from VP meats, but they only produce hydrogen sulphide under appropriate conditions of oxygen and glucose availability (Egan et al., 1989).

VP beef during storage at 5°C developed increased concentrations of diamines, including putrescine and cadaverine (Edwards et al., 1987). Dainty et al. (1986) tested a number of strains of lactobacilli, leuconostocs, *Enterobacteriaceae* and *B. thermosphacta* and only *Hafnia alvei* and *Serratia liquefaciens* showed production of cadaverine and putrescine during growth of pure cultures on VP beef stored at 1°C. There have only been two recorded instances of diamine production by LAB: *Lactobacillus brevis* (Zee et al., 1991) and an oral *Lactobacillus* (Lagerborg and Clapper, 1952). Cadaverine is produced by decarboxylation of lysine which does not require the input of metabolic action from other organisms. However, putrescine formation requires growth of LAB and metabolism of arginine to produce ornithine which is then decarboxylated by the *Enterobacteriaceae* (Dainty et al., 1986). Another biogenic amine that has been shown to increase in VP meats is tyramine (Edwards et al., 1987). Tyramine is also formed by the decarboxylation of tyrosine. Tyramine production has been associated with the growth of *Carnobacterium divergens* and *Carnobacterium piscicola* strains during prolonged storage (Edwards et al., 1987). The significance of tyramine on the sensory properties of meat is not known but its low concentration suggests that it does not play a role in the spoilage of fresh meats (Edwards et al., 1987; Dainty and Mackey, 1992).

Detecting the spoilage of VP meats is normally done by the time-consuming method of sensory evaluation. Shelf-life is determined by odour or appearance. Studies have been done on the sensory quality of meat with pure cultures of LAB added aseptically to study their spoilage patterns during anaerobic storage. Smith et al. (1980) added pure cultures of lactobacilli to steaks at high and low concentrations and, after extended storage, concluded that the inoculated steaks had lower acceptance scores and that the added cultures were not beneficial to the appearance, odour and flavour compared with the uninoculated controls. Ockerman and Cahill (1977) also reported that the inoculation of a *Leuconostoc* sp. onto VP meat resulted in lower flavour scores

than the uninoculated control samples. Egan and Shay (1982) conducted a study in which they added pure cultures of LAB to VP beef and noted increases in the rate of spoilage, largely due to the development of sour, acid and bitter flavours. Off-flavours became significant some time after reaching maximum population of 10^8 LAB/cm^2 after 13 to 28 days even with films of low gas permeability (Egan and Shay, 1982). They also demonstrated that the sterile control samples eventually spoil after 28 days of extended storage showing that meat spoils not only as a result of microbial growth but also due to intrinsic changes in the meat. Inoculating meat with a strain of *Lactobacillus* caused decreased flavour scores after maximum population was reached, but the addition of a strain of leuconostoc resulted in decreased flavour scores before maximum numbers were reached (Borch and Agerhem, 1992). These studies suggest that spoilage patterns differ among LAB genera and that the addition of pure cultures of LAB result in spoilage. However, a recent study on the effect of a bacteriocin-producing LAB on the storage life of normal pH beef stored anaerobically (under vacuum) and aerobically showed that *Leuconostoc gelidum* UAL187 did not contribute any adverse changes in appearance or odour (Leisner et al., 1995).

1.6. LAB AS BIOPRESERVATIVES

The result of VP and MAP of fresh meats under chilled storage is the development of a highly competitive microflora consisting primarily of LAB. The LAB dominate the microflora of meats that have been chill stored and anaerobically packaged because they are psychrotrophic and they are able to grow in an environment that lacks oxygen and contains carbon dioxide. In addition to their ability to dominate the microflora of meat, some LAB produce antimicrobial substances that may aid in their dominance. The antimicrobial substances that are produced by LAB include: hydrogen peroxide, diacetyl, organic (lactic and acetic) acids and bacteriocins. Hydrogen peroxide is produced by LAB but only during aerobic growth and therefore would not contribute to their ability to dominate during anaerobic storage. However, the

production of hydrogen peroxide may play a role when anaerobically packaged meats are transferred to an aerobic environment for retail display. Diacetyl is produced but it is only formed by the oxidation of acetate. Organic acids and bacteriocins are the metabolites that are most important for use of LAB as biopreservatives.

1.6.1. Organic Acids

Reduction of pH *in situ* by microbial growth as a result of the production of lactic and acetic acids is inhibitory to some of the microorganisms that are present on meat. Lowering of pH causes an extension of the lag time, and reduces growth and total microbial population. The effect of pH on microbes is complex and there are a number of theories for the actual mechanism of action. Formation of lactic and acetic acids by the LAB causes meat pH to drop and results in antagonism of other bacteria. Reduction of pH by LAB in fermented sausages plays a major role in safety as well as the production of desirable sensory attributes. However, in VP or MAP fresh meats, organic acids inhibit other microorganisms on the meat, but over production of these acids results in undesirable sensory changes.

B. thermosphacta was not able to grow at a pH below 5.8 and this was initially attributed to the decrease in pH, but Grau (1980) found that *B. thermosphacta* could grow at a pH of 5.5 in the absence of lactate. In the presence of lactate at the same pH inhibition of the organism occurs. The growth of the gram-negative spoilage organisms is also inhibited during extended VP storage and it is known that they are sensitive to organic acids produced during LAB fermentation (Ray and Sandine, 1992). Rozbeh et al. (1993) found that the application of sodium lactate to vacuum packaged beef significantly reduced the growth of the adventitious microflora. A study by Grau (1981) on the effect of pH, lactate and anaerobic environment on the growth of the *Enterobacteriaceae* showed that the *Enterobacteriaceae* are inhibited and that this inhibitory action depends on the maintenance of an anaerobic environment, low pH (5.4 to 5.6) and the production of acetate. The role of acetic acid has not been studied

to a sufficient extent to define its role in controlling the growth of the spoilage organisms during growth under vacuum. Currently the use of organic acids is being used to decontaminate carcasses after slaughter, but its efficacy is still in question (Dickson and Anderson, 1992).

1.6.2. Bacteriocins

Bacteriocins were first described as a heterogeneous group of antibacterial peptides produced by gram-negative and gram-positive bacteria (Tagg et al., 1976). Klaenhammer (1988) further defined the bacteriocins produced by gram-positive LAB as "proteins or protein complexes with bactericidal activity directed against species that are closely related to the producer bacteria" and can be further defined by their mode of action as bactericidal, bacteriostatic or bacteriolytic. There are a number of bacteriocin-producing LAB that have been isolated from meat and meat products (Table 1.1). The use of bacteriocins creates the potential to replace the chemical preservatives with a more "natural" means of preservation. In the past twenty years there has been considerable research on the genetic characterization of bacteriocins and their production. Information on the influence of bacteriocin-producing LAB on the indigenous flora of foods is very limited.

The major focus of the application of bacteriocin-producing LAB to meats has been on their ability to restrict the growth of the foodborne pathogens to enhance the safety of the product. The study of the action of bacteriocins against foodborne pathogens has been focused mainly on their ability to inhibit *Listeria monocytogenes* and *Clostridium botulinum*. The use of bacteriocin-producing LAB as antimicrobials has been successful in some meat systems. Control of growth of *Listeria monocytogenes* has been demonstrated in fresh meats (Motlagh et al., 1992; Nielson et al., 1990; Rozbeh et al., 1993; Skyttä et al., 1991) and processed meats (Berry et al., 1991; Degnan et al., 1992; Foegeding et al., 1992, Luchansky et al., 1992; Motlagh et

Table 1.1. Bacteriocins associated with bacteriocin-producing lactic acid bacteria isolated from meat.

Species	Bacteriocin	Reference
Lactobacillus		
sake	Sakacin A	Schillinger and Lücke(1989)
sake	Sakacin M	Sobrino et al. (1992)
sake	Sakacin P	Tichaczek et al. (1992)
sake	Lactocin S	Mørtvedt and Nes (1989)
curvatus	Curvacin A	Tichaczek et al. (1992)
plantarum	Plantaricin BN	Lewus and Montville (1992)
bavaricus	Bavaricin MN	Lewus and Montville (1992)
Carnobacterium		
piscicola	Carnobacteriocin	Ahn and Stiles (1990)
divergens	Divergicin A	Worobo et al. (1995)
Leuconostoc		
gelidum	Leucocin A	Hastings and Stiles (1991)
carnosum	Carnosin	van Laack et al. (1992)
paramesenteroide:	Leuconocin S	Lewus et al. (1992)
Pediococcus		
acidilactici	Pediocin PA-1/AcH	Gonzalez and Kunka (1987); Ray et al. (1989)

al., 1992). *Lactobacillus sake* Lb706 produces sakacin A and inhibits the growth of *L. monocytogenes* in fresh sausage and pasteurized ground beef (Schillinger et al., 1991).

Some bacteriocin-producing LAB inhibit the outgrowth of *Clostridium botulinum* spores (Okereke and Montville, 1991). Crandall and Montville (1993) studied the inhibition of *C. botulinum* by nisin-producing strains of *Lactococcus lactis* and *Pediococcus pentosaceus* in a model gravy system. They concluded that the inhibition was a result of the production of lactic acid and not the bacteriocin. However, the inhibition of *C. botulinum* in a model gravy system (Crandall et al., 1994) and in chicken salad (Hutton et al., 1991) by *Pediococcus* spp. was achieved as a result of its bacteriocin production. The inhibition of foodborne pathogens by bacteriocins in foods varies considerably with the bacteria and food system.

There are five selection criteria that are required for a bacteriocin-producing LAB to be used as a biopreservative in meats: the bacteriocin-producing LAB must be able to grow in meats during chill storage; the production of the bacteriocin must be reliable; the bacteriocin must be active and stable in the meat environment; early production of a bacteriocin is desired for more effective control of growth of the foodborne pathogens and spoilage organisms; and, most important, it must not cause any undesirable sensory changes. The final selection criterion is very important because no matter how effective the bacteriocin-producing strain is at controlling or eliminating the pathogens and spoilage organisms, any undesirable sensory changes would negate the preservative attribute.

There are only five studies that report the sensory consequences of bacteriocin action (Raccach et al., 1979; Schillinger et al., 1991; Leisner et al., 1993; McMullen, 1994; Leisner et al., 1996). Sensory analyses were cursory and not presented in the studies by Raccach et al. (1979) and Schillinger et al. (1991). However, a study by Leisner et al. (1995) reported the effect of inoculation of sterile beef with *C. piscicola, Leuc. gelidum* or *L. sake*. *Leuc. gelidum* UAL187 was found to be the most suitable

antagonistic strain because of its ability to extend the storage life of beef packaged anaerobically or aerobically. A subsequent study by Leisner et al. (1996) showed that growth of *Leuc. gelidum* UAL187 (Bac$^+$) with *L. sake* 1218 (an overt sulphide-producing strain) could control the spoilage by the sulphide-producing strain whereas the Bac$^-$ isogenic variant (UAL187-13) failed to inhibit production of the sulphide odour. The control of spoilage has been studied in model systems but the action of these bacteriocin-producing strains must be further studied to see if they can compete and effectively control the spoilage of the indigenous microflora associated with meats and also to study the sensory consequences by determining the flavour consequences, in addition to odour and appearance of the meat.

1.7. OBJECTIVES OF THE RESEARCH

Over the past 80 years the microbiological quality of ground beef has not changed because the production of ground beef has not changed. However, there is now a movement towards centralized preparation of retail meats and centralized production has been shown to be able to produce fresh meats of high microbial quality. The centralized preparation of fresh meats involves preservative packaging in either modified atmosphere or vacuum packaging. This form of production and packaging extends the shelf-life of fresh meats compared with the past method of production, but the shelf-life is still limited by the prevalence of the LAB that dominate in this environment.

Currently, there is interest for the use of bacteriocin-producing LAB and their bacteriocins as biopreservatives in fresh meats. The use of bacteriocin-producing LAB may give vacuum packaged meats a predictable and reliable storage life, and furthermore the bacteriocinogenic LAB could act as an additional hurdle to the growth of the pathogenic bacteria.

The objectives of this study were:

1. To determine the hygienic efficiency of a commercial process of centralized production, transport and storage of coarse ground beef based on the bacteriological profile and temperature evaluation; and to assess the bacteriological profile and storage life under conditions of retail display (Chapter 2).

2. To evaluate the ability of a bacteriocin-producing LAB to control the growth of the adventitious spoilage microflora and to assess the storage life based on odour and appearance of vacuum packaged coarse ground beef (Chapter 3).

3. To evaluate the sensory consequences of a bacteriocin-producing LAB on the flavour of the vacuum packaged coarse ground beef after extended storage (Chapter 4).

1.8. BIBLIOGRAPHY

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

Beebe, S.D., C. Vanderzant, M.O. Hanna, Z.L. Carpenter, and G.C. Smith. 1976. Effect of initial internal temperature and storage temperature on the microbial flora of vacuum packaged beef. J. Milk Food Technol. 39:600-605.

Bem, Z., H. Hechelmann, and L. Leistner. 1976. The bacteriology of DFD meat. Fleischwirtschaft 56:986-987.

Bendall, J.R. 1973. Post mortem changes in muscle, pp. 87-103. *In* G.H. Bourne (ed.) Structure and function of muscle, vol. 2, 2nd ed. Academic Press, New York.

Berry, E.D., R.W. Hutkins, and R.W. Mandigo. 1991. The use of bacteriocin-producing *Pediococcus acidilactici* to control postprocessing *Listeria monocytogenes* contamination of frankfurters. J. Food Prot. 54:681-686.

Blickstad, E., S.-O. Enfors, and G. Molin. 1981. Effect of high concentrations of CO_2 on the microbial flora of pork stored at 4°C and 14°C, pp. 345-357. *In* T.A. Roberts, G. Hobbs, J.H.B. Christian and N. Skovgaard (ed.), Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Academic Press. New York.

Blickstad, E., and G. Molin. 1983. Carbon dioxide as a controller of the spoilage flora of pork, with special reference to temperature and sodium chloride. J. Food Prot. 46:756-763.

Blickstad, E., and G. Molin. 1984. Growth and endproduct formation in fermenter cultures of *Brochothrix thermosphacta* ATCC 11509 and two

psychrotrophic *Lactobacillus* spp. in different gaseous atmospheres. J. Appl. Bacteriol. 57:213-220.

Bogh-Sorensen, L., and P. Olsson. 1990. The chill chain, pp. 245-267. *In* T.R. Gormley (ed.), Chilled Foods: The State of the Art. Elsevier Applied Science, London.

Borch, E., and H. Agerhem. 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.

Borch, E., and G. Molin. 1988. Numerical taxonomy of psychrotrophic lactic acid bacteria from prepacked meat and meat products. Antonie van Leeuwenhoek 54:301-323.

Borch, E., and G. Molin. 1989. The aerobic growth and product formation of *Lactobacillus, Leuconostoc, Brochothrix* and *Carnobacterium* in batch cultures. Appl. Microbiol. Technol. 30:81-88.

Borch, E., and E. Nerbrink. 1989. Shelf-life of emulsion sausage stored in vacuum or modified atmospheres, pp. 470-477. *In* Proc. 35th International Congress of Meat Science and Technology, Copenhagen, Denmark, Vol. II.

Collins, M.D., J.A.E. Farrow, B.A. Phillips, S. Ferusu, and D. Jones. 1987. Classification of *Lactobacillus divergens, Lactobacillus piscicola,* and some catalase-negative, asporogenous, rod-shaped bacteria from poultry in a new genus, *Carnobacterium*. Int. J. System. Bacteriol. 37:310-316.

Crandall, A.D., and T.J. Montville. 1993. Inhibition of *Clostridium botulinum* growth and toxigenesis in a model gravy system by coinoculation with bacteriocin-producing lactic acid bacteria. J. Food Prot. 56:485-488, 492.

Dainty, R.H. 1981. Volatile fatty acids detected in vacuum-packed beef during storage atchill temperature, pp. 688-690. *In* Proceedings of the 27th Meeting of European Meat Workers, Vienna.

Dainty, R.H. 1982. Biochemistry of undesirable effects attributed to microbial growth on proteinaceous foods stored at chill temperatures. Food Chem. 9:103-113.

Dainty, R.H., and C.M. Hibbard. 1980. Aerobic metabolism of *Brochothrix thermosphacta* growing on meat surfaces and in laboratory media. J. Appl. Bacteriol. 48:387-396.

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

Dainty, R.H., R.A. Edwards, and C. Hibbard. 1989. Spoilage of vacuum-packed beef by a *Clostridium* sp. J. Sci. Food Agric. 49:473-486.

Dainty, R.H., R.A. Edwards, C.M. Hibbard, and S.V. Ramantanis. 1986. Bacterial sources of putrescine and cadaverine in chill stored vacuum-packaged beef. J. Appl. Bacteriol. 61:117-123.

Dainty, R.H., B.G. Shaw, C.D. Harding, and S. Michanie. 1979. The spoilage of vacuum-packed beef by cold tolerant bacteria, pp. 83-100. *In* A.D. Russell and R. Fuller (ed). Cold Tolerant Microbes in Spoilage and the Environments, Academic Press, New York.

Dainty, R.H., B.G. Shaw, and T.A. Roberts. 1983. Microbial and chemical changes in chill-stored red meats, pp. 151-178. *In* T.A. Roberts and F.A. Skinner (ed.), Food Microbiology: Advances and Prospects. Academic Press, Toronto.

Daniels, J.A., R. Krishnamurthi, and S.S.H. Rizvi. 1985. A review of effects of carbon dioxide on microbial growth and food quality. J. Food Prot. 48:532-537.

Degnan, A.J., A.E. Yousef, and J.B. Luchansky. 1992. Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. J. Food Prot. 55:98-103.

Dempster, J.F., and D.H. Cody. 1978. Bacteriological and chemical status of minced beef. Irish J. Food Sci. Technol. 2:1-11.

de Pablo, V., M.A. Asensio, B. Sanz, and J.A. Ordonez. 1989. The D(-)lactic acid and acetoin/diacetyl as potential indicators of the microbial quality of vacuum-packed pork and meat products. J. Appl. Bacteriol. 66:185-190.

Dickson, J.S., and M.E. Anderson. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: a review. J. Food Prot. 55:133-140.

Duitschaever, C.L., D.R. Arnott, and D.H. Bullock. 1973. Bacteriological quality of raw refrigerated ground beef. J. Milk Food Technol. 36:375-377.

Edwards, R.A., and R.H. Dainty. 1987. Volatile compounds associated with the spoilage of normal and high pH vacuum-packed pork. J. Sci. Food Agric. 38:57-66.

Edwards, R.A., R.H. Dainty, C.M. Hibbard, and S.V. Ramantanis. 1987. Amines in fresh beef of normal pH and the role of bacteria in changes in concentration observed during storage in vacuum packs at chill temperatures. J. Appl. Bacteriol. 63:427-434.

Egan, A.F., and B.J. Shay. 1982. Significance of lactobacilli and film permeability in the spoilage of vacuum-packaged beef. J. Food Sci. 47:1119-1122, 1126.

Egan, A.F., B.J. Shay, and P.J. Rogers. 1989. Factors affecting the production of hydrogen sulphide by *Lactobacillus sake* L13 growing on vacuum-packed beef. J. Appl. Bacteriol. 67:255-262.

Emswiler, B.S., C.J. Pierson, and A.W. Kotula. 1976. Bacteriological quality and shelf life of ground beef. Appl. Environ. Microbiol. 31:826-830.

Enfors, S.-O., G. Molin, and A. Ternström. 1979. Effect of packaging under carbon dioxide, nitrogen or air on the microbial flora of pork stored at 4°C. J. Appl. Bacteriol. 47:197-208.

Erichsen, I., and G. Molin. 1981. Microbial flora of normal and high pH beef stored at 4°C in different gas environments. J. Food Prot. 44:866-869.

