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Extended Storage Life of Retail Cuts of Pork

by 

Lynn Marie McMullen

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN

PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

IN

Food Microbiology

Department of Food Science

Edmonton, Alberta

Spring 1994

ISBN 0-612-11294-2

Canada

UNIVERSITY OF ALBERTA

RELEASE FORM

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TITLE OF THESIS: Extended Storage of Retail Cuts of Pork

DEGREE: Doctor of Philosophy

YEAR THIS DEGREE GRANTED: 1994

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Permission is hereby granted to Lynn M. McMullen to use the joint publications with me as part of her thesis "Extended Storage Life of Retail Cuts of Pork", including:

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"Microbial Ecology of Fresh Pork [illegible] Modified Atmosphere at -1, 4.4 and 10°C" McMullen [illegible] *Journal of Food Microbiology* 18: 1-14.

"Quality of Fresh Retail Pork [illegible] Atmosphere Under Temperature Conditions Simulating [illegible] Markets" submitted to Meat Science, 19[illegible]

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DEDICATION

Dedicated to the memory of my father,

L.H. McMullen, Ph.D.

ABSTRACT

Application of modified atmosphere packaging for retail marketing of pork cuts was studied. Experiments were designed to determine: (1) effects of storage conditions on keeping quality and the prevailing microflora on the meat cuts; (2) the potential to access distant markets with retail-ready cuts using this technology; and (3) the effect of inoculation of retail cuts with selected lactic acid bacteria (LAB) on keeping quality and the use of headspace gas analysis to monitor spoilage.

To examine the effects of storage conditions pork loin cuts prepared with two levels of initial bacterial load were packaged in three films of different gas transmission in an atmosphere containing 40% CO_2/60% N_2 and stored at -1, 4.4 and 10°C. Temperature was the overriding factor influencing storage life. Spoilage at each storage temperature could be attributed to the growth of different groups of bacteria and was influenced by package type. Storage life of pork cuts in packages with low oxygen transmission rates was 5 or 8 weeks at 4.4 or -1°C, respectively. Listeriae were detected as part of the prevalent microflora on samples stored at -1°C, but not on samples stored at 4.4 or 10°C. A total of 162 (30%) of LAB isolated from the meat samples produced inhibitory substances against a range of indicator strains.

Samples for studies to simulate storage conditions to access distant markets with retail-ready cuts of pork were packaged in 100% CO_2 in plastic film with extremely low gas transmission and stored at -1.5°C for three weeks. Reference samples were held at -1.5°C for the duration of the study. After transfer of samples to 4 and 7°C, samples remained acceptable for retail sale for 2 and 1 weeks, respectively. Appearance of the cuts was the main factor limiting storage life; however, confinement odour became a potential problem for consumer acceptance of the product with extended storage.

Studies of inoculated retail-ready cuts of pork packaged in 100% CO_2 and stored at 4°C revealed that the type of bacteriocinogenic LAB affected the storage life of the meat. Sulphur odours were detected on meats inoculated with *Carnobacterium piscicola* LV17 or *Leuconostoc gelidum* UAL187 but not with *Lactobacillus sake* Lb706. Detection of sulphur compounds in the headspace gas at the time that the sensory panel detected off-odours, indicated that monitoring of these compounds may be useful as an objective measure of spoilage.

The studies demonstrated that there is good potential to apply modified atmosphere packaging technology to retail cuts of pork. With adequate temperature control, storage life can be extended for weeks beyond what is possible with aerobic packaging. Assessment of the spoilage potential of selected strains of LAB is imperative before they can be exploited as biopreservatives for achieving a predictable storage life of retail-ready products.

ACKNOWLEDGMENTS

I would like to express my appreciation to my supervisor, Dr. M.E. Stiles, for sharing his knowledge and experience, and for his advice and patient assistance during my graduate studies. I am especially thankful to him for his sincere interest in me both as an individual and as a professional. I am grateful that he had the courage and persistence to turn a sensory scientist into a food microbiologist.

I would like to thank the other members of my supervisory committee, especially Dr. G.G. Greer, for their advice and support.

For her guidance, patience and friendship during the course of my graduate studies, I would like to acknowledge Dr. Lynn Elmes. I would like to extend my sincere gratitude to Mira Fenton, Department of Animal Science, for her time and assistance with the GC/MS. Special thanks are extended to Randy Worobo, Mirella Cerrone, Alison Poon and Hanna Vaheri for their technical assistance and friendship. The time and assistance of the members of the sensory panels is gratefully acknowledged.

A special thanks goes to Mr. R. Yeung for his assistance at Gainers Ltd., Edmonton. I would also like to thank the Food Processing Development Centre of Alberta Agriculture for allowing me access to their facilities.

I would also like to acknowledge the never-ending support of my friends and family, especially my mother, Iris McMullen. Without their love and support this endeavor would not have been possible.

Financial assistance for this research was provided by the Alberta Agriculture Research Institute matching funds research program in association with Gainers Ltd., Edmonton and the Natural Sciences and Engineering Research Council. The donations of packaging film from Condor Laminations, Ontario, Packall Packaging, Ontario and Walki-Pak, Finland are gratefully acknowledged.

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1. Literature Review

1.1. INTRODUCTION

Vacuum and modified atmosphere packaging has long been applied and has enjoyed tremendous success for the retail marketing of sliced processed meats. In the past 30 years, the development of new meat packaging systems has revolutionized the fresh meat industry. The traditional movement of carcasses between the producer and the retail outlet has gradually been replaced by the distribution of vacuum packaged primal (wholesale) cuts to the retailer for further cutting and repackaging for retail sale. Fresh meat is a highly perishable food product with a storage life of only 4 to 5 days when packaged aerobically for sale at the retail level. It has been estimated that over 55% of the economic loss due to meat spoilage occurs in the retail marketplace (Breidenstein, 1986). In recent years, researchers have examined alternate means of packaging meats for retail sale to extend storage life and decrease economic loss. The most attractive means for extending the storage life of fresh retail meats is with centralized prepackaging using modified atmospheres. In addition to decreasing the economic losses due to spoilage, centralized prepackaging of fresh meats offers many other benefits for the meat processor.

Implementation of a centralized prepackaging system has the potential to increase efficiency in the fresh meat industry through better utilization of resources (labour, raw materials and packaging). Centralized prepackaging of fresh meat for the domestic retail market leads to an opportunity for brand name products. In addition, such a system would decrease the amount of product handling as product moves through the food chain with the associated benefit of reduced cross-contamination with pathogens. With the extended storage life that can be achieved by packaging in a modified atmosphere there is the potential for Canadian pork manufacturers to access distant markets with fresh, nonfrozen

product. With the application of modified atmosphere packaging, meat producers will be able to demand a higher return for their fresh product.

Controlled atmosphere packaging is being applied in Europe for marketing of fresh meats to the consumer. According to Lioutas (1988) 42% of all fresh meat for retail sale in Denmark is packaged in controlled atmospheres. The packaging system used relies on an atmosphere with a high O_2 concentration and gives meat a storage life of 8 to 10 days. This is sufficient marketing time for product in Europe where distribution systems are geographically small. However, for success in the North American market a storage life of 21 days is the minimum which would be accepted by the meat industry for retail marketing (Lioutas, 1988). Anaerobic modified atmosphere packaging will extend the storage life of fresh meat for weeks. However, anaerobic modified atmosphere packaging has not been applied to fresh red meats for retail sale mainly because the purplish red colour of meat held under anaerobic conditions has not been accepted by consumers. Pork is a relatively pale meat and may be suited for packaging in anaerobic atmospheres.

Modified atmosphere packaging (MAP) has been defined as "the enclosure of food products in high gas-barrier materials, in which the gaseous environment has been changed to slow respiration rates, reduce microbiological growth, and retard enzymatic spoilage - with the intent of extending shelf life" (Young et al., 1988). This broad definition includes vacuum packaging where the storage atmosphere is a vacuum, as well as gas flush packaging systems where the atmosphere may include CO_2 singly or in combination with O_2 and/or N_2. For this review, a limited definition of MAP that refers only to gas flushed product will be used because this is the basis of the experimental work of this thesis. The storage life of fresh meat packaged in MA is influenced by a number of factors, including: storage temperature, packaging film, gas atmosphere, and initial meat quality in terms of both microbial load and pH. Effects of each of these factors on the quality of primal cuts of

meat have been reported but researchers have not examined the combined effects of these factors on the storage life of MAP retail cuts of pork.

When chilled, fresh meat is stored in an atmosphere with elevated levels of CO_2 a microflora develops in which lactic acid bacteria prevail. Lactic acid bacteria (LAB) could be exploited as biopreservatives in fresh meat stored in MAP because they have the potential to control the growth of the adventitious LAB microflora that may contain strains of LAB that can cause spoilage. Before the application of LAB as biopreservatives for fresh meat can be practiced by the meat industry, suitable strains must be examined for their potential to prevent spoilage in MAP fresh meat.

Currently, the only means of determining the storage life of a MAP fresh meat is by examining the sensory quality of the meat. Not only are sensory panels very time consuming but their application in the meat industry for evaluation of storage life of meat is impractical. Researchers have been searching for an objective means of monitoring meat spoilage but no suitable chemical analysis is sufficiently correlated with sensory data to give a reliable measure of spoilage. Development of a rapid objective measure of spoilage would be of value for use by the meat industry.

This study was designed to determine the storage life of retail cuts of fresh pork under different conditions of storage, examining closely the composition of the adventitious microflora and the spoilage potential of selected strains of LAB. In addition, the use of headspace volatile analysis was examined as part of the search for an objective measure of spoilage. A number of reviews on the technical aspects of modified atmosphere packaging are available in the literature, thus this review will focus on the microbiology of anaerobically packaged fresh meat, examining the effects of lactic acid bacteria on meat spoilage, and the biopreservation of chilled, fresh meat.

1.2. MEAT MICROBIOLOGY DURING ANAEROBIC STORAGE

At the time of slaughter, muscle tissue of healthy animals is virtually sterile. The level and incidence of bacteria on the surface of fresh meat depends on the level of hygiene maintained during slaughter and processing. However, the development of a particular microflora during storage is mainly controlled by the conditions of storage (Dainty et al., 1983). When fresh meat is stored at chill temperatures under aerobic conditions spoilage occurs as a result of the growth of a putrefactive aerobic microflora consisting mainly of *Pseudomonas* sp. (Ingram, 1962; Gill and Newton, 1977; Dainty and Mackey, 1992). When fresh meat is stored in an atmosphere with elevated levels of CO_2, the growth of pseudomonads and other Gram-negative, putrefactive aerobes is inhibited and a microflora develops in which lactic acid bacteria prevail (Enfors et al., 1979). It is the change from the aerobic, putrefactive microflora to the lactic microflora that is responsible for the extension of storage life of fresh meat.

The term "lactic acid bacteria" (LAB) refers to a group of Gram-positive, nonsporeforming, microaerophilic bacteria that produce lactic acid as the main product of carbohydrate metabolism (Kandler, 1983). The genera associated with the LAB include: *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Carnobacterium, Streptococcus* and *Enterococcus* spp. Of these genera, the *Lactococcus* spp. are the only ones that are not commonly associated with meats (Holzapfel, 1992). Other genera that are closely associated with the LAB are *Bifidobacterium, Aerococcus* and *Brochothrix* spp. For the purposes of this review, *Bifidobacterium* spp. will not be considered because they are not true lactics and they are generally associated with human intestinal microflora and with dairy foods (Kandler, 1983). *Aerococcus* spp. are excluded because their application in foods is limited (Holzapfel, 1992). *B. thermosphacta* is an important aerobic spoilage organism of meat. The main product of its carbohydrate metabolism is lactic acid. *B. thermosphacta* is classified among the *Clostridium-Lactobacillus-Bacillus* branch in

Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986). *B. thermosphacta* is associated with lactic acid bacteria because the main product of glucose metabolism is lactate; however, it differs from other lactic acid bacteria because it produces catalase.

Traditionally, LAB were considered to be nonpathogenic organisms. Nonpathogenicity was an important criterion because they make a beneficial contribution to the preservation and quality of many foods (Blickstad, 1983). However, *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus* and *Enterococcus* spp. have all been associated with clinical infections (Aguirre and Collins, 1993). In most cases, an underlying disease, antibiotic therapy or immunosupression was associated with the clinical infection. There is an increasing volume of evidence that LAB are opportunistic pathogens; however, whether these organisms will one day be considered opportunistic foodborne pathogens remains to be seen. According to Aguirre and Collins (1993) the enterococci represent the greatest risk for human infection. The association of enterococci with modified atmosphere packaged meats is unclear. The lack of reports on their isolation from vacuum and modified atmosphere packaged fresh meats suggests that they do not grow on these products; however, enterococci and carnobacteria have very similar phenotypic characteristics and few studies have attempted to distinguish between the two groups (Enfors et al, 1979; Blickstad and Molin, 1983; Borch and Molin, 1988).

Lactic acid bacteria on meats can be viewed as having a preservative effect or as spoilage agents. When stored at chill temperatures in anaerobic modified atmospheres with elevated levels of CO_2 fresh meat undergoes a slow "fermentation" process that eventually results in souring of the meat due to the production of organic acids by LAB. However, some LAB are potent spoilage agents of meat stored in anaerobic environments. Some *Lactobacillus* spp. produce sulphur compounds when they grow on meats stored in

anaerobic environments (Hitchener et al., 1982; Egan et al., 1989) and dramatically reduce storage life (see section 1.3).

Studies of the LAB of chilled, fresh meat stored under vacuum or in MAP revealed the prevalence of homo- and heterofermentative lactics, mainly lactobacilli, carnobacteria or leuconostocs (Hitchener et al., 1982; Shaw and Harding, 1984; Schillinger and Lücke, 1987a,b; Borch and Molin, 1988). Traditionally, the LAB found on chilled, stored fresh meats were classified as "streptobacteria" or homofermentative lactobacilli, betabacteria or heterofermentative lactobacilli and leuconostocs according to the classification of Orla-Jensen (1919). The terms homo- and heterofermentative divide LAB into two groups based on the endproducts of carbohydrate metabolism. With an unlimited carbohydrate supply, homofermentative lactics ferment hexose sugars via the glycolytic pathway to produce lactic acid as the sole endproduct of metabolism. Under the same conditions, heterofermentative lactics ferment hexose sugars via the 6-phosphogluconate pathway to produce equimolar amounts of CO_2, lactate and acetate or ethanol. The ratio of acetate to ethanol depends on the oxidation potential of the system. Under anaerobic conditions, the production of ethanol is favoured (Blickstad and Molin, 1984).

Few attempts to define the species of LAB growing on chill stored, fresh meat were reported until Sutherland et al. (1975b) found 24 LAB in a group of 105 organisms isolated from vacuum packaged beef. They classified 11 of the LAB as lactobacilli and the other 13 as "lactic cocci". Enfors et al. (1979) reported that 85% of the LAB isolated from pork loins stored in 100% CO_2 for 21 days at 4°C were *Lactobacillus plantarum*, a homofermentative LAB, while the remaining LAB were heterofermentative lactobacilli. After 35 days of storage the proportion of heterofermentative lactobacilli had increased to 55% (Enfors et al., 1979). A change in the type of LAB that prevail in the microbial population of vacuum packaged beef was also reported by Vanderzant et al. (1982). They found that the initial prevailing microflora was a combination of homo- and

heterofermentative lactobacilli but after storage for 12 or 24 days at 2°C, a single heterofermentative species, *Lactobacillus cellobiosus*, prevailed. Leuconostocs also prevail in the microbial flora of MAP fresh meat (Savell et al., 1981; Schillinger and Lücke, 1987a; Nortjé and Shaw, 1989; Jackson et al., 1992c). However, homofermentative species have also been found to be the major group of LAB on MAP fresh meat (Blickstad and Molin, 1983; Schillinger and Lücke, 1987a). In a numerical taxonomic study of 94 strains of LAB isolated from a variety of refrigerated, prepacked meat and meat products, Borch and Molin (1988) found that the majority of the strains (60%) were homofermentative lactobacilli.

Hitchener et al. (1982) reported that the majority of isolates from vacuum packaged beef stored for 9 weeks at 0 - 1°C were lactobacilli. However, none could be identified to the species level and were considered to be atypical "streptobacteria" or "betabacteria". A taxonomic study of the LAB from vacuum packaged beef, pork and lamb by Shaw and Harding (1984) indicated the prevalence of "atypical streptobacteria" that could be divided into aciduric and nonaciduric lactobacilli. They tentatively identified the aciduric strains as *Lactobacillus sake* or *Lactobacillus bavaricus* (Shaw and Harding, 1984). The nonaciduric strains were subsequently divided into two species, *Lactobacillus divergens* (Holzapfel and Gerber, 1983) and *Lactobacillus carnis* (Shaw and Harding, 1985) and reclassified by Collins et al. (1987) as *Carnobacterium divergens* and *Carnobacterium piscicola*, respectively.

The types of LAB that prevail in the microbial population of fresh meat stored for extended periods could have a significant effect on the storage life of the product. However, no consensus is apparent from the literature as to which group of LAB prevail on MAP fresh meat stored for extended periods. Differences in the composition of the initial microbial population could account for differences during storage. In addition, the media used for selection of isolates could play a significant role in the types of LAB isolated (see Chapter 3).

Brochothrix thermosphacta is a Gram-positive, facultative anaerobe that can grow on meats stored under vacuum and modified atmosphere conditions and can be responsible for the spoilage of packaged meats. Under strict anaerobic conditions the low pH of meat, the presence of lactate, the presence of a competitive LAB microflora and low temperature storage interact to inhibit the growth of *B. thermopshacta* (Roth and Clark, 1975; Grau, 1980). However, if anaerobic conditions are not maintained, for example with the use of films which allow oxygen to permeate into the package, *B. thermosphacta* will grow and spoil meat (Grau, 1980; Blickstad and Molin, 1983). Under aerobic conditions, *B. thermosphacta* metabolizes glucose to yield acetic acid and acetoin, which is oxidized to diacetyl and causes the "dairy" odours of meat. The production of isovaleric and isobutyric acids from leucine and valine also contributes to the spoilage that this organism causes in aerobically stored fresh meats (Dainty and Hibbard, 1980). Under anaerobic conditions, *B. thermosphacta* produces mainly lactic acid from carbohydrates (Gardner, 1980).

Enterobacteriaceae have also been isolated from MAP fresh meats. Enterobacteriaceae are facultatively anaerobic organisms that include a number of potentially pathogenic bacteria that can be found on raw meats. Enterobacteriaceae are found as part of the prevalent microflora on high pH meat stored at chill temperatures under vacuum (Patterson and Gibbs, 1977; Erichsen and Molin, 1981); however, storing high pH meat in 100% CO_2 suppresses the growth of these organisms (Erichsen and Molin, 1981; Rousset and Renerre, 1991). Temperature control is the key to controlling the growth of these organisms on meat with a normal pH because most of these organisms do not grow at temperatures below 5 to 7°C. However, some Enterobacteriaceae such as *Enterobacter*, *Hafnia* and *Serratia* spp. and *Yersinia enterocolitica* grow at temperatures below 5°C and they have been isolated from vacuum and modified atmosphere packaged meats (Hanna et al., 1976; Patterson and Gibbs, 1977; Vanderzant et al., 1982; Manu-Tawiah et al., 1993). Psycrothrophic Enterobacteriaceae have been detected on MAP meats stored below 2°C for extended periods of time (Newton et al., 1977; Blickstad and Molin,

1983; Gill and Harrison, 1989). However, it is of interest to note that in all of these cases, although Enterobacteriaceae were present initially, they were not detected during the early stages of storage but became part of the prevalent microflora after extended periods of time. This phenomenon was also noted by Patterson and Gibbs (1977) on vacuum packaged beef with a high pH that was stored below 2°C. The control of growth of Enterobacteriaceae is imperative for extending the storage life and ensuring the safety of MAP fresh meats. These organisms produce malodorous compounds and spoil meat rapidly under conditions that allow them to grow.

The isolation of *Clostridium* sp. from anaerobically packaged beef is unusual but there have been isolated reports of vacuum packaged beef spoiling due to the growth of a *Clostridium* sp. (Dainty et al., 1989; Kalchayanand et al., 1989). In both cases, the *Clostridium* sp. isolated was capable of growing below 0°C and was responsible for the development of offensive odours and severely bloated packages. Clostridia generally do not grow well in the presence of a competitive microflora (Hauschild, 1989); however, when Dainty et al. (1989) isolated the clostridia from vacuum packaged beef, a LAB microflora was prevalent. This was confirmed by Kalchayanand et al. (1989) who reported that the LAB had reached 10^6 CFU/ml in the purge of vacuum packaged beef spoiled by *Clostridium* sp. The *Clostridium* sp. isolated by Kalchayanand et al. (1989) had different biochemical characteristics to other clostridia and was tentatively classified as *C. laramie* (Kalchayanand et al., 1993). This strain was nontoxigenic to mice, proteolytic and able to grow at temperatures as low as -3°C in tryptic soy broth supplemented with 0.1% haemin and 0.001% vitamin K_1. Inoculation studies using 1 ml of purge from spoiled vacuum packaged beef as the inoculum on aseptically prepared beef, indicated that spoilage occurred within 1 to 2 weeks (Kalchayanand et al., 1989). It is not known if a LAB microflora developed on the inoculated, vacuum packaged meats. The lack of a competitive microflora would likely enhance the growth of the *Clostridium* sp. Some strains of *Clostridium botulinum* can grow in meat under anaerobic conditions at 25°C (Hauschild et

al., 1985); however, the potential hazard for refrigerated fresh meat is small as the incidence of botulinal spores is low and spoilage precedes toxigenesis (Hauschild, 1989).

1.3. MODIFIED ATMOSPHERE STORAGE OF FRESH MEAT

The role of modified atmosphere and vacuum packaging in extending the storage life of fresh meat has become of major importance to the meat industry. Vacuum and MAP of primal cuts of fresh meat is currently used for transportation of fresh meat to the retail market. Storage life is influenced by a number of interrelated factors including both intrinsic properties of the meat as a substrate for microbial growth and extrinsic factors relating to storage conditions, for example the storage atmosphere and temperature. Failure to control these factors will result in detrimental changes in the colour, odour, flavour and(or) texture of meat, all of which are considered to be manifestations of spoilage. The role of intrinsic and extrinsic factors in extending storage life of fresh meats with MAP has been extensively reviewed by Gill and Molin (1991), Lambert et al. (1991) and Stiles (1992).

Spoilage of fresh meat is mainly a result of microbial activity on the meat surface. An exception to this is the change in meat colour, which in some cases can be attributed to microbial activity, but is also a manifestation of change in the oxidation state of myoglobin that is brought about by variations in the availability of oxygen. In addition, some deterioration of meat occurs in the absence of microorganisms (Egan and Shay, 1982; Jackson et al., 1992c). This implies that the metabolic processes of the meat tissue may contribute to spoilage, but this aspect of meat spoilage under anaerobic conditions has yet to be thoroughly defined.

1.3.1. Meat as a Substrate for Microbial Growth

Meat is highly susceptible to microbial spoilage. It has a high water activity (a_w = 0.99), a favourable pH (5.6-5.8) and has virtually all of the nutrients, growth factors and minerals required for optimum microbial growth (Hammes et al., 1990). The hygienic condition of fresh meat at the time of packaging markedly affects its storage life. High initial microbial counts decrease the time for spoilage to occur (Sutherland et al., 1975a; Christopher et al., 1979).

Bacterial spoilage of meat is thought to be a result of the breakdown of the low molecular weight soluble components listed in Table 1.1 (Dainty et al., 1975; Gill and Newton, 1978). Proteolysis is not a factor in the spoilage of meat and is considered to be a postspoilage phenomenon (Dainty, 1982; Greer, 1989). According to Thomas and Pritchard (1987) LAB do not secrete significant amounts of proteinases or peptidases and the enzymes involved in degradation of extracellular proteins are bound to the bacterial cell wall. The highly structured organization of muscle proteins may limit proteolysis (Law and Kolstad, 1983).

According to Gill (1985) glucose and arginine are the only substrates in meat that are utilized for growth by lactobacilli. However, reports on the formation of H_2S by lactic acid bacteria on meats stored under anaerobic conditions indicate that the catabolism of amino acids other than arginine may play a role in anaerobic spoilage. A large number of *Lactobacillus* spp. and some carnobacteria (Schillinger and Lücke, 1987b; Egan et al., 1989; Leisner, 1992) are capable of producing H_2S.

Meat pH and its interrelationship with glycogen levels in fresh meat plays a significant role in the spoilage of MAP meats, influencing the types of microorganisms that proliferate which in turn influences the rate at which spoilage occurs. Muscle with a high final pH, described as dark firm and dry (DFD), has little or no carbohydrate to act as an

Table 1.1. Concentration of the soluble, low molecular weight components of post-rigor beef.[1]

Substance	Concentration (mg/g)
Creatine	6.5
Inosine monophosphate	3.0
Glycogen	1.0
Glucose	0.1
Glucose-6-phosphate	0.2
Lactic acid	9.0
Amino acids	3.5
Dipeptides	3.0

[1] Adapted from Gill (1985)

energy source for bacterial growth and under aerobic conditions, amino acid catabolism results in the early onset of spoilage (Newton and Gill, 1978). When DFD meat is stored under vacuum, *B. thermosphacta* and Gram-negative organisms including pseudomonads and Enterobacteriaceae are detected in high numbers on the meat (Patterson and Gibbs, 1977; Erichsen and Molin, 1981; Shay and Egan, 1986; Rousset and Renerre, 1991). However, packaging DFD meat in an atmosphere of 100% CO_2 controls the growth of these microorganisms and a lactic microflora prevails (Erichsen and Molin, 1981; Gill and Penney, 1986; Rousset and Renerre, 1991). The storage life of high pH beef stored under CO_2 in oxygen impermeable films is comparable to that of normal pH beef stored under the same conditions (Gill and Penney, 1986). Reports on the storage life in either vacuum or MAP of meat with pale, soft and exudative (PSE) characteristics are lacking. Under aerobic storage conditions, the storage life of PSE pork is limited because of deterioration of appearance (Greer and Murray, 1988). Gill and Harrison (1989) in their study on MAP pork, noted that initially pale pork loses colour during storage. However, their study was not specifically designed to examine the storage life of PSE meat.

1.3.2. Storage Atmosphere

The success of MAP for extending the storage life of fresh meats relies on the bacteriostatic effect of CO_2 (Coyne, 1933; Clark and Lentz, 1969, 1972; Ledward et al., 1971; Gill and Tan, 1980). Carbon dioxide inhibits the growth of the putrefactive Gram-negative organisms (Sutherland et al., 1977; Enfors et al., 1979). The mechanism of bacterial inhibition by CO_2 is not clear. Several theories have been proposed but none has been conclusively proven. The theories of inhibition of bacterial growth by CO_2 were reviewed in detail by Daniels et al. (1985). Initially it was thought that the displacement of O_2 was responsible for inhibition. However, this was discounted by the fact that N_2 atmospheres do not have the same effect as CO_2 (Pierson et al., 1970; Huffman, 1974; Huffman et al., 1975; Seideman et al., 1979b). It has been suggested that the penetration

of CO_2 through the cell wall, the subsequent alteration in intracellular pH due to the formation of carbonic acid and interference with intracellular enzyme activity could play a role (Wolfe, 1980). Carbon dioxide inhibits production of extracellular lipase by *Pseudomonas fluorescens* (Rowe, 1988). Inhibition of decarboxylating enzymes by CO_2 has been suggested as the mode of action (King and Nagel, 1975). The disruption of cell membranes and membrane function by dissolution of CO_2 has also been considered to be responsible for the inhibitory effect of CO_2 (Enfors and Molin, 1981). This theory is supported by the work of Tan and Gill (1982), who postulated that inhibition of substrate uptake may be responsible for growth inhibition. To date there is no conclusive evidence of a single mode of action of CO_2. It is likely that CO_2 has multiple effects on bacterial cells and that the mode of action could be species dependent. Although LAB are resistant to the bacteriostatic effects of CO_2, it increases the lag phase and reduces the growth rate of these organisms (Sutherland et al., 1977; Blickstad and Molin, 1984).

