SENSORY AND MICROBIAL CHANGES IN RETAIL-DISPLAYED FRESH PORK PREVIOUSLY STORED UNDER CONTROLLED (CO_2) ATMOSPHERES

By

Melanie A. Wasney

A Thesis
Submitted to the Graduate Studies
In Partial Fulfillment of the Requirements
For the Degree of

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Department of Food Science
University of Manitoba
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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

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ABSTRACT

The research for this Masters thesis was divided into two parts. The first part was concerned with the evaluation of a refrigerated container previously designed to store fresh pork chops at −1.5 °C under 100% CO_2. To assess the ability of the container to delay spoilage, measurements of metmyoglobin content, sensory changes and microbial populations of the pork chops were conducted. Tests were conducted on chops after a period of storage (weeks) in the container followed by aerobic display at 4°C for up to 4 days. It was determined that fresh pork chops could be stored for up 9 weeks with 4 days of subsequent retail display with minimal changes in appearance, odour, and palatability.

The second half of the work concerned the development of a selective medium for the isolation of carnobacteria. Cresol Red Thallium Acetate Sucrose (CTAS) agar was previously devised as a selective medium for enumeration of carnobacteria; however, poor recovery of carnobacteria and interference by other genera, has precluded its use. The aim of this study was to improve CTAS agar by broadening the spectrum of *Carnobacterium* spp. recovery and increasing specificity through systematic improvement. Ten *Carnobacterium* strains (5 ATCC cultures and 5 isolates from fresh pork) and 20 other genera were used in testing the agar. A wider range of *Carnobacterium* spp. recovery was obtained by modifying concentrations of sucrose, manganese sulphate and thallium acetate. Additions of inulin and thiamine hydrochloride improved growth response, additions of vancomycin and Chrisin® (nisin) eliminated interference from other genera and a two-temperature incubation procedure improved

characteristic growth. The final medium was designated Cresol Red Thallium Sucrose Inulin (CTSI) agar. Lactic acid bacteria (LAB) and *Enterobacteriaceae* were unable to grow on CTSI incubated aerobically. Growth of carnobacteria on CTSI yielded pink colonies, except for *Cb. mobile*, which formed gray colonies. In some instances, a red precipitate formed in the center of the colony. Yellowing and clearing of the growth medium was observed frequently. Recovery of carnobacteria using CTSI was identical to that obtained with All Purpose Tween (APT) agar.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ABBREVIATIONS

CAP	Controlled atmosphere packaging
MAP	Modified atmosphere packaging
PVC	Polyvinylchlride
OTR	Oxygen transmission rate
ATCC	American Type Culture Collection
PCA	Plate Count Agar
BHI	Brain Heart Infusion
APT	All Purpose Tween
MRS	deMan Rogosa Sharpe
VRBG	Violet Red Bile Glucose
CFC	Cetrimide Fucidin Cephaloridine
CTAS	Cresol Red Thallium Acetate Sucrose
TTC	Triphenyl Tetrazolium Chloride
CTSI	Cresol Red Thallium Acetate Sucrose Inulin
CFU	Colony forming units
LAB	Lactic acid bacteria
CO_2	Carbon dioxide
O_2	Oxygen
r	Correlation coefficient
SD	Standard deviation

UNITS

°C	Celsius
d	day
h	hour
min	minute
s	second
m	metre
cm	centimetre
kg	kilogram
g	gram
L	litre
ml	millilitre
ppm	parts per million
w/w	weight/weight
RH	relative humidity

SECTION I: INTRODUCTION

The research for this Masters thesis was divided into two parts. The first part was concerned with the evaluation of a refrigerated container previously designed to store fresh pork chops at −1.5 °C under 100% CO_2. In order to predict consumer acceptance of fresh pork chops stored under such a system, those attributes which determine meat quality were assessed. It was therefore the objective of this study to examine the microbiological, sensory and pigment changes that occur in fresh pork chops stored in the container and during subsequent retail display. In order to avoid residual O_2 effects during primary storage, individual chops were repacked in retail format before display. The study was therefore a "simulation" of the commercial use of the container.

The second half of the research concerned the development of a selective medium for the recovery of carnobacteria from fresh pork. CTAS agar was previously devised for this purpose but proved inadequate. Through stepwise changes to CTAS agar, a new medium which was designated CTSI agar was developed. This growth medium showed improved selectivity for all *Carnobacterium* spp. tested while at the same time preventing growth of the other genera.

SECTION II: LITERATURE REVIEW

PART I

A. Introduction

Manitoba has experienced steady growth of the hog industry in recent years. Continued expansion will require innovative methods for processing, shipment and distribution of fresh meat products. It is imperative that there be techniques available to delay the onset of spoilage in fresh pork so as to widen the window of opportunity for distribution of this highly perishable product to distant markets.

B. Available Fresh Pork Preservation Techniques

At 4 °C, under aerobic conditions, pork loin slices have an expected shelf life of six days (Holley et al., 1994b). Shipping of fresh pork from a slaughtering plane to a retail buyer, however, may take up to several weeks. The role of preservation techniques is to extend the storage life of meat beyond that which is attainable during retail display. Such techniques must delay the onset of meat spoilage by slowing down microbial growth and inhibiting biochemical reactions so as to prolong the time allotted for distribution of the product. Since chemical additives and preservatives are not permitted for use in or on fresh meat, other means must be used to achieve shelf life goals.

1. Freezing Meat

The freezing of fresh meat is an effective method of meat preservation whereby water becomes unavailable for microbial growth and biochemical deterioration. When pork is frozen for 168 days, the subsequent retail display life is that of pork without previous frozen storage (Jeremiah, 1982). The freezing of pork can allow for longer storage periods; however, the technique is not without problems. Visual acceptance of pork significantly declines when pork is subjected to freeze/thaw treatments (Greer and Murray, 1991). Consumers also distrust frozen meat (Gill, 1989) because the product's age is not immediately apparent.

2. Vacuum Packaging

Vacuum packaging is a more attractive method of pork preservation. It involves the placement of meat in a package of low oxygen permeability (< 15 ml/ (m^2•day), 23 °C, 75% RH at atmospheric pressure), followed by air removal and subsequent sealing of the package (Gill, 1990). Growth of aerobic spoilage organisms on vacuum packaged meat is precluded by low oxygen conditions. As such, the shelf life of vacuum packaged meat can be extended to 42 days (Jeyamkondan et al., 2000). Vacuum packaging of fresh pork is, however, problematic due to the precipitation of metmyoglobin in pockets of exudate on the meat surface (Jeremiah et al., 1992; Gill, 1996). Because of the anoxic conditions within vacuum packages, meat turns a purple colour due to deoxymyoglobin formation (Gill, 1990). This colour of meat is not deemed as appealing as meat of a bright, red colour which is characteristic of aerobic storage conditions.

C. Application of CAP

Controlled atmosphere packaging (CAP) is an alternative form of pork preservation which uses the CAPTECH (Chilled Atmosphere Packaging Technology) process (Gill, 1989). This process heavily relies on hygienic processing conditions, low storage temperatures, low residual O_2 levels and high concentrations of CO_2 within storage atmospheres as well as gas impermeable packaging (Greer et al., 1993). In the CAPTECH process, pork chops with a low initial microbial load are placed on retail trays and overwrapped with an oxygen-permeable film having an OTR (Oxygen Transmission Rate) between 5000 and 10000 ml/ (m^2 ·day) at atmospheric pressure and 23 °C. Four to six retail trays of meat are then placed into a "motherbag" (Jeyamkondan et al., 2000) or master package which is virtually impermeable to oxygen. After placing retail trays into gas impermeable "master packages", a CAPTRON (or similar machine) (Gill, 1989) is used to evacuate the air within a package and fill the remaining space with a saturating volume of CO_2 gas. In order to offset spoilage for as long as possible, the OTR of master packages should be < 1 ml/ (m^2 ·day) at atmospheric pressure and 23 °C (Church, 1993) so that the CO_2-saturated atmosphere will be maintained while permeation of oxygen will be prevented. After master packages are flushed and filled with CO_2, they are then heat-sealed and placed in refrigerated containers capable of maintaining temperature at -1.5 ± 0.5 °C (Gill, 1989). Following storage, packages are removed from the container and their seals broken. Retail trays are removed from the packages and without further manipulation, are directly placed in a refrigerated case for display.