Foegeding, P.M., A.B. Thomas, D.H. Pilkington, and T.R. Klaenhammer. 1992. Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. Appl. Environ. Microbiol. 58:884-890.

Foster, J.F., J.L. Fowler, and W.C. Ladiges. 1977. A bacteriological survey of raw ground beef. J. Food Prot. 40:790-794.

Gardner, G.A. 1980. *Brochothrix thermosphacta (Microbacterium thermosphactum)* in the spoilage of meats: a review, pp. 139-173. *In* T.A. Roberts, G. Hobbs, J.H.B. Christian and N. Skovgaard (ed.), Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Academic Press, Toronto.

Gill, C.O. 1976. Substrate limitation of bacterial growth at meat surfaces. J. Appl. Bacteriol. 41:401-410.

Gill, C.O. 1985. The control of microbial spoilage in fresh meats, pp. 49-88. *In* A.M. Pearson and T.R. Dutson (ed.), Advances in Meat Research, vol. 2. Meat and Poultry Microbiology. AVI Publishing Co., Inc., Connecticut.

Gill, C.O., and J.C.L. Harrison. 1989. The storage life of chilled pork packaged under carbon dioxide. Meat Sci. 26:313-324.

Gill, C.O., and C. McGinnis. 1993. Changes in the microflora on commercial beef trimmings during their collection, distribution and preparation for retail sale as ground beef. Int. J. Food Microbiol. 18:321-332.

Gill, C.O., and G. Molin. 1991. Modified atmospheres and vacuum packaging, pp. 172-199. *In* N.J. Russell and G.W. Gould (ed.), Food Preservatives. Blackie and Sons Ltd., Glasgow.

Gill, C.O., and K.G. Newton. 1977. The development of aerobic spoilage flora on meat stored at chill temperatures. J. Appl. Bacteriol. 43:189-195.

Gill, C.O., and K.G. Newton. 1978. The ecology of bacterial spoilage of fresh meat at chill temperatures. Meat Sci. 2:207-217.

Gill, C.O., and K.G. Newton. 1979. Spoilage of vacuum-packaged dark, firm, dry meat at chill temperatures. Appl. Environ. Microbiol. 37:362-364.

Gill, C.O., and N. Penney. 1986. Packaging conditions for extended storage of chilled dark, firm, dry beef. Meat Sci. 18:41-53.

Gill, C.O., and K.H. Tan. 1980. Effect of carbon dioxide on growth of meat spoilage bacteria. Appl. Environ. Microbiol. 39:317-319.

Gonzalez, C.F., and B.S. Kunka. 1987. Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. Appl. Environ. Mirobiol. 53:2534-2538.

Grau, F.H. 1980. Inhibition of the anaerobic growth of *Brochothrix thermosphacta* by lactic acid. Appl. Environ. Microbiol. 40:433-436.

Grau, F.H. 1981. Role of pH, lactate and anaerobiosis in controlling growth of some fermentative gram-negative bacteria on beef. Appl. Environ. Microbiol. 42:1043-1050.

Greer, G.G., C.O. Gill, and B.D. Dilts. 1994. Evaluation of the bacteriological consequences of the temperature regimes experienced by fresh chilled meat during display. Food Res. Int. 27:371-377.

Hammes, W.P., A. Bantleon, and S. Min. 1990. Lactic acid bacteria in meat fermentation. FEMS Microbiol. Rev. 87:165-174.

Hanna, M.O., J.W. Savell, G.C. Smith, D.E. Purser, F.A. Gardner, and C. Vanderzant. 1983. Effect of growth of individual meat bacteria on pH, color and odor of aseptically prepared vacuum-packaged round steaks. J. Food Prot. 46:216-221.

Hanna, M.O., D.L. Zink, Z.L. Carpenter, and C. Vanderzant. 1976. *Yersinia enterocolitica*-like organisms from vacuum-packaged beef and lamb. J. Food Sci. 41:1254-1256.

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

Hauschild, A.H.W. 1989. *Clostridium botulinum*, pp. 111-189. *In* M.P. Doyle (ed.), Foodborne Bacterial Pathogens. Marcel Dekker, Inc., New York.

Hauschild, A.H.W., L.M. Poste, and R. Hilsheimer. 1985. Toxin production by *Clostridium botulinum* and organoleptic changes in vacuum-packaged raw beef. J. Food Prot. 48:712-716.

Hitchener, B.J., A.F. Egan, and P.J. Rogers. 1982. Characteristics of lactic acid bacteria isolated from vacuum-packaged beef. J. Appl. Bacteriol. 52:31-37.

Hjörleifsdottir, S., S. Seevaratnam, O. Holst, and B. Mattiasson. 1990. Effects of complete cell recycling on product formation by *Lactobacillus casei* spp. *rhamnosus* in continuous cultures. Curr. Microbiol. 20:287-292.

Holzapfel, W. H. 1992. Culture media for non-sporulating gram-positive food spoilage bacteria. Int. J. Food Microbiol. 17:113-133.

Holzapfel, W.H., and S.E. Gerber. 1983. *Lactobacillus divergens* sp. nov., a new heterofermentative *Lactobacillus* species producing L(+)-lactate. System. Appl. Microbiol. 4:522-534.

Hudson, W.R., T.A. Roberts, A.R. Crosland, and J.C. Casey. 1986. The bacteriological quality, fat and collagen content of minced beef at retail level. Meat Sci. 17:139-152.

Hutton, M.T., P.A. Chehak, and J.H. Hanlin. 1991. Inhibition of botulinum toxin production by *Pediococcus acidilactici* in temperature abused refrigerated foods. J. Food Safety 11:255-267.

Ingram, M., and R.H. Dainty. 1971. Changes caused by microbes in spoilage of meats. J. Appl. Bacteriol. 34:21-39.

Ingram, M. 1962. Microbiological principles in prepacking meats. J. Appl. Bacteriol. 25:259-281.

James, S.J., and C. Bailey. 1990. Chilling Systems for Foods, pp. 1-36. *In* T.R. Gormley (ed.), Chilled Foods: The State of the Art. Elsevier Applied Science, London.

Kalchayanand, N., B. Ray, and R.A. Field. 1993. Characteristics of psychrotrophic *Clostridium laramie* causing spoilage of vacuum-packaged refrigerated fresh and roasted beef. J. Food Prot. 56:13-17.

Kalchayanand, N., B. Ray, R.A. Field, and M.C. Johnson. 1989. Spoilage of vacuum packaged refrigerated beef by *Clostridium*. J. Food Prot. 52:424-426.

Kandler, O. 1983. Carbohydrate metabolism in lactic acid bacteria. Antonie van Leeuwenhoek 49:209-22.

Kandler, O., B.J. Shay, and A.F. Egan. 1988. Identification of atypical streptobacteria from Australian vacuum-packaged beef, pp. 550-551. Proceedings of the 34th International Congress of Meat Science and Technology, Brisbane, Part B.

Klaenhammer, T.R. 1988. Bacteriocins of lactic acid bacteria. Biochemie 70:337-349.

Lawrie, R.A. 1974. Chemical and biochemical constitution of muscle, pp. 70-124. *In* Meat Science. Pergamon Press, Oxford.

Lagerborg, V.A., and W.E. Clapper. 1952. Amino acid decarboxylases of lactic acid bacteria. J. Bacteriol. 63:393-397.

Lee, B.H., and R.E. Simard. 1984. Three systems for biochemical characterization of lactobacilli associated with meat spoilage. J. Food Prot. 57:937-942.

Leisner, J.J., G.G. Greer, B.D. Dilts, and M.E. Stiles. 1995. Effect of growth of selected lactic acid bacteria on storage life of beef stored under vacuum and in air. Int. J. Food Microbiol. 26:231-243.

Leisner, J.J., G.G. Greer, and M.E. Stiles. 1996. Control of spoilage of beef by a sulfide-producing *Lactobacillus sake* with bacteriocinogenic *Leuconostoc gelidum* UAL187 during anaerobic storage at 2°C. Appl. Environ. Microbiol. 62:2610-2614.

Lewus, C.B., and T.J. Montville. 1992. Further characterization of bacteriocins plantaricin BN, bavaricin MN and pediocin A. Food Biotechnol. 6:153-174.

Lewus, C.B., and T.J. Montville. 1992. Production of an amylase-sensitve bacteriocin by an atypical *Leuconostoc paramesenteroides* strain. Appl. Environ. Microbiol. 58:143-149.

Luchansky, J.B., K.A. Glass, K.D. Harsono, A. J. Degnan, N.G. Faith, B. Cauvin, G. Baccus-Taylor, K. Arihara, B. Bater, A.J. Maurer, and R.G. Cassens. 1992. Genomic analysis of *Pediococcus* starter cultures used to control *Listeria monocytogenes* in turkey summer sasusage. Appl. Environ. Microbiol. 58:3053-3059.

Manu-Tawiah, W., D.J. Myers, D.G. Olson, and R.A. Molins. 1993. Survival and growth of *Listeria monocytogenes* and *Yersinia enterocolitica* in pork chops packaged under modified atmospheres. J. Food Sci. 58:475-479.

McMullen, L.M. 1994. Extended storage of retail cuts of pork. Ph.D. thesis. University of Alberta, Edmonton, Canada.

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

Mørtvedt, C.I., and I.F. Nes. 1989. Bacteriocin production by a *Lactobacillus sake* strain isolated from fermented meat. Eur. Food Chem. Proc. 1:336-341.

Motlagh, A.M., S. Holla, M.C. Johnson, B. Ray, and R.A. Field. 1992. Inhibition of *Listeria* spp. in sterile food systems by pediocin AcH, a bacteriocin produced by *Pediococcus acidilactici* H. J. Food Prot. 55:337-343.

Newton, K.G., and C.O. Gill. 1978a. Storage quality of dark, firm dry meat. Appl. Environ. Microbiol. 36:375-376.

Newton, K.G., and C.O. Gill. 1978b. The development of the anaerobic spoilage flora of meat stored at chill temperatures. J. Appl. Bacteriol. 44:91-95.

Newton, K.G., J.C.L. Harrison, and K.M. Smith. 1977. The effect of storage in various gaseous atmospheres on the microflora of lamb chops held at -1°C. J. Appl. Bacteriol. 43:53-59.

Newton, K.G., and W.J. Rigg. 1979. The effect of film permeability on the storage life and microbiology of vacuum-packed meat. J. Appl. Bacteriol. 47:433-441.

Nicol, D.J., M.K. Shaw, and D.A. Ledward. 1970. Hydrogen sulphide production by bacteria and sulfmyoglobin formation in prepacked chilled beef. Appl. Microbiol. 19:937-939

Nielson, J.W., J.S. Dickson, and J.D. Crouse. 1990. Use of a bacteriocin produced by *Pediococcus acidilactici* to inhibit *Listeria monocytogenes* associated with fresh meat. Appl. Environ. Microbiol. 56:2142-2145.

Nychas, G.J., A. Robinson, and R.G. Board. 1992. Microbiological and physico-chemical evaluation of ground beef from retail shops. Fleischwirtschaft Int. 1:49-53.

Ockerman, H.W., and V.R. Cahill. 1977. Microbiological growth and pH effects of bovine tissue inoculated with *Pseudomonas putrefaciens, Bacillus subtilis* or *Leuconostoc mesenteroides*. J. Food Sci. 42:141-145.

Okereke, A., and T.J. Montville. 1991. Bacteriocin-mediated inhibition of *Clostridium botulinum* spores by lactic acid bacteria at refrigeration and abuse temperatures. Appl. Environ. Microbiol. 57:3423-3428.

Patterson, J.T., and P.A. Gibbs. 1977. Incidence and spoilage potential of isolates from vacuum-packaged meat of high pH value. J. Appl. Bacteriol. 43:25-38.

Penney, N., and R.G. Bell. 1993. Effect of residual oxygen on the colour, odour and taste of carbon dioxide-packaged beef, lamb and pork during short term storage conditions. Meat Sci. 33:245-252.

Pierson, M.D., D.L. Collins-Thompson, and Z.J. Ordal. 1970. Microbiological, sensory pigment changes of aerobically and anaerobically packaged beef. Food Technol. 24:1171-1175.

Raccach, M., R.C. Baker, J.M. Regenstein, and E.J. Mulnix. 1979. Potential application of microbial antagonism to extended storage stability of a flesh type food. J. Food Sci. 44:43-46.

Ray, B., and W.E. Sandine. 1992. Acetic, propionic, and lactic acids of starter culture bacteria as biopreservatives, pp. 103-137. *In* B. Ray and M. Daeschel (ed.), Food Biopreservatives of Microbial Origin. CRC Press, London.

Ray, B., M.C. Johnson, and B. Ray. 1989. Bacteriocin plasmids of *Pediococcus acidilactici*. J. Ind. Microbiol. 4:163-171.

Renerre, M. 1990. Review: factors involved in the discoloration of beef meat. Int. J. Food Sci. Technol. 25:613-630.

Rhee, S.K., and M.Y. Pack. 1980. Effect of environmental pH on fermentation balance of *Lactobacillus bulgaricus*. J. Bacteriol. 144:217-221.

Roth, L.A., and D.S. Clark. 1972. Studies on the bacterial flora of vacuum-packaged fresh beef. Can. J. Microbiol. 18:1761-1766.

Roth, L.A., and D.S. Clark. 1975. Studies on the bacterial flora of vacuum-packaged fresh beef. Can. J. Microbiol. 18:1761-1766.

Rozbeh, M., N. Kalchayanand, R.A. Field, M.C. Johnson, and B. Ray. 1993. The influence of biopreservatives on the bacterial level of refrigerated vacuum packaged beef. J. Food Safety 13:99-111.

Schillinger, U., and F.-K. Lücke. 1987a. Lactic acid bacteria on vacuum-packaged meat and their influence on shelf-life. Fleischwirtschaft 647:1244-1248.

Schillinger, U., and F.-K. Lücke. 1987b. Identification of lactobacilli from meat and meat products. Food Microbiol. 4:199-208.

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

Schillinger, U., M. Kaya, and F.-K. Lücke. 1991. Behaviour of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. J. Appl. Bacteriol. 70:473-478.

Seideman, S.C., and P.R. Durland. 1983. Vacuum packaging of fresh beef: a review. J. Food Quality 6:29-47.

Seideman, S.C., C. Vanderzant, B.C. Smith, M.O. Hanna, and Z.L. Carpenter. 1976. Effect of degree of vacuum and length of storage on the microflora of vacuum-packaged beef wholesale cuts. J. Food Sci. 41:738-742.

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

Shaw, B.G., and C.D. Harding. 1985. Atypical lactobacilli from vacuum- packaged meats: comparison by DNA hybridization, cell compostion and biochemical tests with a description of *Lactobacillus carnis* sp. nov. System. Appl. Microbiol. 6:291-297.

Shay, B.J., and A.F. Egan. 1981. Hydrogen sulfide production and spoilage of vacuum-packaged beef by a *Lactobacillus*, pp. 241-251. *In* T.A. Roberts, G. Hobbs, J.H.B. Christian and N. Skovgaard (ed.), Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Academic Press, Toronto.

Shay, B.J., A.F. Egan, M. Wright, and P.J. Rogers. 1988. Cysteine metabolism in isolate of *Lactobacillus sake*: plasmid composition and cysteine transport. FEMS Microbiol. Lett. 56:183-188.

Shewan, J.M. 1974. The biodeterioration of certain proteinaceous foodstuffs at chill temperatures, pp. 475-490. *In* Spencer, B. (ed.), Industrial Aspects of Biochemistry, vol. 30, part 1. Amsterdam, Holland.

Shoup, J.B., and J.L. Oblinger. 1976. Microbiological evaluation of retail ground beef: centralized and traditional preparation. J. Milk Food Technol. 39:179-183.

Sinell, H.-J., and K. Luke. 1979. D(-)lactat als Parameter fur die mikrobielle Belastung von vacuum-verpacktem Bruhwurstaufschnitt. Fleischwirtschaft 59:547-550.

Skyttä, E., W. Hereijgers, and T. Mattila-Sandholm. 1991. Broad spectrum antibacterial activity of *Pediococcus damnosus* and *Pediococcus pentosaceus* in minced meat. Food Microbiol. 8:231-237.

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

Sobrino, O.J., J.M. Rodriguez, W.L. Moreira, L.M. Cintas, M.F. Fernandez, B. Sanz, and P.E. Hernandez. 1992. Sakacin M, a bacteriocin-like substance from *Lactobacillus sake* 148. Int. J. Food Microbiol. 16:215-225.

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

Sutherland, J.P., P.A. Gibbs, J.T. Patterson, and J.G. Murray. 1977. The effect of several gaseous environments on the multiplication of organisms isolated from vacuum-packaged beef. J. Food Technol. 12:249-255.

Sutherland, J.P., P.A. Gibbs, J.T. Patterson, and J.G. Murray. 1976. Biochemical changes in vacuum packaged beef occurring during storage at 0-2°C. J. Food Technol. 11:171.

Sutherland, J.P., and J.G. Murray. 1975b. Changes in the microbiology of vacuum-packaged beef. J. Appl. Bacteriol. 39:227-237.

Tagg, J.R., A.S. Dajani, and L.W. Wannamaker. 1976. Bacteriocins of gram-positive bacteria. Bacteriol. Rev. 40:722-756.

Taylor, A.A., and B.G. Shaw. 1977. The effect of meat pH and package permability on putrefaction and greening in vacuum packed beef. J. Food Technol. 12:515-522.

Tichaczek, P.S., J. Niessen-Meyer, I.F. Nes, R.F. Vogel, and W.P. Hammes. 1992. Characterization of the bacteriocins curvacin A from *Lactobacillus curvatus* LTH1174 and sakacin P from *L. sake* LTH673. System. Appl. Microbiol. 15:460-468.

Thomas, T.D., D.C. Elwood, and V.M.C. Longyear. 1979. Change from homo- to heterolactic fermentation by *Streptococcus lactis* resulting from glucose limitation in anaerobic hemostat cultures. J. Bacteriol. 138:109-117.

Vanderzant, C., M.O. Hanna, J.G. Ehler, J.W. Savell, G.C. Smith, D.B. Griffin, R.N. Terrel, K.D. Lind, and D.E. Galloway. 1982. Centralized packaging of beef loin steaks with different oxygen barrier films: microbiological characteristics. J. Food Sci. 47:1070-1079.

van Laack, R.L.J.M., U. Schillinger and W.H. Holzapfel. 1992. Characterization and partial purification of a bacteriocin produced by *Leuconostoc carnosum* LA44A. Int. J. Food Sci. Technol. 16:183-195.

Worobo, R.W., M.J. van Belkum, M. Sailer, K.L. Roy, J.C. Vederas, and M.E. Stiles. 1995. A signal peptide secretion-dependent bacteriocin from *Carnobacterium divergens*. J. Bacteriol. 177:3143-3149.

Young, L.L., R.D. Reviere and A.B. Cole. 1988. Fresh red meats: a place to apply modified atmospheres. Food Technol. 42(9):65-69.

Zee, J.A., R.E. Simard, R. Vaillancourt, and A. Boudreau. 1981. Effect of *Lactobacillus brevis, Saccharomyces uvarum* and grist composition on amine formation in beers. Can. Inst. Food Sci. Technol. J. 14:321-325.

2. Bacteriology and Storage Life of Ground Beef Produced under Centralized, Commercial Conditions

2.1 INTRODUCTION

Ground beef produced at retail level traditionally uses store trim and/or commercial beef trimmings that are vacuum packed and distributed to retailers for fine grinding and aerobically packaged for the retail display case. Ground beef produced in this manner has a very variable total bacterial load, ranging from 10^3 to 10^7 colony forming units (CFU) per gram and coliform bacteria usually exceeding 10^3 CFU per gram (Egbert et al., 1992). Retail ground beef often exceeds 10^7 CFU per gram (Duitschaever et al., 1973; Hudson et al., 1986; Nychas et al., 1992) and these bacterial loads have changed little over the past eighty years (Foster et al., 1977). The bacterial load of ground beef produced in this manner depends on the bacterial quality of trimmings, process hygiene, and temperature during transit, storage and retail display. At these levels it is not surprising that retail stores do not expect as much as 24 hours case life of their ground beef. The commercial process for ground beef production has been studied in detail by Gill and McGinnis (1993).