The minimum concentration of CO_2 required to inhibit the growth of Gram-negative organisms is 20% (Gill and Tan, 1980). Both O_2 and N_2 have been used as the balance of gas in MAP for fresh meat. Modified atmospheres containing high levels of oxygen are used to retain the cherry-red colour of oxygenated fresh meat; however, storage life compared with packaging under anoxic conditions is compromised due to the growth of *B. thermosphacta* and *Pseudomonas* spp. (Christopher et al., 1979; Seideman et al., 1979a; Nortjé and Shaw, 1989; Jackson et al., 1992c). With high-O_2 atmospheres, there is concern that oxidative rancidity could reduce storage life. Lamb stored in high-O_2 atmospheres becomes rancid after 3 weeks (Newton et al., 1977). However, oxidative rancidity is not a problem when pork (Asensio et al., 1988; Taylor et al., 1990) and beef (Nortjé and Shaw, 1989; Taylor et al., 1990) are stored in high-O_2 atmospheres.

From a microbiological point of view, atmospheres of 100% CO_2 are being recommended for the storage life of fresh meats. According to Blickstad and Molin (1984), the presence of O_2 in a modified atmosphere for fresh meat storage reduces storage life due to fast microbial growth rates and the production of microbial metabolites that are not detected under anaerobic conditions. When comparing the storage life of pork chops stored at 2°C in different gas atmospheres, Spahl et al. (1981) found that an atmosphere of 30% CO_2/70% N_2 was as effective in extending storage life as 100% CO_2.

Controlling the amount of residual O_2 in a "100% CO_2" pack is important for colour stability of meat. At low partial pressures of O_2 the formation of metmyoglobin is favoured resulting in browning of the meat surface. Myoglobin does not oxidize to metmyoglobin at oxygen concentrations below 600 ppm (Penney and Bell, 1993). According to Gill and Molin (1991) residual O_2 in excess of 0.1% will cause the formation of metmyoglobin. In a recent study on the effects of residual O_2 levels in MAP on colour of different fresh meats after removal from the pack, Penney and Bell (1993) found that pork muscle tolerates 1% O_2 in the pack without the formation of metmyoglobin when the meat is exposed to air, while beef and lamb develop metmyoglobin on exposure to O_2 when more than 0.15% O_2 is present in the modified atmosphere.

The storage atmosphere maintained within a package is directly related to the oxygen transmission rate of the film used, which is inversely related to storage life. Egan and Shay (1982) found little difference in shelf life of vacuum packaged meat stored in films with O_2 transmission rates (OTR) of 1 and 25 ml/m^2/24 h, but storage life was reduced when a film with an OTR of 1000 ml/m^2/24 h was used.

1.3.3. Storage Temperature

Control of storage temperature is critical for the extension of storage life with modified atmospheres. As storage temperature increases, the duration of the lag phase decreases and the growth rate increases, thereby reducing the time for spoilage to occur (Gill, 1985). In addition, the efficacy of CO_2 inhibition of organisms from fresh meats is inversely related to storage temperature (Gill and Tan, 1980). The underlying principle that could be responsible for the temperature dependence of the efficacy of CO_2 is the greater solubility of CO_2 in muscle tissue at low temperatures (Gill, 1988). Several researchers have examined the effect of temperature on the storage life of MAP fresh meat, all with the same conclusion that increases in storage temperature decrease the storage life.

Storage temperature also affects the composition of the microflora that grows on MAP meats. As storage temperature increases, the number of organisms that can grow increases. The most dramatic effect is seen with the growth of Enterobacteriaceae on meat at high storage temperatures. Increasing the storage temperature of vacuum packaged beef to 5°C, which is not an unrealistic temperature in the retail counter, increases the chance that Enterobacteriaceae will grow (Beebe et al., 1976). When meat is stored anaerobically at ambient temperatures, which would be considered gross temperature abuse for fresh meat, *Clostridium* spp. can grow and spoilage is rapid (Hauschild et al., 1985).

1.4. LACTIC ACID BACTERIA AS SPOILAGE AGENTS IN MAP MEATS

Lactic acid bacteria are generally viewed as preservative agents on MAP meats. However, with time they can cause spoilage of fresh meat packaged in MA. The metabolism of meat constituents results in the production of a number of compounds that influence the sensory quality of MAP fresh meat stored for extended periods of time. It

was suggested that the measurement of metabolic by-products may give a better measure of food quality than microbial numbers (Sharpe, 1979). This is especially true with MAP fresh meat where spoilage occurs after maximum bacterial numbers have been reached.

A limited number of studies have investigated the chemistry of spoilage of anaerobically packaged fresh meats. According to Gill and Newton (1978) bacteria grow on the surface of meat utilizing the low molecular weight components. The use of substrates available in meat for growth of a single *Lactobacillus* sp. was determined by Gill (1976). The growth of a *Lactobacillus* sp. in beef juice medium or on the surface of sterile lamb loin samples was limited by the availability of glucose and no change in lactic acid or ammonia concentrations was detected (Gill, 1976). At the time that maximum cell number was reached on the surface of intact muscle, glucose at the muscle surface was depleted and the concentration of arginine had decreased.

In a study of the chemical changes in beef inoculated with either a homofermentative *Lactobacillus* sp. or a *Leuconostoc* sp. and stored at 4°C in an atmosphere of 95% N_2 and 5% CO_2, Borch and Agerhem (1992) found that the growth of these organisms caused a decrease in muscle glucose concentration. These researchers found that D-lactate concentrations increased when meat was inoculated with either of these organisms, and a decrease in L-lactate was only detected when beef was inoculated with *Lactobacillus* sp. (Borch and Agerhem, 1992). Increases in the D-lactate concentration that corresponded to high numbers of LAB on vacuum packaged and MAP pork were also reported by De Pablo et al. (1989) and Ordóñez et al. (1991), respectively. Nassos et al. (1983) correlated an increase in lactate concentration with spoilage of refrigerated ground beef stored in casings with low oxygen permeability. Determination of D-lactate concentration may be useful as an objective measure of spoilage; however, more research correlating D-lactate concentration with sensory changes in meats is required.

The metabolic by-products of glucose metabolism by lactic acid bacteria vary depending on the type of LAB and the availability of oxygen and glucose. Under anaerobic conditions with unlimited glucose, homofermentative lactic acid bacteria produce lactate as the sole end-product of glucose metabolism. However, under conditions of limiting carbohydrate, as occurs on the surface of meat when maximum bacterial population has been reached, some homofermentative lactics may become heterofermentative, producing ethanol, acetate, formate and CO_2 (Condon, 1987; Borch et al., 1991). It is not clear if glucose is the sole carbon source for the production of the additional metabolites. Glucose is not the sole carbon source for the production of acetate and lactate by *Carnobacterium divergens* (de Bruyn et al., 1988).

In a study of the growth of a homo- and a heterofermentative *Lactobacillus* spp. in batch culture with glucose as the carbon source, Blickstad and Molin (1984) found that under anaerobic conditions (100% CO_2) lactic acid and ethanol were the only metabolites detected, whereas under aerobic conditions acetic acid and small amounts of H_2O_2 and acetoin were also produced. Under anaerobic conditions, leuconostocs ferment glucose to produce lactate, ethanol and CO_2; whereas under aerobic conditions, acetate is produced in place of ethanol (Condon, 1987).

The souring of vacuum packaged meat has been attributed to the production of acetate (Sutherland et al., 1976). In batch cultures under anaerobic conditions, acetate is not detected as a metabolite of LAB but ethanol is produced (Borch et al., 1991). However, the growth of a *Leuconostoc* sp. on anaerobically stored meat resulted in the production of ethanol whereas a *Lactobacillus* sp. produced acetate (Borch and Agerhem, 1992). No attempt was made by the researchers to monitor the gas atmosphere within the packages during storage, thus it is not clear if anaerobic conditions were maintained in the packages throughout storage. When meat is held under strict anaerobic conditions the production of acetate is not expected, unless carbon sources other than glucose are being

utilized for acetate production. Some homofermentative lactics produce acetate from pyruvate via the formate-lyase pathway under anaerobic and glucose-limiting conditions (Condon, 1987). The role of this pathway in the metabolism of LAB from meats needs further investigation. The utilization of ribose as a carbon source has been documented for *B. thermosphacta* growing on meat (Grau, 1988), but it has not been reported for *Lactobacillus, Carnobacterium, Pediococcus or Leuconostoc* spp. growing on meat.

The low glucose concentration of meat favours the catabolism of amino acids to form pyruvate. The sensory consequences of the formation of pyruvate from amino acids can be significant as the breakdown involves the production of ammonia and, in the case of cysteine, H_2S. The presence of sulphur odours in meat when a lactic microflora is present has been reported by a number of researchers (Shay and Egan, 1981; Hanna et al., 1983; Edwards and Dainty, 1987; Schillinger and Lücke, 1987a). Shay and Egan (1981) isolated a strain of *Lact. sake* from vacuum packaged meat that produces sulphur compounds from cysteine. They indicated that the only sulphur compound detected in the headspace volatiles was H_2S (Shay and Egan, 1981). The production of H_2S is dependent on the maintenance of anaerobic and glucose-limiting conditions (Egan et al., 1989). They found that over time their strain lost its ability to produce large amounts of H_2S and it was subsequently discovered that the active uptake of cysteine may be a plasmid-mediated trait (Shay et al., 1988).

The sensory significance of deamination of amino acids in MAP stored meats appears to be small because ammonia-like odours have not been reported for these products. Ammonia concentrations increase only slightly in ground beef of normal pH stored in 100% CO_2 (Nychas and Arkoudelos, 1990). Free amino acids could also be dissimilated through transamination, decarboxylation and reduction reactions to form branched chain aldehydes and alcohols. A number of branch chain aldehydes and alcohols have been detected in the headspace of aerobically and anaerobically stored meat (Dainty

and Mackey, 1992). Amino acids could also be the carbon source for acetate production under anaerobic conditions. Deamination of alanine, cysteine and serine result in the formation of pyruvate which could be converted to acetate.

Another property of LAB that may have significance in spoilage of fresh meats is the decarboxylation of amino acids to form biogenic amines. The only biogenic amines that have been shown to increase in concentration during storage of vacuum packaged meats are putrescine, cadaverine and tyramine (Edwards et al., 1987). The production of tyramine is associated with the growth of *Carnobacterium divergens* and *C. piscicola*, and it is not associated with the growth of the *Leuconostoc* sp. (Edwards et al., 1987). The significance of tyramine production on the storage life of vacuum or MAP fresh meats has not been tested. However, due its lack of distinct sensory properties, Dainty and Mackey (1992) suggested that it does not play a role in the spoilage of fresh red meats. The formation of putrescine and cadaverine have been associated with the growth of Enterobacteriaceae (Dainty et al., 1986; Edwards et al., 1987).

It is apparent that the metabolites produced by LAB growing on MAP stored fresh meat are numerous. Possibilities exist for the use of chemical detection of metabolites as a means of measuring spoilage. The detection of D-lactate may be useful in this regard; however, more research is necessary to correlate D-lactate concentration with spoilage. Not all LAB produce acetate when growing on chill stored fresh meat which limits the usefulness of acetate detection as a measure of spoilage with an adventitious LAB microflora. Acetate detection has potential for use as a research tool in the detection of spoilage; however, as with D-lactate concentrations, more research is needed. The detection of sulphur compounds may also serve as a useful measure of spoilage; however, not all LAB produce sulphur compounds and their absence may cause misleading assumptions.

Currently, the only reliable method of detecting spoilage of chill stored MAP meats is by sensory analysis. As this is expensive, time consuming and impractical in the field, the search continues for a metabolite or group of metabolites that may be used as an objective measure of spoilage. The detection of volatile compounds in the headspace of MAP meats may prove to be an efficient measure of spoilage.

Very little research has been reported on the detection of volatile compounds produced by LAB growing on meat stored in anaerobic environments. Edwards and Dainty (1987) were the first to examine the volatiles in the headspace of vacuum packaged normal and high pH fresh pork. After storage at 5°C, lactic acid bacteria were the prevalent organisms found on normal pH pork, with Enterobacteriaceae and *B. thermosphacta* each comprising approximately 1% of the total population. After 20 days of storage small amounts of hydrogen sulphide, methanethiol, methylthioacetate, dimethyldisulphide and 3-methylbutanol were detected. The odour of the normal pH meat was described as slightly sour. In contrast, the high pH pork had a prevalent microflora comprised of Gram-negative organisms (tentatively identified as *Shewanella putrefaciens*) and *B. thermosphacta*. In addition to the compounds detected in the headspace of normal pH meat, a number of additional sulphur compounds and four esters of short-chain fatty acids was detected in the headspace of the high pH meat. After storage, the odour of the high pH pork was described as sulphury, obnoxious or faecal. The authors (Edwards and Dainty, 1987) tentatively attributed the production of sulphur compounds in the headspace of normal pH meat to the growth of lactic acid bacteria. However, the greater quantities of sulphur compounds detected in the headspace of high pH meat were attributed to the growth of Enterobacteriaceae.

In a comparison of the volatile compounds produced in the headspace of beef packaged under vacuum, 100% CO_2, 40% CO_2/60% N_2 or 20% CO_2/80% O_2 with an adventitious microflora, Jackson et al. (1992c) found that a large number of the volatile

compounds present in the headspace gases originated from the packaging film. The strongest off-odours, described as cheesy and rancid, were detected in the headspace of samples stored in the high-O_2 atmosphere. At the time that off-odours were detected from beef stored in high-O_2 MA the presence of 1-hexene, 3-hexene, methyl thiirane, ethyl acetate, benzene and 1-heptene were the compounds found in the headspace and the microflora was comprised of *Leuconostoc* and *Pseudomonas* spp. (Jackson et al., 1992c). Of the volatile compounds detected, methyl thiirane, ethyl acetate and 1-heptene were associated with the high-O_2 atmosphere. The only other sulphur compound detected was dimethyl sulphide and it was detected throughout storage in the headspace gas of all samples. Samples stored under vacuum developed a moderate off-odour after 14 days of storage whereas samples stored in 100% CO_2 or 40% CO_2/60% N_2 developed a moderate off-odour after 21 days storage. The volatile compounds detected in the headspace of vacuum packaged beef after 14 days of storage at 3°C were identical to those detected in the 100% CO_2 atmosphere but a greater number of volatile compounds was detected in the headspace of the 40% CO_2/60% N_2 package (Jackson et al., 1992c). However, differences in the prevalent microflora were evident, with *Lactobacillus* sp. prevailing on samples stored under vacuum and 40% CO_2/60% N_2, and *Leuconostoc* sp. prevailing on samples packaged in 100% CO_2.

The growth of pure cultures of LAB on sterile or aseptically prepared meat and the subsequent effects on sensory quality have been used to define the role of individual strains of LAB in the spoilage of anaerobically stored fresh meat. In a study comparing the sensory quality of vacuum packaged beef steaks inoculated with 4 different strains of *Lactobacillus* sp. with the quality of uninoculated steaks, Smith et al. (1980) reported that inoculation resulted in a higher incidence of off-odour, discoloration and poorer flavour ratings than when uninoculated steaks were tested. Based on numerical differences in the data, the authors concluded that inoculation with the cultures used in their study would not enhance storage life; however, statistical analyses of their data indicated that very few of the

differences between inoculated and uninoculated steaks were significant, thus their conclusion may not be valid. In a subsequent study using 9 strains of *Lactobacillus* and *Leuconostoc* spp. inoculated on aseptically prepared meat, these researchers (Hanna et al., 1983) again based their conclusion on numerical differences that inoculated steaks had greater off-odours than uninoculated control samples, although there were few significant differences between the off-odours of the inoculated and the control steaks. In fact, significant differences in off-odour between the controls and steaks inoculated with *Lactobacillus coryneformis* were noted. However, there were differences in the description of the odours produced by the different species of LAB. Off-odours were generally described as sour, buttermilk, sulphur-like or H_2S. The sulphur odours were detected on steaks inoculated with *Lactobacillus viridescens, Lact. coryneformis, Leuc. dextranicum* and *Leuc. paramesenteroides.*

Spoilage of vacuum packaged beef by pure cultures inoculated onto meat was also investigated (Egan and Shay, 1982). They inoculated beef slices with a strain of *Leuconostoc* and 2 homofermentative strains of *Lactobacillus* and found that the growth of the *Leuconostoc* caused an off-odour to develop, whereas this was not a problem with meat inoculated with the homofermentative *Lactobacillus* spp. However, when the rate of spoilage was measured by the development of off-flavour, the different strains of LAB had similar rates of spoilage, all causing sour, bitter and liver off-flavours (Egan and Shay, 1982). In contrast, in a study of beef stored in an anaerobic atmosphere, Borch and Agerhem (1992) found that inoculation with a *Leuconostoc* sp. caused more rapid flavour changes than inoculation with homofermentative *Lactobacillus* sp.

In an effort to determine the impact of LAB on the volatile compounds in the headspace of MAP fresh meat, Jackson et al. (1992a) inoculated sterile slices of beef with *Lact. plantarum* and *Leuc. mesenteroides* subsp. *mesenteroides*. In a companion paper, Jackson et al. (1992b) determined the volatile compounds present in the headspace of

sterile pork inoculated with *Lact. plantarum* and *Lactobacillus fermentum*. The volatiles detected in the headspace of inoculated and sterile samples after storage at 3°C for 21 days in glass bottles are listed in Table 1.2. Most of the volatile compounds were detected throughout storage, with the exception of the C_7 hydrocarbon and trichloromethane in the headspace of the beef samples. The detection of these volatile compounds in the headspace of the sterile control samples, coupled with the fact that sterile samples eventually soured, indicate that microbial metabolism may not be the only source of volatile compounds. The source of the volatiles from sterile meat remains to be discovered. According to Dainty and Mackey (1992) the possibility of microbial involvement in the spoilage of "sterile" meat exists even though no causative organisms can be detected.

Many questions remain unanswered about the spoilage of chill stored, MAP fresh meats. The spoilage of sterile meat stored for extended periods of time needs further investigation. Spoilage in this case may be due to unknown intrinsic metabolic processes in meat. The contribution of LAB to spoilage also requires further research. The spoilage potential of LAB such as *Carnobacterium* sp. and *Leuconostoc gelidum* remains to be examined in MAP meats.

1.5. LACTIC ACID BACTERIA AS BIOPRESERVATIVES IN MAP MEATS

It is well established that the prevalent microflora on MAP fresh meats is composed primarily of lactic acid bacteria. The lactic acid bacteria have a number of important characteristics that allow them to compete successfully in anaerobic environments and become the prevalent organisms. Lactic acid bacteria grow rapidly at chill storage temperatures, they are tolerant of the antimicrobial effects of CO_2 and they produce a number of antimicrobial substances. These antimicrobial substances include, organic acids (acetate, formate and lactate), diacetyl (formed from the oxidation of acetate under aerobic

Table 1.2. Volatile compounds detected in the headspace of sterile beef loin inoculated with *Lact. plantarum* or *Leuc. mesenteroides* subsp. *mesenteroides* and in the headspace of sterile pork loin inoculated with *Lact. plantarum* or *Lact. fermentum* stored for 21 days at 3°C[1].

Compound	Compounds detected in Beef	Compounds detected in Pork
acetic acid	+	
acetone	+	+
benzene	+	+
cyclohexane		+
dichloroethane	+	
ethyl acetate	+	
C_5 hydrocarbons		+
C_6 hydrocarbons		+
C_7 hydrocarbon	+	
trichloromethane	+	+
toluene	+	+

[1] Adapted from Jackson et al. (1992a,b).

conditions), hydrogen peroxide (aerobic conditions) and bacteriocins. Of these antimicrobial metabolites, the production of organic acids and bacteriocins would be of significance for biopreservation of fresh meats stored under anaerobic MAP conditions.

1.5.1. Organic Acids

The use of lactic acid bacteria to produce organic acids *in situ* for preservation of meats is a well known and extensively exploited property of these organisms. The production of organic acids in fermented sausages is critical to the safety of these products and they are important for development of desirable sensory properties. The application of organic acids for decontamination of carcasses has been reviewed (Dickson and Anderson, 1992). The topical application of sodium lactate significantly reduced the growth of the adventitious microflora of vacuum packaged beef (Rozbeh et al., 1993). However, in this study the beef samples were exposed to CO to stabilize meat colour, so the effect of the topical application of sodium lactate on meat colour could not be evaluated.

It is well documented that a number of Gram-negative spoilage organisms are sensitive to the antimicrobial effects of lactate and acetate (Ray and Sandine, 1993). However, only a limited number of reports concerning the production of organic acids in MAP fresh meats and their role in controlling the growth of meat spoilage organisms has been published.

Evidence for the inhibition of *B. thermosphacta* growth by lactate was provided by Grau (1980) who found that this organism could grow anaerobically at pH 5.5 in the absence of lactate but that in the presence of lactate growth was inhibited. In a study on the effects of lactate, pH and anaerobiosis on the growth of Enterobacteriaceae on beef, Grau (1981) found that the inhibition of these organisms was dependent on the maintenance of anaerobic conditions, a low pH (5.4-5.6) and the presence of lactate. The role of acetate in controlling the growth of spoilage organisms on MAP meat has not been clearly defined.

1.5.2. Bacteriocins

Bacteriocins are a heterogeneous group of antibacterial proteins produced by a variety of Gram-positive and Gram-negative bacteria (Tagg et al., 1976). Bacteriocins normally inhibit the growth of closely related organisms and may play a role in the dominance of lactic acid bacteria in vacuum and MA packaged meats (Klaenhammer, 1988; Hastings and Stiles, 1991). A number of lactic acid bacteria isolated from meat and meat products have been identified as bacteriocin producers (Table 1.3). However, few researchers have examined the incidence of bacteriocin-producing lactic acid bacteria on modified atmosphere packaged meats throughout storage, and their role in the prevalence of lactic acid bacteria on chill stored meats is unclear.

In the past 10 years there has been an exponential growth in the number of published reports on the production of bacteriocins by lactic acid bacteria and the potential of bacteriocin-producing LAB for use as biopreservatives. The emphasis on bacteriocins as biopreservatives has been concentrated on the control of foodborne pathogens, especially *Listeria monocytogenes*. Bacteriocin-producing lactic acid bacteria inhibit the growth of *L. monocytogenes* in vacuum-packaged wieners (Berry et al., 1991; Degnan et al., 1992), ground beef and a sausage mix (Motlagh et al., 1992), dry fermented sausages (Foegeding et al., 1992) and model gravy systems (Winkowski and Montville, 1992).

Some bacteriocinogenic strains of LAB isolated from meats inhibit *Clostridium botulinum* spores (Okereke and Montville, 1991) and have the potential for use as either a co-preservative with or replacement for nitrite in processed meat products. However, in a recent study on the inhibition of *C. botulinum* growth in a model gravy system by *Lactococcus lactis* (a nisin producer) and *Pediococcus pentosaceus*, Crandall and Montville(1993) concluded that it was the production of lactic acid and not bacteriocin production that was responsible for the inhibition that they observed.

Table 1.3. Some bacteriocin-producing lactic acid bacteria isolated from meat and their associated bacteriocins.

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)
Leuconostoc		
gelidum	Leucocin A	Hastings and Stiles (1991)
carnosum	Carnosin	van Laack et al. (1992)
paramesenteroides	Leuconocin S	Lewus et al. (1992)
Pediococcus		
acidilactici	Pediocin PA-1/AcH	Gonzalez and Kunka (1987); Ray et al. (1989)

There is a lack of information on the use of bacteriocin-producing LAB as biopreservatives to control spoilage in meat products. To date, LAB used in pure culture experiments to determine their effect on spoilage of anaerobically stored fresh meats have not been chosen for their ability to produce bacteriocins, although they may in fact be bacteriocin producers. There are only three reports in the literature on the use of bacteriocin-producing LAB in meat products that include any evaluation of the sensory quality of the product (Raccach et al., 1979; Schillinger et al., 1991; Leisner et al., 1993). However, in the reports by Raccach et al. (1979) and Schillinger et al. (1991) the sensory analysis was cursory and results were not presented. Leisner et al. (1993) reported that inoculation of sterile beef with strains of *C. piscicola*, *Leuc. gelidum* or *Lact. sake* did not cause deterioration of appearance or odour. If a bacteriocin-producing LAB inhibits the growth of "all" foodborne pathogens and its bacteriocin was proven to be safe for human consumption, but the sensory consequences of growth of this hypothetical LAB on meat are offensive, then its application in food systems would not be acceptable. The control of spoilage of MAP foods by bacteriocin-producing LAB along with experiments designed to determine the spoilage potential of these organisms requires more attention by researchers promoting the use of bacteriocin-producing LAB as biopreservatives.

1.6. CONCLUSION AND OBJECTIVES OF RESEARCH

The application of MAP for centralized prepackaging and marketing of retail-ready pork cuts would be a highly desirable, value-added process for the Alberta pork industry. The extended storage life achieved by packaging fresh pork in modified atmospheres would reduce economic losses due to spoilage in domestic markets and would allow processors to access distant markets with fresh, nonfrozen meat products. The success of MAP of retail cuts will ultimately depend on consumer acceptance. However, before successful marketing can be undertaken, a number of problems require attention. It has been established that the storage life of fresh meat packaged in modified atmospheres can be extended for weeks past the storage life which is currently achieved with aerobic packaging at the retail level. However, only limited research has been done on the storage life and microbiology of retail-ready pork cuts.

The prevalence of lactic acid bacteria on anaerobically packaged fresh meats is well established. Many questions remain about the exact composition of the mixed population of LAB that develops and their role in the eventual spoilage of MAP fresh meats. The spoilage of MAP meats by H_2S-producing LAB is a concern and the control of these LAB in the adventitious microflora is desirable. The use of bacteriocinogenic LAB as biopreservatives may significantly improve the quality and safety of MAP pork.

The objectives of this study were:

1. To evaluate the effects of storage temperature, gas permeability of packaging film, and initial microbial load on the changes in the gas atmosphere and storage life of MAP retail cuts of pork (Chapter 2).

2. To determine the nature of the prevailing microflora during storage of retail cuts of pork at different storage temperatures in two packaging films and examine the prevalent lactic acid bacteria for antimicrobial activity and bacteriocin production (Chapter 3).

3. To examine the effect of a change of storage temperature after MA storage at -1.5°C on the storage life of MA packaged retail cuts of pork and to describe and quantify confinement odour compared with the meat odour as possible barriers to consumer acceptance of MA packaged retail-ready cuts of pork (Chapter 4).

4. To evaluate the spoilage potential of three bacteriocin-producing lactic acid bacteria and to evaluate the potential of headspace gas analysis as a nondestructive and rapid method of evaluating spoilage (Chapter 5).

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2. Changes in Microbial Parameters and Gas Composition During Modified Atmosphere Storage of Fresh Pork Loin Cuts[1]

2.1. INTRODUCTION

Centralized prepackaging of meat in modified atmospheres using mixtures of carbon dioxide, oxygen, and nitrogen has been proposed as an effective method of packaging for retail sale. Modified atmosphere packaging (MAP) systems will not improve the quality of fresh meats; however, economic benefits, including the possibility of an extended storage life, could make MAP an alternative to current retail packaging (Young et al., 1988). Extension of storage life with MAP depends on several factors, including the initial microbial load of the meat and the storage atmosphere and temperature. Studies have indicated that the lower the initial microbial load, the longer the storage life (Van Garde et al., 1975; Christopher et al., 1979).