1. Importance of Temperature

The minimum temperature at which fresh meat can be stored without freezing is -1.5 ± 0.5 °C (Gill and Molin, 1991). Problems with maintaining this narrow temperature range have precluded the use of CAP systems by the meat industry in North America; however, such a temperature range is critical if the storage life of fresh meat is to be maximized. When refrigeration temperatures approach 0, 2, or 5 °C, storage life decreases to 70, 50 or 30 %, respectively of that at -1.5 ± 0.5 °C (Gill et al., 1988).

2. Importance of Anaerobic Conditions

In contrast to vacuum packaging, CAP involves the use of a defined gaseous atmosphere which remains invariant during the life of the package (Gill, 1990). For the long-term storage of fresh meats, the gaseous atmosphere should consist of CO_2 at a volume of 2 L per kg of meat in the package (Jeremiah et al., 1996). At an optimal gas volume to meat mass ratio, CO_2 fills the volume of the package and remains at a constant concentration despite saturation of meat tissue with the gas (Gill, 1991). The solubilization of CO_2 in meat tissue is achieved through hydration of the gas and eventual formation of carbonic acid (Stiles, 1991b):

$$CO_2 + H_2O \leftrightarrow \underset{\text{carbonic acid}}{H_2CO_3} \leftrightarrow HCO_3^- + H^+$$

The inhibitory effects of CO_2 can be attributed to the impairment of cell membrane function, the inhibition of metabolic processes and the disruption of enzyme activity (Stiles, 1991b). When anaerobic atmospheres comprised solely of CO_2 are used in CAP technology, growth of strict aerobes is inhibited while growth of facultative anaerobes is permitted. The bacteriostatic nature of CO_2 results in the inhibition of microbial respiration and the extension of generation times (Gill and Tan, 1980). The extent of bacterial growth inhibition by a constant concentration of CO_2 increases with decreasing temperatures of storage (Gill and Tan, 1980) due in part to increased solubility of the gas. The use of refrigeration temperatures in CAP technology also functions to slow down the growth of CO_2 tolerant species.

3. Combination of Low Temperatures and Anaerobic Conditions

The combination of anaerobic conditions (100% CO_2) and low temperature storage (-1.5 ± 0.5 °C) allows the initial quality of fresh pork chops to be maintained for periods of time greater than that possible with vacuum packaging. The extension of shelf life of fresh meat is about 8 to 15 fold in 100% CO_2 at -1.5 ± 0.5 °C, as opposed to a two-fold increase in shelf life as obtained with vacuum packaging (Gill, 1990). At refrigeration temperatures in an atmosphere of 100% CO_2, pork spoilage is offset in terms of microbiology and physieochemistry effects (Holley et al., 1994a).

4. Residual Oxygen Levels

Although an atmosphere totally free of oxygen is desired for maximization of shelf life, traces of O_2 will remain after air evacuation of master packages (Gill, 1996). McMullen and Stiles (1994) found that fresh pork was rejected for loss of acceptable appearance after 7 weeks of CAP storage (-1.5 °C, 100% CO_2) when residual O_2 levels were < 200 ppm. The deterioration in appearance was not attributed to discolouration but rather to an increase in purge. In contrast, Greer et al. (1993) ensured that residual O_2 levels were < 300 ppm when fresh pork loins were stored under CAP (-1.5 °C, 100% CO_2). Under such conditions, it was determined that loins could be stored for up to 7 weeks under CAP with an additional 3 to 4 days of retail display (as chops). Adverse effects on pork colour have been shown to occur at O_2 levels > 1% (10 mL O_2/ 1 L CO_2) (Penney and Bell, 1993).

D. Advantages of CAP Technology

1. Increased Efficiency and Monetary Savings

It is standard practice for the meat industry to ship vacuum packaged primals and sub-primals (pork loins) to retail outlets where they are made into retail cuts (chops) by an in-store butcher and packaged (McMullen and Stiles, 1994). As such, the current distribution system of fresh meat is inefficient. Space, time and money are wasted at the retail level because retail cuts of meat have to be prepared from primal/subprimal cuts (Jeyamkondan et al., 2000). However, CAP storage systems can allow for the

preparation of "retail ready" packages of meat within a centralized meat processing operation (Farris et al., 1991) which can then be shipped to the retail level. Because "retail ready" trays can be immediately displayed without further preparation, the need for butchers at the retail level would be eliminated. Waste would be reduced because of the abandonment of intermediate packaging associated with shipment of larger "primal" cuts (Gill, 1996). There would also be the elimination of product waste due to the direct display of retail ready trays without prior trimming or cutting. The removal of trays from the container to meet consumer demand (Jeyamkondan et al., 2000) would eliminate the amount of meat wasted due to the overstocking of store display cases. The elimination of needless waste and the lowered in-store labour costs could allow grocers to sell fresh meat to customers at a lower cost.

2. Natural Preservation Method

With the interest in "natural foods", CAP technology offers an attractive way of preserving meat freshness without the addition of chemical additives or preservatives (Gill, 1989). The use of low temperatures and anaerobic atmospheres reduce the opportunity for product abuse during distribution.

3. Overseas Markets

If fresh pork chops are handled in a sanitary manner at the processing level, CAP technology can maintain the quality of this highly perishable product for extended time periods. By delaying the onset of pork spoilage, the amount of time available to reach the

consumer increases; therefore, making distant markets (Armstrong, 1996) a more viable option. It was previously determined that fresh pork loins could be stored at −1.5 °C in 100% CO_2 for up to 7 weeks before appearance started to deteriorate (McMullen and Stiles, 1994).

E. Metmyoglobin Adversely Affects Fresh Pork Quality

The acceptability of fresh meat by consumers is largely determined by the appearance of muscle tissue. Overall, tissue colour is determined by the various chemical forms of myoglobin pigment, although muscle structure, surface moisture and fat content do play a part (American Meat Science Association, 1991). While the degree of oxygenation of myoglobin determines the degree of redness which will be perceived by consumers, colours may vary from extremely pale to extremely dark (Agriculture Canada, 1984). The particular form of myoglobin is largely determined by the state of the iron atom within the heme moiety of myoglobin. In low oxygen conditions, myoglobin will be in the deoxy state resulting in dark purple-red meat due to the ferrous (Fe^{+2}) state of the iron atom. In high oxygen conditions, deoxymyoglobin is converted to oxymyoglobin whereby the iron moiety is also in the ferrous (Fe^{+2}) state (Jeyamkondan et al., 2000). The ferrous atoms within deoxymyoglobin and oxymyoglobin differ by their orientation about the globin component. The oxygenation of myoglobin to oxymyoglobin results in meat with bright red colour. As can be seen under retail conditions, this process requires 15 to 30 min (Agriculture Canada, 1982)

and is reversible (Forrest et al., 1975). Through exposure to atmospheric air, there is gradual conversion of oxymyoglobin to metmyoglobin whereby meat takes on a brown colour. When this happens, the iron atom within myoglobin is in the ferric (Fe^{+3}) state (Jeyamkondan et al., 2000). While the bright red colour of oxymyoglobin is deemed attractive by consumers, overall preference decreases with increases in the extent of discolouration due to metmyoglobin formation (Brewer at al., 1998). Consumers erroneously perceive brown meat as being bacterially spoiled or originating from older animals (Jeyamkondan et al., 2000).

1. Effect of Storage Temperature

Although the colour of meat is affected by bacterial contamination, relative humidity, time and retail display lighting (Agriculture Canada, 1982), it is also sensitive to storage temperature. During retail display where temperatures are > 3 °C, myoglobin is more readily oxidized to metmyoglobin (Walters, 1975). Although metmyoglobin may be slowly reduced back to deoxymyoglobin through enzymatic action, the likelihood decreases after lengthy storage (Moore and Gill, 1987). When pork is stored at 4 °C aerobically for 4 d, approximately 40 to 50% of the meat pigment is normally metmyoglobin (Zhu and Brewer, 1998).

2. Effect of Anaerobic Atmospheres

When packaged under anaerobic atmospheres, the majority of myoglobin in pork will be converted into the deoxy state, thereby imparting a purple colour to the meat. During CAP storage in CO_2, most of the residual O_2 within master packages will be scavenged by meat tissue. Any remaining oxygen tends to cause the formation of metmyoglobin, but this initial discolouration is transient and is usually resolved within 2 to 4 days (Gill, 1996) provided levels are not excessive (< 1 % residual O_2). The rate of myoglobin oxidation decreases as storage temperature decreases (Gill and McGinnis, 1995). The presence of metmyoglobin reductase in the meat tissue converts metmyoglobin into deoxymyoglobin; however, under CO_2-saturated atmospheres, meat tissue may eventually be exhausted of its myoglobin-reducing capabilities. This results in the irreversible formation of metmyoglobin. Conservation of metmyoglobin reductase activity through the elimination of residual oxygen is important because it is necessary for any metmyoglobin to be reduced to myoglobin before meat can bloom by formation of oxymyoglobin after meat is exposed to oxygen. Chops with previous CAP storage in CO_2 have a smaller display time because of the previous formation of metmyoglobin and lowered metmyoglobin-reducing capacity during storage. When fresh pork is removed from anaerobic atmospheres for retail display, there is oxygenation of myoglobin to the bright red oxy form. During prolonged display, there is eventual conversion to metmyoglobin.