Centralized preparation of vacuum packaged, coarse ground beef offers an alternative to the traditional method. Centralized preparation is defined as "an autonomous facility in which all preparation operations are completed before the packaged product is transported to retail outlets" (Shoup and Oblinger, 1976). Ground beef produced in a centralized operation has a lower microbiological load than ground beef prepared in the traditional way (Shoup and Oblinger, 1976), because centralized preparation allows for more effective control of process hygiene, and improvement in product safety and storage stability.

This study was undertaken to determine if centralized preparation of vacuum packaged coarse ground beef could result in a high quality product with a markedly improved storage life in the retail counter.

2.2. MATERIALS AND METHODS

2.2.1. The Production Process

Meat trimmings were collected during fabrication of carcasses from animals that were slaughtered the previous day at a large beef slaughtering plant processing 2000 carcasses per day. Trimmings were collected on a common belt and sorted for various uses. Trimmings for coarse ground beef production were collected in large stainless steel bins and standardized for desired fat content. Production of coarse ground beef was done in batches by dumping the contents of a single bin into a hopper from which it was passed through a 5/8-inch grinder and into a mixing bin. The ground meat was mixed and cooled to 0 to 1°C (32 to 34°F) by blowing carbon dioxide (CO_2) through a tunnel as the meat was mixed. The chilled meat was then passed through a 3/8-inch grinder, immediately prior to vacuum packaging (Multivac). The vacuum packs (chubs) contained approximately 6 kg of coarse ground beef per package. Four chub packs were placed into a cardboard box, sealed and transferred to chill storage to await dispatch in refrigerated trailers to a centralized distribution warehouse 50 km (30 miles) from the production plant. The boxed chubs were then shipped by refrigerated trailer to retailers for fine grinding and aerobic packaging for retail sale. For this study the meat was shipped 175 km (110 miles) from the distribution warehouse to a local retailer where the product was stored overnight. The next morning the meat was taken to the research laboratory for study of extended vacuum storage and retail case life. The distance between the retailer and the laboratory was not sufficient to alter product temperature.

2.2.2. Temperature Profiles During Transportation and Storage

Temperature profiles of the coarse ground beef in the chub packages were obtained by placing a temperature data logger (MIRINZ Delphi Tru-Test, Auckland, New Zealand) at the center of each box between the chub packs. The temperature data loggers were kept in the boxes throughout the study to monitor the temperature during

extended storage. The temperature history of the product during retail display was monitored with external probes at the product surface with a recording multipoint strip chart recorder (Honeywell, Ltd.) equipped with saber-type thermocouples.

2.2.3. Vacuum Storage and Retail Case Life Studies

After storage under vacuum at 4°C (39.2°F) for 0, 4, 8, 12, 16 and 20 days, a 25 kg box of coarse ground beef was removed from storage and fine ground (1/8 inch). The fine ground beef was subdivided into 0.5 kg (1.1 lb) amounts, placed on styrofoam trays (Scott National, Calgary, AB, Canada), overwrapped with 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 and placed under simulated retail conditions in a horizontal-type retail case with fan-circulated air (model LPM12T, Hill Refrigeration of Canada, Ltd., Barrie, ON). The display case was illuminated for 12 hours per day with 750 lux of incandescent lighting. Five meat samples were placed at each storage point from the front to the back of the case. On each sampling day, one tray of ground beef from each storage point was evaluated for bacterial load, appearance and odour.

2.2.4. Sensory Evaluation

Odour and appearance of the vacuum packaged coarse ground beef was evaluated 15 minutes after opening the vacuum bag at each testing time. Finely ground, retail ground beef was also evaluated subjectively on each day of retail display by an experienced five-member sensory panel for appearance (with a 7-point hedonic scale: 1=extremely undesirable to 7=extremely desirable) and odour (with a 5-point acceptability scale: 1=acceptable to 5=unacceptable). Retail case life was estimated by assuming that rejection would occur at the time in days when the mean scores of the five-member sensory panel had declined to 3.5 for acceptance and (or) exceeded scores of 3.5 for odour (Greer and Jones, 1991).

2.2.5. Bacteriological Sampling

The meat was sampled to determine bacterial content during commercial production (meat trim, first and second coarse grinds), after each vacuum storage interval and daily during retail display. A 25 g meat sample was aseptically removed and placed in a sterile Stomacher bag followed by the addition of 225 ml of sterile, 0.1% peptone water and homogenized for 1 minute using a Colworth Stomacher (Baxter Diagnostics Corp., Canlab Division, Edmonton, Alberta, Canada). Four samples of vacuum packaged coarse ground beef and five samples of fine ground retail beef were tested at each sampling time using standard dilution and plating techniques.

The total population of psychrotrophs was determined on plate count agar (PCA, Difco Laboratories, Detroit, Mich.) incubated at 4°C for 10 days. *Pseudomonas* spp. were determined on cephaloridine-fucidin-cetrimide (CFC) agar incubated at 25°C for 48 hours. Lactic acid bacteria (LAB) were determined using De Man-Rogosa-Sharpe (MRS, Difco) agar incubated anaerobically (BBL Anaerobic System with 5 to 10% CO_2 ; Becton and Dickinson Co., Cockeysville, MD, USA) at 25°C for 72 hours. *Enterobacteriaceae* (enteric bacteria) were determined on violet red bile glucose (VRBG, Difco) agar incubated at 35°C for 18 to 21 hours. The selective properties of the preceding media have been described in detail (Baird et al., 1987). Coliform bacteria were determined on Lactose Monensin Glucuronate (LMG) agar (QA Laboratories; Toronto, Canada) incubated at 35°C for 24 hours. *E. coli* were determined by transferring the hydrophobic grid membrane after counting coliforms to buffered 4-methylumbelliferyl-ß-D-glucuronide (BMUG) agar (QA Laboratories), incubated at 35°C for 2 hours and examined under long wavelength ultraviolet light to enumerate the fluorescent colonies. *Brochothrix thermosphacta* was determined on streptomycin-indole-neutral red (SIN) agar incubated at 25°C for 72 hours (Shillinger and Lücke, 1987). The enterics, *B. thermosphacta*, coliform and *E. coli* were enumerated by hydrophobic grid membrane filtration technique to give a sensitivity of detection of 1

organism per gram. Other bacterial groups were enumerated by standard plating technique with a minimum detection level of a 100 organisms per gram. Bacterial numbers were reported as Colony Forming Units per gram (CFU/g) or were transformed to logarithms (log_{10}) for calculation of geometric means.

2.3. RESULTS

2.3.1. Meat Temperatures during Shipment and Storage

A period of 3 days elapsed between the time that the chubs of coarse ground beef were boxed at the processing plant and they arrived at our laboratory, including storage overnight in the cold room of a local retailer. Two shipments were completed for the study of temperature profiles during transportation from the slaughter plant to the retailer and research laboratory. For both shipments the temperatures ranged from -1.0 to 4.8°C and had an average of +0.5°C. An example of the temperature history of the product during shipment is shown in Figure 2.1. During storage under vacuum at our laboratory the temperature of the meat in the chub packs was 4.0°C (range 3.5 to 4.75°C) during the 20 days of storage. In the retail display case, the mean internal temperature of the ground beef was 5.9°C (range 1 to 10°C) when the temperatures at the five locations in the retail case were averaged. The five storage locations were distributed from the rear to the front of the retail case with the meat placed below the load limit line specified by the manufacturer.

2.3.2. Bacterial Contamination of Coarse Ground Beef during Commercial Preparation and Distribution.

The data in Table 2.1 are the mean log counts of the different bacterial groups on the meat during commercial preparation and transportation to the research laboratory. The microbial load of the coarse ground beef in the "chub" packs indicated that very hygienic processing conditions were achieved at the meat plant (Table 2.1). Total bacterial load was of the order of 10^3 CFU/g and the organisms consisted mainly of *Pseudomonas* spp. and lactic acid bacteria (LAB). *E. coli* was present at levels

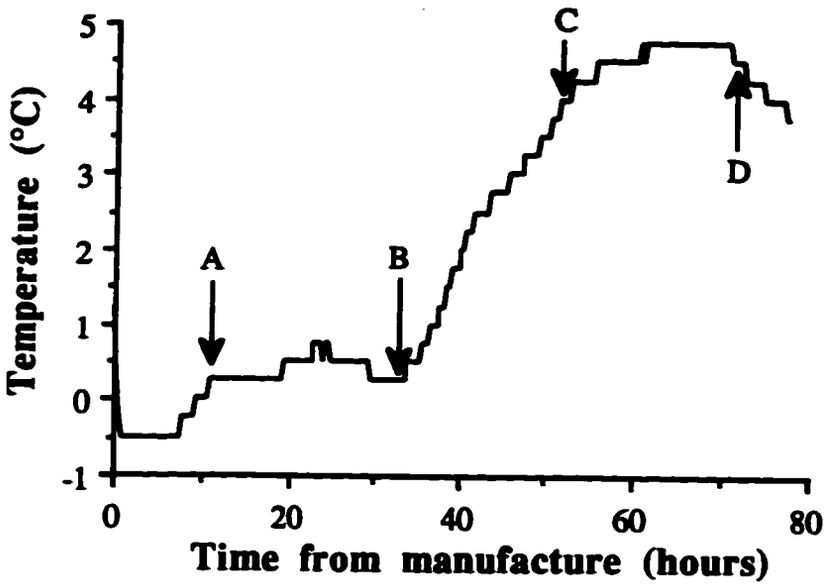


Figure 2.1. Temperature profile of vacuum packaged coarse ground beef produced at a centralized processing facility and transported to a retail store. The arrows indicate temperature: (A) on arrival at the distribution warehouse; (B) at time of shipping from the distribution warehouse; (C) on arrival at the retail store; and (D) on arrival at research laboratory.

Table 2.1. Bacterial counts (log CFU/g or cm^2) in coarse ground beef during commercial production and shipping from the processor to the research laboratory.

	Sample			
Organism	Trim [a]	First Grind	Second Grind	Research Lab [b]
Enterics	1.16	1.46	1.43	1.33
E. coli	0.43	0.25	0.86	0.54
Pseudomonads	2.36	2.76	2.92	2.77
Lactics	2.73	2.76	2.07	2.12
Psychrotrophs	2.91	3.32	3.29	3.17

[a] Log CFU reported per cm^2; remaining counts are log numbers per gram

[b] Arrival at research laboratory after three days of transportation and storage

below 10 CFU/g. No marked changes in bacterial counts were observed from the time of preparation of the trim at the processing plant to the time of arrival at the laboratory.

2.3.3. Bacterial Growth in Coarse Ground Beef and Sensory Changes

The effect of time of storage under vacuum on bacterial growth in the chub packs of coarse ground beef at 4°C is shown in Figure 2.2. Growth of the important spoilage bacteria (*Pseudomonas* spp., LAB and enterics) and *E. coli* is compared with the total population of psychrotrophs. *B. thermosphacta* and coliform bacteria were also enumerated but their population did not reach numbers greater than 10 CFU/g. The data show that the main bacteria enumerated during vacuum storage at 4°C were LAB and that their growth was similar to that of the total bacterial population. After 20 days of storage at 4°C under vacuum the population density of LAB was 10^8 CFU/g. The number of *Pseudomonas* spp. remained unchanged at 10^2 to 10^3 CFU/g until day 16, but by day 20 of storage they had increased 100-fold to 10^4 to 10^5 CFU/g. *Enterobacteriaceae* were not detected during the first 8 days of vacuum storage at 4°C. The *Enterobacteriaceae* reached approximately 10^4 CFU/g by day 12 and 10^5 CFU/g by day 20. In contrast, the number of *E. coli* and coliform bacteria remained at less than 10 CFU/g throughout storage. Acceptability of the appearance and odoUr of the coarse ground beef changed little during vacuum storage at 4°C. None of the samples reached a point of rejection by the panel, even at the end of the 20-day storage period.

2.3.4. Bacterial Growth in Retail Ground Beef

The effect of aerobic retail display on bacterial growth of ground beef prepared from chub packs of the vacuum packaged coarse ground beef stored at 4°C for up to 20 days is shown in Figure 2.3. The data illustrate the growth of each group of spoilage bacteria during retail display for 5 days. LAB account for the majority of the psychrotrophic bacteria growing on the meats (Figs. 2.3.1.A and 2.3.1.B), even on day 0 when the meat was received at the research laboratory (after three days of vacuum storage in the chub packs). LAB dominated the microbial population by a factor of at

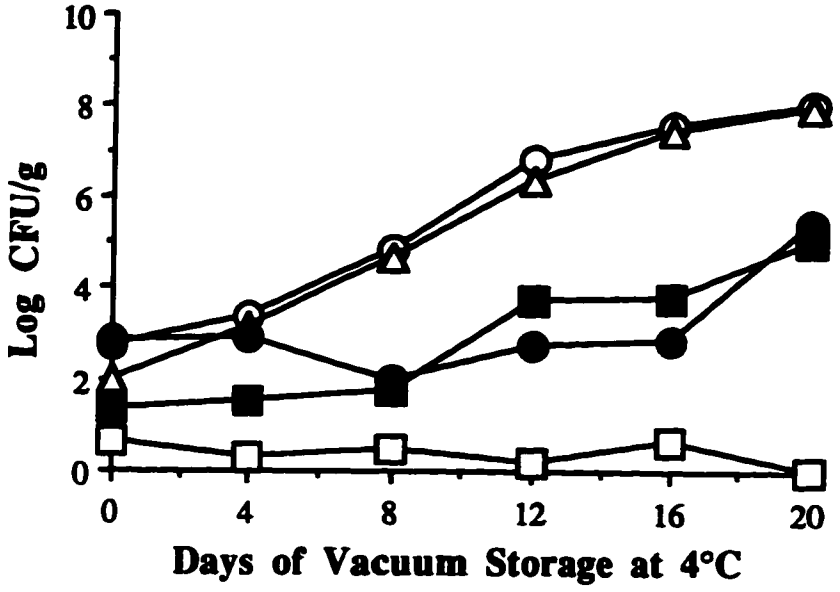


Figure 2.2. The effect of vacuum storage at 4°C on growth of total psychrotrophic bacteria (○), lactic acid bacteria (△), *Pseudomonas* spp. (●) enteric bacteria (■) and *E. coli* (□) in coarse ground beef. Each data point represents the mean log CFU/g of four vacuum "chub" packs.

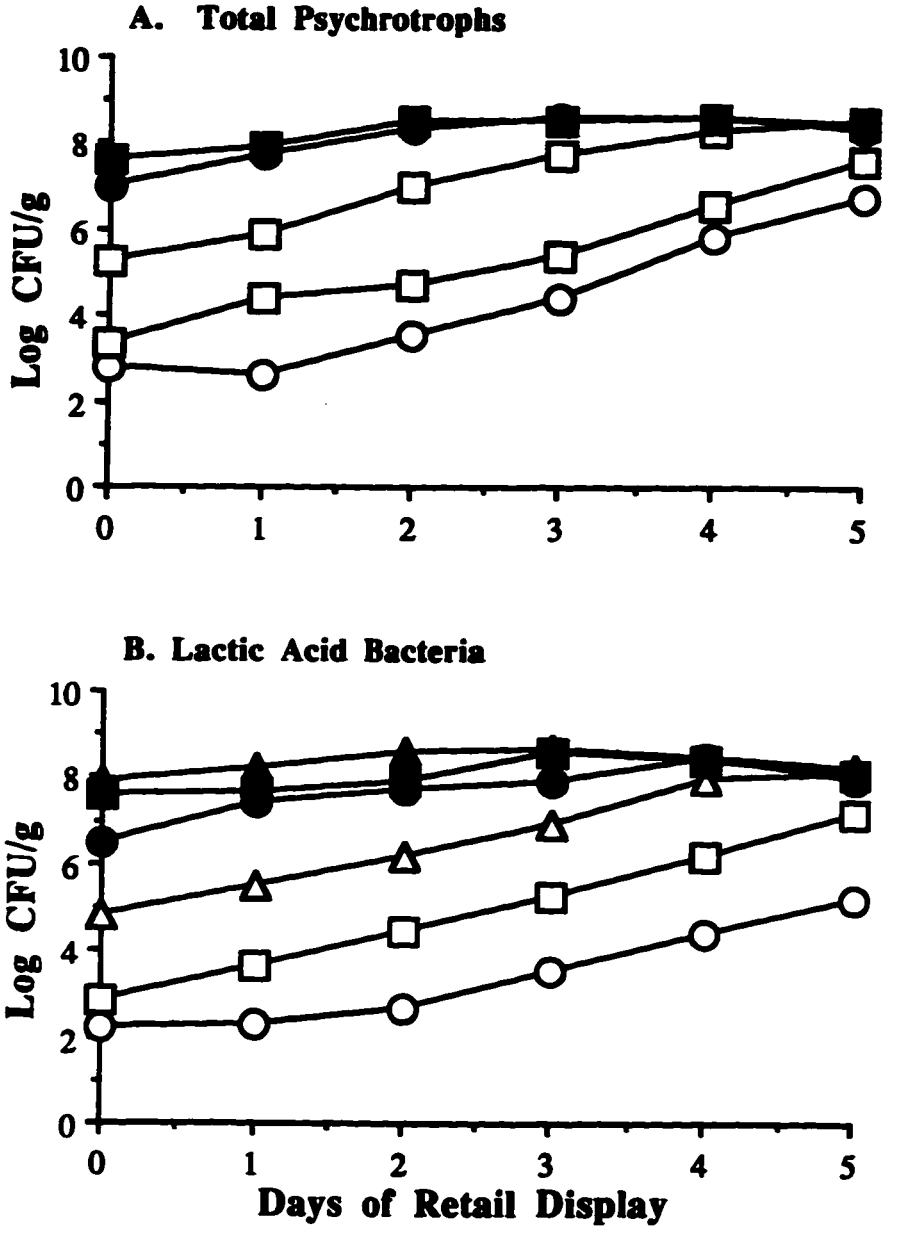


Figure 2.3.1. Effect of time of vacuum storage of coarse ground beef on bacterial populations in retail packaged ground beef. Each data point represents the mean of five samples stored aerobically on retail display with a mean temperature of 6°C. Growth of (A) total psychrotrophic bacteria and (B) lactic acid bacteria at each interval of storage under vacuum, day 0 (○), day 4 (□), day 8 (△), day 12 (●), day 16 (■) and day 20 (▲) of vacuum storage.