Packaging with elevated levels of CO_2 retards the growth of aerobic spoilage microorganisms, permitting lactic acid bacteria and other CO_2-resistant organisms to dominate the microbial population (Enfors et al., 1979; Blickstad and Molin, 1983). It is generally accepted that a minimum of 20% CO_2 is needed to extend the storage life of MAP fresh meats (Clark and Lentz, 1969; Wolfe, 1980). Christopher et al. (1980) found that the growth of the spoilage microflora of pork loins was effectively inhibited during storage in an atmosphere containing 40% CO_2. The sensory characteristics of retail cuts prepared from the stored loins were not affected by storage in 40% CO_2 (Hall et al., 1980). However, retail cuts of pork, beef, or lamb stored at 2°C up to 21 days in 20 or 40% CO_2 became severely discoloured (Seideman et al., 1980). The presence of 5 to 10% O_2 in the

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[1] A version of this chapter has been published. McMullen and Stiles, 1991. Journal of Food Protection 54:778-783.

retail packages may have promoted the development of metmyoglobin. An atmosphere of 100% CO_2 effectively extends the storage life of pork provided that O_2 is kept at very low concentrations throughout storage (Gill and Harrision, 1989). However, Spahl et al. (1981) found that a gas mixture of 30% CO_2/70% N_2 was as effective in extending storage life as 100% CO_2 when pork chops were stored at 2°C.

The minimum storage temperature for fresh meat without freezing is approximately -1.5°C. Modified atmosphere storage at temperatures close to -1°C markedly extends the storage life of fresh meat (Adams and Huffman, 1972; Carpenter et al., 1976; Simard et al., 1985; Gill and Harrison, 1989). Pork cuts stored in an atmosphere of 100% CO_2 at -1.5°C have a storage life of 12 weeks (Gill and Harrison, 1989). Absorption of CO_2 by meat, respiration of meat and bacteria (with the evolution of CO_2), and gas exchange through the package contribute to changes in the gas atmosphere during storage (Gardner et al., 1967; Daun et al., 1971). Reports on how the gas composition changes during storage in packs of MAP pork are limited and conflicting. Seideman et al. (1980) reported that the CO_2 concentration decreases during storage of MAP retail pork cuts at 2°C in gas mixtures of either 20 or 40% CO_2 with 5 to 10% O_2. However, Spahl et al. (1981) found that CO_2 levels increased during storage of MAP retail pork cuts.

This study examines the combined effects of storage temperature, gas permeability of packaging films, and microbial load on changes in the gas atmosphere and the storage life of MAP retail cuts of pork.

2.2. MATERIALS AND METHODS

2.2.1. Preparation of Meat Samples

Hogs from a selected producer were killed at a federally inspected packing plant on each of three successive days (replicates). Each replicate consisted of hogs from one producer. The carcasses were chilled to 0°C overnight before the *longissimus dorsi* muscles were excised and skinned of all fat. The pH of the excised lean muscles was determined by placing a glass electrode on the muscle surface. To avoid PSE (pale, soft, and exudative) meat, muscles with an appropriate physical appearance and a pH between 5.8 and 6.4 were selected. Each muscle was divided into two portions. At the packing plant, one portion was cut into six pieces approximately 1.5 cm thick (commercial product). The uncut portion was bagged separately, and all samples were held in a laboratory cooler at 4°C for packaging (not more than 6 h). In the laboratory the uncut half-loin was placed on a stainless steel tray, flamed with alcohol twice on each side, and aseptically cut into 6 x 1.5 cm portions with a sterile knife (aseptic product). The surface area of each cut was determined by aseptically tracing the outline on a sheet of aluminium foil and measuring the surface area with an Area Meter (model LI-3100, LiCor Inc., Lincoln, NB). The mean surface area of the pork loin cuts was 30.5 ± 5.4 cm^2. The meat cuts were placed on the bottom dish of a 150 x 15 cm sterile petri plate and packaged. Duplicate samples of commercial and aseptic product from each loin were randomly assigned to each of three package types.

2.2.2. Packaging and Storage

The packaging materials used were (1) 12.2 μm polyester/ 9μm foil/ 76 μm polyester laminate (Packall Packaging, Mississauga, Ontario, Canada; F1), (2) a 17.8 μm nylon/ 71.1 μm polyvinylchloride coextrusion laminated to 48 gauge metalized polyester (Condor Laminations, Progressive Packaging Ltd., Aurora, Ontario, Canada; F2), and (3)

a 50.8 μm nylon/ 76.2 μm polyvinylchloride coextrusion bag (Condor Laminations, Progressive Packaging Ltd.; P3) with the following manufacturer's reported oxygen transmission rates (OTR): 0.0, 12.6, and 26.5 ml/m^2/24 h at 23°C, 0% relative humidity under 1 atmosphere pressure. Packages (20 x 26 cm) were evacuated, flushed with a gas mixture of 40% CO_2 and 60% N_2, and heat-sealed using a Bizerba Packaging Machine (model D66; Bizerba Inc., Mississauga, Ontario, Canada). Gases were mixed with a Smith Proportional Gas Mixer (model 299-029; Smith Equipment, Watertown, SD). During preliminary work the volume of gas injected was *ca.* 500 ml which resulted in a gas/meat weight ratio of *ca.* 5 l/kg. Packaged samples were stored at -1, 4.4, and 10°C on shelves to ensure good air flow around the packages. Bags with the modified atmosphere, but without meat, were stored at each storage temperature and at 25°C. Sufficient samples were prepared for duplicate weekly microbial and gas analyses over the 10-week storage period. A spot of silicone adhesive sealant (Dow Corning Canada Inc., Mississauga, Ontario, Canada) applied to the exterior of each package served as a gas-sampling port.

2.2.3. Analyses

Gas samples were drawn with a 100 μl gas-tight syringe (Hamilton Co., Reno, NV) through the silicone sampling port. Gas composition (CO_2, O_2, and N_2) was determined by gas chromatography using a Varian Aerograph Chromatograph (model 90P, Varian Instrument Group, Palo Alto, CA) equipped with the 2-column system described by Smith et al. (1983). Helium at a flow rate of 40 ml/min was used as the carrier gas. Data were interpreted using a Hewlett-Packard integrator (model 3390A, Hewlett-Packard Canada Ltd., Mississauga, Ontario, Canada). When the packages were opened for microbial analyses, objectionable changes in the appearance and odour of the meat were recorded. The plastic carrier dish was removed from the package, 99 ml of sterile, 0.1% peptone water was added to the package, and the contents were blended for 1 min in a Colworth 400 Stomacher (Seward and Co., London, England). Serial dilutions were

prepared with 0.1% peptone water for determination of "total" aerobic colony forming units (CFU) and presumptive counts of lactic acid bacteria, coliform bacteria, total Enterobacteriaceae, pseudomonads, and *Brochothrix thermosphacta*. Total aerobic, lactic acid bacteria, pseudomonads, and *B. thermosphacta* counts were determined by placing 20 µl drops of appropriate dilutions onto prepoured plates of Plate Count Agar (PCA; Difco Laboratories, Detroit, MI), Lactobacilli MRS agar (MRS; Difco), cephaloridine, fusidic acid, cetrimide agar (CFC; Mead and Adams, 1977), and Gardner's medium (STAA; Gardner, 1966), respectively. Inoculated plates were incubated at 25°C for 48 h. MRS plates were incubated anaerobically in an atmosphere of 10% CO_2 and 90% N_2. To determine coliform bacteria and total Enterobacteriaceae counts 50 µl of appropriate dilutions were streaked onto prepoured plates of violet red bile agar (VRBA; BBL, Cockeysville, MD) and VRBA with 10% added glucose (VRBG), respectively, overlaid with approximately 5 ml of the same growth medium, and incubated at 35°C for 24 h. The pH of the blended meat slurry was determined using a Fisher Acumet pH meter (Fisher Scientific, Edmonton, Alberta, Canada).

2.2.4. Experimental

Three replicates of the study were done. Samples for each replicate were prepared on each of three consecutive days. For each replicate, initial (week 0) microbial load was determined on five meat samples taken at random. Packaged samples were taken from storage at weekly intervals and analyzed for microbial content. Gas analyses were determined on the same sample throughout the testing period. Bacterial counts (PCA, MRS, CFC, STAA) were converted to colony forming units (CFU)/cm^2 and geometric means were calculated. Microbial and pH data were statistically analyzed using ANOVA procedures. Where appropriate, means were ranked by Student Newman Keul's multiple range test (Steele and Torrie, 1980).

2.3. RESULTS

Preliminary work indicated the pH of the meat slurry to be within 0.1 pH unit of the pH on the meat surface. Means for the initial muscle pH of each replicate were 5.87 ± 0.19, 5.94 ± 0.17, and 6.03 ± 0.03 for replicates 1, 2, and 3, respectively. At all three storage temperatures, the mean pH of the samples decreased to *ca.* 5.5 during the first week of storage. No significant changes in pH occurred after this initial decrease, either with length or temperature of storage or with type of packaging.

Packages filled with 40% CO_2 and 60% N_2 without meat had similar amounts of gas exchange at -1, 4.4, and 10°C and slightly greater gas exchange at 25°C. At the lower storage temperatures, for example at 4.4°C, the decrease in CO_2 in F1 packs was marginal after 9 weeks storage; whereas in F2 packs, CO_2 decreased to 35%, and in P3 packs, to approximately 30%. Oxygen levels in the foil packs remained below 1% throughout storage, but in the plastic (P3) packs, O_2 was detected (2-3%) after 4 weeks and reached 4.5% after 9 weeks of storage.

Changes in the composition of headspace gases of meat samples stored at -1 and 4.4°C were similar. At -1 and 4.4°C there was little difference in the change in CO_2 during storage between the CP and AP samples. Figure 2.1 shows the changes in CO_2 of headspace gases of meat samples stored at 4.4°C. After a 3-5% drop in CO_2 during week 1 of storage, there was a greater loss of CO_2 in the P3 packs than in F1 or F2 over time. The percentage of O_2 detected in the F1 and F2 packs was less than 1% during 9 weeks of storage; in P3 packs O_2 levels increased from less than 1% during the first 4 weeks of storage to *ca.* 4% after 9 weeks of storage. Figure 2.2 shows the change in CO_2 of headspace gases of meat samples stored at 10°C. At 10°C package and sample type (AP vs CP) affected the change in the gas composition over time. After 2 weeks of storage at 10°C, the CO_2 content of F1 and F2 packages with CP samples was much higher than AP samples.

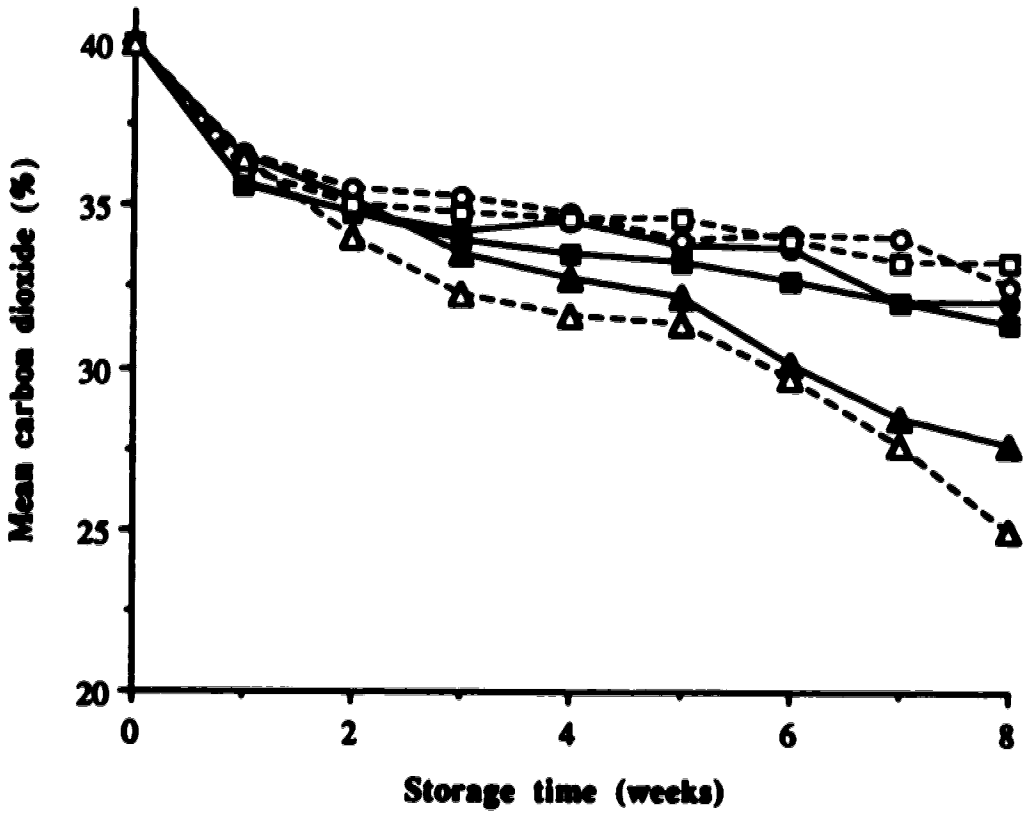

Figure 2.1. Changes in carbon dioxide concentration in head space gases of pork cuts stored in different packaging films at 4.4°C for 8 weeks.

●, ○ F1 foil laminate package 0.0 OTR;

■, □ F2 metalized polyester package 12.5 OTR;

▲, △ P3 plastic package 26.5 OTR.

Closed symbols = commercially prepared product.

Open symbols = aseptically prepared product.

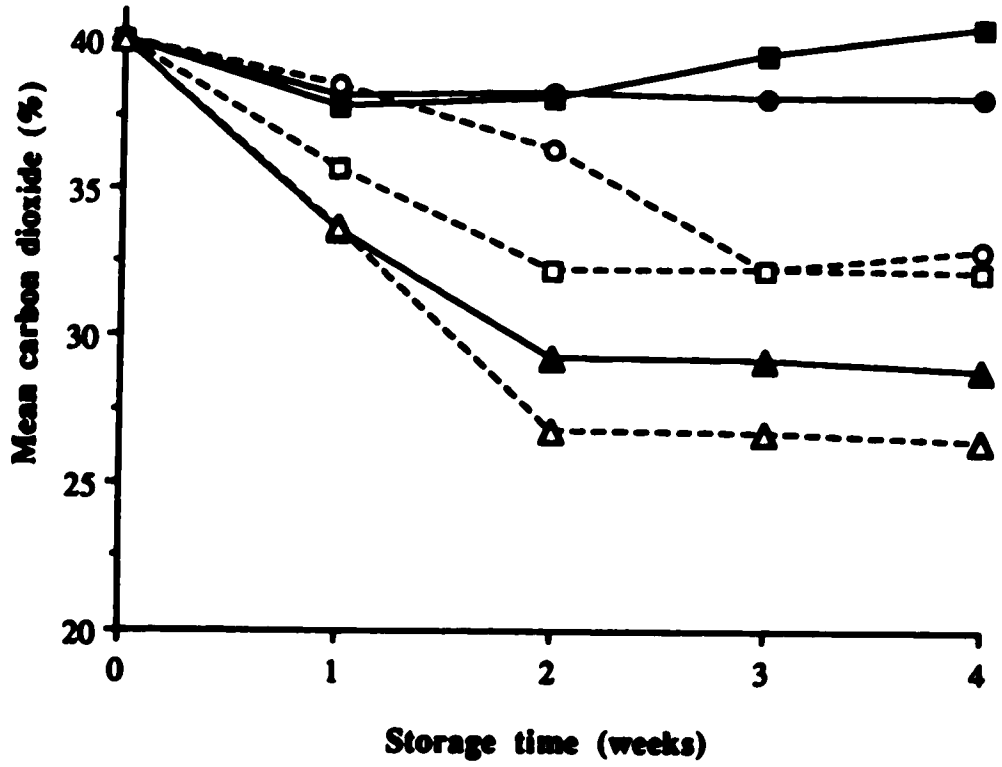

Figure 2.2. Changes in carbon dioxide concentration in head space gases of pork cuts stored in different packaging films at 10°C for 4 weeks.
●, ○ F1 foil laminate package 0.0 OTR;
■, □ F2 metalized polyester package 12.5 OTR;
▲, △ P3 plastic package 26.5 OTR.
Closed symbols = commercially prepared product.
Open symbols = aseptically prepared product.

The microbial quality of the meat samples was monitored at weekly intervals for 4 weeks and, for samples stored at -1 and 4.4°C, at two week intervals thereafter. Samples stored at 10°C became unacceptable after 2 to 3 weeks of storage and testing was discontinued after 4 weeks. Samples stored at 4.4 and -1°C remained acceptable for 5 and 8 weeks, respectively, and were tested up to 9 and 10 weeks of storage, respectively. Statistical analyses of microbial data for the pork samples indicated that storage time and temperature, package type, and initial microbial load (CP vs AP samples) were important factors affecting the microbial quality and hence the storage life of the meat samples. For a large proportion of the microbial data, there was a significant difference among replicates. The storage life of samples in replicate 2 was 1 to 2 weeks shorter than that of samples in replicates 1 and 3.

Table 2.1 lists initial mean counts for each microbial parameter for pork cuts prepared commercially and aseptically. There was a 1 to 2 log difference in total initial microbial load between meats prepared commercially and those prepared aseptically. This difference in microbial load was maintained throughout storage at -1 and 4.4°C, because microbial populations were only approaching a maximum at the end of the storage period. Samples stored at 10°C had a maximum bacterial population within 2 to 3 weeks and there was no difference between pork samples prepared commercially or aseptically.

Figure 2.3 shows the means for total aerobic and presumptive lactic acid bacteria, Enterobacteriaceae and pseudomonad counts, for CP samples stored at -1°C. Package type generally had little influence on these bacterial counts, therefore data were pooled across package types. The total aerobic microorganisms had a lag phase of about 4 weeks; thereafter the population increased with a generation time of approximately 2 days. After 6 and 8 weeks of storage, samples packaged in P3 had significantly higher total aerobic counts than samples packaged in F1 or F2, but the differences were less than 1 log unit. Presumptive lactic acid bacteria counts of samples stored at -1°C were 1 to 2 orders of

Table 2.1. Initial mean log bacterial counts (CFU/cm^2) obtained from each replicate for commercially (CP) and aseptically prepared (AP) pork cuts.

Replicate	1		2		3	
Organisms	CP	AP	CP	AP	CP	AP
Total aerobic count	3.5	2.6	3.5	1.3	4.1	2.8
Lactic acid bacteria	<1.8	<1.8	1.2	<1.8	1.8	<1.8
Coliforms	1.2	<1.4	1.7	<1.4	1.4	<1.4
Enterobacteriaceae	1.6	<1.4	1.1	<1.4	2.2	<1.4
Pseudomonads	2.7	0.8	2.5	1.3	3.4	1.3
Brochothrix thermosphacta	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8

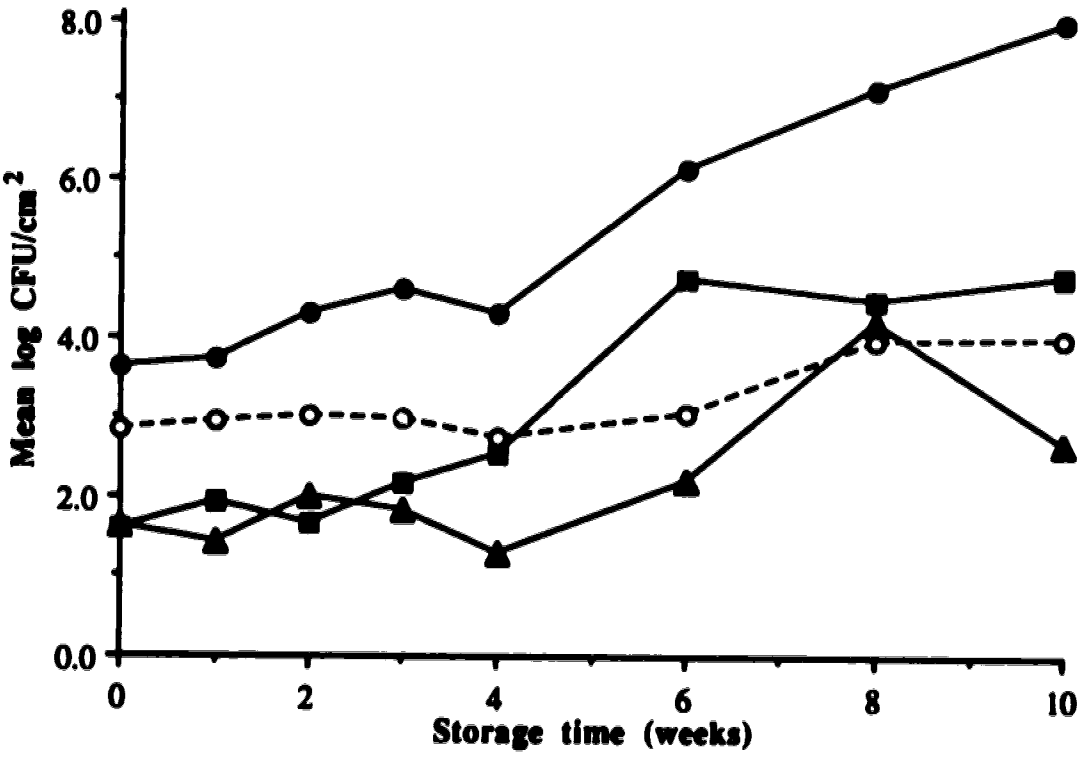

Figure 2.3. Changes in mean log counts of bacteria on commercially prepared pork cuts in modified atmosphere of 40% carbon dioxide and 60% nitrogen during 10 weeks of storage at -1°C.

- ● Total aerobic colony count determined on plate count agar at 25°C;
- ■ presumptive lactic acid bacteria determined on lactobacilli MRS agar;
- ▲ presumptive Enterobacteriaceae determined on violet red bile agar with added glucose;
- □ presumptive pseudomonads determined on Cefaloridine Fusidic acid Cetrimide agar.

magnitude lower than total counts and represented only 1% of the bacterial population. After a lag phase of approximately 4 weeks, Enterobacteriaceae numbers increased to a maximum population of 10^4 CFU/cm^2 after 8 weeks of storage. There was only limited growth of pseudomonads during storage at -1°C. Growth of *B. thermosphacta* in MAP pork cuts stored at -1°C (Figure 2.4) represented the principal change in microbial load. For both CP and AP samples stored for 6 or 8 weeks, those packaged in P3 had *Brochothrix* counts 1 to 2 log units higher ($P<0.05$) than those packaged in F1 and F2. CP samples stored for 8 weeks had a slight sour meat odour and some discoloration. In contrast, AP samples only developed a slight sour odour at 10 weeks.

At 4.4°C, sample preparation method (CP vs AP) had a significant effect on all bacterial counts. Package type had no influence on total aerobic and presumptive lactic acid and Enterobacteriaceae counts for either CP or AP products. For the CP products the estimated generation time for the total aerobic population at 4.4°C was approximately 2 days, with little or no lag in bacterial growth. Total aerobic counts reached a maximum of 10^5 CFU/cm^2 at 5 weeks. Generation time during the first 4 weeks of storage was 2 days for the lactic acid bacteria and 3 days for Enterobacteriaceae. By 4 weeks, lactic acid bacteria and Enterobacteriaceae reached maximum populations of 10^6 and 10^4 CFU/cm^2, respectively. Package type had a significant effect on the counts of pseudomonads and *B. thermosphacta*. Pseudomonads (Figure 2.5) failed to grow on CP samples packed in F1 or F2; however, after a 3-week lag in growth, pseudomonad bacteria increased in numbers on products packaged in P3. This increase in pseudomonads coincided with an increase in O_2 to between 2 and 3%. Data obtained for *B. thermosphacta* followed the same pattern as that shown for pseudomonad growth, with increases in *Brochothrix* numbers in P3 after 4 weeks of storage. After 7 weeks of storage at 4.4°C, sulphur gas as well as sour meat odours were detected when the meat packages were opened. After 9 weeks of storage

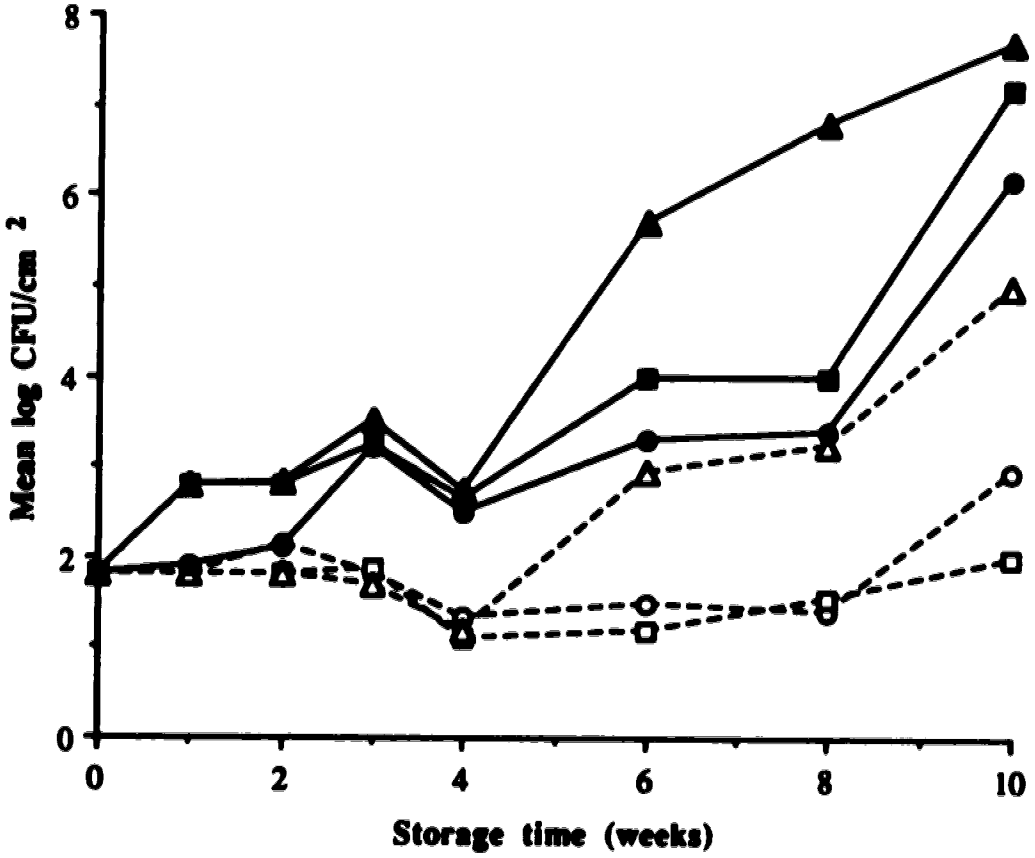

Figure 2.4. Change in presumptive *Brochothrix thermosphacta* count during storage in modified atmosphere at -1°C in packages with different gas transmission rates.
●, ○ F1 foil laminate package 0.0 OTR;
■, □ F2 metalized polyester package 12.5 OTR;
▲, △ P3 plastic package 26.5 OTR.
Closed symbols = commercially prepared product.
Open symbols = aseptically prepared product.