F. Organoleptic Changes

The storage life of fresh pork stored in 100% CO_2 at -1.5 °C is limited by the deterioration of appearance after 7 weeks of storage. There is also a decrease in odour acceptability under such conditions, however, it can be well below the rejection level after 17 weeks of storage (McMullen and Stiles, 1994). As does vacuum packaging, CAP storage of fresh pork selects for the growth of LAB. As this population evolves, there can also be a decrease in flavour acceptability (Jeremiah et al., 1996).

Although CAP storage extends the storage life of fresh pork, it does not extend the case life during subsequent aerobic retail display. Retail cuts of pork without previous CAP storage do not have established bacterial populations and therefore have the maximum display life. The expected shelf life of pork loin slices at 4 °C is six days (Holley et al., 1994b) due to rapid bacterial growth under aerobic conditions. Previous CAP storage of chops allows bacterial populations to evolve over time which shorten the display time under aerobic conditions. For every six weeks of loin storage under 100% CO_2 at −1.5 °C, there is reduction in display life at 8 °C by one day (Greer et al., 1993).

G. Microflora of Fresh Pork

The initial bacterial populations on freshly-slaughtered pork are extremely low. Meat surfaces will generally have between 10^2 and 10^3 CFU/cm^2 surface (Borch et al., 1996). As storage time progresses, bacterial numbers increase. Spoilage is brought on by the aerobic growth of bacteria which degrade meat tissue and produce end-products of catabolism which result in aberrant changes in colour, odour and palatability. Aerobic spoilage organisms are typically pseudomonads or *Enterobacteriaceae* (at temperatures > 4 °C), which produce off- odours and flavours from the breakdown of amino acids (Gill and Greer, 1993). In order to minimize the amount of time needed for maximum microbial numbers to be reached, microbial contamination of pork chops should be minimized during slaughter and processing of pork (Sheridan, 1998) as well as at the retail level.

1. Microflora Under Retail Display

During chilled, aerobic display of pork chops, pseudomonads grow rapidly and dominate the microbial flora (Gill and Molin, 1991). Numbers of pseudomonads on pork the day after slaughter range in between 10 and 10^2 per cm^2 if hygienic practices are in place (Grau, 1986). At spoilage levels, pseudomonads reach numbers of 10^9/cm^2 (Stiles, 1991a; Gill and Greer, 1993) whereby they limit shelf life of pork by producing off odours from protein breakdown and amino acid metabolism (Stiles, 1991a) as well slime over the meat surface. *Enterobacteriaceae* do not contribute significantly to aerobic

spoilage flora because of their slow aerobic growth rates (Gill and Greer, 1993); however, they will dominate on meats displayed at poor refrigeration temperatures (10 °C) and limit shelf life through the production of off-odours (Stiles, 1991a).

2. Microflora Under Anaerobic Conditions and Refrigeration Temperatures

The use of anaerobic atmospheres in CAP technology functions to inhibit growth of strict aerobes and permit growth of facultative anaerobes, such as the fermentative LAB, which tolerate CO_2 saturation. The bacteriostatic nature of CO_2 results in the inhibition of microbial respiration and the extension of generation times (Gill and Tan, 1980). Blickstad et al. (1981) found that the time it took for the microbial flora to reach $10^7/cm^2$ at 4 °C was only 10 d in air compared to 40 d for pork within a 100% CO_2 atmosphere. Under refrigeration temperatures, the growth of psychrotrophic bacteria, which can grow at temperatures as low as – 3.0 °C (Gill and Molin, 1991), is permitted while the growth of CO_2 tolerant mesophiles is inhibited. Under refrigeration temperatures and CO_2 saturation, LAB emerge as the dominant population (Egan, 1983; Greer et al., 1993). As LAB emerge as the dominant species, they produce lactic acid as the end product of carbohydrate metabolism. Maximum numbers of LAB usually do not exceed 10^8 CFU/cm^2 under anaerobic atmospheres (Gill and Greer, 1993) whereby they can cause deterioration of flavour (Gill, 1996). After nine weeks of storage at –1.5 °C in 100% CO_2, LAB populations between 10^3 and 10^4 CFU/cm^2 can be expected (McMullen and Stiles, 1994).

3. Microflora Under Subsequent Retail Display

The microbial flora which evolves during retail display can vary between pork with and without previous CAP storage. CAP storage under low temperatures selects for the growth of psychrotrophs and selects against mesophiles. If one population dominates under 100% CO_2 at −1.5 °C, it effectively populates the meat surface which could otherwise have been populated by strict aerobic spoilage organisms. Because LAB dominate the flora of pork stored under 100% CO_2 at −1.5 °C, they dominate the flora during subsequent retail display (Greer et al., 1993). Pseudomonads do not tolerate the high concentrations of CO_2 during CAP storage (McMullen and Stiles, 1991); therefore, they do not dominate the flora during subsequent retail display (Greer et al., 1993), although population numbers will increase rapidly in the presence of oxygen.

H. Previous Studies

It is common practice for primal and sub-primal cuts of meat to be shipped to the retail level whereby they are cut, trimmed and repackaged for retail display (McMullen and Stiles, 1994). Previous studies on the CAP storage of pork at low temperatures used sub-primal cuts (Greer et al., 1993; McMullen and Stiles, 1994) and these were further broken, trimmed and repacked before final presentation. While the shelf life expectation of CAP-stored primal and sub-primal cuts can be > 90 days, the shelf life of "retail ready" cuts can be expected to be lower even though stored under CAP because they are not trimmed, sliced or repacked before retail sale. It was anticipated that the CAP storage

life of chops would be lower than that of sub-primals because on chops that have been cut before long-term storage, there is increased opportunity for microbial growth "final surfaces" which are not further trimmed before presentation. With sub-primals, there is opportunity to trim away discoloured surfaces, undesirable edges and surfaces with a high microbial load. This option does not exist with "retail-ready" products which are already reduced to consumer-size cuts (Jeyamkondan et al., 2000).

I. Objective of CAP Study

A specialized shipping container cooled by liquid nitrogen to maintain temperature at -1.5 ± 0.5 °C (Jeyamkondan, 1999) was previously designed to store "retail-ready" fresh meat in 100% CO_2 for extended periods of time. To evaluate the storage system, previously CAP-stored pork chops were subsequently displayed in a retail display case. To assess the storage life of CAP-stored pork chops (with and without retail display), microbiology, metmyoglobin formation and sensory changes were assessed.

PART II

J. Introduction

It has been shown that carnobacteria tend to dominate on fresh meats stored at 100% CO_2 at -1.5°C (McMullen and Stiles, 1994). In 1987, Collins proposed the new genus *Carnobacterium* to include "atypical" non-aciduric lactic acid bacteria (LAB) which are unable to grow on acetate-containing agar, like Rogosa medium, commonly used in the isolation of LAB (Collins et al., 1987). Carnobacteria have been isolated from a number of sources, including cheese (Milliere et al., 1994; Milliere and Lefebvre, 1994; Morea et al., 1999), fish (Gancel et al., 1997; Gonzalez et al., 1999; Lyhs et al., 1999), fresh meat and poultry products (Grant and Patterson, 1991; Ahn and Stiles, 1992; McMullen and Stiles, 1993; Samelis et al., 1998) as well as from Antarctic waters (Franzmann et al., 1991). Typically, LAB dominate on meats stored anaerobically under refrigerated storage, due to their psychrotrophic nature. Being facultatively anaerobic, LAB are able to grow during anaerobic storage (Blickstad et al., 1981; Greer et al., 1993; Penney et al., 1993; Venugopal et al., 1993). There is evidence, however, that *Carnobacterium* spp. grow faster and may initially dominate typical LAB on fresh meat stored at –1.5 °C in an atmosphere of 100% CO_2 (McMullen and Stiles, 1994; Nadon, 1998).