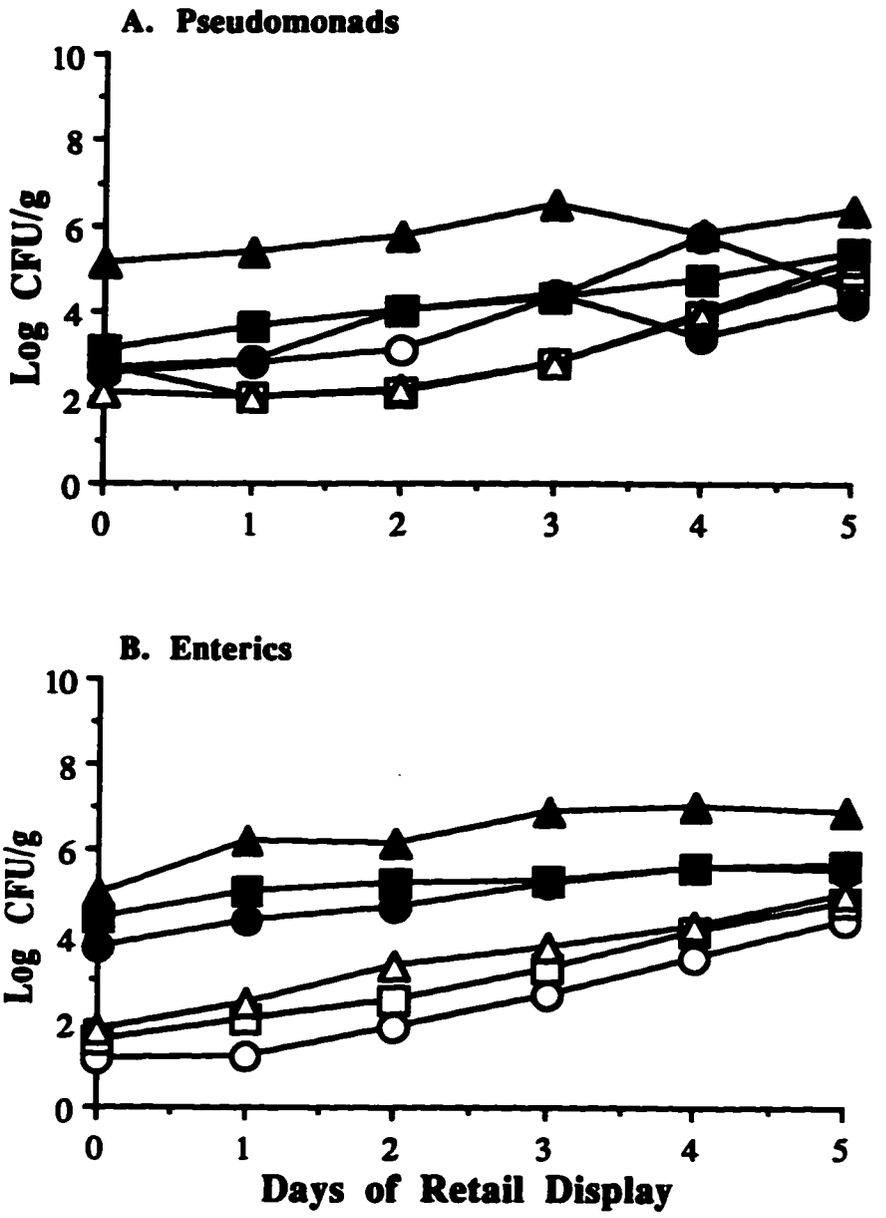


Figure 2.3.2. Effect of time of vacuum storage of coarse ground beef on bacterial populations in retail packaged ground beef. Each data point represents the mean of five samples stored aerobically on retail display with a mean temperature of 6°C. Growth of (A) *Pseudomonas* spp. and (B) enteric bacteria at each interval of storage under vacuum, day 0 (○), day 4 (□), day 8 (△), day 12 (●), day 16 (■) and day 20 (▲) of vacuum storage.

least 100-fold throughout the 5 days of retail display. LAB reached their maximum population of 10^8 CFU/g by day 16 of storage.

Pseudomonas spp. (Fig. 2.3.2.A) and enterics (Fig. 2.3.2.B) comprised a minor component of the bacterial population in retail meat prepared from chub packs stored under vacuum at 4°C for up to 16 days. In retail meat prepared from chub packs stored for 20 days these bacteria were present at 10^5 to 10^6 CFU/g, accounting for less than 1% of the total population of the meat. The number of *E. coli* did not exceed 10^2 CFU/g during retail display, and *B. thermosphacta* did not reach detectable numbers (>10^2 CFU/g). As expected, the bacterial load of the retail product increased with the time of storage under vacuum.

2.3.5. Case Life Studies

The effect of time of storage of the chub packs of coarse ground beef at 4°C on retail case life of aerobically packaged ground beef is shown in Table 2.2. Case life was assessed using both odour and appearance as acceptance criteria. Acceptability of the odoUr of the meat decreased from 5 days (after 0 days of vacuum storage) to 2.5 days after 20 days of vacuum storage at 4°C (Table 2.2). In contrast, retail case life determined by appearance (colour) was only 1.5 days for ground beef prepared from the chub packs at day 0, but thereafter the case life ranged between 2.2 and 3.3 days for ground beef prepared from chub packs stored for up to 20 days.

Table 2.2. Storage life of aerobically packaged ground beef assessed by appearance and odour of meat prepared after vacuum storage of coarse ground beef at 4°C for up to 20 days.

Time of vacuum storage (days)	Case Life (days)	
	Odour[a]	Appearance[b]
0	>5.0	1.5
4	4.9	2.6
8	4.4	3.3
12	2.8	2.2
16	2.7	2.3
20	2.5	2.5

[a] Odour was assessed using a 5-point hedonic scale (1=acceptable, 5=unacceptable). Samples were considered unacceptable when the mean panel score was >3.5 on the hedonic scale.

[b] Appearance was assessed using a 7-point hedonic scale (1=extremely undesirable, 7=extremely desirable). Samples were considered unacceptable when the mean score was <3.5 on the hedonic scale.

2.4. DISCUSSION

Ground beef provides a highly favourable environment for microbial growth. The quality depends on three main factors: initial bacterial load of meat used for grinding, sanitation of equipment and time-temperature of storage. Traditional preparation of ground beef for retail sale utilizes meat trimmings accumulated at retail store level and from a centralized producer. It is well documented that bacterial load of traditional ground beef is high and often exceeds 10^7 CFU/g and as a result, ground beef has a short retail shelf life (Duitschaever et al., 1973; Hudson et al., 1986; Nychas et al., 1992). With traditional preparation of ground beef, in which grinding, packaging and other operations are carried out by the retail store the quality of the ground beef depends on the quality of the trim (Shoup and Oblinger, 1976). Ground beef produced by a centralized producer was of better microbiological quality than that prepared at retail level or by traditional preparation (Shoup and Oblinger, 1976). Only one sample of the centrally produced ground beef had *E. coli* greater than 50 CFU/g compared with levels as high as 10^4 CFU/g in ground beef prepared by the traditional method.

Gill and McGinnis (1993) examined the microbial quality of traditionally prepared ground beef. Meat plant trimmings at the slaughter plant had levels between 10^3 and 10^5 CFU/g with a mean of 10^4 CFU/g. Little or no change in microbial load occurred from the slaughter plant to arrival at the retail store (Gill and McGinnis, 1993). However, ground beef displayed at retail had bacterial loads ranging from 10^4 to 10^7 CFU/g, with 75% of the samples containing 10^6 CFU/g. They reported an increase in mesophilic gram-positive bacteria in meat. This is expected due to the increase in numbers of lactic acid bacteria in vacuum packaged meat during refrigerated storage (Newton and Gill, 1978). The factors primarily responsible for loss of microbial quality at retail level are: wholesalers and retailers maintain stocks of meat that

allow them to deal with fluctuations in supply and demand (Gill and McGinnis, 1993), and temperature control is lost in retail display cases (Greer et al., 1994).

An alternative method of ground beef production is described in this study. Beef trim was collected on a common belt during meat cutting with the specific objective of preparing vacuum packaged, coarse ground chub packs for retail grinding. Results showed that coarse ground beef produced in this manner and shipped under commercial conditions maintained constant bacterial numbers of 10^3CFU/g during three days of transit and storage. Emswiler et al. (1976) also studied the bacteriological quality and case life of ground beef in 1.5 kg chub packs that were fabricated commercially and supplied unfrozen to retailers. They showed that fresh ground beef produced and shipped under their conditions contained $<10^5$ CFU/g. The superior bacterial quality of ground beef in the current study was likely related to the low levels of bacterial contamination on the beef trim and temperature control during shipment and storage (average 0.5°C).

The results of the present study showed that even after 20 days of vacuum storage of coarse ground beef at 4°C, the resulting fine ground product had a retail case life equal to or better than that observed under commercial conditions. Bacteria predominating during vacuum storage and retail display were lactic acid bacteria and these organisms generally have a low spoilage potential. Pseudomonads would normally be expected to predominate during aerobic storage and spoil the meat. It is possible that the prevailing lactic acid bacteria had consumed the available glucose in the meat, enabling the pseudomonads to cause spoilage at lower cell densities (i.e. 10^5 to 10^6 CFU/g) than would normally be expected. The lactic acid bacteria may also have mediated spoilage through their own metabolic activities. The number of coliform bacteria remained constant at 10 CFU/g during vacuum storage but increased to 10^3 CFU/g during retail display. Growth of *E. coli* under retail display conditions would be expected (Greer et al., 1994).

In summary, the present study illustrates that vacuum packaged coarse ground meat prepared and distributed through a commercial system, stored under vacuum for up to 20 days at 4°C can yield a retail product with excellent case life. This would allow considerable marketing flexibility and it would be revolutionary in terms of retail trade norms by reducing retail fabrication.

2.5. BIBLIOGRAPHY

Baird, R.M., J.E.L. Corry, and G.D.W. Curtis (ed). 1987. Pharmacopoeia of culture media for food microbiology. Int. J. Food Microbiol. 5:187-299.

Duitschaever, C.L., D.R. Arnott, and D.H. Bullock. 1973. Bacteriological quality of raw refrigerated ground beef. J. Milk Food Technol. 36:375-377.

Egbert, W.R., D.L. Huffman, C.M. Chen, and W.R. Jones. 1992. Microbial and oxidative changes in low-fat ground beef during simulated retail distribution. J. Food Sci. 57:1269-1293.

Emswiler, B.S., C.J. Pierson, and A.W. Kotula. 1976. Bacteriological quality and shelf life of ground beef. Appl. Environ. Microbiol. 31:826-830.

Foster, J.F., J.L. Fowler, and W.C. Ladiges. 1977. A bacteriological survey of raw ground beef. J. Food Prot. 40:790-794.

Gill, C.O., and C. McGinnis. 1993. Changes in the microflora on commercial beef trimmings during their collection, distribution and preparation for retail sale as ground beef. Int. J. Food Microbiol. 18:321-332.

Greer, G.G., C.O. Gill, and B.D. Dilts. 1994. Evaluation of the bacteriological consequences of the temperature regimes experienced by fresh chilled meat during display. Food Res. Int. 27:371-377.

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

Hudson, W.R., T.A. Roberts, A.R. Crosland, and J.C. Casey. 1986. The bacteriological quality, fat and collagen content of minced beef at retail level. Meat Sci. 17:139-152.

Newton, K.G., and C.O. Gill. 1978. The development of anaerobic spoilage flora of meat stored at chill temperatures. J. Appl. Bacteriol. 44:91-95.

Nychas, G.J., A. Robinson, and R.G. Board. 1992. Microbiological and physico-chemical evaluation of ground beef from retail shops. Fleischwirtschaft Int. 1:49-53.

Schillinger, U., and F.K. Lücke. 1987. Lactic acid bacteria on vacuum packaged meat and their influence on shelf life. Fleischwirtschaft 67:1244-1248.

Shoup, J.G., and J.L. Oblinger. 1976. Microbiological evaluation of retail ground beef: centralized and traditional preparation. J. Milk Food Technol. 39:179-183.

3. Biopreservation of vacuum packaged coarse ground beef with *Leuconostoc gelidum* UAL187

3.1. INTRODUCTION

The microbial quality of fresh meats is of increasing consumer concern and it calls for innovative methods of preservation. Lactic acid bacteria (LAB) are the dominating bacteria on fresh meats when it is chill stored under anaerobic conditions (Egan, 1983; Dainty and Mackey, 1992). Packaging of meat under vacuum or in modified atmosphere with elevated levels of carbon dioxide dramatically increases its storage life compared with aerobic storage because the normal aerobic spoilage bacteria are replaced by LAB; however, the meat is ultimately spoiled by LAB, usually after it has reached maximum microbial load (Egan and Shay, 1982; Borch and Nerbrink, 1989). The main genera of LAB that dominate in anaerobically packaged meats are *Lactobacillus*, *Leuconostoc* and *Carnobacterium* (Shaw and Harding, 1984; Dainty and Mackey, 1992; McMullen and Stiles, 1993). For a strain of LAB to be successful in the biopreservation of fresh meat it must compete with the indigenous microorganisms on raw meats; inhibit the spoilage and pathogenic microflora; but it must not impart undesirable sensory changes. *L. gelidum* UAL187 was proposed as a possible biopreservative in fresh meats because it meets these criteria (Leisner et al., 1995).

Biopreservation with a bacteriocin-producing LAB could extend and achieve a predictable storage life of anaerobically packaged meats. Bacteriocins are small peptides or proteins produced by bacteria which are generally active against closely related species (Klaenhammer, 1988). There has been interest in using bacteriocinogenic LAB isolated from raw meat to inhibit pathogens on meat packaged under vacuum or in modified atmosphere (Lewus et al., 1991). Initial studies on the extension of storage life of meat using a strain of *Lactobacillus sake* were not encouraging (Schillinger and Lücke, 1987a); however, Leisner et al. (1995) showed that bacteriocinogenic *L. gelidum* UAL187 extended the storage life of beef, even in the

presence of a sulphide-producing strain of *Lactobacillus sake* (Leisner et al., 1996). Further study is required to determine the potential of *L. gelidum* UAL187 to extend the storage life of commercially produced raw meats.

L. gelidum UAL187 is a wild type bacteriocin-producing strain isolated in our laboratory from modified atmosphere packaged meats (Hastings and Stiles, 1991). It produces a bacteriocin, leucocin A, that is plasmid mediated and has been well characterized (Hastings and Stiles, 1991; van Belkum and Stiles, 1995). Leucocin A is active against a broad spectrum of LAB and strains of *Listeria monocytogenes* and *Enterococcus* spp. In this study we report the biopreservative capability of the wild type bacteriocinogenic (bac^+) and the nonbacteriocinogenic variant (bac^-) strain of *L. gelidum* against the adventitious microflora of commercially prepared ground beef and their effect on the sensory qualities of odour and appearance of commercially produced vacuum packaged and aerobically packaged product.

3.2. MATERIALS AND METHODS

3.2.1. Ground Beef Preparation

Fresh, vacuum packaged neck and shoulder trim (85% lean) was obtained from a federally inspected, meat plant that slaughters up to 2000 cattle daily (Chapter 2). Beef trim for use in the experiments was collected on two separate occasions in the morning immediately after the start of carcass fabrication. The meat that was collected was vacuum packaged and transported under cold storage to our laboratory where it was held overnight in a cooler at –1°C. The following morning the trim was cut into similar sized strips in a research abbatoir setting and the cut trim was placed in sterile autoclavable plastic buckets (Nalgene) that held the required 7.5 kg batch of meat. Separate batches of trim were used for each of three treatments. The trim was coarse ground through a 15 mm plate and ground again through a 10 mm plate (Magic Mill DLX Kitchen Machine, Sweden). A subsequent fine grind was done with a 3 mm plate

after each interval of vacuum storage and 250 g samples were packaged for aerobic retail display.

3.2.2. Inoculation of Ground Beef

The test strains used in this study were *L. gelidum* UAL187 the bac$^+$ wild type strain with all three of its native plasmids, pLG5.0, pLG7.6 and pLG9.2 and the bac$^-$ variant *L. gelidum* UAL187-13 containing only plasmid pLG9.2 (Hastings and Stiles, 1991; van Belkum and Stiles, 1995). The strains were maintained in Cooked Meat medium (Difco Laboratories Inc., Detroit, Mich.) at 4°C, and they were subcultured (0.1% inoculum) at least twice in APT broth (All Purpose Tween; Difco) and incubated at 25°C for 18 h before use in experiments.

L. gelidum UAL187 and UAL187-13 were grown in 50 ml of APT broth and incubated at 25°C for 18 h before 10 ml of the *L. gelidum* UAL187 and UAL187-13 cells were harvested by centrifugation (5000 x *g* for 10 min at 2°C), washed twice with 0.1% peptone-water and resuspended in 10 ml of the sterile 0.1% peptone water. The bacterial suspensions were diluted 1:1000 with sterile 0.1% peptone water (10^6 cells/ml). The 1 L bacterial suspensions and sterile solution were poured over the batches of trim (7.5 kg), mixed and allowed to stand for 1 min, drained and coarse ground (15 mm and 10 mm plates).

3.2.3. Ground Beef Storage

For each treatment, 1.5 kg of coarse ground beef was weighed into high barrier PVDC/ polyethylene vinyl alcohol bags with a oxygen permeability of 50 cc/m^2/24 h and vacuum packaged using a Multivac (Sepp Hagenmuller KG, Wolfertschwenden, Germany). The vacuum packaged coarse ground beef was stored at 4°C for up to 35 days. Samples were removed for analysis after 0, 10, 21 and 35 days of storage. At each of the storage intervals two 1.5 kg packages of coarse ground beef for all three treatments was removed and fine ground (3 mm) for aerobic retail display at 2°C. Approximately 250 g of fine ground beef was placed on a styrofoam tray and

overwrapped with oxygen permeable polyvinyl chloride film (Vitafilm Choice Wrap, Goodyear Canada Inc., Toronto, ON) with oxygen transmission rate of 8000 cc/m^2/24 h. Duplicate samples of each treatment were withdrawn for analysis on day 0, 1, 3, 5, 7 and 9 of aerobic storage.

3.2.4. Bacteriology

Duplicate samples from two vacuum bags and from two aerobically stored samples of each treatment were used for microbial analysis by standard dilution and plating techniques. A 25 g sample of ground beef was weighed, placed in a stomacher bag and homogenized in 225 ml of sterile 0.1% peptone water for 1 min using a Colworth Stomacher (Baxter Diagnostics Corp., Canlab Division, Edmonton, AB, Canada). Indigenous LAB were enumerated on MRS agar containing 5% sucrose after anaerobic incubation (BBL Anaerobic System with 5-10% CO_2; Becton and Dickinson Co., Cockeysville, MD, USA) at 25°C for 72 h. The colonies that did not produce dextran (slime) were enumerated as the indigenous population. A preliminary study compared the enumeration of the indigenous LAB on MRS agar and the reformulated MRS agar containing sucrose and the results concluded that the reformulated MRS media would be suitable for the enumeration of the indigenous LAB. *L. gelidum* strains were enumerated using Lactobacilli MRS agar according to the Difco formulation except that dextrose was replaced with 5% sucrose. The *L. gelidum* strains were enumerated by counting the slime-forming colonies after 48 h of anaerobic incubation at 25°C. Control samples were also checked for slime-producing colonies to ensure that the indigenous slime-producing LAB were not high enough to affect the numbers of *L. gelidum* enumerated. The *Pseudomonas* spp. were enumerated on cephaloridine-fucidin-cetrimide (CFC) agar incubated at 25°C for 48 h (Baird et al., 1987). *Enterobacteriaceae* were determined on overlayed plates of violet red bile agar (Difco) with 1% added glucose (VRBG) and counted after 18 to 21 h of incubation at 35°C. The surface plating technique has a minimum detection level of 100 colony

forming units (cfu)/g, and the pour plate method used for enumeration of *Enterobacteriaceae* has a sensitivity of 10 cfu/g.

3.2.5. Storage Life, Beef Colour and pH

Appearance and odour of vacuum and aerobically stored beef was evaluated at each storage interval by an experienced five-member panel based on established criteria (Greer and Jones, 1991; Greer and Murray, 1991). Vacuum packaged samples were evaluated 15 min after the package was opened. Meat colour (CIE, 1976) was determined using a Minolta Chroma Meter II (Minolta Camera Co., Ramsey, NJ) which measures the reflectance coordinates (L*, a*, b*). The L* coordinate represents the brightness or paleness of the meat, higher values indicate lighter colours; the a* coordinate measures the red-green spectrum, higher values indicate a redder colour; and the b* coordinate measures the yellow-blue spectrum, higher values indicate a more yellow colour. A saber-type pH probe was used to measure the internal pH of the ground beef using an Oakton microprocessor WD-00605-00 (Anachemia Scientific, Calgary, Alberta).