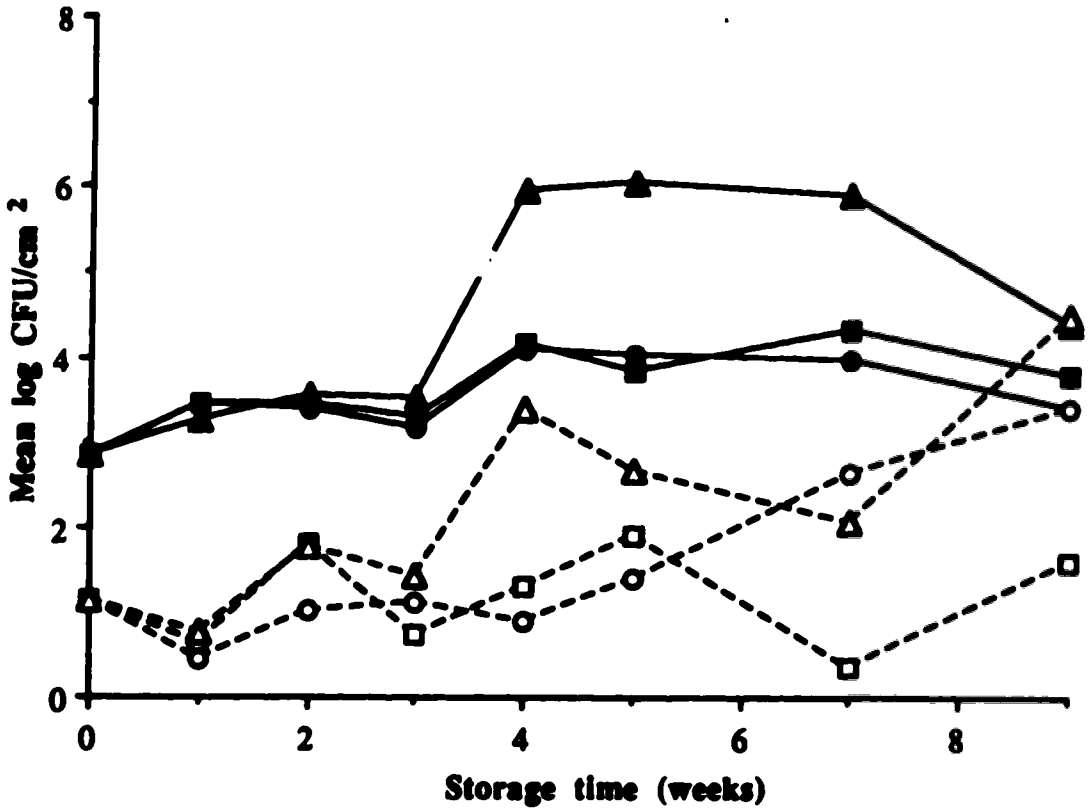

Figure 2.5. Change in presumptive pseudomonad count of pork cuts in modified atmosphere of 40% carbon dioxide and 60% nitrogen during 9 weeks of storage at 4.4°C.

●, ○ F1 foil laminate package 0.0 OTR;

■, □ F2 metalized polyester package 12.5 OTR;

▲, △ P3 plastic package 26.5 OTR.

Closed symbols = commercially prepared product.

Open symbols = aseptically prepared product.

samples stored at 4.4°C showed gross spoilage, characterized by strong off-odours and severe discoloration.

Bacteria grew rapidly on both CP and AP pork samples stored at 10°C. Off-odours were detectable after 2 weeks of storage. At this storage temperature, the principal group of bacteria was Enterobacteriaceae, which reached a maximum population of 10^8 CFU/cm^2 by 2 weeks of storage. In contrast, lactic acid bacteria and pseudomonads reached maximum populations of 10^6 CFU/cm^2 in the same period. Commercially prepared pork samples had a maximum *B. thermosphacta* count of 10^4 to 10^5 CFU/cm^2 after 1 week of storage. In contrast, *Brochothrix* counts for aseptically prepared samples increased gradually to 10^6 to 10^7 CFU/cm^2 over 4 weeks of storage.

2.4. DISCUSSION

A decrease in CO_2 in headspace gases of MAP meats is common and has been attributed to diffusion through the package (Hall et al., 1980, Seideman et al., 1980) and absorption by meat (Gill, 1988). Changes in the gas composition of the packs without meat samples were proportional to the theoretical oxygen transmission rates of the packaging materials. In the meat packs, CO_2 absorption by meat and gas exchange across the package were responsible for the major proportion of the change in the composition of the headspace gases. In the P3 packs (OTR 26.5) without meat, *ca.* 25% of the CO_2 was lost during storage at 4.4°C for 9 weeks. In the same package containing *ca.* 100 g of meat, after *ca.* 12.5% of the CO_2 was absorbed by the meat, a further *ca.* 25% CO_2 was lost over the 9-week storage period. The greatest difference in the composition of the headspace gases between packages with and without meat samples was noted at 10°C in that the CO_2 in the packs with meat either increased (as in the case of CP samples packed in F1 and F2) or decreased to a lesser extent (in P3 packs) than in packages without meat.

Increases in CO_2 during storage can result from a lowering of CO_2 solubility in the meat tissue at higher temperatures, which results in an increase in CO_2 in the atmosphere (Daun et al., 1971; Gill, 1988). Carbon dioxide in meat packages can also increase due to microbial metabolism because Enterobacteriaceae and heterofermentative lactic acid bacteria produce CO_2 during growth. It has been suggested that for gas composition to change as a result of bacterial metabolism, a high bacterial count (10^8 organisms/g) is required (Gardner et al., 1967). In the present study, the Enterobacteriaceae count reached 10^8 CFU/cm^2 at the time increases in CO_2 were noted.

The difference in storage life of samples "between" replicates is not readily explained. All samples from replicate 2 spoiled more rapidly than comparable samples from replicates 1 and 3. A possible reason for the difference among replicates could be variable muscle pH. High pH muscle spoils more rapidly than normal pH muscle because the carbohydrate available for microbial metabolism is limited (Gill, 1985). When growing on muscle with a high pH, compared with muscle at normal pH 5.3 to 5.7, bacteria use amino acids as an energy source and produce organoleptically obnoxious compounds much earlier during storage. However, the initial mean muscle pH of samples from the three experimental replicates differed by <0.2 pH units, and muscle samples from replicate 3 had the highest mean pH. During week 1 of storage, the pH in all samples dropped to between pH 5.3 and 5.7 and did not change significantly thereafter. Moreover, Gill and Harrison (1989) reported that large differences in the initial pH of pork did not affect the development of spoilage during modified atmosphere storage. Thus small differences in muscle pH in the present study should not account for the faster spoilage of the meat samples in replicate 2. Another possible explanation for the difference could be a higher initial microbial load or the types of microorganisms predominating on the meat. Higher microbial loads do reduce storage life of MAP meats (Ayres, 1960; Reagan et al., 1973); however, the initial microbial load on samples from the three replicates (Table 2.1) were similar, and samples from replicate 3 had a higher load than samples from replicate 2.

Thus, variation in the initial microbial load is not responsible for the difference in storage life of replicate 2.

Differences among replicates could represent differences in product from different producers, because samples for each replicate were taken from hogs obtained from a different producer each day. Perhaps, as a result of some factor in the feeding or raising of the hogs, the meat samples selected for replicates 1 and 3 contained an inhibitory substance not present in the samples for replicate 2. It was not possible to test this hypothesis. The failure of samples from replicate 2 to achieve the storage life of samples from replicate 1 and 3 raises questions about the reliability of obtaining an extended storage life for MAP pork. If such differences in the storage life of MAP pork are a result of production practices, then MAP of pork cuts may be of limited applicability.

The initial microbial load of the "commercial" samples was at the high end of the range normally acceptable in meat (Gill and Harrision, 1989). Total aerobic counts of 10^3 to 10^4 CFU/cm^2, with presumptive Enterobacteriaceae counts of 10^2 CFU/cm^2, indicate that improvements in plant sanitation could decrease these numbers. Aseptic laboratory preparation of samples reduced total bacterial load by 1 to 2 log counts and extended the storage life of the pork cuts by 1 week at 10°C and 2 weeks at 4.4 and -1°C. The success of MAP in extending the storage life of fresh meat relies heavily on low initial bacterial loads (Gilbert et al., 1983).

Storage in CO_2-enriched atmospheres inhibits or retards the growth of the aerobic and facultatively anaerobic spoilage microflora of fresh meats, such as pseudomonads and Enterobacteriaceae, and permits lactic acid bacteria and other CO_2 insensitive organisms to dominate the microbial population. The determining factor as to which organisms will predominate the bacterial population is dependent on storage temperature. At higher storage temperatures, Enterobacteriaceae grow in anaerobic environments (Grau, 1981). In this study, Enterobacteriaceae dominated the microbial population of pork samples stored at

10°C and by week 2 spoilage was noticeable. This confirms the work of Gardner et al. (1967), who found that Enterobacteriaceae dominated the microflora of pork stored at 16°C in modified atmospheres. In meat samples stored at 4.4°C, lactic acid bacteria dominated the microbial population regardless of package type, although growth of pseudomonads and *B. thermosphacta* occurred in P3 packs. In contrast, in samples stored at -1°C, *B. thermosphacta* dominated the microbial population of samples packaged in P3 once a small amount of O_2 (2 to 3%) was present. The presence of CO_2, combined with low temperature storage, should inhibit the growth of *B. thermosphacta* (Blickstad and Molin, 1983; Gill and Harrision, 1989). In samples stored at -1°C in foil packs, which contained very low amounts (<1%) of O_2, growth of *B. thermosphacta* was limited even after 8 weeks and lactic acid bacteria tended to dominate. It would appear that even at -1°C, the presence of small amounts of O_2 in the packs allowed *B. thermosphacta* to dominate the microbial population. This emphasizes the need to ensure that an anaerobic environment is maintained throughout storage to maximize the storage life of pork, as *B. thermosphacta* is a major spoilage organism of MAP and vacuum packaged meats (Gill and Harrision, 1989).

An increase in temperature from -1 to 4.4°C shortened the storage life of the pork cuts by 3 weeks. The potential for temperature abuse of MAP fresh pork cuts sold at the retail level raises concerns regarding the safety of such products. The temperature of household refrigerators is often above that recommended for safety (Van Garde and Woodburn, 1987). In the current study, after 2 weeks of storage at 10°C, the pork cuts had obvious signs of spoilage that would make them unacceptable for consumption. During storage at good refrigerator temperatures (5°C) potential pathogens, such as *Yersinia enterocolitica, Listeria monocytogenes,* and *Aeromonas hydrophila* could grow in an anaerobic environment and possibly develop a health hazard prior to the development of spoilage. This problem has yet to be studied for fresh pork products.

This study indicates that a storage life of 10 weeks for individually packaged retail cuts of pork can be achieved by packaging in modified atmospheres with elevated CO_2. Low temperature storage (-1°C) is the over-riding control for long-term extension of the storage life. A low level of initial microbial contamination also contributes to the extension of storage life. The use of O_2 impermeable films provides control of the dominating microflora which ultimately influences the storage life. Investigation of the reasons for differences in storage life between replicates is required to improve the reliability of obtaining an extended storage life of MAP pork cuts.

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3. Microbial Ecology of Fresh Pork Stored Under Modified Atmosphere at -1, 4.4 and 10°C[1]

3.1. INTRODUCTION

Storage life of chilled fresh pork is markedly extended when retail cuts are packaged in plastic film with low gas transmission rate, under vacuum or in modified atmosphere with elevated carbon dioxide (MAP) and lowered oxygen (Spahl et al., 1981; Gill and Harrison, 1989; McMullen and Stiles, 1991). Initial microbial load, gas permeability of the packaging film and storage temperature significantly affect the storage life of the meat (Gardner et al., 1967; Adams and Huffman, 1972; McMullen and Stiles, 1991). Prevalence of lactic acid bacteria in place of the putrefactive microflora of aerobically packaged meats is responsible for the extended storage life. However, the lactics and other bacteria such as *Brochothrix thermosphacta* and members of the Enterobacteriaceae have been implicated in the spoilage of MAP meats (Egan et al., 1989; Gill and Harrison, 1989; Lambert et al., 1991). Increased knowledge of the microbiology of MAP meats is essential for a more complete understanding of the dynamics of extended storage and safety of these products.

Studies of the lactic acid bacteria of chilled, vacuum packaged fresh meats revealed the prevalence of homo- and heterofermentative lactic acid bacteria, mainly *Lactobacillus*-type bacteria or leuconostocs (Hitchener et al., 1982; Shaw and Harding, 1984; Schillinger and Lücke, 987). Some strains were identified as atypical lactobacilli that have been established as a new genus, *Carnobacterium* (Collins et al., 1987). Lactic acid bacteria are the prevalent microflora of modified atmosphere packaged (MAP) meats because of their

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[1] A version of this chapter has been published. McMullen and Stiles, 1993. International Journal of Food Microbiology 18:1-14.

resistance to the CO_2 environment, their growth rate at chill temperatures and production of numerous inhibitory substances including organic acids, hydrogen peroxide and naturally produced proteinaceous antagonistic substances (bacteriocins). In recent years the antagonistic activity of a variety of lactic acid bacteria has been studied with major emphasis on the production of bacteriocins by these organisms (Harris et al., 1989; Ahn and Stiles, 1990; Schillinger and Holzapfel, 1990; Hastings and Stiles, 1991). It has been suggested that bacteriocins play a role in the prevalence of lactic acid bacteria in vacuum and modified atmosphere packaged meats (Klaenhammer, 1988; Hastings and Stiles, 1991). However, few researchers have examined the incidence of bacteriocin-producing lactic acid bacteria on modified atmosphere packaged meats throughout storage, and their role in the prevalence of lactic acid bacteria on chill stored meats is unclear. In this study we determined the nature of the prevailing microflora during storage of pork cuts at different storage temperatures in two packaging films and examined the prevalent lactic acid bacteria for antimicrobial activity and bacteriocin production.

3.2. MATERIALS AND METHODS

3.2.1. Strain Isolation

The method of meat packaging and storage has been described (see Chapter 2; McMullen and Stiles, 1991). Strains were isolated from aseptically and commercially prepared pork samples stored in either a foil laminate or nylon/PVC package and stored at -1, 4.4 and 10°C. At weekly intervals for up to 4 weeks for samples stored at 10°C, and up to 9 or 10 weeks for samples stored at-1 and 4.4°C, samples were removed from storage and prepared for microbial analysis, including: total aerobic plate count on Plate Count agar (PCA; Difco Laboratories Inc., Detroit, Michigan) incubated aerobically at 25°C; and presumptive lactic acid bacteria count on nonacidified Lactobacilli MRS agar (MRS; Difco)

incubated at 25°C in an atmosphere of 10% CO_2 and 90% N_2. Colonies for identification in this study were selected from samples stored in foil laminate with negligible oxygen transmission and in plastic film with oxygen transmission of 25 ml/m^2/24 h/atm at 23°C and 0% relative humidity. Colonies were picked from the greatest dilution of the samples that generally contained 20 to 50 colonies, and were selected to represent the principal colony types growing on the plates. Where appropriate, two colonies of each type were picked.

3.2.2. Strain Identification

The selected colonies were inoculated into soft APT agar plugs (0.4% agar; Difco). Strains were subcultured in basal medium (BM) broth (Wilkinson and Jones, 1977), examined for purity, and differentiated by Gram stain and catalase and oxidase reactions. Catalase-positive strains were characterized according to the criteria in Tables 3.1 and 3.2. Motility was tested in APT plugs made with 0.4% agar and incubated at 25°C. Flagella staining was done using Ryu stain (Heimbrook et al., 1989). Oxidative/fermentative utilization of glucose was done using OF medium (Difco) containing 1% filter-sterilized glucose. Strains were inoculated into 2 tubes, one tube was overlayered with approximately 2 ml of sterile mineral oil. For Gram-positive strains, additional tests included the determination of oxygen requirement by observation of growth under an APT plug, ability to grow in BM broth at 35°C, and ability to grow on streptomycin thallous acetate agar (STAA; Gardner, 1966)

The Gram-positive, catalase-negative strains were subdivided according to the scheme proposed by Schillinger and Lücke (1987) with the following changes: (1) carnobacteria were differentiated from heterofermentative lactobacilli by the formation of predominantly L(+)-lactate (Collins et al., 1987), and were further differentiated by the carbohydrate fermentation patterns shown in Table 3.3; (2) strains that produced gas from

Table 3.1. Tests used to classify Gram-negative, catalase-positive bacteria to the generic or Family level.[a]

Genus or Family	Oxidase	Motility	Flagellation pattern	Fermentation of glucose[b]
Pseudomonas	+/-	+	polar	O
Flavobacterium	+	-	-	O
Moraxella	+	-	-	NS
Alcaligenes	+	+	peritrichous	NS
Acinetobacter	-	-	-	NS
Shewanella	+	+	single polar	O
Aeromonas	+	+	single polar	O/F
Enterobacteriaceae	-	+/-	peritrichous	O/F

[a] Based on Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1986).

[b] O, oxidative; NS, nonsaccharolytic; O/F, oxidative and fermentative.

Table 3.2. Tests used to differentiate Gram-positive, catalase-positive bacterial species.[a]

Bacterial genus or species	Oxidase	Motility	Flagellation pattern	Oxygen requirement	Growth at 35°C	Growth on STAA[b]
B. thermosphacta	-	-	-	facultative	-	+
Listeria spp.	-	+	peritrichous	facultative	+	-
Kurthia spp.	+	+	peritrichous	strict aerobe	+	-
Micrococcus spp.	-	-	-	facultative	+	-

[a] Based on Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1986).

[b] Streptomycin thallous acetate agar (Gardner, 1966) for selection of *B. thermosphacta*.

Table 3.3. Carbohydrate fermentation patterns used to differentiate *Carnobacterium* species.[a]

Carbohydrate[b]	*C. divergens*	*C. gallinarum*	*C. mobile*	*C. piscicola*
Inulin	-	-	+	+
Mannitol	-	-	-	+
Melibiose	-	-	-	+
Melezitose	v	+	-	v
Xylose	-	+	-	-

[a] Adapted from Collins et al. (1987).

[b] - no acid production; + acid production; v variable acid production.

glucose, no ammonia from arginine, and greater than 80% of lactate as the D(-)-isomer, were classified as leuconostocs and subdivided according to the scheme in Figure 3.1; and (3) homofermentative lactobacilli were differentiated on the basis of fermentation patterns for arabinose, cellobiose, gluconate, lactose, maltose, mannitol, melibiose, raffinose, rhamnose and trehalose, based the criteria of Kandler and Weiss (1986).

The methods of Wilkinson and Jones (1977) were used to test for growth in 6.5% NaCl, for growth at 10, 15, 35 and 45°C, and for production of dextran (slime) from sucrose. Gas production from glucose, carbohydrate fermentation tests and production of ammonia from arginine were done according to methods described by Shaw and Harding (1985). To determine the proportion of the lactate isomers formed, strains were grown in BM broth for 48 h, heated at 80°C for 15 min, cooled, centrifuged at 14,900 x *g* for 5 min and the supernatant analyzed for D(-)- (Gawehn, 1984), and L(+)- lactate (Noll, 1984) using D- and L-lactate dehydrogenase, NAD and glutamate pyruvate transaminase (Boehringer Mannheim, Montreal, Que.). The method of Kempler and McKay (1980) was used for detection of citrate dissimilation.

3.2.3. Antagonistic Activity

All strains classified as lactic acid bacteria were screened for antibacterial activity by deferred antagonism technique (Hastings and Stiles, 1991) against the indicator strains listed in Table 3.4. To broaden the range of lactic acid bacteria used as indicators, each set of 12 strains was screened against the other lactic strains within the set. To test the nature of the inhibitory substance, a 24-h culture grown in APT broth was centrifuged at 6,000 x *g* for 3 min, the supernatant was neutralized to pH 6.5 with 10 N NaOH and either unheated (control) or heated at 65°C for 30 min, divided, and untreated (control) or treated with pronase E (Sigma; 1 mg /ml) or catalase (Sigma; 100 units/ml). After 1 h incubation, 10 µl of each sample was spotted onto the surface of APT plates that had been overlayered

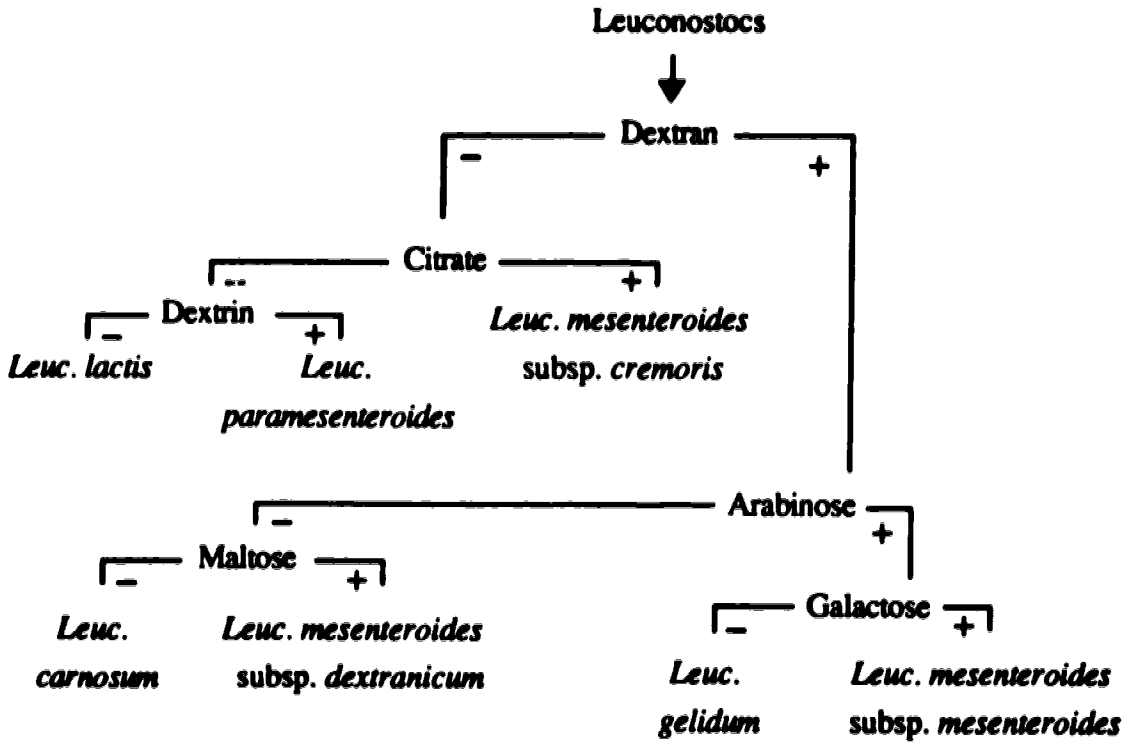

Figure 3.1. Scheme used for the differentiation of *Leuconostoc* spp., adapted from Shaw and Harding (1989) and Garvie (1986).

Dextran: production of dextran from sucrose;

citrate: dissimilation of citrate;

arabinose, dextrin, maltose, galactose: production of acid from the respective carbohydrate sources.

Table 3.4. Bacterial strains used in this study and their sources.

Species	Strain and Source
Carnobacterium piscicola	LV17 (Shaw)[a]
Carnobacterium divergens	LV13 (Shaw)
Aciduric *Lactobacillus* strain	LV69 (Shaw)
Lactobacillus plantarum	ATCC[b] 4008
Pediococcus parvulus	ATCC 19371
Enterococcus faecalis	ATCC 19433
Enterococcus faecium	ATCC 19434
Brochothrix thermosphacta	ATCC 11509
Brochothrix thermosphacta	UA Meat Isolate 41
Listeria monocytogenes	ATCC 15313
Listeria monocytogenes	Scott A
Listeria sp.	UA42[c]
Listeria innocua	ATCC 33090
Staphylococcus aureus	S6[d]
Staphylococcus aureus	ATCC 13565
Bacillus macerans	ATCC 7048
Bacillus cereus	ATCC 14579
Clostridium bifermentans	ATCC 19299

[a] Strains obtained from Dr. B.G. Shaw, Institute of Food Research, Bristol UK.

[b] American Type Culture Collection, Rockville, MA.

[c] University of Alberta Food Microbiology Culture Collection.

[d] Obtained from Dr. P. Ewan, Health Protection Branch, Ottawa.

with soft APT agar inoculated with 1% of an overnight culture of either *Carnobacterium piscicola* LV17 or *C. divergens* LV13. Inhibitory activity was estimated by spotting doubling dilutions of supernatant onto overlayered APT agar as described above. The reciprocal of the highest dilution showing inhibition was taken as a measure of arbitrary activity units (AU) adjusted per ml of supernatant fluid (Hastings and Stiles, 1991).

3.3. RESULTS

Treatment variables for the pork samples for isolation of strains for this study included two packaging types, aseptic and commercial meat preparation and three storage temperatures. A total of 1,220 strains was selected, of which 1,049 strains (86%) remained viable and were characterized. The method of strain selection ensured that the selected strains are those prevalent in the meat microflora, but they do not represent the proportions of these strains on the samples. Generally, the types of bacteria growing on the samples prepared either aseptically or commercially and stored in the two packaging films were similar, therefore data were pooled across method of meat preparation and package type.

Of the characterized strains, a total of 652 (62.1%) was lactic acid bacteria. Strains isolated from MRS included 20% nonlactics that were primarily identified as yeast, based on microscopic identification; whereas strains isolated from PCA included 49% nonlactics, of which 52 (19%) of 278 strains were identified as yeast.

Classification of nonlactic strains isolated from PCA is shown in Table 3.5. From samples stored at -1 and 4.4°C, the frequency of detection of Gram-negative bacteria and micrococci decreased with time of storage, whereas the frequency of detecting aeromonads

Table 3.5. Classification of the number of nonlactic acid bacteria isolated from Plate Count agar plates from pork samples stored at -1, 4.4 and 10°C.

Storage time[a] (weeks)	Number of Strains																	
	Gram-negative bacteria			Aeromonads			Listeriae			Micrococci			Enterobacteriaceae			*Brochothrix thermosphacta*		
	-1°	4.4°	10°	-1°	4.4°	10°	-1°	4.4°	10°	-1°	4.4°	10°	-1°	4.4°	10°	-1°	4.4°	10°
1	6	7	0	0	0	0	3	0	0	9	6	0	0	0	4	0	2	0
2			2			10			0			0			3			0
3	5	0	0	1	1	18	2	0	0	2	2	0	0	0	13	3	3	0
4			1			3			0			0			9			0
5/6	3	0		2	0		2	0		0	0		0	4		5	7	
7/8	0	1		3	3		0	0		1	0		0	10		8	9	
9/10	0	0		6	9		2	0		0	0		0	0		16	9	

[a] Bacterial strains were obtained from meat samples stored at -1°C at week 1, 3, 6, 8, and 10; at 4.4°C at week 1, 3, 5, 7, and 9; and at 10°C for the first four weeks of storage.

and *Brochothrix thermosphacta* increased. The majority of the *B. thermosphacta* strains were isolated from samples stored in plastic film with the higher oxygen transmission rate. At -1°C listeriae were detected as one of the prevalent strains isolated from the samples throughout storage, indicating that they grew in proportion to the total population of the meat; however, listeriae were not detected among the strains isolated from samples stored at 4.4 or 10°C. Enterobacteriaceae were isolated as part of the prevalent microflora of samples stored at 4.4 and 10°C, but they were not isolated from samples stored at -1°C.

The classification of the lactic strains isolated from PCA and MRS are summarized in Table 3.6. From PCA the majority of the strains (85%) isolated were carnobacteria and homofermentative lactobacilli; whereas on MRS the majority of strains (62%) isolated were homofermentative lactobacilli, while carnobacteria were infrequent isolates.

Temperature of storage had little effect on the types of lactic acid bacteria present on the pork samples, with the exception of the homofermentative lactobacilli and pediococci. The majority of the homofermentative strains isolated on PCA and MRS from pork stored at -1°C had carbohydrate fermentation patterns that matched *Lactobacillus alimentarius, Lact. farciminis* and *Lact. sake*. From samples stored at 4.4°C there was a greater variety of homofermentative lactobacilli including: *Lact. alimentarius, Lact. curvatus, Lact. farciminis, Lact. plantarum* and *Lact. sake*. From samples stored at 10°C the homofermentative lactobacilli included: *Lact. alimentarius, Lact. casei* subsp. *casei, Lact. coryneformis* subsp. *coryneformis, Lact. farciminis, Lact. plantarum* and *Lact. sake*. A large proportion (89 strains; 38%) of homofermentative strains could not be identified from their fermentation patterns. The heterofermentative lactic acid bacteria isolated from both PCA and MRS were *Lact. viridescens* and *Lact. minor*. All of the carnobacteria isolated were identified as *C. divergens*. The leuconostoc strains were *Leuconostoc gelidum* except for a few strains of *Leuc. mesenteroides* subsp. *mesenteroides*. A few strains of

Table 3.6. Classification of the number of lactic acid bacteria isolated on Plate Count and Lactobacilli MRS agar from pork samples stored at -1, 4.4 and 10°C[a].