K. Characterization

Phenotypically, both carnobacteria and LAB are Gram-positive, non-sporeforming, catalase- and oxidase-negative. Both species produce lactic acid from the fermentation of sugars (Collins et al., 1987). Unlike LAB, carnobacteria optimally grow at pH 8-9 (Holzapfel, 1992). The presence of meso-diaminopimelic acid in cell walls, exclusive production of L-lactate (as opposed to D- or DL-lactate) further distinguish LAB from carnobacteria. Montel et al. (1991) noted that carnobacteria were unable to grow on acetate agar, whereas LAB can. Ouattara et al. (1997) confirmed the antimicrobial nature of organic acids (acetic-, propionic-, lactic- and citric acid) on the growth of *Carnobacterium* (*Cb.*) *piscicola*. They also noted that lactobacilli were relatively resistant to the action of the same organic acids. Although the heterofermentative carnobacteria and heterofermentative LAB use the pentose-phosphate pathway to ferment sugars to L-lactic acid, CO_2, acetic acid and/or ethanol, some LAB also have the ability to produce lactic acid through glycolysis during homofermentative catalysis (Bottazzi, 1988). Despite the similarities with LAB, evidence based on 16S rRNA homology (Wallbanks et al., 1990) suggests that carnobacteria are more closely related phylogenetically to the genus *Enterococcus*. Physiological similarities between carnobacteria and species of *Enterococcus* include vitamin requirements for growth and the ability to grow under alkaline conditions or in the presence of thallous acetate (Stiles and Holzapfel, 1997).

L. Methods Available to Enumerate Carnobacteria

Although they are time-consuming, molecular methods which utilize genomic probes for identification of carnobacteria (Nissen et al., 1994) are available. Various media are also available for the recovery of carnobacteria (Holzapfel, 1992) but have proven to be only semi-selective.

When attempting to isolate carnobacteria from fresh meats, McMullen and Stiles (1993) found that counts obtained on Plate Count Agar (PCA) were higher than those obtained on deMan Rogosa Sharpe (MRS) agar, a medium commonly used in the isolation of LAB from fresh meats. They attributed the lower recovery rate on MRS agar to its acetate content. It was also mentioned in their paper that the use of either PCA or MRS agar in previous investigations might have led to conflicting reports as to the type of LAB which evolve on MAP/CAP-stored meats. McMullen and Stiles (1993) noted that in instances when MRS agar was used, the principal isolates on fresh meats stored under modified atmospheres were homofermentative lactic acid bacteria, whereas when PCA was used, carnobacteria were mainly found. Although tedious, the standard approach for enumerating carnobacteria thus far has involved differential counting. By subtracting total counts obtained on acetate-agar from total counts obtained on PCA, carnobacteria populations can be determined (McMullen and Stiles, 1994; Nadon, 1998). Nadon (1998) also used Rogosa agar – an acetate containing medium – in the enumeration of carnobacteria by differential counting. However, an evaluation of this method was not undertaken.

CTAS agar was previously devised for the selective recovery of *Carnobacterium* spp. (Baird et al., 1989), however, problems with low recovery and interference by other genera prevented this medium from becoming widely accepted (Holzapfel, 1992; Nadon, 1998).

M. Objective of Carnobacteria Research

Most studies on fresh meat storage at refrigeration temperatures under anaerobic atmospheres (Greer et al., 1993; Penney et al., 1993; Holley et al., 1994a; Holley et al., 1994b) have not examined the role of carnobacteria amongst the microflora, due to a lack of a selective growth medium. Because of a need to determine the role of carnobacteria in the spoilage of fresh meats, the objective of this study was to improve the selective recovery of CTAS agar to allow its use by direct plating.

SECTION III: MATERIALS AND METHODS

PART I

A. Pork Sample Preparation and Treatment

1. Preparation of Pork Samples

Boneless pork loins (slaughter occurring the day before) were commercially obtained from Fletcher's Fine Foods Inc., Red Deer, AB. Loins were packed in a cooler with bagged ice before being transported to the experimental site (0.5 h away). Loins were cut into 2.5 cm thick portions in a highly sanitary, Federally-inspected research abattoir (Agriculture and Agri Food Canada, Lacombe). Eight randomly-chosen chops were placed on a sanitized cafeteria tray. Ten trays were used in total. All trays were placed in master packages (Winpak ESOPAEV2E 121575 R, Winpak, Winnipeg, MB) with an oxygen transmission rate of 0.55 ml/ (m^2·day). Air was evacuated from the bags, flushed with 100% CO_2 and then replaced with 4.4 L of 100% CO_2 atmosphere (average weight of 8 chops being 2 kg) using a Captron model # 2R897 (Captech, Hamilton, NZ) packaging machine. Residual O_2 levels in the bags were analyzed periodically (Mocon MS-750, Modern Controls Inc., Minneapolis, MN, USA) to monitor and ensure 100 ppm O_2 (1ml O_2 / 1000ml CO_2) or less was present.

2. Treatment of Samples at CAP Container

Master packages were randomly allocated to shelves within the CAP storage unit (Jeyamkondan, 1999). Pork chops, however, were not "retail ready" during CAP storage as they were not individually wrapped on separate trays. This was done to avoid residual

O_2 effects during primary storage. Individual chops were repacked in retail format after CAP storage, before retail display. The study was therefore a "simulation" of the commercial use of the container. The "simulated" master packages which contained a 100% CO_2 atmosphere remained in the storage unit at -1.5 ± 0.5°C, for a maximum of nine weeks with removal at weekly intervals.

3. Treatment of Samples at Retail Display

At the beginning of each trial week, a master package was removed from the jacketed CAP storage unit. Residual oxygen levels were analyzed (Mocon MS-750, Modern Controls Inc., Minneapolis, MN, USA) and recorded before opening a master package. Each chop was then placed on a clear 15 x 9 cm plastic retail tray (Show Off Meat Trays 93272, Western Paper and Food Distributors Ltd., Calgary, AB) lined with a 12 x 6 cm commercial soaker pad (Dri-Loc®, Sealed Air Ltd., Saddle Brook, NJ). A shrinkable polyvinylchloride, PVC, film having an O_2 transmission rate of 8000 ml/(m^2· day) (Vitafilm 'Choice Wrap', Huntsman Corp., Toronto, ON) was used to over-wrap individual trays. The meat was allowed to bloom for approximately one hour to allow oxygenation of myoglobin. Three digit numbers were randomly assigned to each retail tray. Trays were then placed in a retail display case (Model LPM12T, Hill Refrigeration of Canada, Ltd., Barrie, ON). Pork chops were illuminated with fluorescent lights for 24 h at an average of 1024 lux at the tray surface as measured by a lux meter (Model 401025 Davis Instruments, Baltimore, MD). Display case temperature was

approximately 4.0 °C, as monitored by a temperature data logger (Model 861-1TP, Tru-Test, Auckland, New Zealand).

4. Layout of Analyses

Retail trays remained in the retail case for the duration of the trial week (Monday through to Friday). The number of chops used for each analysis are listed in Table 1. The first analysis conducted on Monday morning of each week of retail display consisted of spectophotometric measurements of metmyoglobin content on all chops in the display case. The sensory panel was then brought in to evaluate all chops in the display case for visual and odour desirability. Two chops were randomly chosen for both microbial analysis and evaluation of palatability. These two chops were discarded after testing. As such, six chops remained in the display case on Tuesday through to Friday where analyses consisted of metmyoglobin measurements and evaluation of visual acceptability. On Friday, all chops were evaluated for odour acceptability. Odour was not evaluated between Tuesday and Thursday because it was desired that odour should saturate the atmosphere of the retail pack so as to allow easier evaluation on Friday morning. Evaluation of palatability and microbial analyses were also conducted on two randomly chosen chops on Friday.

Table 1: Number of chops designated for various analyses during a retail display period.

Analysis	Number of chops analyzed				
	Monday	Tuesday	Wednesday	Thursday	Friday
Metmyoglobin content	8 / 8 [a]	6 / 6	6 / 6	6 / 6	6 / 6
Sensory (visual)	8 / 8	6 / 6	6 / 6	6 / 6	6 / 6
Sensory (odour)	8 / 8	*	*	*	6 / 6
Sensory (palatability)	2 / 8	*	*	*	2 / 6
Microbiology	2 / 8	*	*	*	2 / 6

* Denotes that the analysis was not conducted on that particular day.
[a] Number of chops used for analysis / Total chops on retail display that day.