3.2.6. Experimental Design and Statistical Analyses

The experiment consisted of three treatments in which meat was inoculated with *L. gelidum* UAL187 (bac^+) or *L. gelidum* UAL187-13 (bac^-) and an uninoculated control. The experiment was repeated twice with separate batches of meat. Each treatment was tested in duplicate on samples taken at each interval of vacuum and aerobic storage. Microbial counts, beef colour coordinates (L*, a*, b*) and pH were subjected to analysis of variance according to the General Linear Models procedure of the Statistical Analyses System (SAS Institute, 1985).

3.3. RESULTS

3.3.1. Growth and antagonism by *L. gelidum* in vacuum and aerobically packaged ground beef

Inoculation of beef trim with *L. gelidum* UAL187 (bac^+) and UAL187-13 (bac^-) resulted in coarse ground beef with 10^4 to 10^5 CFU of *L. gelidum* per g. Both variants of *L. gelidum* had grown to maximum population of 10^8 CFU/g by day 10 of vacuum storage at 4°C and remained at that level at 21 and 35 days of vacuum storage (Fig. 3.1) with standard deviations less than ± log 0.5 cfu/g at each storage interval. The antagonistic effects of *L. gelidum* UAL187 and UAL187-13 on the adventitious spoilage microflora of meats (lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* spp.) compared with the uninoculated samples are shown in Fig. 3.2. The uninoculated control samples had counts that were significantly ($p< 0.05$) higher by a factor of 1 to 2 log cycles for adventitious spoilage bacteria after 21 and 35 days of vacuum storage compared with the inoculated samples. After 10 days of vacuum storage there was a significant ($p<0.05$) antagonistic effect exhibited by *L. gelidum* UAL187 and UAL187-13 compared with the uninoculated samples, but there was less than a 1 log lower count for each of the bacterial groups (Fig. 3.2). The reduced count with either of the *L. gelidum* strains compared with the control treatments was virtually identical during vacuum storage except after 35 days storage, when samples inoculated with *L. gelidum* UAL187 showed a greater reduction of *Enterobacteriaceae* than *L. gelidum* UAL187-13 (Fig. 3.2.B).

Throughout the 35 days of vacuum storage the indigenous LAB were the dominating bacterial population in all the treatments. The indigenous LAB reached a maximum level of 10^8 CFU/g in the uninoculated control and 10^7 CFU/g in the inoculated samples. The *Enterobacteriaceae* reached 10^7 CFU/g in the uninoculated samples after 35 days of vacuum storage, whereas in the inoculated treatments they reached levels of 10^6 and 10^5 CFU/g in the presence of *L. gelidum* UAL187-13 and *L.*

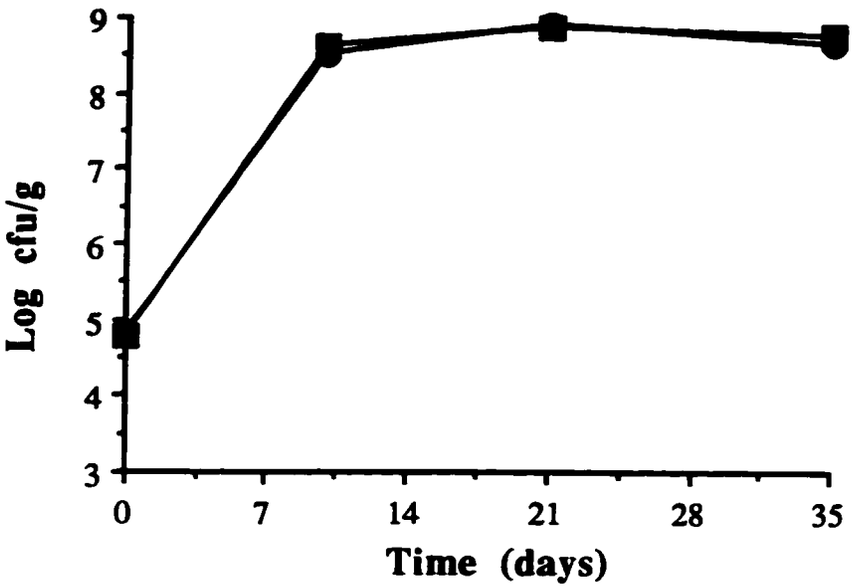


Figure 3.1. Growth of *L. gelidum* UAL187 (●) and UAL187-13 (■) in commercially produced vacuum packaged coarse ground beef at 4°C.

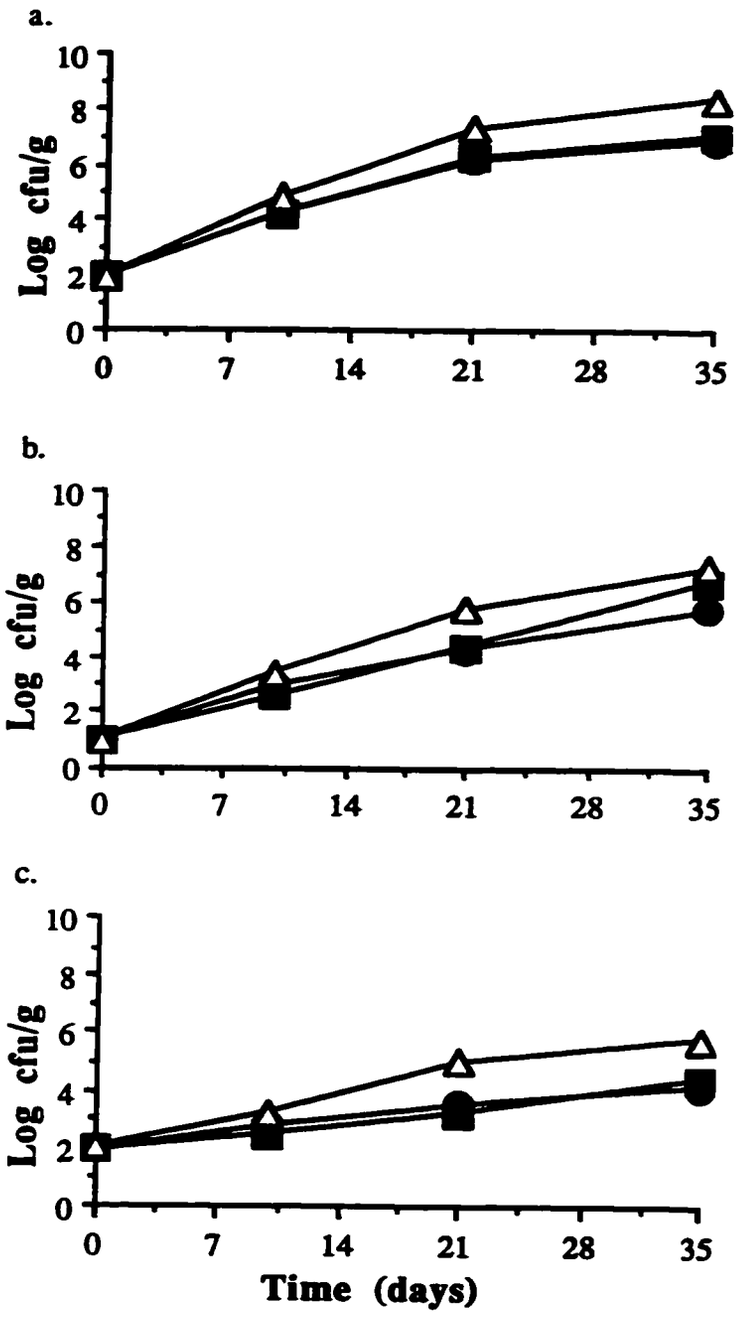


Figure 3.2. Effect of *L. gelidum* UAL187 (●), *L. gelidum* UAL187-13 (■) or the uninoculated control sample (Δ) on the growth of a) lactic acid bacteria; b) *Enterobacteriaceae*; and c) *Pseudomonas* spp. in vacuum packaged coarse ground beef at 4°C.

gelidum UAL187, respectively. *Pseudomonas* spp. reached a maximum level of 10^5 CFU/g in the uninoculated control while in the inoculated samples they only reached a level of 10^4 CFU/g after 35 days of vacuum storage.

The effect of *L. gelidum* UAL187 and UAL187-13 compared with the uninoculated control on retail-ready ground beef prepared from vacuum packaged coarse ground beef stored for 21 days at 4°C is shown in Fig. 3.3. The data illustrate that the microbial load of the retail product increased for each of the spoilage groups during aerobic display, but that the same antagonistic effect of *L. gelidum* UAL 187 and UAL187-13 that was observed in samples stored under vacuum was observed during aerobic retail display at 2°C for up to 7 days. The same antagonistic or competitive growth by *L. gelidum* is exhibited against each spoilage group during retail display at days 10 and 35 of vacuum storage, except that the microbial reduction is not the same as after 10 days compared with 35 days of storage where the microbial reduction was at its greatest (data not shown). LAB remained the dominant indigenous microflora of the inoculated and uninoculated samples throughout aerobic retail display at each of the vacuum storage intervals.

3.3.2. Changes in colour of ground beef

Comparison of the L* (lightness), a* (red-green), b* (yellow-blue) colour coordinates of the uninoculated and inoculated samples for all aerobically stored samples of ground beef at each vacuum storage interval showed a faster deterioration of the a* value during aerobic storage at 2°C after 35 days of vacuum storage in the uninoculated control samples compared with either of the inoculated samples of ground beef (Fig. 3.4.D). After 35 days of vacuum storage, the uninoculated ground beef samples had a significantly lower ($p<0.05$) a* value throughout the 5 days of aerobic retail display (Fig. 3.4.D). The enhanced stability of the a* coordinate during aerobic retail display of samples inoculated with *L. gelidum* UAL187 or UAL187-13 was readily detected compared with the uninoculated control. This was also apparent after

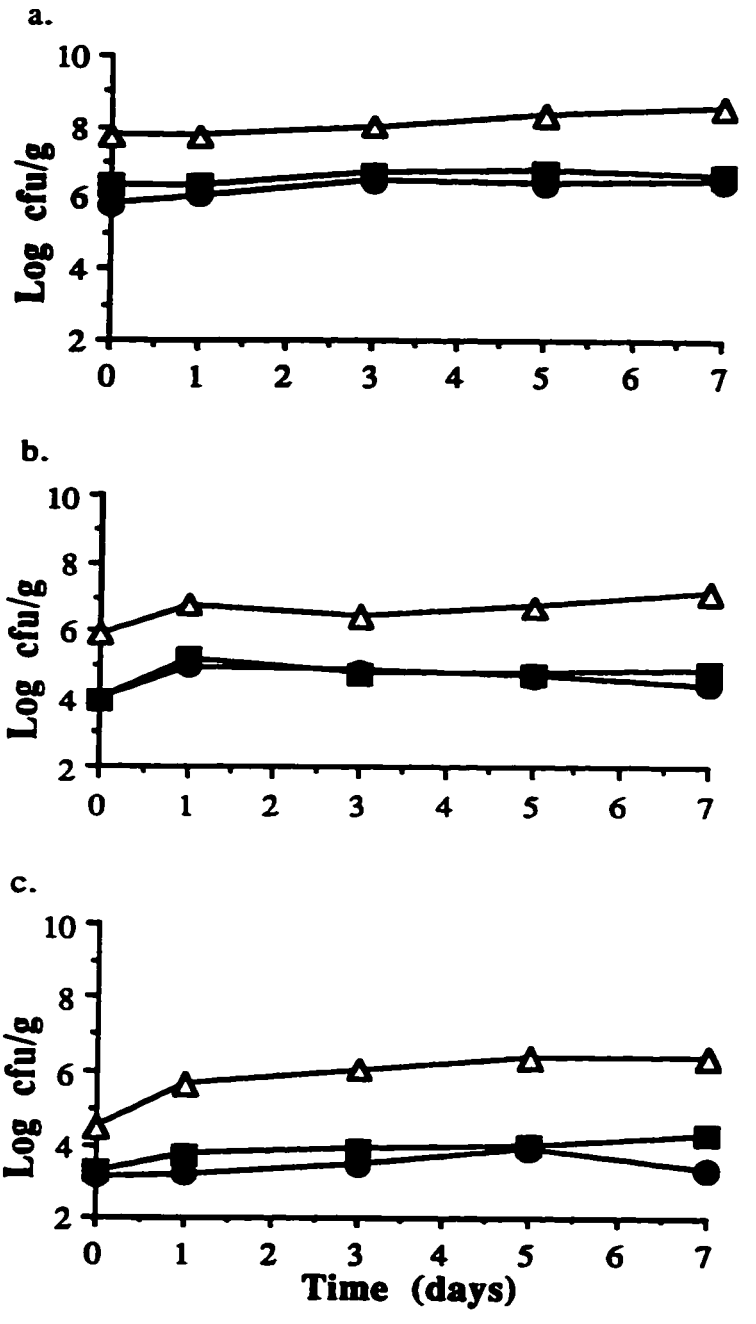


Figure 3.3. Effect of *L. gelidum* UAL187 (●), *L. gelidum* UAL187-13 (■) or the uninoculated control sample (Δ) in aerobically packaged ground beef prepared from vacuum packaged coarse ground beef stored at 4°C for 21 days on the growth of a) lactic acid bacteria; b) *Enterobacteriaceae*; and c) *Pseudomonas* spp. during aerobic storage at 2°C.

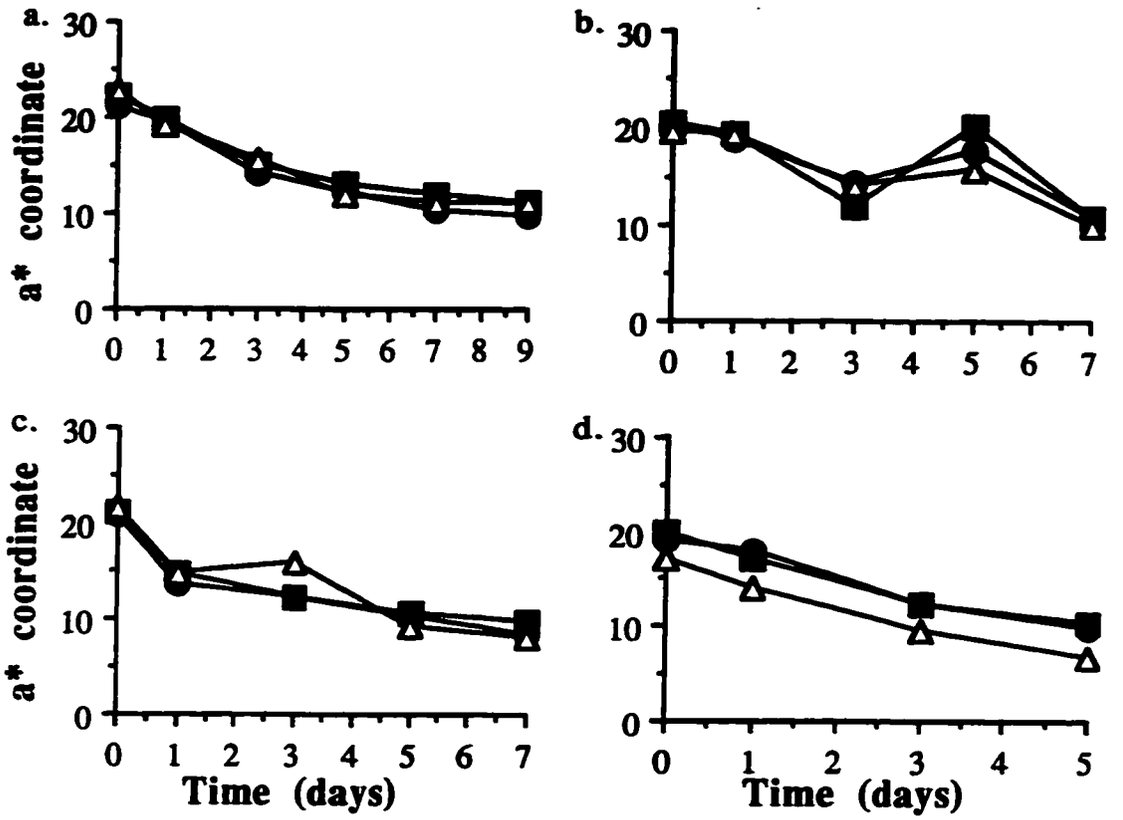


Figure 3.4. Changes in the a* colour coordinate of ground beef inoculated with *L. gelidum* UAL187 (●), *L. gelidum* UAL187-13(■), uninoculated control (Δ) and stored aerobically at 2°C after vacuum storage at 4°C for (a) 0, (b) 10, (c) 21 and (d) 35 days.

21 days of vacuum storage, though there was no significant ($p>0.05$) effect observed in the a* coordinate values during retail display after 21 days of vacuum storage.

3.3.3. Storage life of vacuum packaged and retail (aerobic) ground beef

Acceptability of coarse ground beef based on appearance and odour of inoculated and uninoculated samples changed very little during vacuum storage at 4°C. None of the samples reached a point of rejection for appearance or odour during vacuum storage for up to 35 days, although the uninoculated control was borderline for acceptability of odour based on panel evaluation using a 5-point hedonic scale (1=acceptable, 5=unacceptable). The odour score for the uninoculated control declined to 3.4 and, at a median score of 3.5, odour acceptance is considered unacceptable. A distinct difference in odour was detected in uninoculated samples after vacuum storage for 21 and 35 days. These odour differences were based on comments made by the panelists. The distinct difference in odour is demonstrated by the difference in aerobic odour shelf-life after 35 days of vacuum storage where the uninoculated control was less then 0.5 days compared with greater than 2 days for the inoculated samples. Based on panel evaluations there was no difference in the acceptability or appearance of ground beef inoculated with *L. gelidum* UAL187 or UAL187-13 and the uninoculated ground beef during aerobic storage after any of the vacuum storage intervals (data not shown) although the control samples could be easily identified by the deterioration in the redness of the meat.

The data in Fig. 3.5 illustrate the effects of *L. gelidum* UAL187 and UAL187-13 on the aerobic storage life of ground beef based on odour determined by panel evaluations after extended periods of vacuum storage. The inoculated samples show a trend that *L. gelidum* has a beneficiary effect on the odour. This is indicated after 35 days the inoculated samples have a storage life that is almost 2 days greater than the uninoculated sample (Fig.3.5).

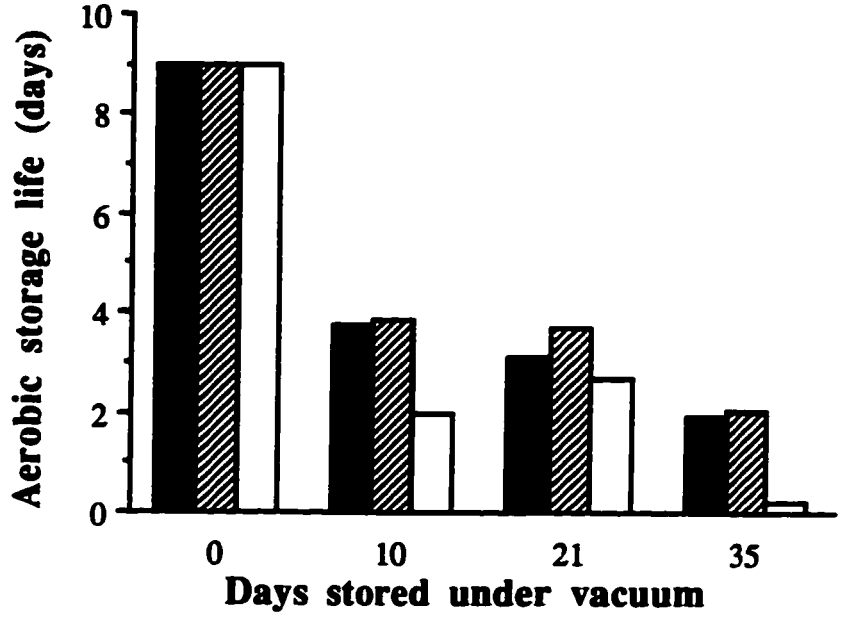


Figure 3.5. Storage life based on acceptance of odour of aerobically packaged ground beef at 2°C prepared from coarse ground beef inoculated with *L. gelidum* UAL187 (black bars), *L. gelidum* UAL187-13 (striped bars) or the uninoculated control (white bars) and stored for up to 35 days under vacuum at 4°C.