Storage time (weeks)	Number of Strains														
	Homofermentative lactobacilli			Heterofermentative lactobacilli			Carnobacteria			Leuconostocs			Pediococci		
	-1°	4.4°	10°	-1°	4.4°	10°	-1°	4.4°	10°	-1°	4.4°	10°	-1°	4.4°	10°
a) Plate Count agar															
1	4	5	7	0	4	5	4	0	1	0	1	1	1	0	0
2			0			0			12			0			1
3	0	7	4	2	10	0	12	16	9	0	2	0	1	1	1
4			2			0			6			1			0
5/6	3	14		0	2		14	21		1	0		0	0	
7/8	11	9		1	0		19	20		0	0		1	0	
9/10	0	8		0	1		22	12		0	6		0	0	
b) Lactobacilli MRS agar															
1	0	0	17	0	0	4	0	0	0	1	5	4	0	1	5
2			26			3			0			3			4
3	4	19	24	2	2	2	2	0	0	10	4	3	0	0	5
4			10			2			1			1			0
5/6	11	15		1	6		1	6		0	6		0	0	
7/8	13	13		2	2		2	3		0	1		0	0	
9/10	13	13		4	3		0	1		0	6		0	3	

[a] Bacterial strains were obtained from meat samples stored at -1°C at week 1, 3, 6, 8, and 10; at 4.4°C at week 1, 3, 5, 7, and 9; and at 10°C for the first four weeks of storage.

Pediococcus sp. were isolated at all 3 storage temperatures, but the majority were from samples stored at 10°C and they were isolated from MRS agar.

A total of 538 lactic cultures was tested for inhibitory activity against the range of indicator organisms listed in Table 3.4, and against different sets of lactic acid bacteria isolated from the pork samples. Deferred antagonism tests detected 162 strains (30%) that produced inhibitory activity against indicator strains used in our study, representing as many as 64 different inhibitory patterns. Antagonistic strains were detected among the prevalent lactic acid bacteria throughout storage at all temperatures. Strains isolated from samples stored at -1°C had narrow spectra and often inhibited only one of the indicator organisms. The percentage of antagonistic strains detected among the homofermentative and heterofermentative lactobacilli, carnobacteria, leuconostocs and pediococci were 18, 27, 37, 66 and 22%, respectively. Of the 162 strains that showed antagonistic activity, only 32 (6% of the total number of lactic acid bacteria tested) were active against at least 50% of the indicator strains used for screening.

Within each group of lactic acid bacteria tested, the activity spectra were diverse. As a group, the antagonistic spectrum of the homofermentative lactobacilli included all of the indicator strains tested (Table 3.7), but individually their activity spectrum was limited and only 12 strains (28%) were antagonistic to greater than 50% of the indicator strains tested. The activity spectra for the heterofermentative group of lactic acid bacteria was narrower than that for the homofermentative group. None of the heterofermentative lactobacilli was antagonistic to *E. faecium*, *B. thermosphacta* ATCC 11509 or *Bac. macerans*. However, the activity spectra were not as diverse and a greater proportion of the strains was antagonistic to the same indicator organisms. The carnobacteria also had narrow activity spectra. None of the carnobacteria was antagonistic to aciduric *Lactobacillus* strain LV69, *E. faecium*, *B. thermosphacta* strains, *Staphylococcus aureus* and *Bac. macerans* and very few inhibited the growth of *Listeria* spp. Although the activity

Table 3.7. Number of strains within each group of lactic acid bacteria that were inhibitory to each of the indicator strains.

Indicator Strains	Homofermentative lactobacilli (n=42)	Heterofermentative lactobacilli (n=15)	Carnobacteria (n=65)	Leuconostocs (n=35)	Pediococci (n=5)
C. piscicola LV17	8 (19)[a]	11 (73)	18 (23)	6 (17)	0
C. divergens LV13	18 (43)	10 (67)	12 (18)	14 (40)	0
Lactobacilli group II LV69	10 (24)	9 (60)	0	5 (14)	1 (20)
Lact. plantarum ATCC 4008	3 (7)	1 (7)	1 (2)	0	0
P. parvulus ATCC 19371	10 (24)	10 (67)	6 (9)	9 (26)	0
Other Lactic acid bacteria[b]	13 (31)	11 (73)	28 (43)	9 (26)	2 (40)
E. faecalis ATCC 19433	8 (19)	12 (80)	4 (6)	6 (17)	0
E. faecium ATCC 19434	8 (19)	0	0	0	0
B. thermosphacta ATCC 11509	14 (33)	0	0	0	1 (20)
B. thermosphacta UA41	11 (26)	1 (7)	0	0	0
L. monocytogenes ATCC 15313	16 (38)	12 (80)	2 (3)	15 (43)	0
L. monocytogenes Scott A	11 (26)	7 (47)	2 (3)	10 (26)	0
Listeria sp. UA42	11 (26)	12 (80)	1 (2)	34 (97)	0
L. innocua ATCC 33090	14 (33)	12 (80)	2 (3)	21 (60)	1 (20)
S. aureus S6	11 (26)	1 (7)	0	0	0
S. aureus ATCC 13565	10 (24)	1 (7)	0	0	0
B. macerans ATCC 7048	9 (21)	0	0	0	0
B. cereus ATCC 14579	13 (31)	1 (7)	0	1 (3)	0
C. bifermentans ATCC 19299	15 (36)	9 (60)	32 (49)	25 (71)	0

[a] Numbers in parentheses represent percentages.

[b] Tested against all inhibitory lactic acid bacteria strains isolated from pork samples (n=48).

spectra for the leuconostoc strains were similar to those for the carnobacteria, a greater proportion of the leuconostocs was inhibitory to *Listeria*. The activity spectrum for each of the *Pediococcus* sp. was limited to one indicator organism.

Of the 162 antagonistic strains, 48 were selected for further characterization of their inhibitory activity. No antimicrobial activity was detected in the supernatant fluids of 14 (29%) of the selected strains. When deferred inhibition tests were repeated for these strains, no antagonism was detected. One strain had activity only in the unheated supernatant. The remaining 33 strains (69%) were sensitive to pronase E, insensitive to catalase and active at pH 6.5, indicating that the activity is due to production of antagonistic proteinaceous substances (bacteriocins). The activity of the antagonistic strains measured against *C. divergens* LV13 varied between strains within each group of lactic acid bacteria and ranged from 100 to 25,600 AU/ml.

3.4. DISCUSSION

The development of a lactic acid bacteria microflora is well established for MAP fresh meats stored below 5°C. Previous studies on the composition of the prevalent microflora of MAP meats have not detailed the specific lactic acid bacteria nor the incidence of bacteriocin-producing lactic acid bacteria throughout storage at different temperatures. When storage temperatures rise above 7 to 10°C, temperature has a significant effect on the composition of the prevalent microbial population. At higher temperatures, lactic acid bacteria do not prevail (McMullen and Stiles, 1991), and Enterobacteriaceae and aeromonads are part of the prevalent microflora of MAP fresh meats stored at temperatures greater than 7°C (Gardner et al., 1967; Lee et al., 1985; Simard et al., 1985). In our study, the prevalent microbial population of fresh pork stored at 10°C included lactic acid bacteria, Enterobacteriaceae and aeromonads in almost equal numbers.

Aeromonads were previously isolated from MAP pork by Blickstad and Molin (1983); however, they were isolated from the fat surfaces and not from the lean. In the present study, aeromonads were isolated as part of the prevailing microflora of lean pork loin cuts packaged in a MA and stored at 10°C, and with prolonged storage at -1 and 4.4°C. Storage temperature was not a factor influencing the presence of aeromonads. In contrast, Gill and Reichel (1989) reported that the growth of *A. hydrophila* inoculated onto high pH beef stored under 100% CO_2 was temperature dependent.

Listeriae have also been isolated from fresh meats (Johnson et al., 1990) but there have been no previous reports of their isolation from MAP fresh meats stored for extended periods of time. Listeriae were detected among the prevalent microflora of samples stored at -1°C. The slow development of a lactic acid bacteria microflora on samples stored at -1°C (McMullen and Stiles, 1991) may have allowed listeriae to grow as part of the prevalent microflora. The *Listeria* strains were not speciated thus the implication of their presence for food safety is not known.

Viable counts for *B. thermosphacta* determined on STAA indicated that this organism is a significant part of the prevalent microbial population of MAP pork cuts stored at -1 and 4.4°C in a plastic film that allowed oxygen levels to increase to 2 to 3% over 9 to 10 weeks (McMullen and Stiles, 1991). This was confirmed by the identification of isolates in this study. *B. thermosphacta* can be a major spoilage organism of MAP meats when O_2 is present in the pack (Blickstad and Molin, 1983; Gill and Harrison, 1989) because its metabolic endproducts are organoleptically unpleasant (Gibbs et al., 1979).

The relatively high proportion of yeast isolates from both PCA and MRS agars was associated with meat samples packaged in the relatively gas permeable film. The increase in O_2 to 2 to 3% during storage (McMullen and Stiles, 1991) may have allowed yeasts to compete in the prevalent microbial population. Yeasts were reported to increase in numbers during storage of minced meat in 100% CO_2 at 3°C (Nychas and Arkoudelos 1990);

however, yeasts were not considered to be a major part of the prevalent microflora based on enumeration on selective agar. Sutherland et al. (1975) recovered yeast from vacuum packaged beef throughout a 9 week storage period at 0 to 2°C. Other studies on the microflora that develops on modified atmosphere packaged fresh meats did not report the presence or growth of yeasts (Enfors et al., 1979; Blickstad and Molin, 1983).

At all three storage temperatures, the population of lactic acid bacteria obtained from PCA agar was principally carnobacteria, whereas the prevalent population based on isolates from MRS agar was homofermentative lactic acid bacteria. Very few carnobacteria were isolated. Differences in growth medium may account for conflicting reports in the literature on the principal types of lactic acid bacteria on MAP fresh meat. No reports on the isolation of lactic acid bacteria with PCA are available; however, when isolating lactic acid bacteria from meat using tryptone glucose extract agar or Tryptic Soy Agar, Enfors et al. (1979) and Vanderzant et al. (1982) reported a change from a predominantly homofermentative population to a predominantly heterofermentative population during storage. In contrast, Schillinger and Lücke (1987) reported that homofermentative lactic acid bacteria prevailed when MRS agar (pH not stated) was used as the medium for isolation. Hitchener et al. (1982) suggested that MRS agar may be selective for certain strains of lactic acid bacteria. The presence of acetate in MRS agar and the relatively low pH could account for the difference in the types of lactic acid bacteria isolated from the two media. Carnobacteria do not grow well in the presence of acetate (Schillinger and Holzapfel, 1990).

Leuconostocs made up a relatively small proportion of the prevailing microflora from the pork samples at all three storage temperatures. The majority of these strains was isolated from MRS agar, indicating the possibility of selection due to growth medium. Almost all of the leuconostocs were identified as *Leuc. gelidum*. This species has been

isolated from meats by other researchers (Shaw and Harding, 1989; Hastings and Stiles, 1991).

Antibacterial activity was detected in 30% of the lactic strains isolated; however, only 6% of the total number of strains screened for activity were inhibitory to a relatively broad spectrum of indicator strains. Few reports are available on the proportion of antagonistic or bacteriocin-producing lactic acid bacteria isolated from foods. Sobrino et al. (1991) found that 16% of the lactic organisms isolated from dry fermented sausages exhibited activity against a single strain of *Lact. farciminis*, whereas Harding and Shaw (1990) found that only 4% of the lactic acid bacteria that they isolated from a variety of foods produced bacteriocins.

The incidence of bacteriocin-producing lactic acid bacteria in foods seems to be low. However, the relatively infrequent isolation of bacteriocin-producing lactic acid bacteria may be due to the use of insensitive indicator organisms or possible differences in microbial metabolism between in vitro and in vivo environments. Tagg et al. (1976) suggested that all bacteria produce bacteriocin. It is possible that we are not detecting all bacteriocin producing lactic acid bacteria during screening. No further characterization of the nature of the inhibitory activity was done on the 14 strains that produced deferred antagonism in initial screening tests, but antagonistic activity could not be demonstrated in the culture supernatant or when deferred tests were repeated. The unexpected loss of antagonism has been noted in other strains of lactic acid bacteria tested in our laboratory. The apparent loss of antimicrobial activity may also contribute to the low frequency of recovery of bacteriocin-producing lactic acid bacteria from foods.

The antimicrobial spectrum of the majority of the bacteriocin-producing strains was limited to other lactic acid bacteria, *Enterococcus faecium* and *Listeria* species. The large number of bacteriocin-producing lactic acid bacteria active against listeriae is not surprising because they are closely related to lactics (Wilkinson and Jones, 1977). The relatively high

sensitivity of listeriae to lactic acid bacteria has been reported by several workers (Harris et al., 1989; Lewus et al., 1991; Schillinger and Lücke, 1991). The sensitivity of listeriae to the antibacterial activity of lactic acid bacteria should preclude their domination of the microflora of MAP meats; however, in this study, listeriae were isolated as part of the prevalent microflora of samples stored at -1°C. This could possibly be due to a lack of growth of bacteriocin-producing lactic acid bacteria at such a low temperature. The production of inhibitory substances in meat systems stored at low temperatures should be examined because the majority of the isolates with a broad spectrum of activity were isolated from samples stored at 4 or 10°C.

Knowledge of the composition of the microflora present on modified atmosphere packaged fresh meats is important to understand spoilage and safety of the products. It may be possible to extend further the storage life and enhance the safety of MAP meats by controlling the fermentation that occurs by inoculation of the meats with bacteriocin-producing lactic acid bacteria to regulate the growth of spoilage organisms and potential pathogens.

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4. Quality of Fresh Retail Pork Cuts Stored in Modified Atmosphere Under Temperature Conditions Simulating Export to Distant Markets[1]

4.1. INTRODUCTION

Modified atmosphere (MA) packaging and chilled storage of fresh meats creates the potential to access distant markets with retail-ready product, provided that an appropriate storage life can be achieved. Storage life under MA depends on many factors, including initial microbial load (Christopher et al., 1979), packaging film (Newton and Rigg, 1979), gas atmosphere (Christopher et al., 1979; Spahl et al., 1981) and of greatest importance, storage temperature (Blickstad and Molin, 1983). According to Gill and Molin (1991), the minimum temperature for nonfrozen storage of meat is -1.5°C. Accurate control of storage temperature of meats can be achieved when meat cuts are prepared in a centralized packaging facility, during storage in a warehouse and transportation to distant markets. Once the meat reaches the retail market temperature control may become compromised and meats may be held at relatively undesirable temperatures. In an earlier study (McMullen and Stiles, 1991), we demonstrated the effect of temperature on storage life and microbiology of fresh pork loin cuts. The effect on storage life of low temperature (-1.5°C) storage prior to storage of meat cuts at typical retail temperatures is not known.

One of the current practices for the distribution of pork to retail markets involves transportation of vacuum packaged primal cuts to the retailer where packs are opened, cut for retail display and repackaged aerobically for sale. This provides the protective effect of MA packaging at the wholesale level of handling, but the advantages are lost with aerobic packaging at the retail level. There have been reports of an objectionable odour, referred to

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[1] A version of this chapter has been submitted for publication. McMullen and Stiles, 1993. Meat Science.

packaging at the retail level. There have been reports of an objectionable odour, referred to as confinement odour, when vacuum packaged primal cuts are opened (Egan and Shay, 1982; Edwards et al., 1985). This odour dissipates quickly and the meat itself does not retain the odour. With current meat handling practices the consumer is not exposed to confinement odours. However, if MAP is applied to retail cuts of fresh meat for sale directly to consumers, confinement odour could be a barrier to consumer acceptance of these products. Objectionable odours such as sour, buttermilk, dairy, cheesy and sulphur-like, have been noted for meats stored under MA for extended periods of time (Hanna et al., 1983; Edwards and Dainty, 1987). Studies to identify compounds in the headspace gases of MA packaged fresh meat have been done (Jackson et al., 1992); however, confinement odour has not been studied by trained sensory panels nor has it been compared with the odour of the meat after removal from the pack.

The objectives of this study were to assess the effect of storage temperature change after MA storage at -1.5°C on the storage life of MA packaged retail cuts of pork and to describe and quantify confinement odour compared with the meat odour to assess their potential as barriers to consumer acceptance of MA packaged retail-ready cuts of pork.

4.2. MATERIALS AND METHODS

4.2.1. Preparation of Meat Samples

Hogs raised on the University of Alberta farm were slaughtered at a federally inspected abattoir, stored at -1°C and broken into primal cuts 24 h postmortem. *Longissimus dorsi* muscles were excised. The pH of each muscle was determined by placing a glass electrode on the surface of the lean tissue. Muscles with a pH between 5.6 and 5.9 that did not show signs of being pale, soft and exudative (PSE) were chosen. Selected loins were packed on ice in coolers, transported to the laboratory, held on ice in a

cooler at 4°C and packaged within 4 h. Each loin was trimmed of visible fat and epimysium and cut into 1.5 cm thick slices with a mean surface area of 75.0 ± 5 cm^2. Surface area was determined by tracing the outline of the meat on aluminum foil, and measuring the surface area with an Area Meter (Model LI-3100, LiCor Inc., Lincoln, NE). Cuts from 12 loins were used for each replicate and randomly assigned to each treatment.

4.2.2. Packaging

Each cut was individually packaged in a 23 x 10 cm Opalen HL75 (Walki-Pak, Valkeakoski, Finland) pouch which has the following construction: 15 μ oriented polyamide/ethylene vinyl alcohol: 60 μ low and linear low density polyethylene. The manufacturers' reported oxygen transmission rate of the film was <1 $cc/m^2/24$ h at 23°C, 0% relative humidity under 1 atmosphere pressure.

Packages were evacuated, flushed with 100% CO_2 and sealed with a Multivac (Model AG500, Sepp Haggenmüller KG, Germany) packaging machine. Packages were double sealed with a second seal applied to the pack inside the first seal (Kwik Seal, Cole Palmer, Chicago IL). The headspace gas was removed from the sealed pack with a vacuum pump (Model S35; Precision Scientific Group, Chicago, IL) through a 25 gauge needle inserted through a silicone (Dow Corning Canada Inc., Mississauga, Ont., Canada) sampling port and replaced with 300 ml of CO_2. Residual O_2 concentration in the pack was measured with a Mocon Oxygen Headspace Analyzer (Model HS-750; Modern Controls Inc., Minneapolis, MN). Packs were evacuated and flushed repeatedly with CO_2 until the residual O_2 concentration was below 200 ppm. Packs which had to be flushed more than 3 times were rejected. The meat samples were stored at -1.5°C for 3 weeks, at which time one-third of the packs was held at -1.5°C and the others were moved to 4 (designated -1.5/4°C) or 7°C (designated -1.5/7°C) for further storage. Packs without meat were flushed with 300 ml of CO_2 and stored in the same manner as the meat packages.

Samples were prepared in triplicate for gas analysis, sensory evaluation and microbial evaluation.

4.2.3. Analyses

Headspace gas samples were drawn from the pack with a 100 µl gas tight syringe (Hamilton Co., Reno NV) and gas composition (CO_2, O_2, N_2) was analyzed as described by McMullen and Stiles (1991).

Microbial analysis was done by aseptically removing half of the meat from the package, placing it in a sterile stomacher bag and proceeding as described by McMullen and Stiles (1991). In addition to the tests for total aerobic count, lactic acid bacteria, pseudomonads, *Brochothrix thermosphacta*, Enterobacteriaceae and coliform bacteria as described by McMullen and Stiles (1991), total aerobic count was also determined on All Purpose Tween agar (APT; Difco) incubated at 25°C for 72 h. Yeast and mould counts were determined on prepoured plates of potato dextrose agar (PDA; Difco) adjusted to pH 3.5 with tartaric acid. PDA plates were inoculated with 50 µl of appropriate dilutions and incubated at 25°C for 48 h. The pH of the blended meat slurry was determined using an Extech pH meter (Model 671, Extech, Boston, MA).

The prevalent strains of bacteria were selected as previously described (McMullen and Stiles, 1993). Gram-positive, catalase negative strains were selected for classification. Strains that were not able to grow on acetate agar (Rogosa et al., 1951) were classified as carnobacteria. Strains that produced gas from glucose and grew on acetate agar but were not able to produce ammonia from arginine were classified as leuconostocs if coccoid-shaped or heterofermentative lactobacilli if rod-shaped. Strains which were homofermentative were classed as either homofermentative lactobacilli or homofermentative coccus-shaped bacteria based on their cell morphology. The homofermentative coccus-shaped bacteria could include: *Enterococcus*, *Lactococcus*, *Pediococcus* and *Streptococcus*

spp. The protocol and methods for production of gas from glucose and ammonia from arginine were described in McMullen and Stiles (1993). In addition, production of gas from glucose was tested in MRS agar with ammonium citrate replaced by ammonium sulphate (Hitchener et al., 1982).

A trained sensory panel consisting of seven or eight individuals evaluated the appearance, confinement odour and meat odour of samples that were stored up to 17 weeks. Panelists were trained for odour profiling according to the procedures outlined by Meilgaard et al. (1987). Initial screening of 18 potential panelists was done using a series of triangle tests to determine if they could detect differences in odour among meat samples that had been inoculated with spoilage organisms found on MA packaged pork. In addition, panelists were asked to describe the odour of spoiled meat samples. After 12 weeks of training, panelist performance was evaluated (Cross et al., 1978), and selection was based on proficiency to discriminate among samples and to consistently describe meat spoilage odours. Group discussion was used to facilitate selection of appropriate descriptors for assessing spoilage in MAP pork. Acceptability of appearance and odour was based on the panelists' projected willingness to purchase or consume the product. In the experimental procedure, samples were removed from storage at weekly or semiweekly intervals, coded and evaluated by each panelist. Samples were evaluated over a three day period. Appearance was evaluated on day 1, confinement odour on day 2 and meat odour on day 3. Sample attributes were scored using 15 cm unstructured line scales.

Appearance in the pack was evaluated under fluorescent lights with samples placed on a white background. The appearance of the samples was evaluated for intensity of red colour, amount of discoloration, amount of purge and acceptability of the meat and of the package contents. The panel was trained to evaluate red colour intensity using pork colour standards (Agriculture Canada, 1984). From the training sessions, a reference point for normal coloured pork was calculated as 7.8 cm based on the mean of panelists' scores

using pork that was packaged in air. At each evaluation panelists assessed the appearance of aerobically packaged and anaerobically packaged fresh pork samples that had been packaged 18 h prior to evaluation.

Confinement and meat odours were evaluated for overall odour intensity, strength of sweet, sour, dairy, sulphur, liver and floral notes and acceptability. Confinement odour was evaluated by withdrawing 5 ml of headspace gas through a silicone sampling port with a SGE gas tight syringe (Mandel Scientific, Guelph, Ontario) equipped with a button lock. Panelists expelled the gas from the syringe over a period of 4 s while taking short, shallow sniffs. For evaluation of meat odour, half of each cut was placed in a glass jar, exposed to air for 15 min before closing and kept on ice in a sensory panel room equipped with green lighting to mask colour differences. For each of the three replicates, panelists evaluated duplicate samples of each storage treatment. Panelists were given a fresh pork sample packaged 18 h prior to evaluation as a reference sample. A coded reference sample was included as a hidden control.

4.2.4. Experimental Design

Three replicates of the experiment were prepared over 3 consecutive weeks. Samples stored at -1.5 and 4°C were tested at semiweekly or weekly intervals for headspace gases and sensory and microbial assessment. Samples stored at 7°C were evaluated semiweekly for headspace gases and microbial changes and weekly for sensory changes. Microbial data were adjusted to colony forming units (CFU)/cm^2 and geometric means were calculated. Microbial and sensory data for appearance, overall odour intensity and acceptability were subjected to ANOVA using the GLM procedure (SAS Institute, 1989). Where appropriate, means were separated using Tukey's Studentized range test (Steele and Torrie, 1980). Intensity values for each odour characteristic were calculated as means across replicates. For a characteristic to be considered important for evaluation of

confinement and meat odour of a sample, 25% of the panelists had to report its presence (Mounts, 1979).

4.3. RESULTS

Samples selected for microbial and sensory evaluation had less than 0.7% O_2 present in the headspace gas. Storage temperatures used for this study had no effect on the ingress of O_2 or loss of CO_2 from the packs. Reference packs without meat had a greater gas exchange than packs containing meat. After 7 weeks of storage, reference packs stored at all three temperatures had 1 to 4% O_2 and 88 to 94% CO_2 in the headspace atmosphere. The mean pH of the pork samples at the time of packaging was 5.69 ± 0.09. After 1 week of storage, the mean pH had dropped to 5.52 ± 0.12 and no further changes in pH occurred during storage.

4.3.1. Microbial Changes

The microbial quality of the pork cuts was determined weekly up to 17 and 11 weeks for samples stored at -1.5 or -1.5/4°C, respectively; and at weekly intervals for 4 weeks and semiweekly up to 9.5 weeks for samples stored at -1.5/7°C. Growth of the adventitious bacterial flora on stored meat samples is shown in Figure 4.1. During the first 3 weeks of storage at -1.5°C counts remained at or below initial levels of *ca.* 10^3 CFU/cm^2. For samples held at -1.5°C for the duration of the experiment there was a 6 week lag phase, thereafter there was a generation time of *ca.* 5 days. Bacterial counts reached maximum population (10^7 CFU/cm^2) by about 17 weeks. For samples stored at -1.5/4°C, after 2 weeks at 4°C counts were significantly greater than counts on samples stored at -1.5°C. The generation time was *ca.* 3.5 days and counts reached 10^7 to 10^8 CFU/cm^2 within 11 weeks. Bacterial counts of samples stored at -1.5/ 7°C increased rapidly at 7°C with a generation time of *ca.* 1.4 days and reached maximum population after

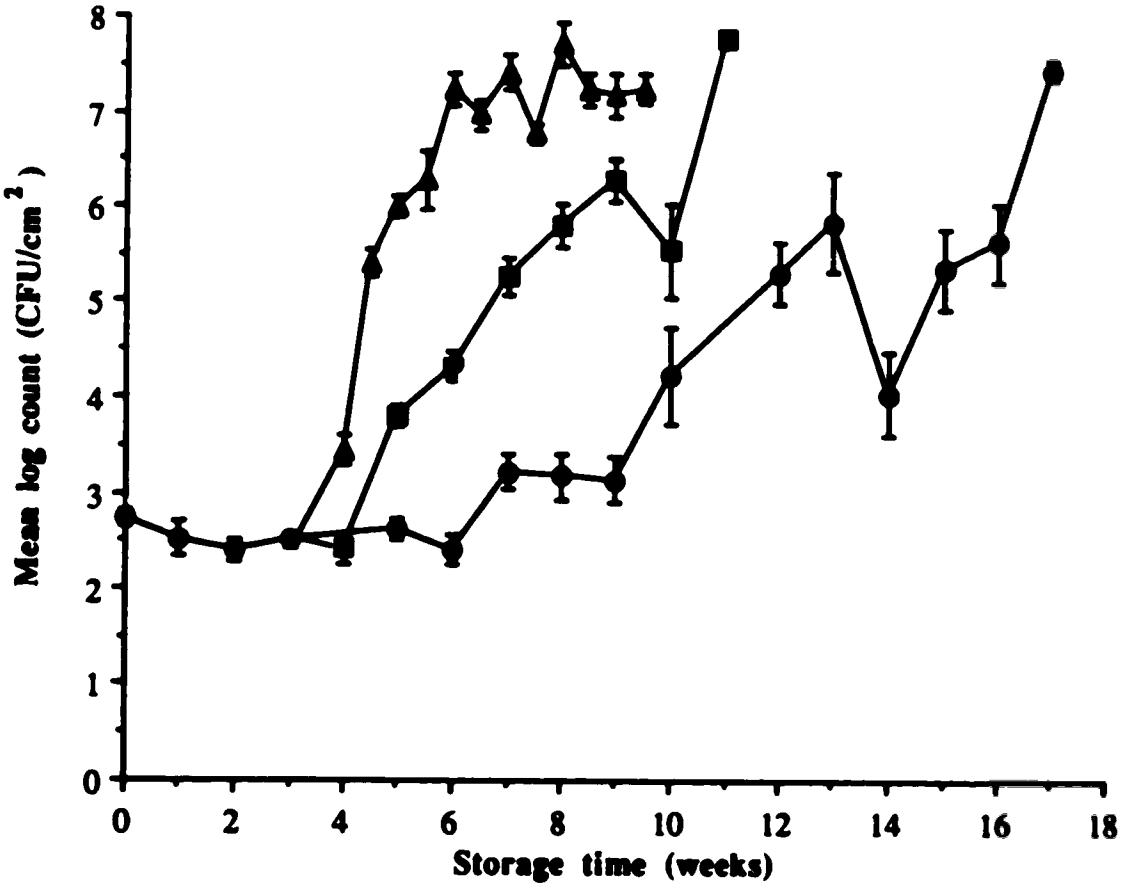

Figure 4.1. Growth of the adventitious microflora of retail cuts of pork stored in 100% carbon dioxide at -1.5 (●), -1.5/4 (■) and -1.5/7°C (▲) determined on PCA plates incubated at 25°C for 4 days. Error bars represent standard errors of mean log counts.