B. Metmyoglobin Measurements

1. Sample K/S Values

At the beginning of each day within each week of retail display, all PVC-overwrapped chops remaining in the retail case were analyzed spectrophotometrically (Macbeth Colour-Eye® M2020PT, Prism Instruments, Whitby, ON) for metmyoglobin content in order to assess changes incurred by CAP storage and retail display (American Meat Science Association, 1991). Each chop was scanned three times in randomly chosen areas and an average reflectance value was obtained. Reflectance values were expressed as K/S ratios to eliminate variation resulting from differences in pigment concentration, marbling and fibre orientation. K/S values were calculated at isobestic wavelengths where there is equal reflectance for two or more myoglobin forms (Judd and Wyszecki, 1963):

$$K/S_x = \frac{(1-R)^2}{2R} \quad \text{and } R = \text{reflectance at a particular wavelength (x)}$$

2. Standard K/S Values

Standard K/S values relating to 100% of either metmyoglobin, deoxymyoglobin or oxymyoglobin were obtained by chemical treatment of pork samples (American Meat

Science Association, 1991). To obtain standard K/S values for 100% metmyoglobin, the heme portion of myoglobin was fully oxidized in a 1.0 % potassium ferricyanide (Fisher Scientific, NJ) solution for 1 min. After removal from the solution, the chop was then blotted to remove excess solution and subsequently packaged in the same film used to over-wrap retail trays of pork (Vitafilm 'Choice Wrap', Huntsman Corp., Toronto, ON). Following placement at 2 °C for 12 h, the chop was then scanned by the spectrophotometer. To obtain standard K/S values for 100% deoxymyoglobin, a fresh pork chop was placed in a 10% dithionite (Fisher Scientific, NJ) solution. Sodium dithionite ($Na_2S_2O_4$), also known as sodium hydrosulfite, is a metal salt which is useful in the reduction of heme moieties in molecules such as myoglobin. The chop was removed from the solution, blotted and then vacuum packaged in order to fully reduce the myoglobin pigment. Following chemical reduction for 1 to 2 h at room temperature, the chop was repackaged in the retail film and scanned by the spectrophotometer. To obtain standard K/S values for 100% oxymyoglobin, a fresh pork chop was placed at 2 °C for 2 min. After packaging in the retail film, the standard was scanned to obtain reflectance values.

3. Calculation of Percent Metmyoglobin

To calculate % metmyoglobin in sample pork chops, standard K/S values and sample K/S values were used (American Meat Science Association, 1991):

$$\% \text{ metmyoglobin} = \frac{\frac{(K/S\ 572)}{(K/S\ 575)_{deox.st.}} - \frac{(K/S\ 572)}{(K/S\ 575)_{samp.}}}{\frac{(K/S\ 572)}{(K/S\ 575)_{deox.st.}} - \frac{(K/S\ 572)}{(K/S\ 575)_{met.st.}}}$$

Where:

K/S (572/575) refers to the K/S values at the specified wavelengths

deox.st. refers to the deoxymyoglobin standard

met.st. refers to the metmyoglobin standard

samp. refers to the meat sample

C. Sensory Evaluations

1. Sensory Panel

A five-member panel (3 novices and 2 experts) evaluated various sensory attributes of fresh pork chops. Members of the sensory panel were chosen on the basis of availability, and reflected diversity in terms of age, sex, race and education. All panelists gave

voluntary and informed consent to participate in the study. All members of the panel were trained by an expert in pork sensory analysis before the trial. Panelists were instructed not to pick up the chops during retail assessments of odour and visual appearance; however, tilting trays within the display case was allowed.

2. Visual Evaluations

At the beginning of each day within each week of retail display, all PVC-overwrapped chops were evaluated for visual acceptability by panelists (Appendix 2). Pictorial colour standards (Agriculture Canada, 1984) were on display to aid panelists in visual assessments of chops. Overall colour of chops (disregarding any discolouration that may have been present) was assessed using a 5-point hedonic scale with extreme points being given as: 1=extremely pale, and 5 = extremely dark. Percent surface discolouration was assessed using a 6-point hedonic scale with extreme points being given as: 1 = 0 % discoloured, and 7 = 100 % discoloured. Retail acceptance was assessed using a 7-point hedonic scale with extreme points being given as: 1 = extremely undesirable, and 7 = extremely desirable (American Meat Science Association, 1991).

3. Odour Evaluations

On days 0 and 4 of retail display, chops were evaluated for odour acceptability by sensory panelists (Appendix 2). To evaluate odour, PVC-overwrap was removed from the retail tray and the chop passed around for assessment by the panelists. For off-odour, a 4-point hedonic scale was used with extreme points being given as: 1 = no off-odour,

and 4 = prevalent off-odour. Odour acceptability was assessed using a 5-point hedonic scale with extreme points being given as: 1 = acceptable, and 5 = unacceptable (American Meat Science Association, 1991). Odour assessments were conducted on day zero and day four of retail display.

4. Preparing Samples for Palatability Testing

The booth area designated for palatability testing was completely separate from the food preparation area, so as to eliminate noise distractions and cooking smells. Booths were adequately ventilated and comfortable, in terms of temperature and humidity. Panelists were assigned to individual booth areas where samples were to be assessed. The person designated to cook the chops was not a member of the palatability panel. For palatability testing, two chops were randomly chosen and placed on a preheated grill (Hamilton Beach 31600, Proctor-Silex Inc., Picton, ON). Iron-constanan microthermocouples (TT-J36) connected to an Omega multipoint digital thermometer (Model 2166A) were inserted into the middle of the pork chops, parallel to the meat surface. Pork chops were flipped over when the internal temperature reached approximately 40°C. Chops were characterized as fully cooked when the internal temperature reached 72°C. In preparation for serving samples, the edges of each chop were cut off. When chops were cut into cubes of approximately 1.5 cm^3, large areas of fat and connective tissue were avoided. Coloured pins were inserted into each cube in order to designate which chop they originally came from. Pork cubes were then individually placed into glass jars and covered with a watch glass. Jars were placed into a 72°C waterbath (Magni Whirl, MW-

1120A-1, Blue Island, IL, USA) for 5 to 7 min. Each tray used for palatability testing contained a glass of distilled water, two unsalted crackers (to remove residual flavours between sampling), one paper napkin, one toothpick, glass jars holding the samples to be evaluated, and an evaluation form. Trays were passed into individual booths, using a "pass through" from the adjacent food preparation area.

5. Palatability Evaluations

On days 0 and 4 of retail display, two chops were evaluated for overall palatability by panelists (Appendix 2). For this set of evaluations, panelists were asked to rate flavour and off-flavour, both of which are based on personal preferences. Overall palatability was also assessed because it encompassed panelists' perceptions of flavour, off-flavour as well as other factors (such as texture and tenderness) which were not directly assessed. Flavour was assessed using a 6-point hedonic scale with extreme points being given as: 1 = undesirable, and 6 = desirable. Off-flavour intensity was assessed using a 4-point hedonic scale with extreme points being given as: 1 = no off-flavour, and 4 = prevalent off-flavour. Overall palatability was assessed with a 6-point hedonic scale with extreme points being given as: 1 = undesirable, and 6 = desirable (American Meat Science Association, 1991). Flavour assessments were conducted on day zero and day four of retail display.

6. Rejection Limits of Sensory Scores

To test the efficacy of the CAP storage container, retail case life was measured by assuming rejection would occur at: "slightly undesirable" for overall palatability; "slightly unacceptable" for overall odour, and "slightly undesirable" for visual acceptance. Numerical endpoints were 3, 4, and 3, respectively.

D. Bacteriological Sampling

1. Preparation of Samples for Microbial Analyses

Bacterial growth was evaluated on randomly chosen pork chops from the retail display case. A sterile corer was used to remove a random 10 cm^2 area of muscle tissue. To obtain samples, corers were pushed approximately halfway through the pork chops. After removal of the corer, a sterile blade was used to cut the core away from the meat tissue. Muscle cores were massaged for 2 min in 10 ml of 0.1% sterile peptone water, using a laboratory blender (Stomacher 400, BA7021, Seward Medical, London, UK), followed by serial ten-fold dilution in sterile 0.1% peptone water. Two chops were sampled on the first and last day of each retail display period. LAB, carnobacteria, pseudomonads and total bacterial numbers were determined using a spread-plate technique. *Enterobacteriaceae* were enumerated using an overlay pour-plate procedure.