3.3.4. Effect of bacterial growth on pH

At the beginning of the experiment the pH of the inoculated and uninoculated samples of ground beef was 5.8 and it dropped to pH 5.5 to 5.6 over the course of the study. Ground beef inoculated with either strain of *L. gelidum* had a significantly ($p<0.05$) lower pH than the uninoculated samples after 10 and 21 days of vacuum storage at 4°C. The pH of the inoculated ground beef dropped by approximately 0.1 pH unit while the pH of the uninoculated control samples remained unchanged (Table 3.1). After 35 days of vacuum storage there was no significant difference ($p>0.05$) noted between the pH of the inoculated and the uninoculated samples. The transfer of coarse ground beef from vacuum to aerobic display resulted in significantly ($p<0.05$) lower pH values for the inoculated samples of ground beef with *L. gelidum* compared with the uninoculated ground beef throughout aerobic retail display after 10 and 21 days of vacuum storage (data not shown).

3.4. DISCUSSION

An earlier study in our laboratory (Leisner et al., 1996) where sterile disks of beef were co-inoculated with sulphide-producing *Lactobacillus sake* 1218 and the bacteriocinogenic or nonbacteriocinogenic strains of *L. gelidum* UAL187, spoilage was controlled by the bacteriocinogenic strain, but not by the nonbacteriocinogenic strain. In this study, commercially prepared coarse ground beef was used to determine the growth, antagonism and sensory consequences of *L. gelidum* UAL187. We had previously shown that coarse ground beef produced at this meat plant was of excellent microbial quality, and that meat from this plant could be delivered to a retail store and have a microbial load $\leq 10^3$ CFU/g three days after production (Chapter 2). Addition of the bacteriocin-producing wild type *L. gelidum* UAL187 or the Bac^- variant *L. gelidum* UAL187-13 resulted in a 1 to 2 log reduction in the adventitious spoilage microflora compared with the uninoculated samples after extended vacuum storage.

Table 3.1. The effect of growth of *L. gelidum* UAL187 and UAL187-13 on pH compared with the uninoculated control samples of vacuum packaged coarse ground beef stored at 4°C for up to 35 days.

	pH*		
Time of vacuum storage (days)	*L. gelidum* UAL187	*L. gelidum* UAL187-13	uninoculated control
0	5.8 ±0.02	5.8 ±0.03	5.8 ±0.04
10	5.7 ±0.07	5.7 ±0.04	5.8 ±0.04
21	5.6 ±0.09	5.6 ±0.07	5.7 ±0.02
35	5.5 ±0.07	5.6 ±0.02	5.6 ±0.09

* log mean of 4 samples showing the standard error of the means

Gilliland (1980) demonstrated a similar inhibitory action by inoculating either *Lactobacillus bulgaricus*, *Lactococcus lactis* or "*Pediococcus cerevisiae*" onto meat. All of these organisms inhibited the growth of the adventitious flora, including growth of the gram-negative bacteria. Antagonistic action against the spoilage microflora of refrigerated meats was also demonstrated for other species of *Leuconostoc* (Daly et al., 1972; Reddy et al., 1970). In a study by Newton and Gill (1978) in which they co-inoculated LAB and *Enterobacteriaceae* into meat juice and stored it anaerobically at 10°C, the LAB outgrew the gram-negative species and an inhibitory effect was evident. The antagonistic action by *L. gelidum* and other LAB can be attributed to antagonistic compounds other than the bacteriocins that they produce or by competition for available energy sources.

The primary source of energy for growth of the dominating LAB under anaerobic conditions is glucose (Gill, 1976; Newton and Gill, 1978). *Leuconostoc* rapidly utilize glucose when stored anaerobically (Borch and Agerhem, 1992). In our study we inoculated the ground beef with 10^5 *L. gelidum* per g. This would give the inoculum a competitive advantage by their rapid uptake of glucose which is the primary growth substrate for LAB and the competition may result in reduced growth of the *Enterobacteriaceae* and *Pseudomonas* spp. compared with the uninoculated samples. The rapid utilization of glucose is evidenced by the significant lowering of pH in the ground beef by *L. gelidum* and this may contribute in part to the inhibition of growth. The end products of glucose fermentation, lactic and acetic acids, can be inhibitory to other bacterial groups including gram-negative spoilage organisms. However, the inhibition that was shown in our study is not likely to be responsible for the significant reduction of the adventitious spoilage groups. Inhibition by *L. gelidum* by the production of hydrogen peroxide would not occur in anaerobically packaged meats because hydrogen peroxide is not formed in the absence of oxygen (Gill, 1976).

Off-odours associated with anaerobically stored meats does not become significant until some time after the maximum population of the LAB is reached (Egan and Shay, 1982; Borch and Nerbrink, 1989), but *L. gelidum* UAL187 or UAL187-13 did not cause any undesirable odours after they reached maximum population at 10 days or for the duration of the 35 days of vacuum storage. These results are unlike Smith et al. (1980) who reported a reduction in microbial populations by the addition of pure cultures of selected LAB, but with the development of more extreme off-odours than in the uninoculated samples after extended storage. Borch and Agerhem (1992) inoculated meat samples with LAB and reported a "rotten egg smell" with the inoculated samples and did not detect off-odours in the control samples after extended vacuum storage.

Extension of the aerobic shelf-life exhibited with the inoculation of either strain of *L. gelidum* compared with uninoculated samples may be due to the reduced growth of the adventitious spoilage microflora in the inoculated samples and spoilage is delayed until the adventitious populations reach high enough numbers. Another possible explanation is that a totally different group of organisms dominate the microflora of the uninoculated samples compared with the inoculated samples. This could be possible because there was an atypical putrid odour that was distinguishable in the control samples compared with either of the inoculated samples. The *Enterobacteriaceae* could be responsible for these atypical putrid off-odours because they reach levels in the control samples that are high enough to result in spoilage, whereas in the inoculated samples they are 1 to 2 log lower. Typically the *Enterobacteriaceae* and the *Pseudomonas* spp. during anaerobic storage do not reach levels this high and they are not among the normal anaerobic spoilage flora, but in some instances they reach levels of 10^3 to 10^6 per cm^2 (Dainty and Hibbard, 1983).

Our study demonstrated that there was a difference in the red meat colour stability, but there was no difference in the aerobic shelf-life based on appearance,

between the inoculated samples and the uninoculated control samples. The deterioration of the appearance acceptability of the samples was not likely to be linked to bacterial growth but rather to the extrinsic factors that are associated with ground beef. Grinding of meat is probably the most detrimental factor that deteriorates the appearance of ground meat because discoloration occurs at a faster rate when a greater surface area of the meat is exposed to air and as a result there is an increase in oxidation of myoglobin (red) to metmyoglobin (brown) (Renerre, 1990). Deterioration of appearance in our study is not likely linked to bacterial growth because during retail display after 0 days of vacuum storage the appearance deteriorated before bacterial numbers were even near levels that result in surface discoloration. Although there was no difference in the aerobic shelf-life, there was a significant effect on the colour stability of the ground beef inoculated with *L. gelidum*. The inoculated samples had a higher a*-coordinate value compared with the uninoculated control, indicating that the meat had a greater "redness" value. There was a distinct difference in the discoloration of the ground beef that had been inoculated and that of the uninoculated sample. This difference in discoloration and maintenance of red meat colour was linked to the bacterial growth of either strain of *L. gelidum* because both of them demonstrated the same results and the uninoculated samples showed none of these same effects. Even though the panelists determined the appearance to be undesirable due to the discoloration which may be attributed mainly to the extrinsic factors associated with ground beef there was recognizable difference in the redness of the meat. Generally meats contaminated with bacteria cause undesirable surface discoloration caused by the metabolites they produce. *L. gelidum* UAL187 and UAL187-13 must produce a metabolite that binds to myoglobin and slows down its oxidation to metamyoglobin. Arihara et al. (1993) found that *Lactobacillus fermentum* JCM1173 which they isolated from the environment generated a red form of oxymyoglobin by the formation of nitric oxide myoglobin. These results were characterized spectrophotometrically. Our results are

similar except you can visually see the difference in the red meat colour in the ground beef that has taken place with a population of microbes. No other known published data show increased stability of red meat colour by the addition of a bacterial strain. This is an important observation if LAB are used as biopreservatives in a meat system since the single most important factor determining the acceptability of meats by consumers is meat colour (Faustman and Cassens, 1990).

The results from this study are interesting from the perspective that *L. gelidum* can reduce the microbial populations, increase the odour stability and also has the ability increase stability of red meat colour. These affects were not related to bacteriocin production. These results look very promising and indicate the potential for biopreservation of vacuum packaged coarse ground beef with *L. gelidum*, but even shows a potential use in other meat systems where the adverse conditions associated with ground beef are not as severe. Further inoculation studies using other meats are required to further evaluate the biopreservation potential. The flavour consequences associated with the addition of *L. gelidum* to vacuum packaged coarse ground beef is currently being investigated.

3.5. BIBLIOGRAPHY

Arihara, K., H. Kushida, Y. Kondo, M. Itoh, J.B. Luchansky, and R. G. Cassens. 1993. Conversion of metmyoglobin to bright red myoglobin derivatives by *Chromobacterium violaceum, Kurthia* sp., and *Lactobacillus fermentum* JCM1173. J. Food Sci. 58:38-42.

Baird, R.M., J.E.L. Corry, and G.D.W. Curtis (ed.). 1987. Pharmacopoeia of culture media for food microbiology. Int. J. Food Microbiol. 5:187-299.

Borch, E., and E. Nerbrink. 1989. Shelf-life of emulsion sausage stored in vacuum or modified atmospheres, pp. 470-477. In: Proc. 35th International Congress of Meat Science and Technology, Copenhagen, Denmark, Vol.II.

Borch, E., and H. Agerhem. 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 Poincare, 75782 Paris Cedex, 16 France.

Dainty, R.H., and C.M. Hibbard. 1983. Precursors of the major end products of the aerobic metabolism of *Brochothrix thermosphacta*. J. Appl. Bacteriol. 55:127-133.

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

Daly, C., W.E. Sandine, and P. R. Elliker. 1972. Interaction of starter cultures and food-borne pathogens: *Streptococcus diacetylactis* versus food pathogens. J. Milk Food Technol. 35:349-357.

Egan, A.F., and B.J. Shay. 1982. Significance of lactobacilli and film permeability in the spoilage of vacuum-packaged beef. Food Sci. 47:1119-1122, 1126.

Egan, A.F. 1983. Lactic acid bacteria of meat and meat products. Antonie van Leeuwenhoek. 49:327-336.

Faustman, C., and R. G. Cassens. 1990. The biochemical basis for discoloration in fresh meat: a review. J. Muscle Food 1:217-243.

Gill, C.O. 1976. Substrate limitation of bacterial growth at meat surfaces. J. Appl. Bacteriol. 41:401-410.

Gilliland, S.E. 1980. Use of lactobacilli to preserve fresh meat. Proc. Recip. Meat. Conf. 33:54-58.

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

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

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

Klaenhammer, T.R. 1988. Bacteriocins of lactic acid bacteria. Biochemie 70:337-349.

Leisner, J.J., G.G. Greer, B.D. Dilts, and M.E. Stiles. 1995. Effect of growth of selected lactic acid bacteria on storage life of beef stored under vacuum and air. Int. J. Food Microbiol. 26:231-243.

Leisner, J.J., G.G. Greer, and M.E. Stiles. 1996. Control of spoilage of beef by a sulfide-producing *Lactobacillus sake* with bacteriocinogenic *Leuconostoc gelidum* UAL187 during anaerobic storage at 2°C. Appl. Environ. Microbiol. 62:2610-2614.

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

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

Newton, K.G., and C.O. Gill. 1978. The development of the anaerobic spoilage flora of meat stored at chill temperatures. J. Appl. Bacteriol. 44:91-95.

Reddy, S.G., R.L. Henrickson, and H.C. Olson. 1970. The influence of lactic cultures on ground beef quality. J. Food Sci. 35:787-791.

Renerre, M. 1990. Review: factors involved in the discoloration of beef meat. Int. J. Food Sci. Technol. 25:613-630.

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

Schillinger, U., and F.K. Lücke. 1987. Lactic acid bacteria on vacuum-packaged meat and their influence on storage life. Fleischwirtschaft 67:1244-1248.

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

4. Sensory consequences of a bacteriocin-producing lactic acid bacterium in vacuum packaged ground beef stored at 4°C.

4.1. INTRODUCTION

Lactic acid bacteria (LAB) are the predominant microflora on vacuum and modified atmosphere packaged red meat stored at refrigeration temperatures (Kitchell and Shaw, 1975; Egan, 1983). The three main genera found on anaerobically stored red meat are *Lactobacillus, Leuconostoc* and *Carnobacterium* (Shaw and Harding, 1984; Dainty and Mackey, 1992; McMullen and Stiles, 1993). The storage of meat in an anaerobic environment extends the storage-life by inhibiting the aerobic gram-negative spoilage organisms and allowing a LAB microflora to develop. LAB cause meat spoilage; however, it is generally not detectable until after maximum cell density is attained and spoilage is often associated with acid and sour odours (Newton et al.,1977; Sutherland et al. 1976). The spoilage is a result of the accumulation of short chain fatty acids that are products of glucose fermentation (Dainty and Mackey, 1992). A strong putrid hydrogen sulphide off-odour has also been associated with spoilage in anaerobically stored meats and this spoilage can be caused by a strain of *Lactobacillus* (Shay and Egan, 1981) that produces H_2S under glucose-limiting conditions (Egan et al., 1989).

Ground beef produced at the retail level uses commercial beef trimmings that are vacuum packaged and supplied to retailers for fine grinding and aerobic display in the retail display case. Meats that are stored aerobically are usually dominated by gram-negative non-sporeforming rod-shaped bacteria of which *Pseudomonas* spp. typically account for >50% of the microflora. These organisms cause meats to spoil rapidly due to the rapid depletion of glucose and the degradation of amino acids to form free amides resulting in putrefactive spoilage (Dainty and Mackey, 1992; Gill and Newton, 1982). However, after extended vacuum storage of meats and then exposure to air for retail display it has been observed that the *Pseudomonas* spp. do not reach a population that

causes spoilage and their growth is somewhat inhibited (see Chapter 2). Spoilage of the meat may occur as a result of the LAB which dominate during anaerobic storage and continue to dominate on the meat when exposed to air. Therefore, it is essential to control the indigenous LAB microflora in the vacuum package to extend the storage life of fresh meats.

There is a great deal of interest in the antagonistic activity of LAB for use as a possible biopreservative in fresh meats. It is well known that some LAB produce antagonistic peptides known as bacteriocins which inhibit closely related bacteria (Tagg et al., 1976; Klaenhammer, 1988). *Leuconostoc gelidum* UAL187 was isolated from vacuum packaged meats and it produces a bacteriostatic bacteriocin known as leucocin A (Hastings and Stiles, 1991). Leucocin A has antimicrobial activity against a broad spectrum of LAB, *Listeria* spp. and *Enterococcus* spp. but it is not active against gram-negative bacteria, *Brochothrix* spp. or *Staphylococcus* spp. (Hastings and Stiles, 1991). *Leuconostoc gelidum* UAL187 was chosen as a possible biopreservative because of its ability to grow on red meat at low storage temperatures without the development of undesirable changes in odour and appearance (Leisner et al., 1995), and for its ability to delay the onset of spoilage by the overt spoilage organism *Lactobacillus sake* 1218 (Leisner et al., 1996).

We previously studied the growth of *Leuconostoc gelidum* UAL187 in commercially produced vacuum packaged coarse ground beef (see Chapter 3). We showed that the bacteriocin-producing *L. gelidum* UAL187 and the isogenic bacteriocin-negative strain *L. gelidum* UAL187-13 inhibited the growth of the indigenous LAB, *Enterobacteriaceae* and *Pseudomonas* spp. The addition of *L. gelidum* UAL187 also increased the odour shelf-life and the colour stability of the ground beef. The objective of this study was to investigate the sensory consequences (odour, flavour) of adding bacteriocin-producing *L. gelidum* to commercially prepared coarse ground beef.

4.2. MATERIALS AND METHODS

4.2.1. Bacterial Strains, Inoculation of Beef and Bacterial Evaluation

The bacterial strains and method of inoculation of meat used in this study are described in detail in Chapter 3. One sample from each treatment was analyzed for bacterial content using standard dilution and plating techniques. The bacterial evaluation included the same microbial spoilage groups and the methodology that are described in Chapter 3.

4.2.2. Ground Beef Preparation

Fresh, vacuum packaged neck and shoulder trim (85% lean) was obtained from a federally inspected, commercial abbatoir. Two trips were made to the slaughter plant and sufficient trim to complete two replicates of the experiment was collected each time. The trim was vacuum packaged and collected immediately after the day's production began on the fabrication line. The meat was transported under cold storage to the laboratory. Immediately after arrival one box of meat trim was cut into a uniform size for grinding and then placed in sterile (autoclaved) plastic buckets (Nalgene) of appropriate batch size (7.5 kg) for inoculation. A batch of trim was inoculated with one of the three treatments and coarse ground through a 15 mm plate followed by a second grind through a 10 mm plate with a Magic Mill DLX Kitchen Machine (Magic Mill, Sweden), vacuum packaged and stored at 4°C. A subsequent 3 mm fine grind was done after each vacuum storage interval to simulate conditions of aerobic retail display. A total of 4 replicates of this experiment was completed.

4.2.3. Ground Beef Storage

For each treatment, 1.0 kg of coarse ground beef was weighed into a high gas barrier PVDC/polyethylene vinyl alcohol bag (Winpak) with an oxygen permeability of 50 cc/m^2/24h and then vacuum packaged using a Multivac vacuum packaging machine (Sepp Hagenmüller KG, Wolfertschwenden, Germany). The coarse ground beef was stored at 4°C for up to 21 days. Samples for each treatment were removed from

vacuum storage after 0, 7, 14 and 21 days and fine ground. The fine ground beef (1.0 kg) was placed on a styrofoam tray and 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 hr and stored for one day at 4°C. After one day of aerobic storage the samples were removed for microbiological and sensory analysis.

4.2.4. Sensory Evaluation

Cooking Procedures. After aerobic storage overnight, approximately 750g of ground beef was weighed into pre-weighed bread pans and cooked as a meat loaf, at 176°C to an internal temperature of 78°C. The temperature of each meat loaf was monitored with a single copper-constantan thermocouple connected to a Honeywell recording potentiometer. The meat loaves were allowed to cool to 55°C, weighed and removed from their pans. The edges of each meat loaf were trimmed off and the loaf was cut into 1 cm slices. Total cooking time (min), volatile loss, drip loss and total cooking losses were recorded. After slicing, the ground beef was mixed in Hobart Kitchen Aid (Model K45SS) food processor, with the whip attachment, first for 30 seconds at low speed and then mixed for two additional times of 15 seconds at high speed.