6 weeks of storage. Even after one week of storage at 7°C, counts were significantly greater ($P<0.01$) than counts for samples stored at -1.5°C. Significant differences between counts for samples stored at 4 or 7°C were found from weeks 4 to 8, inclusive, but by week 9 the counts at these two storage temperatures were similar. Counts obtained on APT and MRS agars (data not shown) were virtually identical to those obtained on PCA.

Presumptive counts of pseudomonads and *B. thermosphacta* (data not shown) on samples stored at -1.5 or 4°C remained at or below detectable levels ($10^{1.8}$ CFU/cm^2) throughout storage. The number of these bacteria on samples stored at 7°C remained below 10^3 CFU/cm^2 up to 6 weeks of storage. The maximum count for both pseudomonads and *B. thermosphacta* after 8 or 9 weeks of storage at -1.5/7°C was 10^4 CFU/cm^2. Storage temperature had a significant effect on the growth of coliform bacteria and Enterobacteriaceae (data not shown). Results for coliforms and Enterobacteriaceae isolated from samples stored at -1.5°C and from samples moved to 4 °C were below detectable levels ($10^{1.5}$ CFU/cm^2) except on 3 occasions (weeks 0, 1 and 10) when counts of less than 10^3 CFU/cm^2 were detected. Coliform and Enterobacteriaceae counts for samples transferred to 7°C increased after 1.5 weeks of storage at 7°C. Counts were quite variable between sampling times but gradually increased to 10^5 CFU/cm^2 by 9.5 weeks, representing less than 1% of the total population. Yeast and mould counts determined on PDA for samples stored at all temperatures remained below 10^3 CFU/cm^2.

The majority of the lactic acid bacteria strains isolated on PCA or MRS were homofermentative lactic acid bacteria and carnobacteria. Very few heterofermentative lactics or leuconostocs were detected. The largest proportion (57%) of the strains isolated from PCA for samples stored at -1.5°C were carnobacteria, whereas samples transferred to 4 or 7°C were dominated by homofermentative lactobacilli and homofermentative coccus-shaped lactics (65 and 75%, respectively). The majority of the strains isolated on MRS were homofermentative lactobacilli, regardless of storage temperature.

4.3.2. Sensory Analysis

Mean scores for intensity of red colour of the stored pork samples are shown in Table 4.1. Mean scores for the freshly packaged anaerobic reference samples ranged from 7.3 to 10.4 and for aerobically packaged reference samples, 7.1 to 11.1, where a higher score indicates a darker red colour. There were no significant differences between the two reference samples at each week of the study. Intensity of red colour decreased as a result of packaging in CO_2, and significant differences were detected from the second week of storage. However, the red colour intensity of the stored samples did not change significantly with storage time and differences among means across storage temperature were limited (see Table 4.1). Scores for discoloration (data not shown) of the cuts indicated that this was not a major factor determining storage life of MA pork samples even at 7°C. Up to six weeks of storage there was no difference ($P>0.05$) between reference and stored sample means. After 6 weeks of storage, a significant degree of discoloration ($P<0.05$) was detected for some samples, but results across storage time were erratic. The mean scores for acceptability of appearance for anaerobically packaged reference samples ranged from 1.5 to 5.9 and for aerobically packaged reference samples ranged from 1.1 to 3.8. The evaluation of the appearance of the stored meat samples indicated that at all times after one week of storage the stored samples were significantly less acceptable than the freshly prepared aerobically or anaerobically packaged reference sample (Table 4.2). Acceptability was judged by a trained panel, therefore these data can only be interpreted from the standpoint of an "expert" opinion regarding the quality of the samples. The rejection point for samples was set at 7.5 ± SE. Based on this interpretation of the data, the first incidence of rejection based on appearance of samples held at -1.5°C and those transferred to 4°C or 7°C occurred at 7, 5 and 4 weeks of storage, respectively. When panelists were asked to score the acceptability of the package contents (meat and purge; data not shown) mean panelist scores indicated that the samples were much less acceptable than when they were evaluated for acceptability of the meat alone.

Table 4.1. Mean scores[1] and standard errors for red colour intensity of pork samples stored for up to 17 weeks at different temperatures.

Storage time (weeks)	-1.5°C	-1.5/4°C	-1.5/7°C	Anaerobic Reference	Aerobic Reference	SEM[2]
1	6.3	6.3	6.3	7.8	7.7	0.24
2	5.2^{b}	5.2^{b}	5.2^{b}	7.8^{a}	8.2^{a}	0.24**
3	6.4^{b}	6.4^{b}	6.4^{b}	8.0^{a}	8.4^{a}	0.23**
4	7.7^{b}	6.0^{c}	4.4^{d}	9.6^{a}	8.8^{a}	0.19***
5	-	5.2^{b}	4.7^{b}	9.1^{a}	8.9^{a}	0.21***
6	4.8^{b}	5.6^{b}	5.4^{b}	8.7^{a}	7.9^{a}	0.25***
7	6.0^{bc}	6.3^{b}	4.8^{c}	8.4^{a}	8.0^{a}	0.24**
8	6.3^{b}	5.3^{b}	5.5^{b}	10.0^{a}	10.1^{a}	0.21***
9	5.1^{b}	6.6^{b}		10.4^{a}	11.1^{a}	0.21***
10	7.8^{b}	6.5^{b}		10.4^{a}	11.1^{a}	0.21***
11	-	6.0^{b}		8.3^{a}	7.8^{a}	0.46*
12	6.1			7.3	7.1	0.45
13	6.6			7.3	7.1	0.60
14	7.1			8.1	7.5	0.30
15	8.6			9.2	8.0	0.43
16	5.9			9.2	8.0	0.49
17	8.2			8.4	9.2	0.8

[1] Mean scores are averages of 48 scores (8 panelists, 3 replications, duplicate samples) determined using a 15 cm line scale where 0=very pale, 15=very dark.

[2] Standard error of the mean.

^{a,b,c} Means in the same row sharing a common superscript are not significantly different at $P>0.05$.

*,**,*** Significant differences among storage treatments at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

- Not tested.

Table 4.2. Mean scores[1] and standard errors for acceptability of appearance of pork samples in retail packs stored at -1.5°C or at -1.5°C for 3 weeks and transferred to 4 or 7°C.

Storage time (weeks)	-1.5°C	-1.5/4°C	-1.5/7°C	Anaerobic Reference	Aerobic Reference	SEM[2]
1	5.6	5.6	5.6	5.9	3.8	0.41
2	6.9^{b}	6.9^{b}	6.9^{b}	2.7^{a}	1.9^{a}	0.30^{***}
3	6.0^{b}	6.0^{b}	6.0^{b}	2.8^{a}	1.8^{a}	0.34^{***}
4	4.2^{ab}	5.7^{bc}	7.5^{c}	1.5^{a}	1.3^{a}	0.33^{***}
5	-	7.4^{b}	9.3^{b}	1.8^{a}	1.1^{a}	0.39^{***}
6	7.0^{b}	6.5^{b}	6.8^{b}	2.0^{a}	1.2^{a}	0.34^{**}
7	8.8^{b}	6.7^{b}	9.0^{b}	2.0^{a}	1.4^{a}	0.34^{***}
8	6.5^{b}	8.8^{bc}	9.5^{c}	2.0^{a}	2.5^{a}	0.31^{***}
9	8.8b	6.3^{b}		2.3^{a}	3.3^{a}	0.35^{***}
10	7.0^{b}	7.9^{b}		2.3^{a}	3.3^{a}	0.42^{***}
11	-	5.4^{b}		2.0^{a}	2.4^{a}	0.60^{***}
12	8.0^{b}			5.2^{ab}	2.0^{a}	0.60^{***}
13	7.1^{b}			5.2^{ab}	2.0^{a}	0.50^{***}
14	8.1^{b}			4.2^{a}	2.4^{a}	0.47^{***}
15	7.1^{b}			2.8^{a}	2.8^{a}	0.78^{***}
16	8.5^{b}			2.8a	2.8^{a}	0.70^{*}
17	8.4^{b}			2.8^{a}	2.8^{a}	0.85^{*}

[1] Mean scores are averages of 48 scores (8 panelists, 3 replications, duplicate samples) determined using a 15 cm line scale where 0=very acceptable, 15=very unacceptable.

[2] Standard error of the mean.

a,b,c Means in the same row sharing a common superscript are not significantly different at $P>0.05$.

*,**,*** Significant differences among storage treatments at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

- Not tested.

Changes in the intensity of the confinement odour are shown in Figure 4.2. There was no difference ($P>0.05$) in confinement odour between the anaerobically packaged, freshly prepared reference sample and samples stored for the first 3 weeks of storage at -1.5°C. For samples stored at -1.5/7°C, no significant change in the intensity of the confinement odour was noted before week 7. No change in confinement odour intensity was detected in samples stored at -1.5/4°C up to 8 weeks of storage; however, at 9 weeks there was a large increase in odour intensity. Intensity of confinement odour of samples held at -1.5°C remained similar to the reference sample until 12 weeks of storage and did not increase significantly throughout the 17 weeks of storage. The acceptability of the confinement odour (data not shown) followed the same profile as intensity of confinement odour. Based on a rejection point at 7.5 ± SE, samples held at -1.5°C were not rejected during 17 weeks of storage; however, for samples transferred to 4 and 7°C the first incidence of rejection of the samples occurred at 9 and 8 weeks, respectively.

The overall intensity of the meat odour (Figure 4.3) was generally stronger than the confinement odour. The first incidence at which the meat odour of the samples stored at -1.5/4 or -1.5/7°C was stronger ($P<0.05$) than the reference or samples held at -1.5°C occurred at 7 or 6 weeks of storage, respectively. After 7 weeks of storage the meat odour of samples stored at -1.5/4°C was quite variable but increased as storage time was extended. The data in Figure 4.4 show the change in the acceptability of the meat odour. Based on a rejection point of 7.5 ± SE, samples held at -1.5°C for 17 weeks were not rejected on the basis of meat odour. For samples stored at -1.5/4°C, the first incidence of rejection occurred at 9 weeks of storage; however, at 10 weeks samples were still acceptable. The meat odour of samples moved to 7°C storage became unacceptable after 6 weeks of storage.

The profile data shown in Figures 4.5 and 4.6 illustrate that confinement and meat odours are similar; yet careful examination of the odour profiles for -1.5/4°C and -1.5/7°C

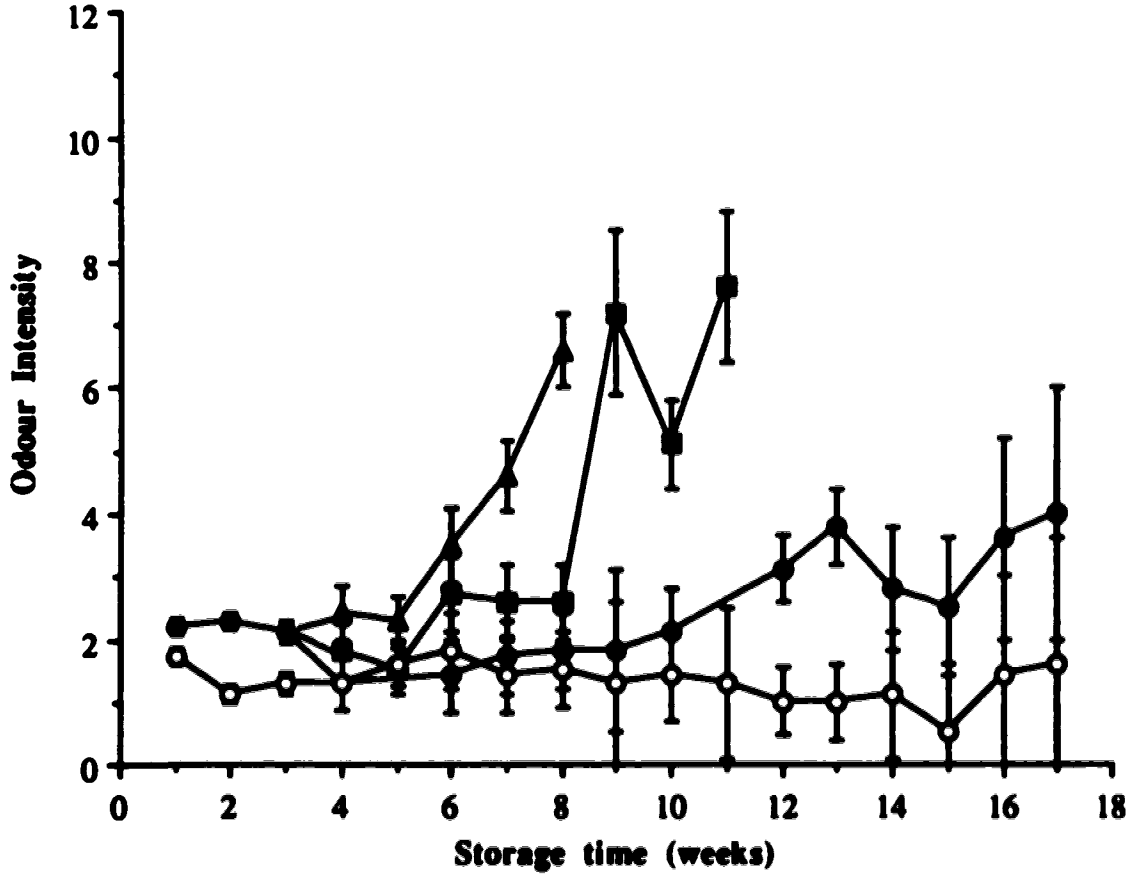

Figure 4.2. Intensity of confinement odour (0=none, 15=very strong) of a freshly prepared, anaerobically packaged reference sample (○) and of retail cuts of pork stored in 100% carbon dioxide at -1.5°C for 3 weeks and transferred to -1.5 °C (●), 4°C (■) or 7°C (▲). Error bars represent standard errors of mean scores (averages of 48 individual scores) for intensity of confinement odour.

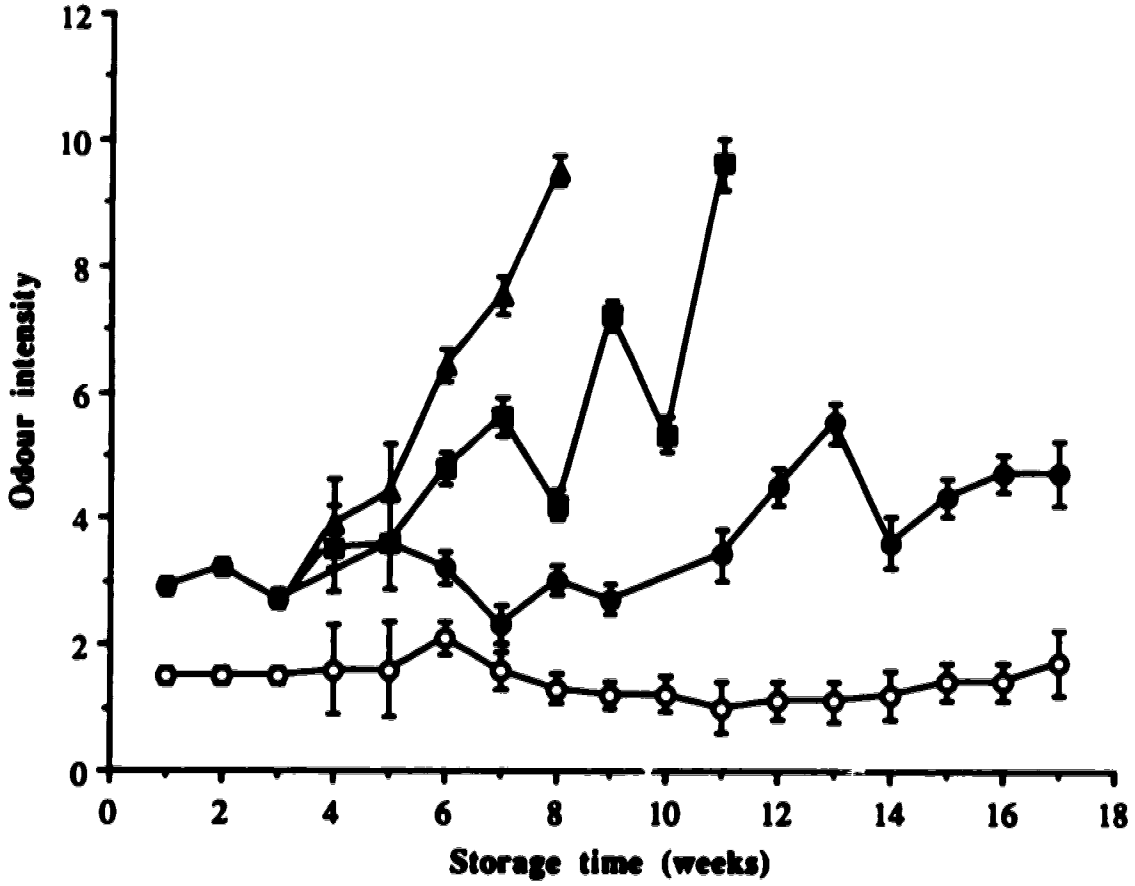

Figure 4.3. Intensity of meat odour (0=none, 15=very strong) of a freshly prepared, anaerobically packaged reference sample (○) and of retail cuts of pork stored in 100% carbon dioxide at -1.5°C for 3 weeks and transferred to -1.5 °C (●), 4°C (■) or 7°C (▲). Error bars represent standard errors of mean scores (averages of 48 individual scores) for intensity of meat odour.

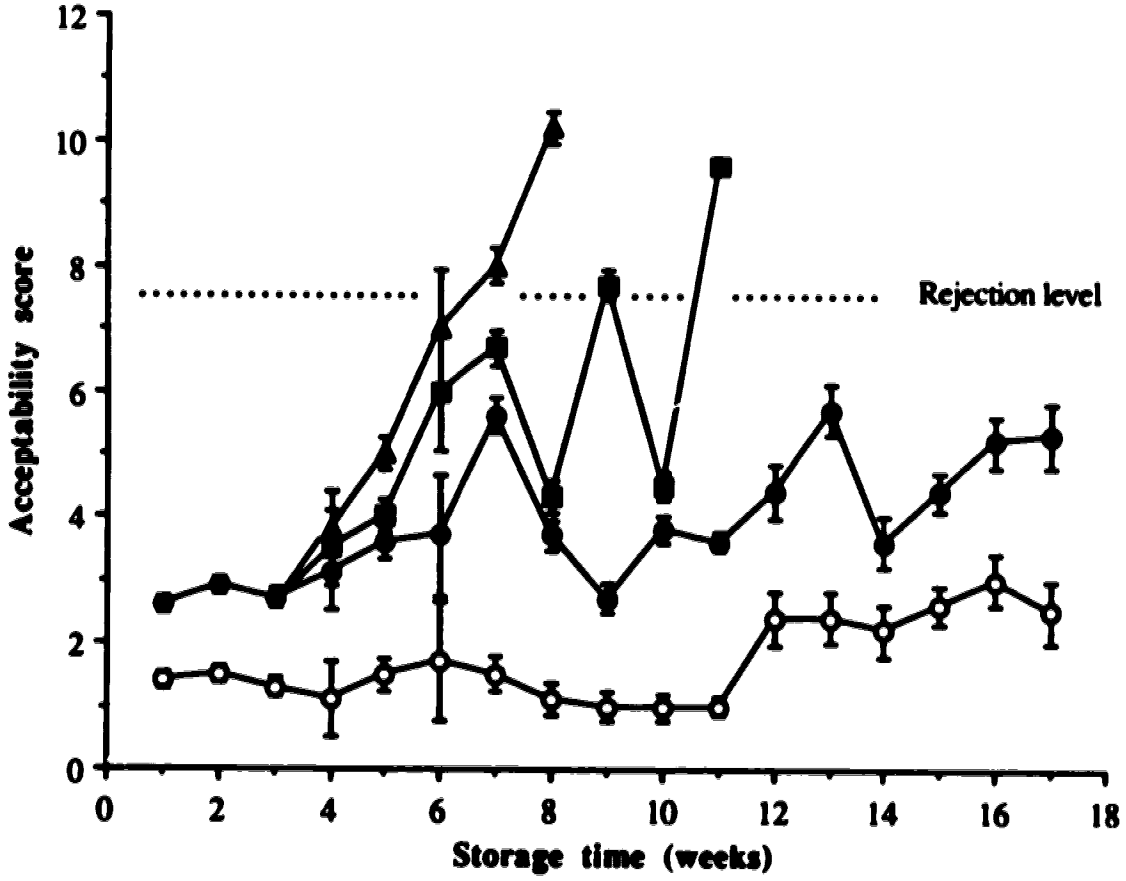

Figure 4.4. Acceptability scores of meat odour (0=very acceptable, 15=very unacceptable) of a freshly prepared, anaerobically packaged reference sample (O) and of retail cuts of pork stored in 100% carbon dioxide at -1.5°C for 3 weeks and transferred to -1.5 °C (●), 4°C (■) or 7°C (▲). Error bars represent standard errors of mean scores (averages of 48 individual scores) for acceptability of meat odour.

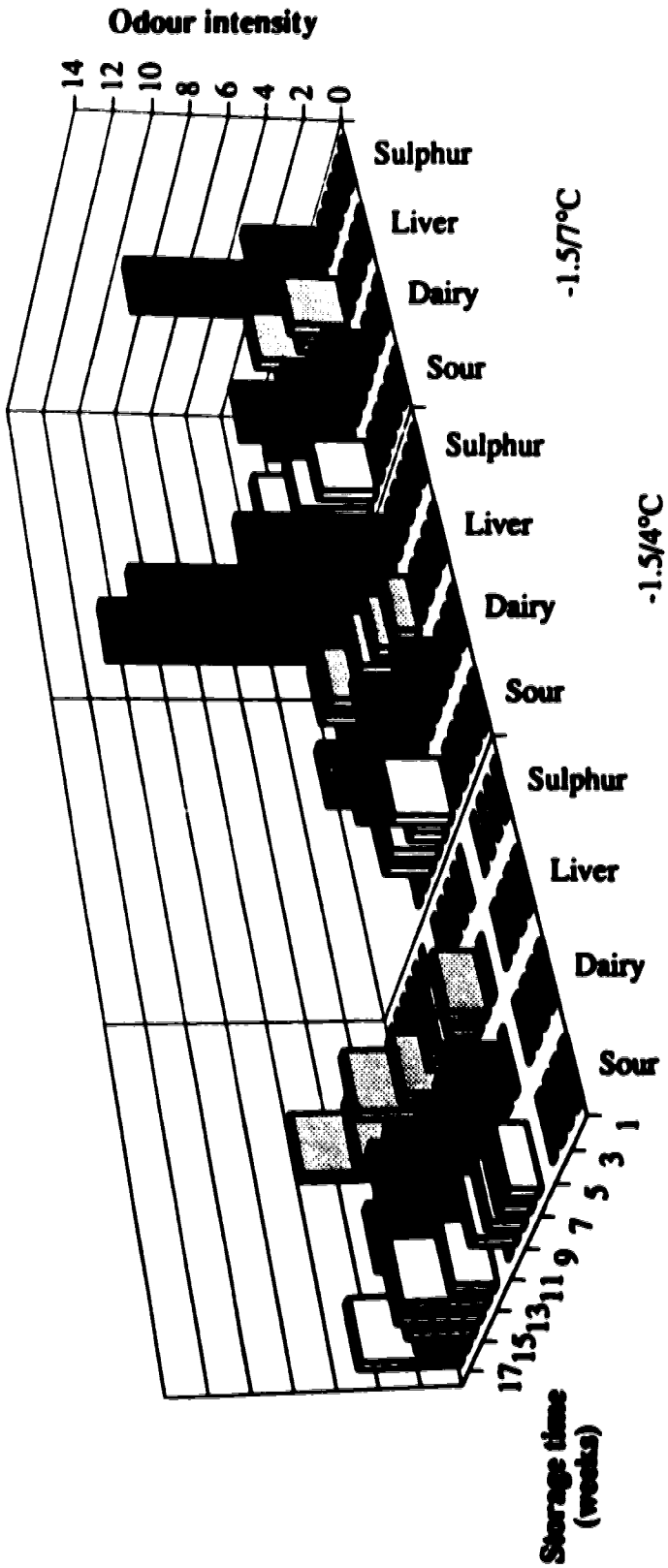

Figure 4.5. Histogram representing development of confinement odour notes (0=none, 15=very strong) of retail cuts of pork stored in 100% carbon dioxide at -1.5°C for 3 weeks and then transferred to -1.5, 4 or 7°C.

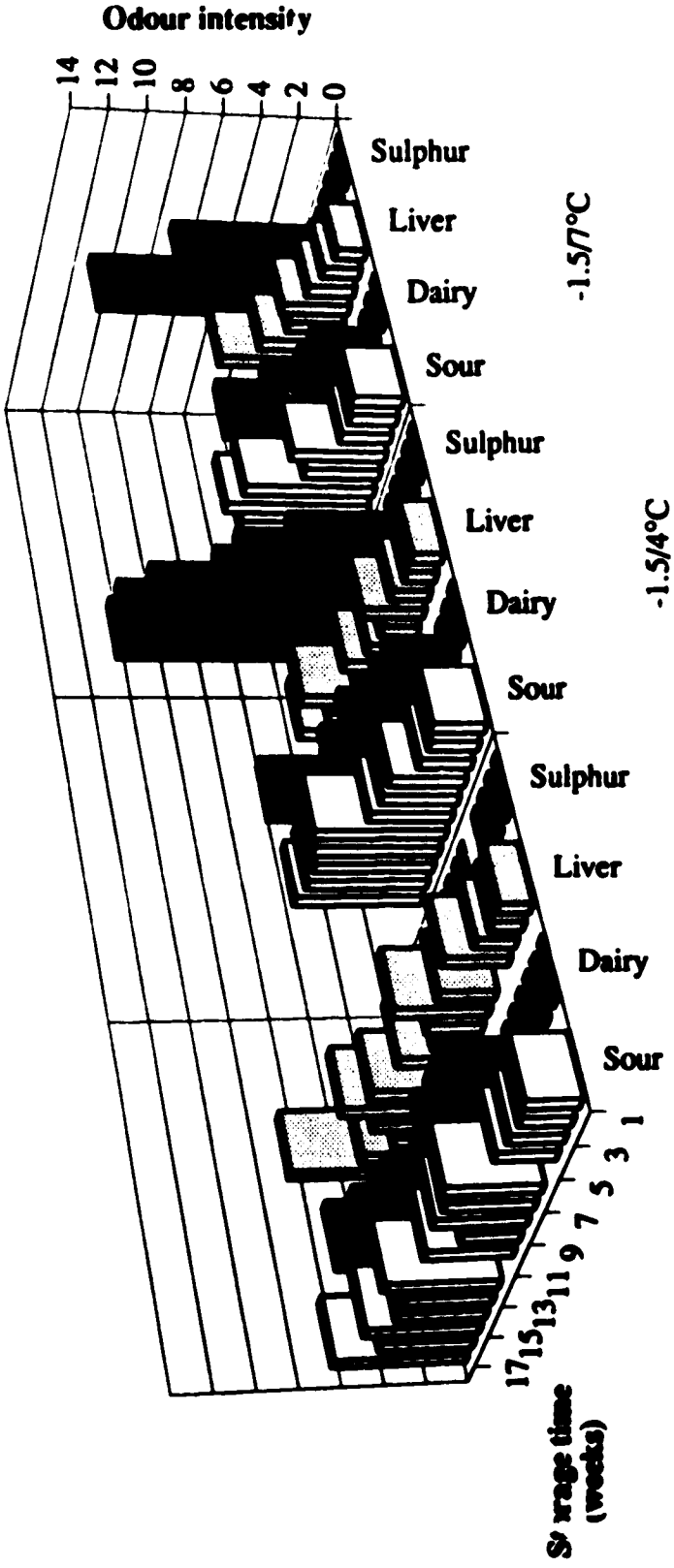

Figure 4.6. Histogram representing development of meat odour notes (0=none, 15=very strong) of retail cuts of pork stored in 100% carbon dioxide at -1.5°C for 3 weeks and then transferred to -1.5, 4 or 7°C.

indicate that the sour, dairy and liver notes for meat odour may be more important than in confinement odour. Nonetheless, sulphur is the principal odour note in both cases; yet for samples stored at 4 and 7°C, 20% of samples had a sulphur note in confinement odour that was not detected in the meat odour. The meat odour of these samples was dominated by sour or liver notes. Sulphur odour was not detected in meat stored at -1.5°C throughout the experiment. The intensity of odour notes in the confinement and meat odours for hidden control samples (data not shown) was less than 1 at all times throughout the experiment.