2. Enumeration of Bacterial Populations

Lactic acid bacteria were enumerated on MRS medium (Difco, Detroit, MI), incubated for 3 d at 25°C in a CO_2-enriched anaerobic atmosphere, using the BBL GasPak Pouch System (Becton and Dickenson Co., Cockeysville, MD). Pseudomonads were enumerated on Cephaloridine Fucidin Cetrimide (CFC) agar incubated for 2 d at 25°C. CFC agar was made using heart infusion (HI) agar (Difco, Detroit, MI), supplemented with cephaloridine, fucidin, cetrimide (Sigma, St. Louis, MO). Carnobacteria were enumerated on CTSI agar at 25°C for 2 d then 8°C for 2 d (Table 2 from Part II of Materials and Methods section). *Enterobacteriaceae* were enumerated using overlaid plates of Violet Red Bile Glucose (VRBG) agar (Difco, Detroit, MI), incubated at 35°C for 2 d. Total bacterial numbers were determined on APT agar (Difco, Detroit, MI), incubated at 25°C, for 2 to 3 d. Colonies from the respective agars were counted and the recovery was determined as colony forming units (CFU) per ml. Bacterial growth on pork chops was reported using semi-logarithmic plots of $\log_{10}$ bacterial numbers (CFU/cm^2) versus CAP storage time (weeks) and $\log_{10}$ bacterial numbers (CFU/cm^2) versus day 4 of retail display for a particular CAP storage period (weeks).

E. Statistical Analysis

Experimental data were subjected to statistical analysis using SAS analytical software (SAS Institute Inc., 1989). The general linear models procedure (Proc GLM) was used to analyze the variance of the collected data.

PART II

F. Preparation of CTSI Agar

Components of the newly developed CTSI agar are given in Table 2. Modification of CTAS (Appendix 1) is based on an earlier recipe (Baird et al., 1989) with changes being a two-fold increase in manganese, a two-fold decrease in the thallium acetate concentration, the additions of thiamine hydrochloride, vancomycin, nisin and the inclusion of a two-temperature incubation procedure. All components of CTSI agar were obtained from Sigma (St. Louis, MO) except for peptone, yeast extract (Difco, St. Louis, MO) and nisin (2.5 g/kg w/w Chrisin, lot # 17127, Canadian Inovatech, Abbotsford, BC). All components except triphenyl-tetrazolium-chloride (TTC) were added to 900 ml distilled water. The solution was boiled to dissolve all components completely, followed by cooling of the solution to 55°C whereby pH was adjusted to 9.1. Agar was then added and the solution heated again. After sterilization at 121°C for 15 min, the medium was placed in a pre-heated 55°C waterbath for approximately 15 to 30 min. TTC was added following its sterilization and the medium was dispensed into petri dishes and allowed to solidify before use.

Table 2: Composition of CTSI agar [a]

Component	g /L
Peptone	10.0
Yeast extract	10.0
Sucrose	10.0
Inulin	10.0
Tween 80	1.0
Sodium citrate	5.0
$MnSO_4 \cdot H_2O$	10.0
Di-potassium hydrogen phosphate	2.0
Thiamine hydrochloride	0.001
Chrisin® (nisin)	0.00125
Vancomycin	0.001
Thallium acetate	0.5
Nalidixic acid	0.04
Cresol red	0.004
pH to 9.1 with 1N NaOH	
Agar	15.0
Distilled water	1000 ml
TTC [b]	0.01

[a] Plates were incubated aerobically at 25 °C for 2 d, followed by 2 d at 8 °C.

[b] Added after autoclaving by filter sterilization.

G. Bacterial Strains

All bacterial strains used in the testing of CTSI agar can be found in Table 3 (Results section). *Pediococcus*, *Weissella* and carnobacteria were grown in APT broth and lactobacilli were grown in MRS broth. All other bacterial strains were grown in Brain Heart Infusion (BHI) broth (Difco, Detroit, MI). All strains were incubated anaerobically in a CO_2-enriched atmosphere using the BBL GasPak Pouch System with the exceptions of *Brochothrix*, *E. coli*, *Listeria* and *Staphylococcus* which were grown under aerobic atmospheric conditions. All strains including the latter group, were grown at 22°C for 1 to 2 d.

H. Qualitative Testing

Ten species of carnobacteria and 18 organisms from 11 other genera were tested for their ability to grow on CTSI and CTAS by a loop streaking method (Reuter, 1985) on prepoured agar. Agar plates were sectioned into quadrants into which an organism was streaked. Organisms were also streaked on either APT, BHI or MRS agar to ensure that metabolically active cells were being used. All plates were incubated at 25°C for 2 d, followed by 2 d at 8°C. Carnobacteria strains were also plated on Rogosa agar (Difco, Detroit, MI) incubated for 2 d at 25°C anaerobically using the BBL GasPak Pouch System to confirm their inability to grow on acidified media (McMullen and Stiles, 1994).

I. Quantitative Testing

To evaluate quantitative recovery on CTSI, dilutions of carnobacteria suspensions were spread in duplicate on either CTSI or APT. Strains used in the quantitative testing of CTSI agar are listed in Table 4 (Results section). Plates were incubated for 25 °C for 2 d, followed by 2 d at 8 °C. Colonies were counted and the recovery was determined as colony forming units (CFU) per ml. Five replicates were conducted.

J. Statistical Analysis

Selective recovery on CTSI was compared to non-selective recovery on APT using SAS analytical software (SAS Institute Inc., 1989). The general linear models procedure (Proc GLM) was used to analyze the variance for each strain tested. An alpha value of 0.05 was used to evaluate differences. Differences between means were compared using Duncan's test.

SECTION IV: RESULTS

PART I

A. Metmyoglobin Formation

Chops were analyzed for metmyoglobin content upon removal from the CAP storage unit and upon each subsequent day of retail display. Metmyoglobin content was significantly affected by the duration of previous CAP storage ($p < 0.05$), yet there was not a linear relationship ($p = 0.88$). Metmyoglobin content was significantly affected by the amount of time spent on aerobic retail display ($p < 0.05$) and this relationship was linear ($p < 0.05$). As chops spent more time on display, metmyoglobin content increased. The interaction between CAP storage and retail display was not significant ($p > 0.05$) therefore indicating that the metmyoglobin content by the end of the display week did not vary among the chops with various storage times in the container. Figure 1 illustrates the erratic formation metmyoglobin on pork chops during CAP storage as well as its formation during subsequent aerobic storage.

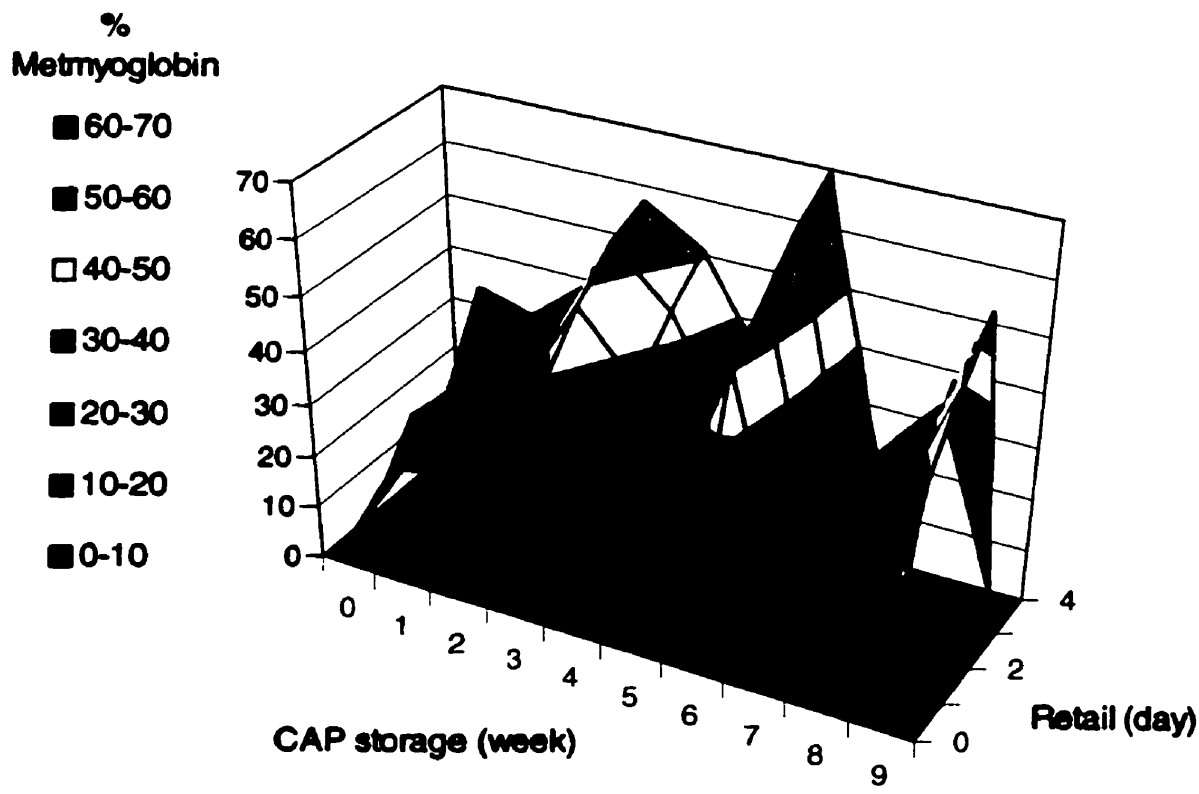

Figure 1: Percent metmyoglobin on pork chops with various storage times under CAP (100% CO_2, -1.5 $\pm$ 0.5 C) and retail display (aerobic, 4 C).