Taste Panels. Potential panelists were screened by the procedure described by AMSA (1995). The fifteen panelists consisting of students and staff in the Department of Agricultural, Food and Nutritional Science at the University of Alberta participated in the initial screening that consisted of 12 triangle tests. Selection of panelists was based on their ability to differentiate among a range of attributes of the cooked ground beef (dairy, liver, sour, aged, warmed-over flavour and fresh). Thirteen panelists were selected for training based on their ability to correctly identify the odd sample in the triangle tests greater than 60% of the time. Panel training was done for 5 weeks with 3 to 4 sessions per week. Panelists were trained to identify and score five attributes: overall intensity, liver, dairy, sour and rancid using a 15 cm line scale anchored with

appropriate descriptors (none to very strong). The first training session was used to familiarize the panelists with sensory evaluation procedures and to acquaint them with the line scaling evaluation procedure. The next 4 weeks of panel training consisted of familiarizing the panelists with the different flavour attributes and to train them to distinguish and consistently rate the intensity of each flavour attribute. After each panel session, all of the results were recorded by the panel leader (researcher) and were further discussed with the panelists. A round-table discussion among panelists ensured that they were consistently evaluating the intensity of each flavour note and also to familiarize and train them to recognize the specific flavour notes. On the fifth week of training the panelists were trained to record results with the Computerized Sensory Analysis (CSA) system (CSA, 1990). After training, panel performance was evaluated using the procedure described by AMSA (1995). The panel evaluation consisted of 3 replicates and 5 treatments that were representative of the main study. The flavour intensity was analyzed by one-way analysis of variance. For each flavour characteristic individual F-values were calculated and were combined as an indication of panelist performance. Panelists that had high F-values indicated their ability to distinguish consistently between treatments. The top 8 panelists were selected on the basis of their F-values while the remaining 5 panelists with the lower F-values were excused from the panel.

Sample Presentation. Panel sessions were conducted twice weekly. Panel evaluation was held in an atmospherically controlled sensory panel room equipped with red lights and 8 individual booths, each equipped with a computer monitor to access the CSA program. Each panelist evaluated 8 samples per session: a reference sample and six coded treatment samples and one coded reference sample to serve as a hidden control. The order of presentation of the samples was randomized for each panelist. Ground beef (10 g) from each treatment was served in small, coded glass jars covered with foil. Prior to evaluation the samples were warmed to 55°C in a water bath. The warmed

samples were placed in the panel booths equipped with Corningware double boiler system on Salton™ hot trays to keep the samples at the appropriate temperature of 50 ± 5°C for panel evaluation. For flavour evaluation the panelists were given distilled water (23°C), a slice of Granny Smith apple and crackers for rinsing and cleansing the palette between samples.

Odour Evaluation. A 7-member panel was used to evaluate the odour of raw ground beef. The panel rated the overall odour intensity and perceived retail acceptability. A 15 cm line scale was used to score the intensity of each attribute. The panel received a brief training period to familiarize them with the procedure for line scaling evaluation. Odour evaluation was done in an atmospherically controlled sensory panel room equipped with individual booths and red lights. The raw ground beef samples were weighed (10 g) into small coded jars, covered with foil and held in a 4°C refrigerator until they were evaluated. Each panelist evaluated 8 samples per session including, 1 reference sample, 6 treatment samples and 1 hidden reference. The sample order for each panelist was randomized.

4.2.5. Experimental Design and Statistical Analysis

Four replicates of the experiment were done over 2 consecutive weeks. The microbial counts were expressed as colony forming units (CFU)/g and the geometric means were calculated from the data. The sensory data for flavour, overall odour intensity and acceptability and the microbial data were subjected to ANOVA using the GLM procedure (SAS Institute, 1989). The intensity values for each flavour and odour characteristic were calculated as means across replicates. Student-Newman-Keuls' Multiple Range Test (Steele and Torrie, 1980) was used to detect significant differences in the flavour and odour intensity attributes among the treatment means.

4.3. RESULTS

4.3.1. Microbial Changes

The bacteriocin-producing *L. gelidum* UAL187 or the isogenic bacteriocin-negative variant *L. gelidum* UAL187-13 were inoculated onto fresh, commercially-produced beef trim and coarse ground to give an initial concentration of log 5 CFU per g. The range was log 4.70 to 5.18 *L. gelidum* per g for the four replicates. Both variants of *L. gelidum* exhibited similar growth patterns throughout the 21 days of vacuum storage at 4°C and both grew with little variability (standard deviations less than ± log 0.5 per gram at each storage interval). Both variants reached maximum population between 7 and 14 days of vacuum storage (Fig. 4.1).

The LAB, *Enterobacteriaceae* and *Pseudomonas* spp. were enumerated in the inoculated treatments and compared with the uninoculated stored control at weekly intervals for 21 days of vacuum storage at 4°C (Fig. 4.2). The samples that were inoculated with either strain of *L. gelidum* had significantly ($P < 0.05$) lower bacterial populations compared with the uninoculated stored control for all of the bacterial spoilage groups enumerated at each of the sampling times. A 0.5 log lower count of the indigenous spoilage population was detected in the inoculated samples after 7 days of vacuum storage and a 1 log lower count was detected after 14 days of vacuum storage. After 21 days of vacuum storage there was only a slightly lower count (<0.5 log) for the LAB and *Enterobacteriaceae* populations when inoculated with *L. gelidum* (Fig. 4.2 A, B), whereas there was a difference of greater than 1 log CFU/g for *Pseudomonas* spp. throughout the 21 days of vacuum storage (Fig. 4.2 C).

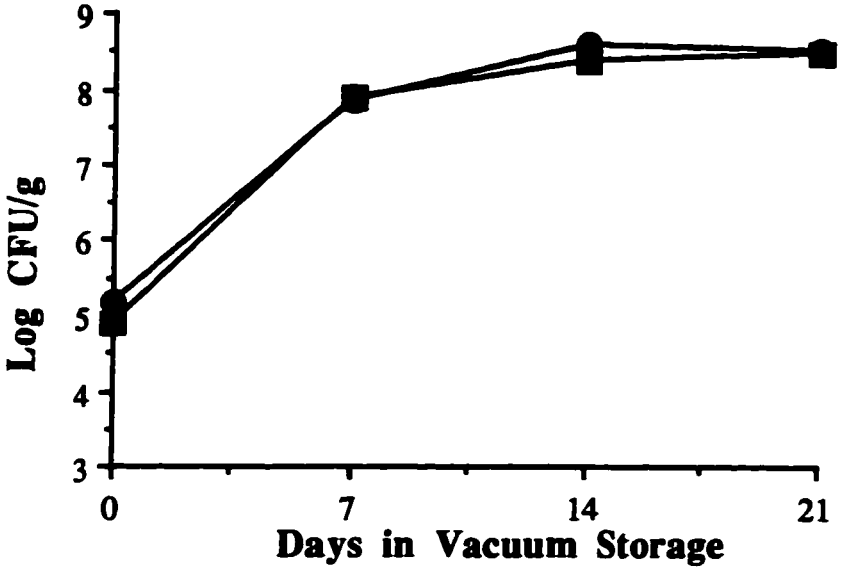


Figure 4.1. Growth of *L. gelidum* UAL187 (●)and *L. gelidum* UAL187-13 (■) under anaerobic storage and transferred to aerobic display for each weekly storage interval for 21 days at 4°C after inoculation at log 5 CFU/g.

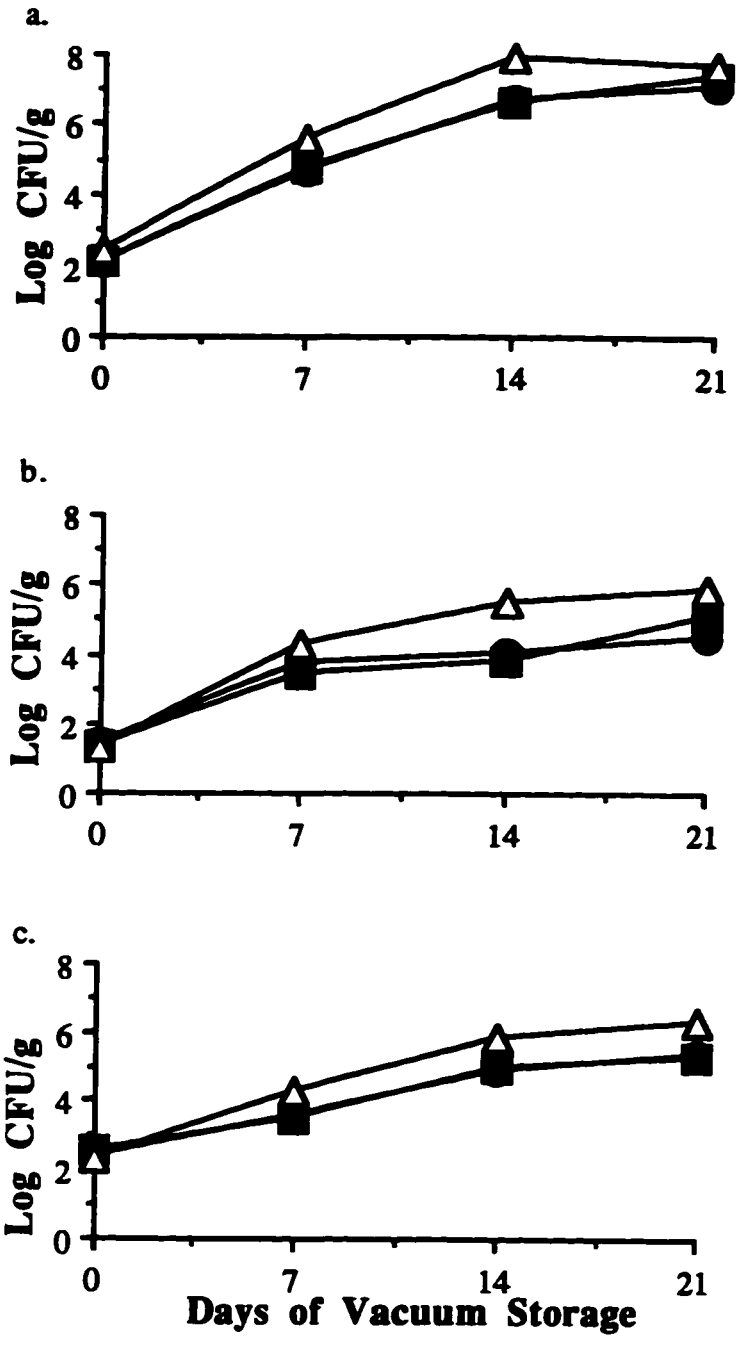


Figure 4.2. Growth of a) lactic acid bacteria, b) *Enterobacteriaceae* and c) *Pseudomanas* spp. during vacuum storage at 4°C following 1 day of aerobic storage at each storage interval for up to 21 days inoculation with either *L. gelidum* UAL187 (●), *L. gelidum* UAL187-13 (■), or the uninoculated stored control (Δ).

4.3.2. Sensory Analysis

There was no significant differences (P>0.05) in drip loss, volatile loss and total cooking losses (data not shown) between the three treatments and the reference sample of ground beef throughout the 21 days of storage.

Profiles for odour intensity (Fig. 4.3) and odour acceptability (Fig. 4.4) for the raw ground beef are virtually identical. There was no significant difference (P >0.05) in the overall odour intensity between the samples inoculated with either variant of *L. gelidum* and the uninoculated samples after 0 and 7 days of vacuum storage. After 14 and 21 days of vacuum storage, the odour intensity of the uninoculated samples was significantly higher (P <0.05) than that of the samples inoculated with either *L. gelidum* UAL187 or UAL187-13, but there was no significant difference (P >0.05) in the overall odour intensity of samples inoculated with either variant of *L. gelidum* throughout the study.

The changes in overall flavour intensity for cooked ground beef inoculated with *L. gelidum* UAL187, *L. gelidum* UAL187-13 and the uninoculated, stored control sample are shown in Fig. 4.5. The overall intensity scores show no significant differences (P >0.05) between the inoculated and the uninoculated control samples on days 0, 7 and 14 of anaerobic storage at 4°C. However, after 21 days of storage the uninoculated sample was scored significantly lower (P <0.05) in overall flavour intensity than the inoculated samples. Sour and rancid flavour notes were the flavours detected by the panelists in the flavour intensity scores during extended vacuum storage of the ground beef samples. No significant differences (P >0.05) were detected in sour flavour intensity scores between the three treatments throughout the 21 days of vacuum storage; however, the rancid flavour intensity was significantly lower (P <0.05) for the uninoculated control compared with the inoculated samples after 14 days of vacuum storage, but not after 21 days of storage. The liver and dairy flavour notes were minor components of the flavour intensity, as indicated by their intensity values, and

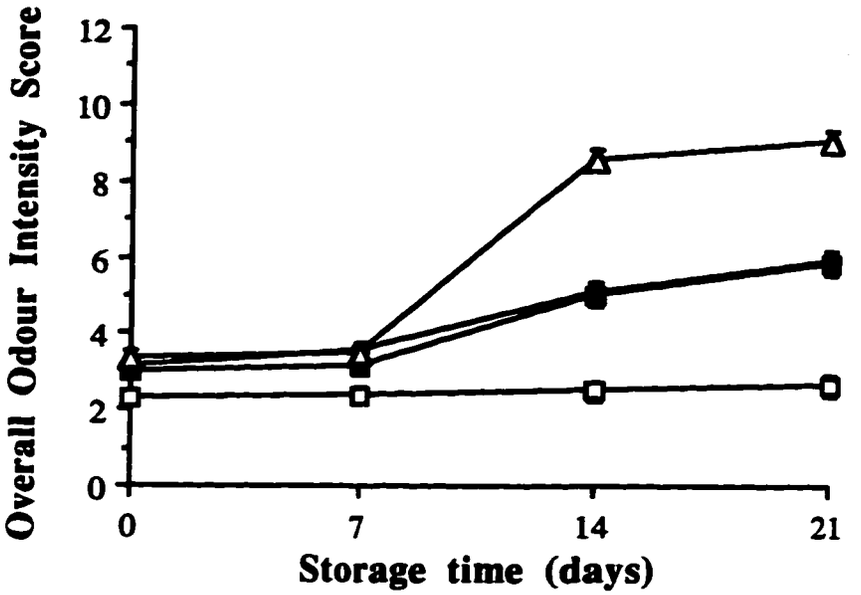


Figure 4.3. Odour intensity (0=none, 15=very strong) of anaerobically packaged ground beef inoculated with strains of *L. gelidum* UAL187 (●), *L. gelidum* UAL187-13 (■), uninoculated stored control (Δ), for up to 21 days at 4°C and a freshly prepared reference sample (□). Error bars represent standard error of mean scores of odour intensity of the ground beef.

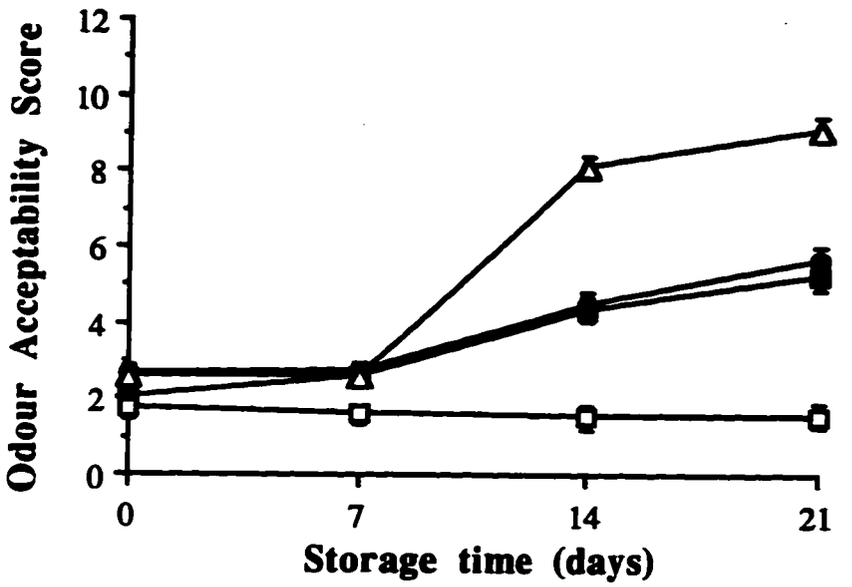


Figure 4.4. Odour acceptability scores (0=very acceptable, 15=very unacceptable) of anaerobically packaged ground beef inoculated with strains of *L. gelidum* UAL187 (●), *L. gelidum* UAL187-13 (■), uninoculated stored control (Δ) for up to 21 days at 4°C and a freshly prepared reference sample (□). Error bars represent standard error of means scores for acceptability of the ground beef odour.

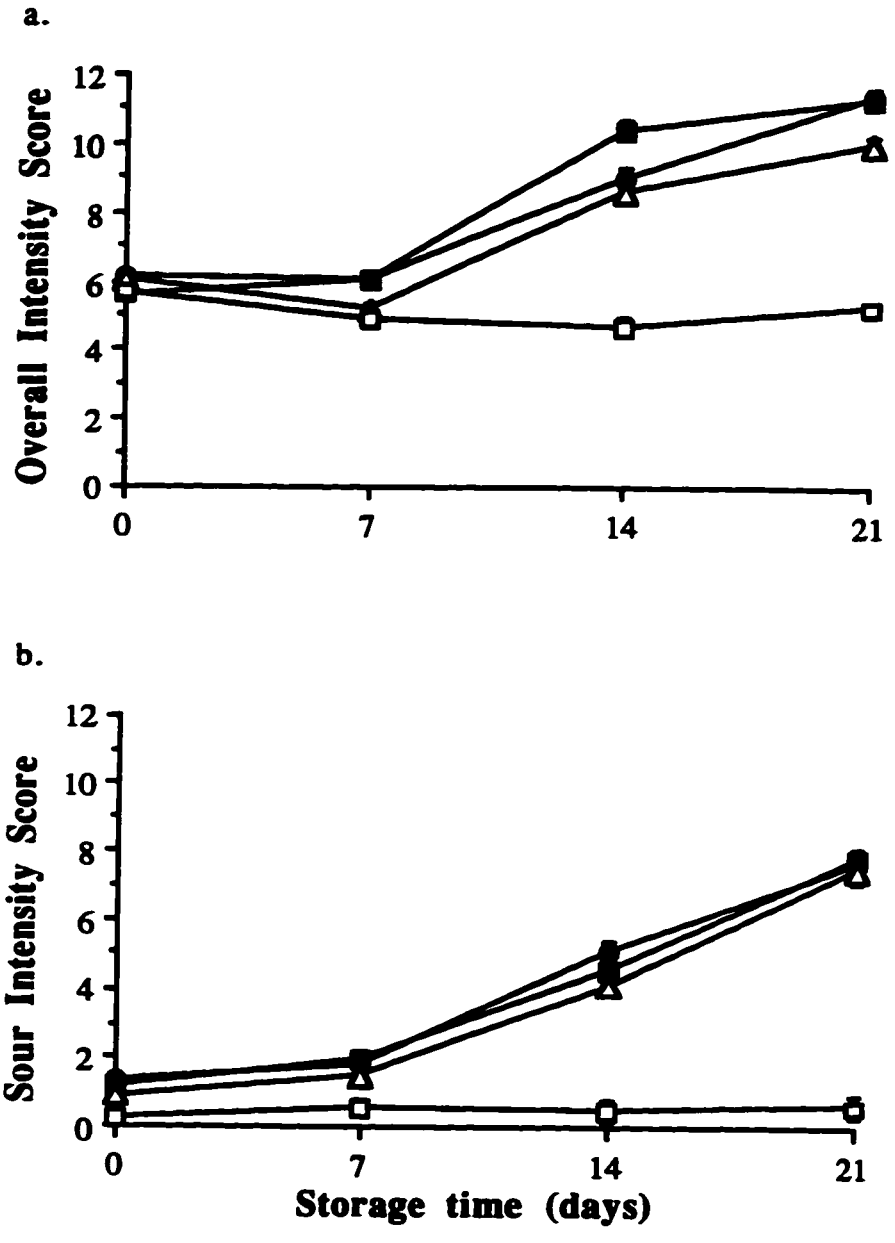


Figure 4.5. Flavour intensity (0=none, 15=very strong) scores for: (a) overall and (b) sour of anaerobically packaged ground beef inoculated with *L. gelidum* UAL187 (●), *L. gelidum* UAL187-13 (■), uninoculated stored control (Δ) for up to 21 days at 4°C and a freshly prepared reference sample (□). Error bars represent standard error of mean scores of odour intensity of the ground beef odour.

no significant differences were detected between any of the treatments (data not shown). The overall intensity of the hidden reference samples (data not shown) were rated consistently throughout the experiment by the panelists.