4.4. DISCUSSION

The microflora of the stored pork samples developed as expected. The initial microbial load of the meat was between 10^2 and 10^3 CFU/cm^2 which is similar to bacterial numbers found on pork loins obtained from the same abattoir in a previous study (McMullen and Stiles, 1991). Lactic acid bacteria dominated the population on all samples throughout storage. The type of lactic acid bacteria isolated from the samples was influenced by storage temperature and growth medium. When PCA was used for isolation, carnobacteria were the prevalent bacteria on samples held at -1.5°C; whereas homofermentative lactics were the principal lactics isolated from samples after transfer to 4 or 7°C. The differences in lactic populations at different storage temperatures were not apparent in our previous study (McMullen and Stiles, 1993) or the work of Blickstad and Molin (1983). When MRS was used as the growth medium, homofermentative lactics were the prevalent species isolated regardless of storage temperature. This confirms the results of our previous study on MAP retail pork cuts (McMullen and Stiles, 1993) in which we reported that growth medium has a selective effect on the type of lactics detected.

Both *Pseudomonas* sp. and *B. thermosphacta* counts remained low throughout storage at -1.5 and 4°C which is to be expected because packaging in atmospheres with

high levels of CO_2 and low O_2 concentrations inhibits the growth of these organisms (Blickstad and Molin, 1983; Gill and Harrision, 1989; McMullen and Stiles, 1991). The absence of Enterobacteriaceae and coliforms on samples stored at -1.5 or -1.5/4°C was also as expected because these organisms do not generally grow on anaerobically packaged meat of normal pH stored at these temperatures (Grau, 1981). An increase in storage temperature to 7°C allowed these organisms to grow on the meats although they represented only a small proportion of the total microbial flora on the samples.

It is well documented that storage temperature is critical for the extension of storage life of fresh meat stored in MAP (Blickstad and Molin, 1983; Gill and Harrison, 1989; McMullen and Stiles, 1991). When meat is moved through the distribution chain to the consumer it is expected that it will be subjected to suboptimal storage temperatures. The present study demonstrated that increased storage temperature after initial, low temperature storage had an expected detrimental effect on the microbial and sensory quality of MAP pork cuts. An increase in storage temperature from -1.5°C to 4°C had a marked effect on the confinement and meat odour of the samples, resulting in a 9 week decrease in storage life based on the development of a confinement odour. At the time that samples stored at -1.5/4°C were rejected on the basis of confinement or meat odour, the microbial load was 10^6 CFU/cm^2, which was below the maximum level achieved. Storage at 4°C is considered to be an acceptable temperature for retail storage of meat but retail storage temperatures as high as 10 to 12°C have been reported (Greer et al., 1993; Sumner et al., 1981). In our study transfer of meat to 7°C resulted in the loss of an additional 2 weeks of storage life compared with meat transferred to 4°C. The microbial load was at a maximum when samples transferred to 7°C were rejected because of confinement or meat odour.

Large amounts of purge accumulated in the packages. This generally detracted from the appearance of the meat in the packs. This problem could probably be alleviated by incorporation of an adsorbant pad in the package. Accumulation of purge has been

reported by other researchers (Jeremiah et al., 1992) and has been implicated as the cause of discoloration of MAP pork (Gill and Harrison, 1989). In this study, discoloration was not identified as a problem, but the appearance of the samples was limited by the pale colour of the pork cuts. Based on appearance data, pork cuts remained acceptable for 1 to 2 weeks after transfer to 4 or 7°C. This should be sufficient time for product to move through the retail marketplace. However, this does not allow for extended storage at temperatures of 4°C or above, thus temperature control at the wholesale level (including transportation) is critical to the success of MAP retail cuts of pork on the retail market.

Vacuum packaged fresh beef has a confinement odour described as sour, dairy, cheesy or sulphur which dissipates rapidly when the packs are opened (Egan and Shay, 1982; Edwards et al., 1985). Gill and Molin (1991) stated that confinement odour is not a problem in controlled atmosphere packaged fresh meats. However, this is a potential problem of MAP packaged meats intended for retail sale that could become a barrier to consumer acceptance. In this study, confinement odour during meat storage was detected, especially after transfer to 4 or 7°C.

For the majority of our samples, the confinement odour profile was similar to that of the meat odour. However, as many as 20% of the samples transferred to 4 or 7°C developed a confinement odour that resulted in rejection of the pack due to sulphur odour notes that dissipated and were not detected as part of the meat odour. Edwards et al. (1985) reported sulphur odours in vacuum packaged beef stored at 5°C when bacterial counts reached 10^7 CFU/cm^2. At the time that our panel detected sulphur odours in the headspace gases of samples stored at 4 or 7°C the total counts were *ca.* 10^4 or 10^7 CFU/cm^2, respectively. The detection of sulphur odours when total counts were only at 10^4 CFU/cm^2 suggests that glucose levels in the meat may have been depleted (Egan et al., 1989) and(or) that a sulphur-producing lactic acid bacterium was present as part of the prevalent microflora. In the selection of pork loins for this study, many were rejected for

use because they exhibited PSE (pale, soft and exudative) characteristics. It is possible that although the pH of the loins that we selected was within the range for normal muscle, some may have been borderline PSE that was not detected by pH or physical appearance. However, PSE pork muscle is extremely sensitive to discoloration (Greer and Murray, 1988), and discoloration was not a problem in this study, thus the presence of sulphur-producing lactic acid bacteria may have been responsible for these off-odours. The presence of sulphur-producing lactic acid bacteria as part of the natural, adventitious lactic microflora of MAP meat cannot be controlled; however, their growth could possibly be controlled by addition of preservative strains of lactic acid bacteria as suggested by Stiles and Hastings (1991). Sulphur-producing lactic acid bacteria have been isolated from MAP meats (Egan et al., 1989; Hitchener et al., 1982; Schillinger and Lücke, 1987) and we isolated sulphur-producing homofermentative lactic acid bacteria from pork stored in MAP that originated from the same abbatoir as the meat used in the current study (McMullen and Stiles, unpublished data). If the absence of sulphur odours at any time during storage at -1.5°C was attributable to inhibition of sulphur-producing strains at this temperature, the importance of storage temperature control throughout the distribution chain is implicit for the successful application of MAP to retail-ready pork cuts.

The criterion used for determining the storage life of any food product should be the quality attribute that exhibits the fastest deterioration with time. In this study, the storage life of pork cuts packaged in 100% CO_2 is limited by the deterioration of appearance. Confinement and meat odours were detected 2 to 4 weeks after samples were rejected on the basis of appearance. The validity of evaluating acceptability criteria with a trained sensory panel can be questioned. However, at some point a decision regarding saleability has to be made by the meat retailer, not the consumer. The study indicated that export of retail-ready pork cuts to distant markets with a three-week time for delivery to market (-1.5°C) can be achieved with 1 to 2 week marketing time on the retail market (4 to 7°C).

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5. Volatile Compounds and Odour Profiles of Pork Inoculated with Lactic Acid Bacteria and Stored Under Modified Atmosphere

5.1. INTRODUCTION

The extended storage of fresh retail-ready meat cuts that can be achieved by packaging with elevated levels of CO_2 and very low levels of O_2 may be limited by the development of a confinement odour, especially when meat is stored at temperatures greater than 4°C (McMullen and Stiles, 1993b). The preservative effect of lactic acid bacteria is partially responsible for the extension of storage life when meat is packaged anaerobically compared with meat packaged in air. However, lactic acid bacteria can cause spoilage of anaerobically packaged fresh meat (Egan and Shay, 1982; Schillinger and Lücke, 1987). In a previous study of pork cuts stored in 100% CO_2, we found that at storage temperatures of 4 and 7°C, a confinement odour developed rapidly in the pack and resulted in rejection of the samples (McMullen and Stiles, 1993b). The adventitious population found on modified atmosphere packaged (MAP) meat consists of many species of lactic acid bacteria (McMullen and Stiles, 1993a), including some that cause rapid spoilage of meat stored at chill temperatures in anaerobic environments (Shay and Egan, 1981). Controlling the growth of these lactic acid bacteria may control development of confinement odour in MAP meats.

Bacteriocin-producing lactic acid bacteria could be added to MAP fresh meats to provide an innovative method for controlling the growth of an adventitious microbial population. Many bacteriocins produced by lactic acid bacteria are antagonistic to a wide range of other lactic acid bacteria. A number of bacteriocin-producing lactic acid bacteria have been isolated from meats, but their suitability for use to control the spoilage of MAP meats has not been studied.

Under aerobic storage conditions meat is considered spoiled when the microbial population reaches 10^7 CFU/g or cm^2 of meat. In contrast, under anaerobic conditions spoilage occurs some time after the microbial population reaches maximum numbers (10^7 CFU/cm^2), as a result microbial counts bear no relationship to spoilage. Currently, sensory analysis is the only means of determining the storage life of MAP fresh meat.

Researchers are seeking a rapid and objective means of monitoring meat spoilage, but no suitable analysis has been discovered that correlates with sensory data and the end of storage life. Static headspace analysis of the volatile compounds contained in the meat package could provide a rapid measure of spoilage but it is relatively insensitive (Maga, 1990). However, concentrating static headspace samples would provide a more sensitive approach for monitoring the development of headspace volatile compounds. Confinement odour dissipates rapidly when the packages are opened and the meat does not retain the odour (Egan and Shay, 1982; Edwards et al., 1985). Comparing the profiles of volatile compounds in the confinement gases with those of the meat after it is removed from the pack may prove useful in developing an objective measure of spoilage.

In this study we report the effect of three bacteriocin-producing lactic acid bacteria, *Leuconostoc gelidum* UAL187, *Carnobacterium piscicola* LV17 and *Lactobacillus sake* Lb706, on the development of confinement and meat odours in MAP pork cuts stored at 4°C for 10 weeks. Concentration and GC/MS analysis of the static headspace volatiles is evaluated as a nondestructive and rapid method to evaluate spoilage.

5.2. MATERIALS AND METHODS

5.2.1. Preparation of Meat Samples

Hogs raised on the University of Alberta farm were slaughtered at a federally inspected abattoir, stored at -1°C, and at 24 h postmortem the *longissimus dorsi* muscles were excised and visually examined for PSE (pale, soft and exudative) characteristics. Muscle pH was determined using a surface electrode on the surface of the lean tissue. Muscles with a pH between 5.6 and 5.9 that did not show signs of PSE were chosen for use in the study. The selected pork loins were packed on ice in coolers for transportation to the laboratory where they were held on ice in a cooler at 4°C.

For use in the study, each loin was immersed in 95% ethanol, suspended on stainless steel meat hooks and flamed. This was repeated twice for each loin. The flamed loin was placed in a laminar flow hood and the outer layer of charred meat was aseptically removed and the sterile inner core was cut into 1 cm slices. To ensure that a consistent surface area of 700 cm^2 of meat was used in each package, the slices were aseptically placed in sterile 15 x 150 cm petri dishes at random until the bottom surface of each petri dish was covered with meat. The slices of meat in each petri dish were treated as one sample for inoculation and packaging.

5.2.2. Preparation of Inoculum

Cultures of *Carnobacterium piscicola* LV17 (B.G. Shaw, Institute of Food Research, Langford, Bristol, UK), *Leuconostoc gelidum* UAL187 (University of Alberta Food Microbiology Lactic Acid Bacteria Culture Collection) and *Lactobacillus sake* Lb706 (U. Schillinger, Institute for Hygiene and Toxicology, Karlsruhe, Germany) were stored at -70°C in APT containing 20% glycerol and subcultured twice in APT broth (Difco Laboratories, Detroit, MI) for use as inocula. Cells were harvested by centrifugation

(15,000 x *g* for 3 min), washed and resuspended in sterile 0.85% NaCl to give 500 ml of bacterial suspension with about 10^5 CFU/ml. Slices of meat for each sample were immersed in the culture suspension for 30 s with gentle agitation. Slices were dried on sterile racks for 1 min. Control samples were prepared in 0.85% saline.

5.2.3. Packaging

Two months prior to packaging of the meat samples, a silicone (Macklanburg-Duncan, Oklahoma City) sampling port was applied to each package. Packages containing a DriLoc50 pad (Price Daxion, Edmonton) were sterilized by exposure to ethylene oxide (12% ethylene oxide in freon, 55 kPa; 40-55% RH) for 2 h at 55°C. Meat slices were aseptically placed on the DriLoc50 meat pad in a sterilized 21 x 22 cm Opalen x 75HL pouch (15 μ oriented polyamide and ethylene vinyl alcohol: 60 μ low and linear low density polyethylene; Walki-Pak, Valkeakoski, Finland). The manufacturers' reported oxygen transmission rate of the film was <1 cc/m^2/24 h at 23°C, 0% relative humidity under 1 atmosphere pressure. Packaging was done as described by McMullen and Stiles (1993b), and each of the packs was filled with 700 ml of CO_2 and had a residual oxygen level below 500 ppm. Bags with a DriLoc50 pad and 700 ml CO_2 were prepared without meat and stored with the meat samples. Meat samples were stored at 4°C for up to 10 weeks. Every two weeks, samples were removed from storage, tested for gas composition and those with O_2 levels below 600 ppm were used for microbial, sensory and volatile compound analyses.

5.2.4. Gas Analysis

Headspace gases (CO_2, O_2 and N_2) were analyzed using a 27.5 m x 0.53 mm fused silica PoraPlot™ Q capillary column (Chrompack International, The Netherlands) in sequence with a 10 m x 0.53 mm fused silica molecular sieve 5Å capillary column (Chrompack International) on a gas chromatograph (HP5890; Hewlett-Packard Ltd.,

Edmonton, Alberta). Helium was used as the carrier gas at a flow rate of 5 ml/min. Analyses were done isothermally at 40°C. The temperature of the thermal conductivity detector was 150°C.

5.2.5. Microbial Analysis

Microbial counts were determined when samples were opened for sensory and GC/MS analysis of the volatile compounds from the meat. Two pieces of meat were aseptically removed from each package, placed separately in sterile Stomacher bags and the outline of each cut was traced on aluminium foil to determine the surface area (McMullen and Stiles, 1991). A sterile peptone water blank (99 ml; 0.1%) was added to each bag and the samples were blended for 1 min in a Colworth 400 Stomacher (Seward and Co., London, England). Serial dilutions were prepared with sterile 0.1% peptone water and either 0.1 ml or 20 µl amounts of appropriate dilutions were inoculated onto prepoured plates of Plate Count agar (PCA; Difco), All Purpose Tween agar (APT; Difco) and Lactobacilli MRS agar (MRS; Difco) for determination of colony forming units (CFU). The pH of the blended meat slurry was determined using an Extech pH meter (Model 671; Extech, Boston, MA).

5.2.6. Sensory Analysis

Analysis of confinement and meat odour intensity was done as described by McMullen and Stiles (1993b) using an eight-member panel trained for descriptive analysis. A piece of meat was cut to 25 cm^2 for sensory analysis of meat volatile compounds. Panelists also evaluated samples for appearance (red colour intensity, discoloration and acceptability) and were asked to indicate reasons for acceptability scores.

5.2.7. Analysis of Headspace Volatile Compounds

Samples of volatile compounds in the headspace gas (confinement volatile compounds) were collected using static headspace sampling (Maga, 1990) by withdrawing 10 ml of headspace gas from the pack with a 20 ml gas-tight syringe equipped with a button lock (SGE; Mandel Scientific, Guelph, Ont.). The sample was concentrated by injecting into a sampling loop fitted on a Tekmar 5010 thermal desorber (Tekmar Corp., Cincinnati, OH). The sample was flushed with helium for 5 min at a flow rate of 20 ml/min onto a 5/8" trap packed with 2 g of 60-80 mesh Tenax TA (Chromatographic Specialties Inc., Brockville, Ont.) held at 40°C.

Headspace volatiles were desorbed from the Tenax TA trap by heating at 190°C for 8 min and collecting the volatiles on a glass bead internal trap cooled to -120°C with liquid nitrogen. Volatiles were collected on a DB-5MS column (30 m x 0.25 mm ID; 0.25 μm film thickness; Chromatographic Specialties Inc.) held in a Tekmar cryofocusing unit at -120°C by transferring for 1.8 min through a heated silica line (210°C). Injection was completed by flash heating the column to 210°C for 0.5 min. The Tenax TA trap was reconditioned between runs by baking at 225°C for 15 min (He flow rate: 20 ml/min).

Purge and trap collection of volatile compounds from the meat was done by placing a 25 cm^2 portion of meat in the bottom of a 500 ml glass jar which was exposed to air for 10 min prior to fitting with a glass headspace sampling device which allowed He to flow over the sample. The sampling device was connected to the sampling loop on the Tekmar thermal desorber. Meat samples were held on ice and equilibrated for 40 min prior to purging with He (20 ml/min) for 20 min onto the Tenax TA trap. Meat volatiles were desorbed as described for the confinement volatiles.

The He carrier gas was purified by filtering through a hydrocarbon trap (SGE; Tekmar Corp.) prior to purging the volatiles. The volatiles were separated and identified

using a gas chromatograph (HP5890 Series II; Hewlett-Packard Ltd.) equipped with a mass selective detector (HP5971A; Hewlett-Packard Ltd.). The column was interfaced with the mass selective detector through a direct inlet. The operating conditions for the gas chromatograph were: initial temperature, 40°C for 2 min; a temperature increase of 5°C/min to 130°C; and a temperature increase of 20°C/min to a final temperature of 175°C for 2 min. Mass selective detector conditions were: 70 eV electron impact; mass range 20-300 *m/z* at 18 scans/s. Artifact formation was detected by making blank runs with and without the glass headspace sampling device. Volatiles eluted from the Tenax TA trap during blank runs were disregarded in sample analyses. Identification of volatile components was achieved by background subtraction and comparison of observed mass spectra with the spectra in the data base library (NIST/EPA/MSDC 54k), *de novo* interpretation of spectra and, where possible, by comparison with spectra of authentic compounds.

5.2.8. Experimental

Samples for three replicates of the study were prepared on 3 separate occasions. Geometric means were calculated for microbial data. Microbial, pH and sensory data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 1989). Where appropriate, means were separated using Student Newman Keul's test (Steele and Torrie, 1980). Intensity values for each odour characteristic were calculated as means across replicates. For a characteristic to be considered important for evaluation of confinement and meat odour, 25% of the panelists had to report its presence (Mounts, 1979).

5.3. RESULTS

The mean pH of the samples at the time of packaging was 5.65 ± 0.08. After storage for 2 weeks at 4°C the mean pH had dropped to 5.56 ± 0.13 and no further changes in meat pH were detected during storage.

5.3.1. Microbial Analysis

Initial microbial loads for the inoculated samples were between 10^4 and 10^5 CFU/cm^2 and control samples had counts below detectable limits ($10^{1.7}$ CFU/cm^2). Samples inoculated with *Leuc. gelidum* UAL187 or *C. piscicola* LV17 reached maximum populations of 10^7 and 10^6 CFU/cm^2, respectively, after 4 weeks of storage. A maximum population of 10^7 CFU/cm^2 was reached at 2 weeks of storage on samples inoculated with *Lact. sake* Lb706. After 2 weeks of storage, only two of the control samples had counts below detectable levels. The microbial counts for the control samples were extremely variable and some of these samples spoiled at a faster rate than the inoculated samples. Because the organisms growing on the control samples were not characterized and the microbial population of these samples may not have been the adventitious microflora, the sensory and GC/MS data for the control samples will not be discussed.

5.3.2 Sensory Analysis.

Changes in red colour intensity of the pork loin cuts stored at 4°C in MAP are shown in Figure 5.1. At week 0, there was no difference ($P>0.05$) in red colour intensity between the freshly prepared reference samples and the experimental samples. However, throughout the remainder of the experiment, the colour of all of the samples stored in 100% CO_2 at 4°C was significantly paler than the colour of the fresh reference samples. Scores for acceptability of appearance of samples stored in 100% CO_2 were similar to those for the anaerobically packaged reference sample up to 8 weeks of storage. At 10 weeks of storage, the acceptability of the inoculated samples stored at 4°C was significantly poorer than for either of the reference samples. When panelists scored samples as unacceptable the reason given was their pale colour. Scores for discoloration of the samples stored for up to 10 weeks were similar to the scores for the reference samples.

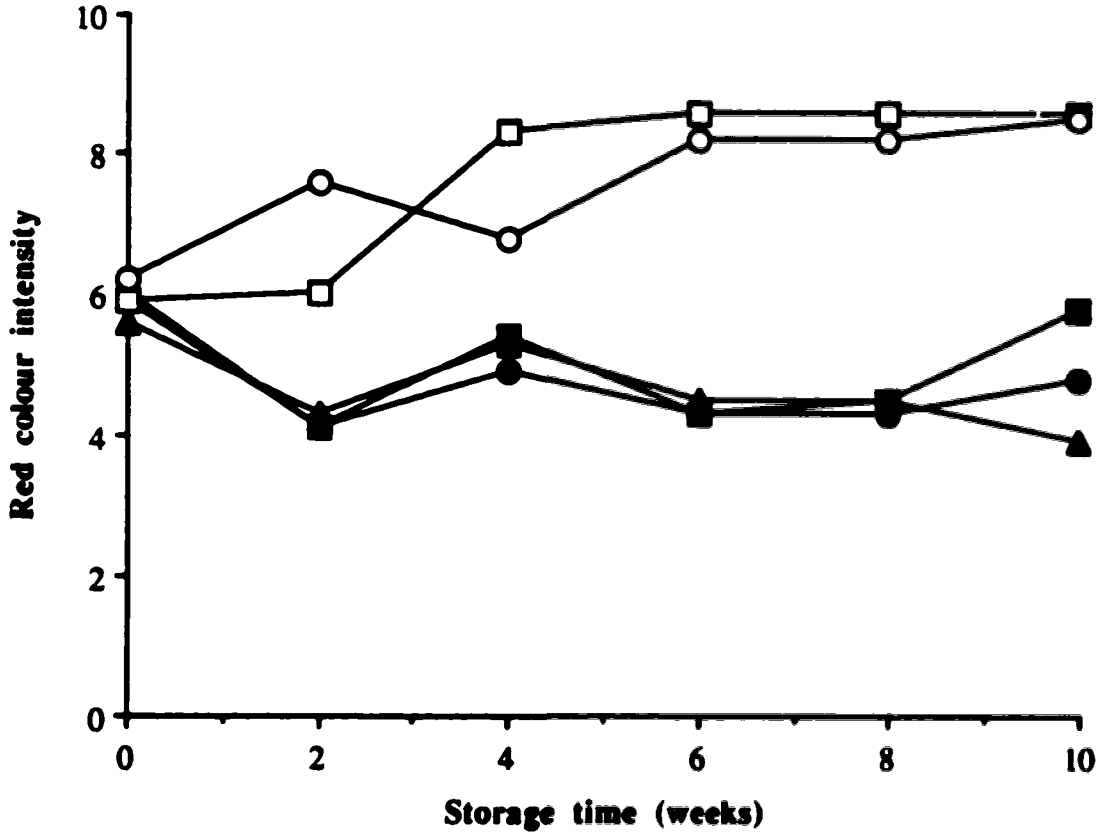

Figure 5.1. Red colour intensity (0=very pale, 15=very dark) of freshly prepared anaerobically (□) and aerobically (○) packaged samples and of retail cuts of pork inoculated with *Leuc. gelidum* UAL187 (▲), *C. piscicola* LV17 (■) and *Lact. sake* Lb706 (●) and stored at 4°C in 100% carbon dioxide for up to 10 weeks.

Changes in the intensity of confinement and meat odours were similar, as a result only data for confinement odour intensity are shown (Figure 5.2). After 2 weeks of storage, and for the remainder of the experiment, the confinement odour intensity of the inoculated samples was significantly greater than the confinement odour of the fresh reference sample. No difference in the confinement odour intensity of the samples inoculated with the three strains of lactic acid bacteria was apparent until 10 weeks of storage when the confinement odour intensity of the sample inoculated with *Lact. sake* Lb706 was significantly ($P<0.001$) lower than the confinement odour intensity of the samples inoculated with *Leuc. gelidum* UAL187 or *C. piscicola* LV17. The scores for acceptability of confinement and meat odours (data not shown) followed the same pattern as that for confinement odour intensity.

Although the confinement and meat odour intensities were similar, differences in the description of the odours were apparent. A sulphur odour was the principal note detected in the confinement odour of pork inoculated with lactic acid bacteria and stored in 100% CO_2 for up to 10 weeks. The intensity of sulphur odours (Table 5.1) was greatest in the confinement odour of samples inoculated with *Leuc. gelidum* UAL187 and *C. piscicola* LV17. Sulphur odour notes were detected less often in the meat samples. Samples inoculated with *C. piscicola* LV17 had the greatest intensity of sulphur notes in the meat odour. The meat odour of all inoculated samples was described as sour and liver.