B. Visual Assessments

Previously CAP-stored chops were visually rated by panelists after removal from the container (Appendix 3) and on each day of subsequent retail display (Appendix 4). Chops without previous CAP storage (controls) were also assessed.

1. Effect of CAP Storage

Ratings of chop colour, % discolouration and overall visual acceptance (Appendix 3) were dependent on the duration of storage within the CAP storage container ($p < 0.05$).

2. Effect of Retail Display

Ratings of % discolouration and overall visual acceptance (Appendix 4) were dependent on the length of retail display ($p < 0.05$) whereas ratings of chop colour (Appendix 4) were not ($p = 0.14$). Multiple comparison tests (Duncan, SNK, SAS Institute, 1989) of % discolouration scores during retail display showed that panelists' perceived differences between subsequent days of retail display.

3. Overall Visual Acceptability

Overall visual acceptance varied significantly with combinations of CAP storage time and retail display ($p < 0.05$). Overall acceptance of chops varied linearly with previous CAP storage ($p < 0.05$) and retail display ($p < 0.05$). Overall visual acceptance of chops (CAP storage) on day 4 of retail display, had a mean score of 4.5. Pork chops with 9

weeks of previous CAP storage had a mean acceptability rating of 4.1 after 4 d of retail display. Visual scores of 4 denoted "slightly desirable" and scores of 5 denoted "moderately desirable". It was evident that after 9 weeks of CAP storage, pork chops still had the visual acceptability of chops without previous CAP storage.

4. Correlation with Metmyoglobin Measurements

Panelists' perceptions of % discolouration correlated somewhat to the actual metmyoglobin content ($r = 0.69$). The actual metmyoglobin content was also negatively correlated with panelists' visual acceptance of the chops ($r = - 0.71$).

C. Palatability Assessments

Previously CAP-stored pork chops were assessed by a sensory panel for changes in palatability on days 0 (Appendix 3) and 4 (Appendix 4) of retail display. Chops without previous CAP storage (controls) were also assessed on day 0 and day 4 of retail display.

1. Effect of Previous CAP Storage

Ratings of flavour, off-flavour intensity and overall palatability varied significantly ($p < 0.05$) from the control (no previous CAP storage) depending on the duration of storage in the CAP container (Appendix 3). As storage of the chops within the container increased, the average ratings of flavour and overall palatability decreased. This indicated that chops were less appealing in flavour and palatability. Ratings of off-

flavour intensity increased as CAP storage increased, therefore indicating that off-flavours were increasingly perceived by panelists. An r value of –0.60 between perceived off-flavour intensity and overall chop palatability was found, therefore suggesting that chops developed off flavours during storage in the container which adversely affect overall chop palatability. After a 9 week storage period in the container, however, chops had an average palatability score of 4.5 (Appendix 3) which was still above the rejection score of 3. Although previous CAP storage adversely affected overall chop palatability, chops were still acceptably palatable after 9 weeks of storage.

2. Effect of Subsequent Retail Display

Analysis of palatability scores (Appendix 4) on day 4 of retail display showed that flavour ($p=0.58$) and overall palatability ($p=0.59$) did not vary significantly among the various weeks of previous CAP storage (Appendix 4). However, average scores for off-flavours did vary significantly on day 4 of retail display depending on the duration of previous CAP storage ($p < 0.05$).

3. Overall Acceptability of Chop Palatability

Overall palatability of chops without previous CAP storage was rated at day 4 of retail display, and the mean score was 4.0 Pork chops with 9 weeks of previous CAP storage had a mean acceptability rating of 4.5 after 4 d retail display (between "slightly" and "moderately" desirable). The purpose of the CAP storage system was not to improve quality of the stored chops, but rather to maintain product quality. It was evident;

therefore, that at 9 weeks of CAP storage, pork chops had the palatability of pork chops without previous CAP storage.

D. Odour Assessments

CAP-stored pork chops were assessed on days 0 (Appendix 3) and 4 (Appendix 4) of retail display by a sensory panel for changes in odour. Chops without previous CAP storage (controls) were also assessed on day 0 and day 4 of retail display.

1. Effect of Previous CAP Storage

Statistical analysis of scores for off-odour intensity (Appendix 3) on day 0 of retail display did not vary significantly with the duration of previous CAP storage ($p=0.32$); however odour acceptability scores did vary significantly ($p < 0.05$).

2. Effect of Subsequent Retail Display

Scores for off-odour intensity as analyzed on day 4 of retail display did vary significantly with the duration of previous CAP storage ($p < 0.05$); however, scores for odour acceptability did not ($p = 0.22$). Overall acceptability of chop odour without previous CAP storage (control) was assessed on day 4 of retail display (Appendix 4), and the mean acceptability rating was 1.3.

3. Overall Acceptability of Odour

Overall acceptability of chop odour without previous CAP storage was assessed on day 4 of retail display, and the mean acceptability rating was 1.3. Chops with 9 weeks of previous CAP storage were also assessed on day 4 of retail display, and the mean rating for these chops was 1.5. Scores of 1 denoted acceptable scores and scores of 2 denoted slightly acceptable scores. It was evident that chops with 9 weeks of previous CAP storage had the same odour acceptability after 4 d as chops without any previous CAP storage.

E. Variability of Sensory Scores

In order to compare the sensory attributes from various weeks of CAP storage after 4 d of retail display, scores were plotted on a spider diagram (Figure 2). This helped to discern what deteriorative changes were occurring when determining product shelf life (Gillette, 1984). Results in Figure 2 show the variability of sensory scores from chops with 4 d of retail display and varying lengths of previous CAP storage. It became apparent that scores did not vary greatly from the control (week 0). During week 4 however, scores inexplicably deviated from those of the control week ($p < 0.05$) for all 3 attributes. Overall palatability scores decreased slightly indicating chops were less palatable. Odour acceptability scores increased, indicating chop odour was less acceptable. Visual acceptability scores decreased indicating chops were less visually appealing to panelists.

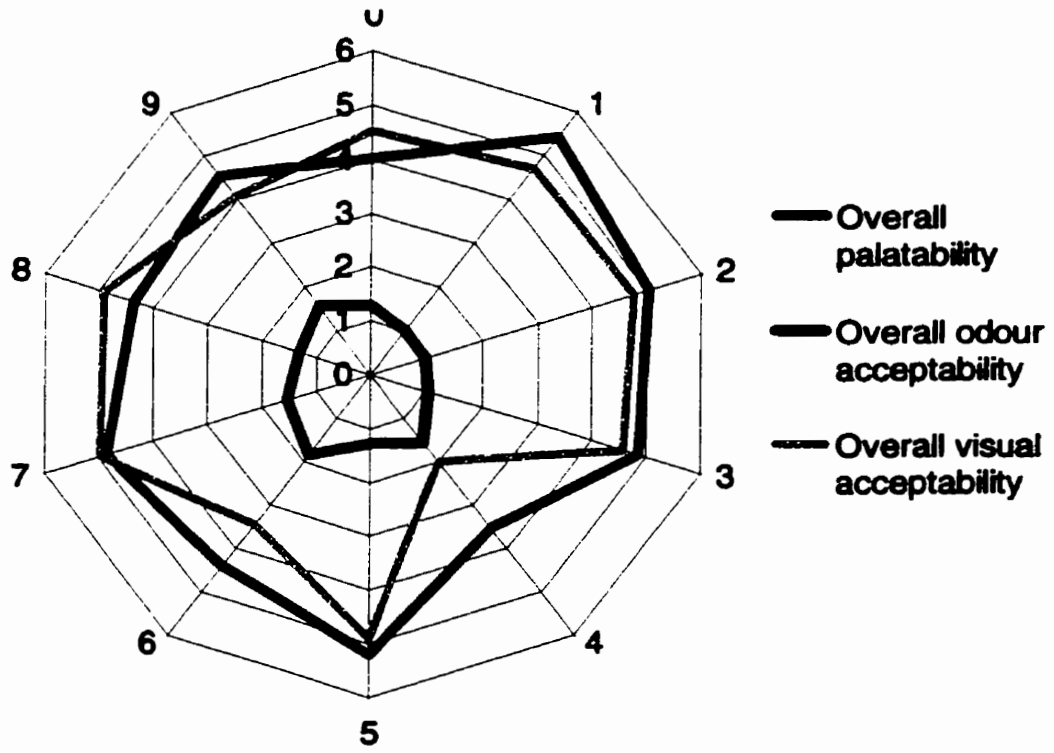

Figure 2: Spider diagram of sensory scores (plotted on radii) obtained from pork chops with 4 d retail display (4 C, aerobic) and various weeks of previous CAP storage at -1.5 $\pm$ .5 C and 100% CO_2 (plotted on perimeter). Sensory scores range from 0 to 6.