4.4. DISCUSSION

Generally, lactic acid bacteria (LAB) act as preservative organisms in modified atmosphere and vacuum packaged meats but there are some strains that cause spoilage in these anaerobic environments. The use of bacteriocin-producing LAB has been investigated to control or eliminate the spoilage LAB in anaerobic environments. A previous study by Leisner et al (1996) reported that bacteriocin production by *L. gelidum* UAL187 could inhibit growth of and prevent the overt spoilage by *Lactobacillus sake* 1218 when they were coinoculated onto sterile beef surfaces and chill stored under vacuum for an extended time. In our previous study (Chapter 3), commercially produced ground beef was inoculated with the same strains of *L. gelidum* and we demonstrated that either strain suppressed the growth of the adventitious microbial population and maintained the colour stability of raw ground beef, thereby extending its odour storage life. The current study was undertaken to determine the impact of the same strains on the odour of the raw ground beef and on the flavour characteristics of cooked ground beef after extended vacuum storage.

The microbial data confirmed the results of the previous study (Chapter 3) showing that growth of the adventitious microbial populations was suppressed by inoculating the meat with either *L. gelidum* UAL187 or UAL187-13. In the previous study (Chapter 3) we also assessed the effect of inoculation of the meat with *L. gelidum* on the odour storage life, by making subjective measurements using a 5-point hedonic scale with a trained sensory panel. The results indicated that the addition of either variant of *L. gelidum* suppressed the development of objectionable odours and increased the odour shelf-life compared with the uninoculated control. In this study the

overall odour intensity and acceptability scores of the raw ground beef were measured by a panel that used line scaling. The results confirmed our previous observation that addition of either strain of *L. gelidum* to ground beef significantly lowered overall odour intensity and increased the acceptability score of the raw product.

Sour or acid odours are common descriptors of meats that have been stored for extended periods at chill temperatures (Sutherland et al., 1976; Dainty, 1981). The sour/acid odours are associated with formation of short chain organic acids. In the previous study (Chapter 3) there was a significant difference reduction of the pH in samples inoculated with *L. gelidum*. Differences in odour intensity and acceptability scores were associated with larger populations of the adventitious microflora in the uninoculated samples. Addition of *L. gelidum* may suppress the growth of certain groups of bacteria that are responsible for the undesirable odour that developed in the uninoculated samples during extended storage. It is well known that some LAB produce sulphur compounds (Hitchener et al., 1982; Egan et al., 1989) buttermilk (Hanna et al., 1983) and painted/rancid odours (Borch and Agerhem, 1992) that greatly reduce storage life of vacuum packaged meat. *Enterobacteriaceae* have been detected on anaerobically packaged meats during chill storage for extended periods (Newton et al., 1977; Blickstad and Molin; 1983; Gill and Harrison; 1989). In our study the *Enterobacteriaceae* reached levels in the uninoculated control that may have caused the undesirable odours. *Enterobacteriaceae* normally do not reach a population as high as in the present study, but on occasion they have been shown to reach levels of this magnitude (Dainty et al., 1983).

The inoculation of coarse ground beef with *L. gelidum* UAL187 or UAL187-13 showed no difference in the flavour profile between the two variants. Results of the flavour intensity scores indicate that there is no difference in the overall flavour intensity after the inoculated strains of *L. gelidum* attain maximum population compared with the uninoculated samples after the same storage period. This is unlike the results

of Borch and Agerhem (1992) in which they reported a rapid decrease in flavour before *Leuconostoc* sp. reached maximum population.

There have been no studies on the consequences of added bacteriocin-producing LAB on meat flavour meat but there have been a number of studies which have determined the effect of the addition of lactic cultures to commercially contaminated raw meats and their impact on flavour (Ockerman and Cahill, 1977; Reddy et al., 1970, 1975; Borch and Agerhem, 1992). Ockerman and Cahill (1977) reported that the flavour scores of meats inoculated with *Leuconostoc* spp. were lower after 14 days of refrigerated storage than the uninoculated samples. Borch and Agerhem (1992) also reported lower flavour scores for meat inoculated with a species of *Leuconostoc*. However, in other studies (Reddy et al. 1970, 1975) flavour scores of ground meat inoculated with LAB were higher, indicating that the meat was more desirable than the uninoculated samples during extended chill storage. Results of our present study indicate a significant deterioration in the flavour scores in the inoculated samples after 21 days of vacuum storage at which time the LAB had reached maximum levels. The deterioration in the flavour is represented by the rancid and overall flavour intensity scores.

Although our study indicates there is no effect on the flavour of cooked ground beef inoculated with *L. gelidum*, there was a significant effect on the odour of the raw ground beef. These results suggest that there is no relationship between the odour of the raw meat and flavour intensity of the cooked meat. In this study the meat had a low odour intensity score, the flavour may still be undesirable, as indicated by the higher overall flavour intensity scores. These results are unlike other studies (Smith et al., 1980; Borch and Agerhem, 1992) in which it was reported that the odour deteriorates at the same rate as the flavour of meat.

Further study on the addition of *L. gelidum* to ground beef is required because an initial level of log 5 *L. gelidum* per gram is high relative to the indigenous LAB and

this could contribute to the higher overall flavour intensity of the ground beef after 21 days of vacuum storage. Studies should be designed to examine the effect of lower inoculation levels of *L. gelidum* on the flavour and odour intensity as well as the antagonistic effect on the indigenous microbial populations in the ground beef. A lower level of inoculum may result in beneficial effects on odour and flavour of the coarse ground beef. Further studies should also determine the effects of *L. gelidum* in different meat systems that may not have the same deteriorating extrinsic factors as those associated with ground beef. It would be beneficial to study the effects of *L. gelidum* at a lower temperature than 4°C because, even at 4°C, the microbial populations in ground beef grow rapidly. A lower storage temperature would slow the growth of the spoilage populations in ground beef and may accentuate the sensory implications of adding *L. gelidum* .

4.5. BIBLIOGRAPHY

AMSA. 1995. Guidelines for cookery and sensory evaluation of meat. *In* American Meat Science Association, pp. 1-16.

Blickstad, E., and G. Molin. 1983. Carbon dioxide as a controller of the spoilage flora of pork, with special reference to temperature and sodium chloride. J. Food Prot. 46:756-763.

Borch, E., and H. Agerhem. 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.

Cross, H.R., R. Moen, and M.S. Stanfield. 1978. Training and testing of judges for sensory analysis of meat quality. Food Technol. 32(7):48-54.

Dainty, R.H. 1981. Volatile fatty acids detected in vacuum-packed beef during storage at chill temperatures, pp. 688-690. In Proceedings of 27th Meeting of European Meat Workers, Vienna.

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

Dainty, R.H., B.G. Shaw, and T.A. Roberts. 1983. Microbial and chemical changes in chill-stored red meats, pp. 151-178. *In* T.A. Roberts and F.A. Skinner (ed.), Food Microbiology: Advances and Prospects. Academic Press, Toronto.

Egan, A.F. 1983. Lactic acid bacteria of meat and meat products. Antonie van Leeuwenhoek 49:327-336.

Egan, A.F., B.J. Shay, and P.J. Rogers. 1989. Factors affecting the production of hydrogen sulphide by *Lactobacillus sake* L13 growing on vacuum-packaged beef. J. Appl. Bacteriol. 67:255-262.

Gill, C.O., and C.L. Harrison. 1989. The storage life of chilled pork packaged under carbon dioxide. Meat Sci. 26:313-324.

Gill, C.O., and K.G. Newton. 1982. The effect of lactic acid concentration on the growth on meat of gram-negative psychrotrophs from a meatworks. Appl. Environ. Microbiol. 43:284:288.

Hanna, M.O., J.W. Savell, G.C. Smith, D.E. Purser, F.A. Gardner, and C. Vanderzant. 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 M.E. Stiles. 1991. Antibiosis of *Leuconostoc gelidum* isolated from meat. J. Appl. Bacteriol. 70:127-134.

Hitchener, B. M., A.F. Egan, and P.J. Rogers. 1982. Characteristics of lactic acid bacteria isolated form vacuum- packaged beef. J. Appl. Bacteriol. 52:31-37.

Kitchell, A.G., and B.G. Shaw. 1975. Lactic acid bacteria in fresh and cured meat, pp. 209-220. *In* Lactic Acid Bacteria in Food (ed). J.G. Carr, C.V. Cutting, and G.C. Whitting. London: Academic Press.

Klaenhammer, T.R. 1988. Bacteriocins of lactic acid bacteria. Biochemie 70:337-349.

Leisner, J.J., G.G. Greer, B.D. Dilts, and M.E. Stiles. 1995. Effect of growth of selected lactic acid bacteria on storage life of beef stored under vacuum and in air. Int. J. Food Microbiol. 26:231-243.

Leisner, J.J., G.G. Greer, and M.E. Stiles. 1996. Control of spoilage of beef by a sulfide-producing *Lactobacillus sake* with bacteriocinogenic *Leuconostoc gelidum* UAL187 during anaerobic storage at 2°C. Appl. Environ. Microbiol. 62:2610-2614.

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

Newton, K.G., and C.O. Gill. 1978. The development of the anaerobic spoilage flora of meat stored at chill temperatures. J. Appl. Bacteriol. 44:91-95.

Newton, K.G., J.C.L. Harrison, and K.M. Smith. 1977. The effect of storage in various gaseous atmospheres on the microflora of lamb chops held at -1°C. J. Appl. Bacteriol. 43:53-59.

Ockerman, H.W., and V.R. Cahill. 1977. Microbiological growth and pH effects on bovine tissue inoculated with *Pseudomonas putrefaciens, Bacillus subtilis* or *Leuconostoc mesenteroides*. J. Food Sci. 42:141-145.

Reddy, S.G., R.L. Henrickson, and H.C. Olson. 1970. The influence of lactic cultures on ground beef quality. J. Food Sci. 35:787-791.

Reddy, S.G., M.L. Chen, and P.J. Patel. 1975. Influence of lactic cultures on the biochemical, bacterial and organoleptic changes in beef. J. Food Sci. 40:314-318.

SAS Institute. 1989. SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC.

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

Shay, B.J., and A.F. Egan. 1981. Hydrogen sulfide production and spoilage of vacuum-packaged beef by a *Lactobacillus*, pp. 241-251. *In* T.A. Roberts, G. Hobbs, J.H.B. Christian and N. Skovgaard (ed.), Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Academic Press, Toronto.

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

Steel, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach, Second Edition. McGraw-Hill Book Co., Toronto.

Sutherland, J.P., P.A. Gibbs, J.T. Patterson, and J.G. Murray. 1976. Biochemical changes in vacuum packaged beef occurring during storage at 0-2°C. J. Food Technol. 11:171-180.

Tagg, J.R., A.S. Dajani, and L.W. Wannamaker. 1976. Bacteriocins of gram-positive bacteria. Bacteriol. Rev. 40:722-756.

5. General Conclusions

Of all of the fresh meat products being produced in the 1990's, even with the advancement in processing technology and knowledge of the beef production, ground beef is still one of the most highly contaminated products. The bacterial load of ground beef sampled at the retail level will be in the order of 10^6 to 10^7 CFU/g, and these numbers have remained unchanged for the past eight decades (Foster et al., 1977; Gill and McGinnis, 1993). Consequently, ground beef is still particularly susceptible to rapid spoilage and deterioration.

Traditionally, ground beef is prepared utilizing commercially produced vacuum packaged trimmings that are vacuum packaged by the processor and blended with trimmings that are collected at the retail level. Recent reports of this process (Gill and McGinnis, 1993) found large numbers of spoilage bacteria and unacceptable levels of coliforms, including *E. coli*. They attributed these results to: poor quality trimmings and retail temperature abuse. The grinding of meat causes disruption of the meat tissue resulting in the release and redistribution of nutrient rich juices which make the product more favourable to bacterial growth. It is a combination of these factors mentioned that results in ground beef with a retail case life that rarely exceeds 1 day.

An alternative to the traditional method of ground beef preparation is centralized preparation of vacuum packaged ground beef. This is an increasing trend in meat production and it has been shown ground beef of superior microbiological quality can be prepared (Shoup and Oblinger, 1976). However, to achieve the desired shelf-life for shipment to distant markets requires effective control of processing hygiene. Even though there are inherent advantages of centralized production of vacuum packaged meats, the shelf-life is often limited and somewhat unpredictable.

The shelf-life of vacuum packaged chilled meats is ultimately limited due to the spoilage caused by the prevailing microflora that dominate under these conditions. The lactic acid bacteria (LAB) are the bacteria which prevail under anaerobic conditions and

they are considered to be of low spoilage potential. Spoilage of VP meats usually occurs sometime after the maximum LAB population is reached. LAB dominate meats because of their ability to grow under anaerobic conditions as well as the inhibitory substances that they produce. LAB are a mixed population and because of this the shelf-life of VP meats is difficult to predict. It is known that high populations of the sulphide-producing *Lactobacillus sake* can reduce the shelf-life of anaerobically packaged meats to less than three weeks (Shay and Egan, 1981).

A novel means of controlling the spoilage of VP chill stored meats that may allow a predictable storage life is to use a competitive strain of LAB to the meat. Competitive LAB that are being suggested and investigated for the control of spoilage are the bacteriocin-producing LAB. As a result, the overall objective of this study was first to evaluate the hygienic efficiency of the a commercial process for ground beef production, transport and storage; second to evaluate the ability of the bacteriocinogenic LAB to control spoilage; and third, to assess the sensorial consequences of added bacteriocin-producing LAB during extended storage.

In the initial study, the centralized production of coarse ground beef at a large meat packing plant was monitored by bacteriological evaluation and temperature assessment. The temperature of the boxed "chub" packs was monitored with a temperature data logger during commercial shipping. The objective assessment of temperature histories was made with real time/temperature data logger which have been effectively used to evaluate commercial production processes in the past (Gill et al., 1991; Gill and Jones, 1992). Bacterial evaluation of the production process showed that the total bacterial load of the trim through the final product remained constant at about 10^3 bacteria per gram. During storage and shipping the temperature averaged +0.5 °C with a range form -1.0 to 4.5°C. This was reflected by no increase in bacterial load in the coarse ground beef during the three days of shipping and storage. The predominant bacterial population of the meat during vacuum storage at 4°C and retail

display was the LAB. This is expected because LAB are known to prevail during refrigerated vacuum storage. The study concluded that centrally produced ground beef could be stored in vacuum for up to 20 days with a resulting retail display case life of 2.5 days. This would be more than sufficient time for storage and distribution from a centralized production operation.

As mentioned earlier, VP meats eventually spoil as a result of the end-products produced by the adventitious LAB microflora that dominate on meats. Because of the mixed population of LAB the storage life of VP meat is unpredictable and in some instances it can be shortened considerably depending on the type of LAB that dominates. A novel means to extend the storage life of meats by controlling the growth of the adventitious LAB microflora would be by inoculating a competitive strain of LAB. LAB produce inhibitory substances that contribute to their dominance, and one of these inhibitory substances that could play an important role in the dominance of a bacterial population is bacteriocin-producing LAB. Focus of bacteriocin-producing LAB research has been on the control of foodborne pathogens with little attention to their ability to control spoilage and the possible sensory consequences of adding these bacteria to foods. The focus of our laboratory has been to study the bacteriocin production by LAB isolated from meats (Ahn and Stiles, 1990; Hastings and Stiles; 1991).

There is increasing interest in the study of bacteriocinogenic LAB for use as biopreservatives in VP meats to further extend the shelf-life of these meats by controlling the growth of the adventitious microflora. *Leuconostoc gelidum* UAL187 was found to be a potential candidate because it had all of the criteria established for a bacteriocin-producing LAB to be used as a biopreservative (Leisner et al., 1995) and it also had the ability to control spoilage of an by and added spoilage strain. This was attributed to its bacteriocin production (Leisner et al. 1996). The present study was designed to determine the ability of *L. gelidum* UAL187 to control the growth and

spoilage of the adventitious spoilage microflora at 4°C for intervals of up to 35 days of extended vacuum storage. After extended storage the levels of the adventitious microflora in the vacuum packaged samples inoculated with either the Bac^+ or Bac^- strain were 1 to 2 log lower than the uninoculated samples. Addition of *L. gelidum* to ground beef extended the retail case life by 2 days even after storage of VP meat for up to 5 weeks. This finding was independent of bacteriocin production. A surprising result was that the red colour of inoculated meats samples was significantly ($P<0.05$) better and more stable compared with uninoculated samples. This is very exciting and could have a major impact on biopreservation potential because the red meat colour is one of the single most important determining factors for retail acceptance (Renerre, 1990).

The single most important determining factor for a LAB strain to be used as a biopreservative in meats is that it must not contribute any undesirable sensory attributes. The objective of the final study was to determine the sensory consequences of the bacteriocin-producing LAB based on objective measurements of flavour and odour made by a trained panel. There have been no studies of this nature reported in the literature. Results from this study again showed that the addition of *L. gelidum* had a beneficial effect on the odour based on intensity and acceptability scores. Results for flavour evaluation indicated that the overall flavour intensity and development of the sour off-flavour notes of ground beef inoculated with *L. gelidum* UAL187 or 187-13 deteriorated at the same rate as the flavour of the uninoculated control samples.

Research on the biopreservation by *L. gelidum* UAL187 and 187-13 showed that the LAB inhibit the growth of the spoilage organisms, improve the odour and colour stability and had little effect on flavour. However, the research showed that the bacteriocin of *L. gelidum* UAL187 had no effect on the adventitious microflora. Research on the centralized production of ground beef indicated the potential for the extension of ground beef storage life and that the addition of a bacteriocin-producing

LAB culture to control the growth and extend the shelf-life was successful. However, the study of the application of these competitive strains as biopreservatives in meats and the role of bacteriocins still requires further investigation.

5.2. BIBLIOGRAPHY

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

Foster, J.F., J.L. Fowler, and W.C. Ladiges. 1977. A bacteriological survey of raw ground beef. J. Food Prot. 40:790-794.

Gill, C.O, S.D.M. Jones, and A.K.W. Tong. 1991. Application of a temperature function integration technique to assess the hygienic adequacy of a process for spray-chilling beef carcasses. J. Food Prot. 54:731-736.

Gill, C.O., and T. Jones. 1992. Assessment of the hygienic efficiencies of two commercial processes for cooling pig carcasses. Food Microbiol. 9:335-343.

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

Leisner, J.J., G.G. Greer, B.D. Dilts, and M.E. Stiles. 1995. Effect of growth of selected lactic acid bacteria on storage life of beef stored under vacuum and in air. Int. J. Food Microbiol. 26:231-243.

Leisner, J.J., G.G. Greer, and M.E. Stiles. 1996. Control of spoilage of beef by a sulfide-producing *Lactobacillus sake* with bacteriocinogenic *Leuconostoc gelidum* UAL187 during anaerobic storage at 2°C. Appl. Environ. Microbiol. 62:2610-2614.

Renerre, M. 1990. Review: factors involved in the discoloration of beef meat. Int. J. Food Sci. Technol. 25:613-630.

Shay, B.J., and A.F. Egan. 1981. Hydrogen sulfide production and spoilage of vacuum-packaged beef by a *Lactobacillus*, pp. 241-251. *In* T.A. Roberts, G. Hobbs, J.H.B. Christian and N. Skovgaard (ed.), Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Academic Press, Toronto.

Shoup, J.B., and J.L. Oblinger. 1976. Microbiological evaluation of retail ground beef: centralized and traditional preparation. J. Milk Food Technol. 39:179-183.