5.3.3. Volatile Compounds

A large number of volatile compounds was detected in the headspace of the CO_2-filled pouch (Table 5.2). These included a number of C_{12-13} branched chain hydrocarbons which were not clearly identified. Each of the C_{13} compounds had a different retention time. The C_{13} branched chain hydrocarbon-1 accounted for more than 50% of the total

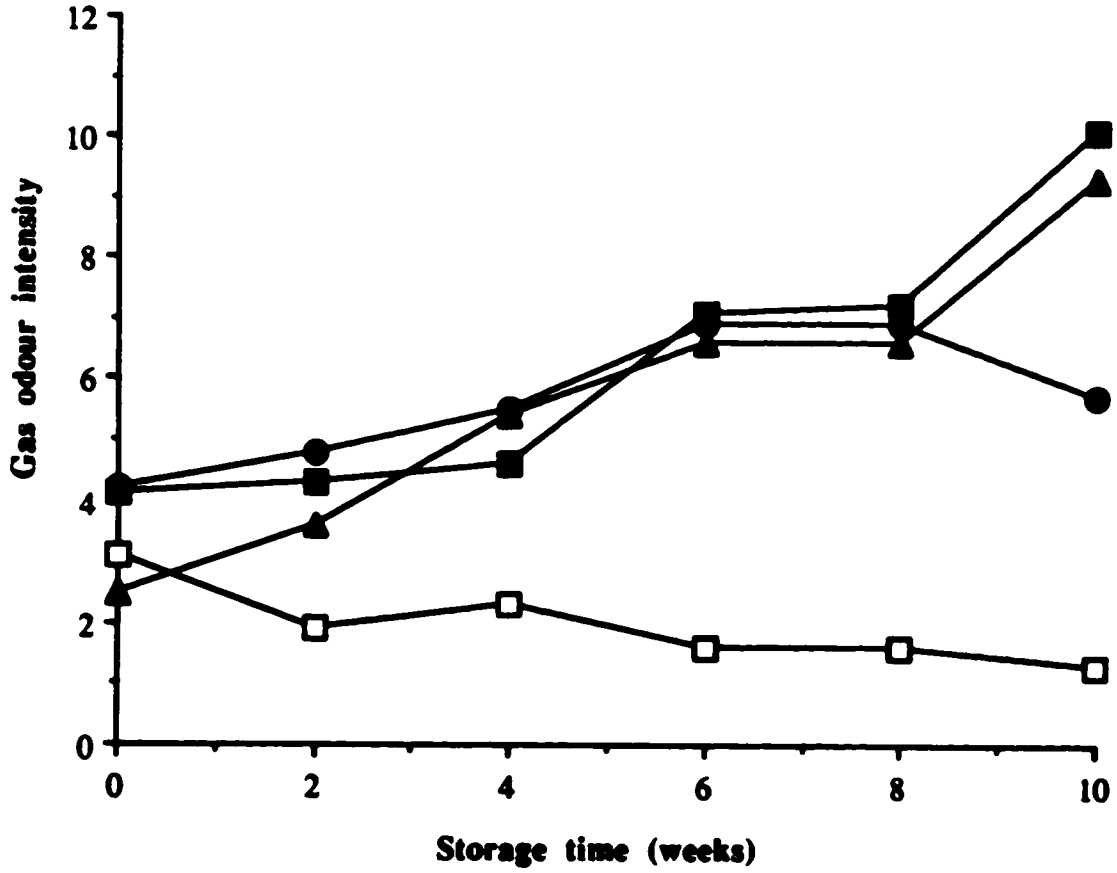

Figure 5.2. Intensity of confinement odour (0=very weak, 15=very strong) of a freshly prepared anaerobically packaged (□) reference sample and of retail cuts of pork inoculated with *Leuc. gelidum* UAL187 (▲), *C. piscicola* LV17 (■) and *Lact. sake* Lb706 (●) and stored at 4°C in 100% carbon dioxide for up to 10 weeks.

Table 5.1. Mean scores[1] for intensity of sulphur odour notes of confinement and meat odours of modified atmosphere packaged retail pork cuts inoculated with lactic acid bacteria and stored at 4°C for 10 weeks.

Storage time (weeks)	*Leuc. gelidum* UAL187	*C. piscicola* LV17	*Lact. sake* Lb706	Reference	SEM[2]
Confinement odour					
0	4.4	2.9	2.0	0.0	1.3
2	3.7	5.1	4.1	1.0	1.8
4	4.0	4.4	5.1	2.9	1.3
6	7.1	8.2	4.1	1.6	1.4
8	5.1^{ab}	8.2^{a}	4.7^{bc}	1.2^{c}	1.1 **
10	9.2^{a}	11.2^{a}	3.7^{b}	1.0^{b}	1.4 ***
Meat odour					
0	0	0	0	0	
2	0	0	0	0	
4	0	0	0	0	
6	2.1^{b}	7.8^{a}	0^{b}	0^{b}	1.3 *
8	0^{b}	5.0^{a}	0^{b}	0^{b}	0.9 *
10	6.7^{b}	12.1^{a}	0^{c}	0^{c}	1.4 *

[1] Mean scores are averages of 24 scores (8 panelists, 3 replications) determined using a 15 cm line score where 0=none, 15=very strong.

[2] Standard error of the mean.

[abc] Means within the same row sharing a common superscript are not significantly different at $P>0.05$.

*,**,*** Significant differences among treatments at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

Table 5.2. Volatile compounds detected in the headspace gas of a CO_2-filled Opalen x 75HL pouch stored at 4°C for 10 weeks.

		Storage time (weeks)					
Compound	ID[1]	0	2	4	6	8	10
ethanol	MS,A		+		+	+	+
hexane	MS,A					+	
hexanal	MS,A					+	
trichloroethane	MS					+	
trichloromethane	MS	+	+	+	+	+	+
1, 3, 5, 7 cyclo-octatetraene	MS	+	+	+	+	+	+
C_{12} branched chain hydrocarbon	MS	+	+	+	+	+	+
C_{13} branched chain hydrocarbon-1	MS	+	+	+	+	+	+
C_{13} branched chain hydrocarbon-2	MS	+	+	+	+	+	+
C_{13} branched chain hydrocarbon-3	MS	+	+	+	+	+	+
C_{13} branched chain hydrocarbon-4	MS	+	+	+	+	+	+
C_{13} branched chain hydrocarbon-5	MS	+	+	+	+	+	+

[1] Identification of the compound based on comparison of observed mass spectra with library spectra (MS) and consistent with the data of authentic compounds (A).

peak area for all samples. The other branched chain hydrocarbons had relatively small peaks. The volatile compounds detected in the headspace gas of the CO_2-filled pouch were consistently detected in the headspace gases of the MAP pork loin cuts and storage-related changes in relative amounts or types of branched chain hydrocarbons were not detected.

The volatile compounds detected in the package headspace of pork cuts inoculated with lactic acid bacteria are listed in Table 5.3. Acetaldehyde, sulphur dioxide, ethanethiol and acetic acid were not detected initially but were detected in the headspace of all samples as storage time progressed. Ethanol was detected in the headspace gases of all meat samples. Based on the average peak area, the amount of ethanol detected in samples stored for 0 to 8 weeks was similar. However, after 10 weeks of storage the average peak area of ethanol detected in the headspace gas of samples inoculated with *Leuc. gelidum* UAL187 was approximately three times that of the average peak area for samples inoculated with *C. piscicola* LV17 and *Lact. sake* Lb706. For samples inoculated with *Leuc. gelidum* UAL187, acetic acid was detected after 2 weeks of storage; however, acetic acid was not detected until 4 and 6 weeks of storage for samples inoculated with LV17 and Lb706, respectively.

Purge and trap concentration of the volatile compounds in the meat after removal from the package (Table 5.4) resulted in the detection of the same volatiles that were detected in the headspace gases, with the exception that heptane was detected on two occasions and the detection of compounds originating from the packaging material was less frequent and fewer compounds were detected. For the majority of the volatile compounds detected in the meat samples, the incidence of detection was storage-related, with the exception of ethanol and the C_{12} branched chain hydrocarbon, which were present throughout storage in all meat samples. The incidence of detection of a number of volatile compounds varied between samples inoculated with the three lactic acid bacteria. Acetaldehyde was detected throughout storage from meat inoculated with *C. piscicola*

Table 5.3. Volatile compounds detected in the headspace of modified atmosphere packaged retail pork cuts i .ulated with strains of lactic acid bacteria and stored at 4°C for up to 10 weeks.

Strain		*Leuconostoc gelidum* UAL187						*Carnobacterium piscicola* LV17						*Lactobacillus sake* Lb706					
		Storage time (weeks)																	
Compound	ID[1]	0	2	4	6	8	10	0	2	4	6	8	10	0	2	4	6	8	10
sulphur dioxide	MS,A				-	+	+				+	+	+				+	+	+
acetaldehyde	MS,A				+	+	+				+	+	+		+	+	+	+/-	+
ethanol	MS,A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ethanethiol	MS,A				-	+	+			+		+/-	+					+	+
2-propanone	MS										+								
1,3 pentadiene	MS									+					+	+			
acetic acid	MS,A		+	+	+	+	+			+	+	+	+				+	+	
toluene	MS,A				+						+		+						+

[1] Identification of the compound based on comparison of observed mass spectra with library spectra (MS) and consistent with the data of authentic compounds (A).

+ Detected in samples from 3 replicates.

+/- Detected in samples from 2 replicates.

- Dectected in samples from 1 replicate.

Table 5.4 Volatile compounds from the meat after removal of inoculated pork cuts from the modified atmosphere package.

Strain		*Leuconostoc gelidum* UAL187						*Carnobacterium piscicola* LV17						*Lactobacillus sake* Lb706					
Compound	ID[1]	0	2	4	6	8	10	0	2	4	6	8	10	0	2	4	6	8	10
acetaldehyde	MS,A				+	+	+	+	+	+	+	+	+		+		+	+	+
sulphur dioxide	MS,A				+		+				+	+	+		+		+	+	+
ethanol	MS,A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ethanethiol	MS,A				+					+		+	+						
hexane	MS,A				+	+	+					+	+				+	+	+
2-propanone	MS										+								
hexanal	MS,A		+												+	+	+		
heptane	MS										+						+		
trichloromethane	MS		+	+	+	+	+		+		+	+	+	+		+	+	+	+
acetic acid	MS,A			+	+	+	+					+	+				+	+	+
toluene	MS,A			+	+	+	+				+	+	+			+			+
1, 3, 5, 7 cyclo-octatetraene	MS		+		+	+	+	+	+	+	+	+	+		+	+	+	+	+
C_{12} branched chain hydrocarbon	MS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C_{13} branched chain hydrocarbon-1	MS		+	+	+	+	+		+		+		+		+		+	+	+
C_{13} branched chain hydrocarbon-2	MS			+	+	+	+		+		+	+					+	+	
C_{13} branched chain hydrocarbon-3	MS			+	+	+	+					+					+	+	
C_{13} branched chain hydrocarbon-4	MS											+							

[1] Identification of the compound based on comparison of observed mass spectra with library spectra (MS) and consistent with the data of authentic compounds (A).

LV17. In contrast, acetaldehyde was detected after 2 and 6 weeks of storage in samples inoculated with *Lact. sake* Lb706 and *Leuc. gelidum* UAL187, respectively. The highest incidence of ethanethiol detection occurred in samples inoculated with *C. piscicola* LV17. Differences attributable to the different bacterial strains were also apparent for sulphur dioxide, hexanal, acetic acid and toluene.

5.4. DISCUSSION

The use of modified atmosphere packaging for fresh meats intended for retail sale can provide the meat industry with economic benefits by allowing for the expansion of markets, reducing packaging costs and reducing spoilage losses in the retail marketplace. In our previous study (McMullen and Stiles, 1993b) we showed that under controlled storage conditions that would allow access to distant markets, retail pork cuts could be marketed for up to 4 to 5 weeks. Although colour of the pork cuts was a limiting factor to acceptance, spoilage of the meats due the confinement odour was identified as a problem after 4 to 5 weeks of storage. Meat colour is an acceptance criterion and not a spoilage factor for meats stored under modified atmosphere conditions. This study was done to determine the influence of bacteriocin-producing lactic acid bacteria on confinement and meat odours.

The pork samples used for this study were typical of fresh meats stored in modified atmospheres (McMullen and Stiles, 1991; Rousset and Renerre, 1991), judging by the normal pH of the meat and the absence of PSE characteristics. Growth of the lactic acid bacteria used for inoculation in this study had no effect on pH of the meat. Low pH causes the denaturation of myoglobin that results in colour fading (Renerre, 1990). In our studies, the pale colour of the pork samples stored in 100% CO_2 was a result of packaging treatment and not low pH.

The purpose of adding lactic acid bacteria to the pork samples was to determine the spoilage potential of selected strains. Bacteriocin-producing lactic acid bacteria were selected because bacteriocins could be a critical factor enabling the inoculated strains to dominate the natural microflora of the meat (Klaenhammer, 1988). Strains used for inoculation must be able to grow on the meat without causing spoilage. The three strains used in this study grew on the pork samples; however, differences in spoilage potential were detected. The confinement and meat odour profiles based on intensity scores were similar. Although the odour of the inoculated samples was more intense than the reference samples, a significant difference was only detected after 4 weeks of storage. Up to 8 weeks there was no difference in odour intensity of the meat inoculated with the different strains. However, there was a difference in the type of odour detected. Samples inoculated with *Leuc. gelidum* UAL187 and *C. piscicola* LV17 developed a sulphur odour that distinguished them from samples inoculated with *Lact. sake* Lb706. At 6 and 8 weeks of storage, the odour intensity of the samples was borderline for acceptance and further study would be necessary to determine whether this intensity of odour would be sufficient for consumer rejection of the product. Samples inoculated with *Lact. sake* Lb706 may still be acceptable up 10 weeks of storage.

Currently, sensory analysis is the only method available for determining the storage life of MAP meats. Volatile compounds of the headspace gases and of the meat after removal from the package were analyzed to determine the feasibility of using volatile analysis to determine storage life. A large proportion of the volatile compounds detected in the headspace gases originated from the packaging film. The presence of these volatile compounds from the beginning of storage when confinement odours were minimal indicates that their impact on confinement odour is limited. In a study of the volatiles associated with modified atmosphere packaged beef loins, Jackson et al. (1992c) also found a predominance of volatiles originating from the packaging film. In the current study, ethanol was detected in the headspace gases and from the meat throughout storage.

The presence of small amounts of ethanol could be attributed to the packaging material but the majority of the ethanol was most probably due to the method of sample preparation. Other studies on the volatile compounds of meat sterilized using ethanol for flaming have not reported the presence of ethanol (Jackson et al., 1992a,b). The presence of ethanol in the headspace gases could be expected as a result of the heterofermentative metabolism of *Leuc. gelidum* UAL187. Ethanol has been detected in beef inoculated with *Leuconostoc* sp. (Borch and Agerhem, 1992). Further study of ethanol in the headspace gases of MAP pork under normal conditions where the meat has not been exposed to ethanol prior to packaging are necessary to determine the significance of ethanol production by *Leuc. gelidum* UAL187 to confinement odour.

Other volatile compounds detected in the headspace gases or from the meats that have potential as indicators of spoilage include acetaldehyde, sulphur dioxide, ethanethiol, hexane and acetic acid; however, compounds such as trichloromethane and the branched chain hydrocarbons could not be associated with storage-related changes. The production of acetaldehyde and acetic acid can be attributed to bacterial metabolism; however, without comparison to sterile control samples, the source of these compounds cannot be confirmed. Acetic acid is detected as a volatile compound in the headspace gases of sterile beef (Jackson et al., 1992b). Hexane was only detected in the volatile compounds of the meat samples and, on one occasion, in the volatiles of the CO_2-filled package. The detection of hexane only after purge and trap analysis of the volatiles of the meat makes it unsuitable for monitoring meat spoilage. However, it is possible that by concentrating the headspace volatiles to a greater extent than was done in this study, hexane may be detected as a headspace volatile. The source of hexane is unclear. It is possible that hexanal, which was detected during analysis of the volatiles compounds in the CO_2-filled packages, was reduced to hexane. However, hexane and hexanal are not metabolites of bacterial growth, and were not consistently detected in the headspace gases of the CO_2-filled package, so

their presence could be attributed to the meat. Analysis of sterile control samples would have clarified the source of these compounds.

A sulphur odour in the headspace gas of modified atmosphere packaged meat would be unacceptable to the consumer. Some lactic acid bacteria are capable of rapid production of sulphur compounds when grown on meat stored in anaerobic environments (Shay and Egan, 1981). The detection of sulphur compounds in the headspace gases and from the meat when the trained panel detected the presence of sulphur odours indicate that these compounds could be used to monitor spoilage. Sulphur dioxide was detected in the headspace gases of samples stored for 6 weeks or longer. However, after two weeks of storage SO_2 was detected by purge and trap analysis of the volatile compounds of the meat samples inoculated with *Lact. sake* Lb706. The sensory panel did not detect sulphur odours in the headspace gases or from the meat of these samples, thus it is unlikely that it made a significant contribution to odour. This confirms the results of Jackson et al. (1992a) who reported the presence of SO_2 in the headspace gases of sterile beef loins and in the headspace gases of beef inoculated with lactic acid bacteria.

Ethanethiol may be more useful than SO_2 for monitoring spoilage. Ethanethiol was detected in the headspace gases at the time that the panelists indicated the presence of sulphur odours. However, ethanethiol was also detected in the headspace gases of pork inoculated with *Lact. sake* Lb706 which had a weak sulphur odour. The absence of ethanethiol in the meat odour of samples inoculated with *Lact. sake* Lb706 and to some extent with *Leuc. gelidum* UAL187 indicate that ethanethiol may have a significant impact on the confinement odour without affecting meat odour. Quantitative analysis of ethanethiol in headspace gases and its correlation to meat odour are needed to clarify this question. The odour threshold of ethanethiol is 20 ppm in air. Other researchers have not reported the presence of ethanethiol (Jackson et al., 1992a,b,c; Edwards and Dainty, 1987)

but have detected methanethiol. The source of ethanethiol is not clear. *Saccharomyces cerevisiae* produces ethanethiol as a byproduct of metabolism (Kadota and Ishida, 1976).

Hydrogen sulphide was detected as a volatile compound of vacuum packaged pork (Edwards and Dainty, 1987) but it was not detected in the current study. Attempts to detect H_2S when it was injected as an authentic compound were unsuccessful. It is possible that the Tenax TA trap was not efficient in trapping the H_2S; however, sulphur odour was not detected at the sampling vent. Tenax TA was chosen for analysis as it is efficient for trapping organic compounds but does not retain water vapour which interferes with GC/MS analysis. The absence of H_2S coupled with the detection of acetaldehyde at the times that ethanethiol was detected could explain the presence of ethanethiol. Under reducing conditions, acetaldehyde can react with H_2S to form a thioaldehyde which will readily oxidize to form ethanethiol. A reaction of ethanol with H_2S may also serve as a source of ethanethiol.

Data from this study indicate that *C. piscicola* LV17 and *Leuc. gelidum* UAL187 are capable of producing sulphur odours and may not be suitable for use to control spoilage of modified atmosphere packaged pork. *Lact. sake* Lb706 did not cause off-odours to develop in the confinement gases or the meat, thus it may be more suitable for use as a biopreservative in MAP meats. Leisner et al. (1993) reported that none of the strains used in the current study caused spoilage of beef stored under anaerobic conditions. However, many differences in experimental methodology (storage temperature: 2 vs 4°C; sensory methodology; sample size: 60 cm^2 vs 700 cm^2) may account for the differences in results. However, further study on the spoilage potential of these organisms is required before unequivocal statements can be made regarding their suitability for use to control spoilage. The inoculation level used in this study was artificially high compared with the levels of lactic acid bacteria naturally present on meat. Inoculation of these organisms on meat at lower levels than used in the current study may sufficiently extend the time before spoilage

occurs to make their use practical. Studies on the interaction of the natural microflora with inoculated lactic acid bacteria are also required before their use for controlling spoilage can be proposed.

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6. General Conclusions

Vacuum packaging in gas impermeable film has been successfully applied to extend the storage life of primal cuts of fresh beef and has had a dramatic effect on the wholesale distribution of beef. However, retail marketing still depends on aerobic packaging and the "bloom" of myoglobin to give the bright red colour of oxymyoglobin (Shay and Egan, 1987). Even greater success has been achieved with the marketing of lamb carcasses, in which chill storage at -1°C in a modified atmosphere of 100% CO_2 with a gas impermeable film has resulted in storage life of 110 to 160 days (Gill, 1986). Such achievements avoid the need for frozen storage that is expensive and has poor consumer appeal. With lamb there is a greater potential to extend the packaging in modified atmosphere to the retail product because U.S. and Canadian consumers are less expectant of a bright red (oxygenated myoglobin) colour.

In the United States and Canada, marketing of the pork carcass is highly differentiated. In the main, only the loin cut enters the retail market; shoulder, leg and belly cuts are processed as bacon, ham and other meat products. Furthermore, the retail marketing of pork loin cuts has not emphasized the red colour of the meat to the same extent as beef. As a result the consumer accepts the light red colour of pork. In recent years, the Alberta meat industry has accessed distant markets for pork. Ideally, they would like to market product that has not been frozen but it must have a good storage life in the retail store. Modified atmosphere packaging has the potential to achieve these goals. Furthermore, as new packaging legislation impacts the food industry, the likelihood exists that there will be increasing pressure on food manufacturers and retailers to reduce or eliminate repackaging of meat between wholesale and retail distribution. This could stimulate interest in the use of modified atmosphere packaging for retail cuts of meat. The principal hurdle will be the education of the retail meat trade and the consumer to accept the colour of unoxygenated meat. For MAP meats, colour is not

related to microbial spoilage and it may therefore be considered an artificial barrier to consumer acceptance. Consumer education regarding the colour of anaerobically packaged fresh meats has the potential to overcome this problem (Lynch et al., 1986).

The meat industry already has a highly developed centralized prepackaging system for fresh meats entering the Hotel and Restaurant trade. Implementation of a centralized prepackaging system for retail cuts of meat using modified atmosphere packaging would give the Canadian meat industry many direct and indirect economic benefits and would help them to survive in an extremely competitive marketplace. As a result, the overall objective of this study was to evaluate the application of modified atmosphere packaging to retail cuts of pork using a multidisciplinary approach, in which microbial, sensory and instrumental analyses are integrated to assess the potential use of this technology for retail marketing.

In the initial study, the effects of packaging film, storage temperature and initial microbial load on the storage life of retail cuts of pork packaged in modified atmosphere was studied. A detailed assessment of the composition of the microbial population found on these meats was included. Each of the parameters examined in this study had an effect on the storage life of MAP fresh pork, but low temperature storage was found to be the overriding control factor for long-term extension of storage. The data from this study indicated that retail cuts of pork could be packaged in oxygen impermeable films and stored at -1°C for up to 8 weeks. This is a major advantage in storage life over the storage life of aerobically packaged pork.. However, the foil-based films used in this study were not considered appropriate for use in the retail marketplace. A clear plastic film with very low oxygen transmission is needed for this purpose. In the assessment of the microbial population prevailing on these meats, some problems were identified that may be associated with low temperature storage (-1°C). In our study, the lactic acid bacteria grew slowly at -1°C and listeriae were isolated as part of the prevailing microbial

population. This was not observed during storage of meat at 4.4 or 10°C. The public health significance of the presence of these strains is unclear because they were not speciated; however, the growth of *Listeria* sp. on MAP fresh meats stored at very low temperatures needs to be evaluated further because their presence as part of the prevailing microflora could be undesirable.

The extended storage life that can be achieved by packaging in MA could allow the meat industry to export fresh, nonfrozen retail-ready products to distant markets at a reasonable cost. However, temperature control is critical for extending storage life and questions were raised about the storage life of MAP fresh meat once it moves into the retail market where temperature control is usually compromised (Sumner et al., 1981). The second study was designed to determine the storage life of retail cuts of pork under conditions that simulate export to distant markets. In addition to microbial and appearance acceptability parameters, confinement odour was assessed as a potential barrier to consumer acceptance of MA packaged retail-ready cuts of pork. Based on trained panel evaluation of odour, retail cuts of pork could be marketed on distant markets with a three-week shipping time at -1.5°C with 1 to 2 weeks marketing time in the retail market at 4 to 7°C. Marketing retail cuts of pork under these conditions is a tremendous advantage over aerobic packaging. Confinement odour was identified as a problem when meat was exposed to storage temperatures at 4 and 7°C. Microbial growth is thought to be the source of the compounds that cause the confinement odour but researchers (Jackson et al., 1992) have shown that sterile meat also develops an odour when it is stored under anaerobic conditions. A number of samples used in our study developed a sulphury confinement odour. Although the cause is unclear, there have been reports of LAB that produce sulphur compounds under anaerobic conditions (Egan and Shay, 1982; Edwards et al., 1985). This study demonstrated that there is good potential to apply modified atmosphere packaging to retail cuts of pork intended for export to distant markets. However, before this technology can be applied to give a reliable

storage life, the development of sporadic sulphur odours needs to be controlled because this would result in consumer rejection of the products.

Controlling the growth of the adventitious microbial population with lactic acid bacteria inoculated onto the meat at the time of packaging would be a novel means of controlling spoilage. Before this can be applied in the meat industry, research is needed to answer many questions regarding the feasibility of application of LAB as biopreservatives. To be suitable for use as biopreservatives the "starter" organisms must not cause meat spoilage and they must be able to dominate the natural lactic acid bacteria that grow on chill stored, MAP meat. Production of inhibitory substances, such as bacteriocins, by LAB could have an important function in domination of the bacterial population by the bacteriocinogenic strains (Klaenhammer, 1988). However, studies on bacteriocin-producing LAB have focused on the control of foodborne pathogens and there is a lack of published information on the use of these organisms to control spoilage. Our laboratory has focused considerable research on bacteriocin production by LAB isolated from meats (Ahn and Stiles, 1990; Hastings and Stiles, 1991). The spoilage potential of three bacteriocin-producing lactic acid bacteria inoculated onto samples of pork was assessed in our laboratory. *Carnobacterium piscicola* LV17 and *Leuconostoc gelidum* UAL187 produce sulphur odours and may not be suitable for use to control spoilage of modified atmosphere packaged pork. *Lactobacillus sake* Lb706 did not cause off-odours in the confinement gases or the meat, thus its application to fresh meats as a biopreservative is more promising. However, further study on the spoilage potential of these organisms is required before unequivocal statements can be made regarding their suitability for use as biopreservatives.

The use of sensory methodology for evaluating storage life is too inefficient for reliable application in the meat industry; however, it is the only method available at present to evaluate spoilage. Detection of volatile compounds as a nondestructive and

rapid means of evaluating spoilage was also evaluated. Concentration of static headspace volatiles was shown to be successful in detecting volatile compounds that have potential for use in this regard. Data for analysis of the headspace volatile compounds of pork samples indicated that sulphur compounds may be useful for monitoring spoilage of MAP fresh pork To be considered useful as an indicator of spoilage, a volatile compound should be detected at a given concentration in the headspace gas at the time that sensory analysis indicates that spoilage has occurred. This requires further study to establish the limits for the concentration of specific volatile compounds that would indicate that the meat has spoiled.

Since these studies were initiated, other researchers have evaluated MAP systems for extending the storage life of retail meat cuts (Fu et al., 1992; Scholtz et al., 1992; Buys et al., 1993; Greer et al., 1993). However, in all cases, MAP was intended for use for bulk storage and meat was rewrapped for retail sale.

Although the storage life of retail cuts of pork can be extended by packaging in modified atmospheres, the greatest barrier to the application of MAP for retail cuts of pork remains the colour of the meat. An alternative approach to marketing retail-ready MAP pork cuts is to use a "master-pack" packaging system. In this case, the meat is packaged in a gas-permeable film and several individual packages are placed in a larger package that is flushed with CO_2. In theory, when the individual meat packages are removed from the master-pack, O_2 permeates the package and the meat "blooms" to its normal colour. This system is used by a small portion of the Alberta pork industry for the distribution of retail-ready cuts.

An alternate approach to ensuring that pork retains a bright red colour might be to chemically alter the myoglobin to form carboxymyoglobin by exposing the meat to carbon monoxide. The use of CO for colour stabilization of beef has been assessed (Gee and Brown, 1976; Clark et al., 1979) but safety concerns with the handling of CO may

override its application in the meat industry. The use of CO to fix the colour of aerobically packaged fresh meat would be considered public deception because it masks the spoilage process (Holland, 1980). However, under anaerobic conditions meat colour is not related to spoilage, thus it could be argued that use of CO to fix meat colour would not constitute consumer deception.

Research on the use of lactic acid bacteria as starter cultures to control spoilage of fresh meat is in its infancy. The use of a starter culture to control spoilage requires that the organism should be able to dominate the adventitious microbial population. This was not addressed in the current study and is an area for future research. Two of the LAB strains used in this study may not be suitable for use as biological preservatives; however, the possibility remains that the ideal strain or strains has yet to be found. Genetic manipulation with the aim of reducing the spoilage potential of lactic acid bacteria remains an area for investigation. The genetic basis for the production of sulphur compounds by LAB has been linked to the presence of plasmids (Shay et al., 1988). Perhaps a LAB strain could be genetically engineered to enhance the desirable properties (bacteriocin production) and, at the same time, reduce the spoilage potential of the organism.

Research on the application of modified atmosphere packaging to retail cuts of pork has illustrated the potential for tremendous extension of storage life with this technology. From an industrial perspective, the economic benefits of application of this technology make further research on application in the retail marketplace worthwhile.

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