F. Microbial Analyses

1. Effect of Previous CAP Storage

Growth of various microbial species during the course of CAP storage (−1.5 ± 0.5 °C, 100% CO_2) was assessed (Appendix 5) and plotted (Figure 3). Analysis of variance determined that all groups enumerated (total numbers, LAB, carnobacteria, enterics and pseudomonads) were significantly affected ($p < 0.05$) by the amount of time spent in the CAP storage container. The total population detected at various CAP storage intervals was found to correlate with the development of the LAB population (correlation coefficient, $r = 0.62$). The correlation between pork storage in the CAP unit and emergence of bacterial species over time was significant ($p < 0.05$) for the LAB, carnobacteria and pseudomonads. The enterobacteria did not develop significantly during CAP storage time ($p = 0.24$).

2. Effect of Subsequent Retail Display

Previously CAP-stored pork chops were displayed in a retail case for a total of 4 d. All microbial species enumerated (Appendix 6) were plotted (Figure 4) to visualize the effect of previous CAP storage on microbial numbers on day 4 of retail display. Statistical analysis showed that growth of all bacterial species was significantly affected by CAP storage time ($p < 0.05$), retail display time ($p < 0.05$) and the interaction between CAP storage time and retail display time ($p < 0.05$). Dominant microbial species at day 4 of

retail display were those that correlated with the total population enumerated. These populations were the LAB ($r = 0.86$) and the pseudomonads ($r = 0.74$).

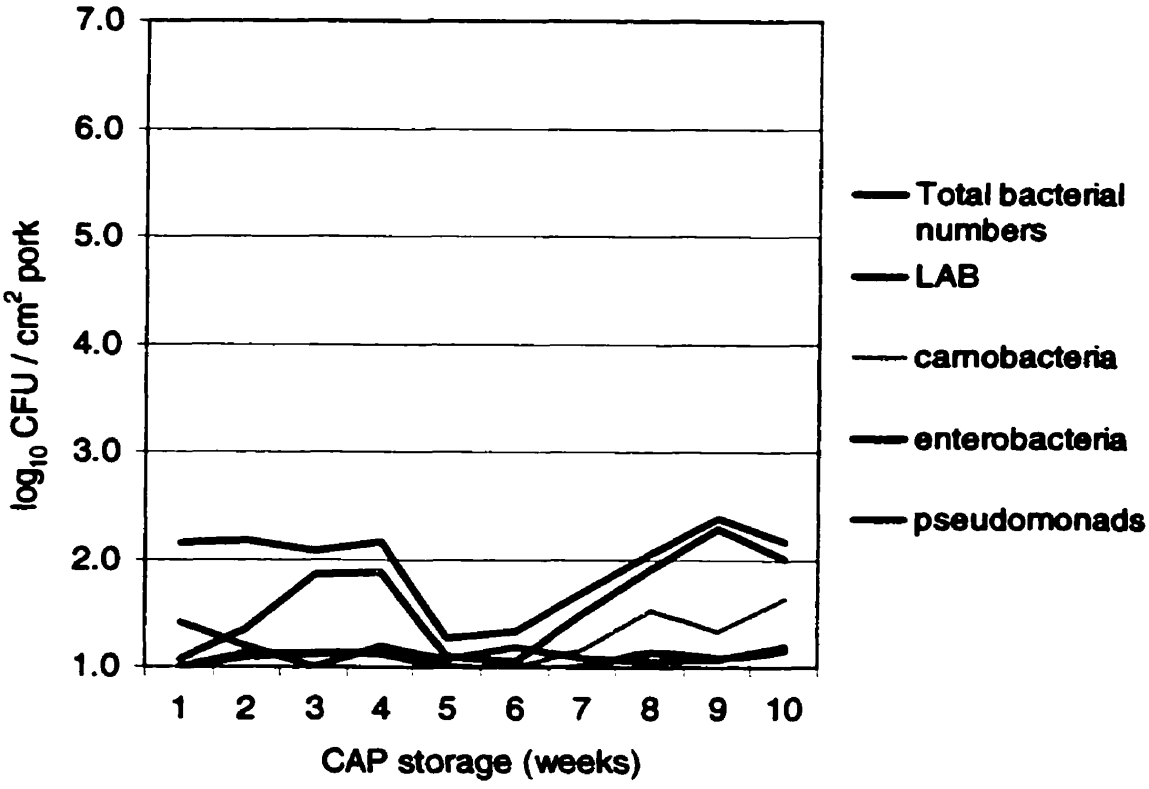

Figure 3: Evolution of microbial populations with various durations of previous CAP storage (100% CO_2, -1.5 $\pm$ 0.5 C)

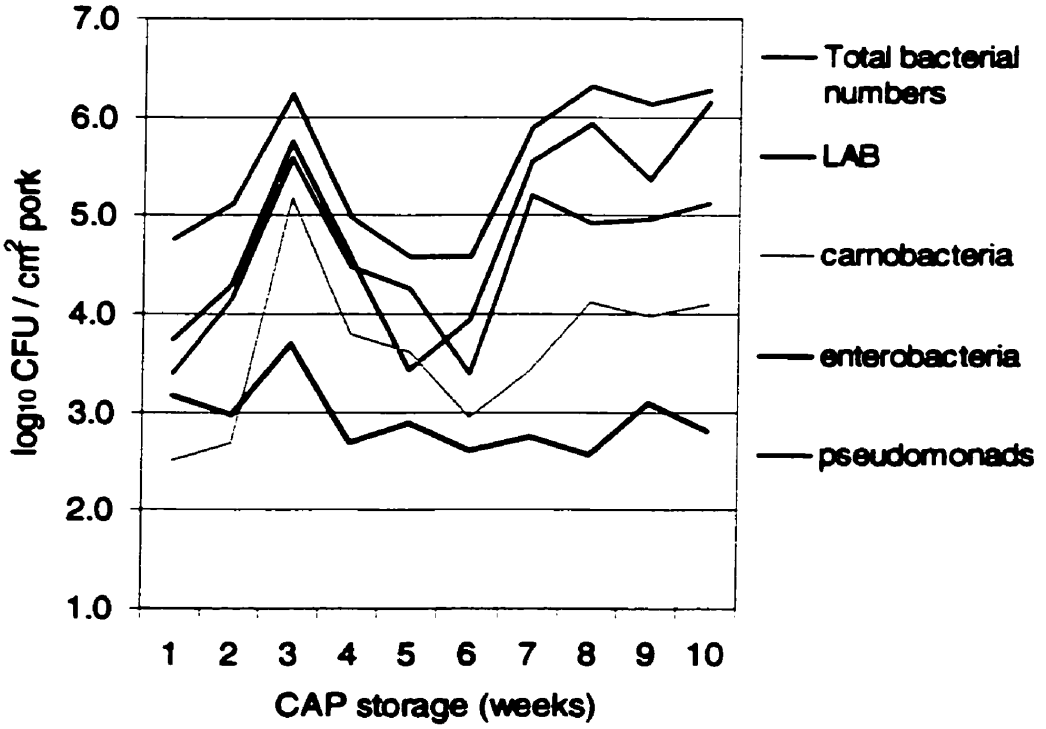

Figure 4: Enumeration of microbial populations after 4 d aerobic retail storage of pork at 4 C. Samples were previously held at -1.5 $\pm$ .5 C under 100 % CO_2 for up to 9 weeks (CAP storage).

G. Correlation of Residual Oxygen Levels with Other Data

Residual oxygen levels within master packages upon removal from the CAP container were measured in order to assess the influence upon various microbial populations and metmyoglobin formation. The amount of oxygen utilized was calculated by subtracting the measured oxygen level of the master package before opening from the average oxygen level of master packages before their placement in the CAP container. After plotting residual oxygen (Figure 5), it became evident that levels of oxygen remaining in the bags during the course of CAP storage were erratic in nature. There was not, however, a strong linear relationship between remaining oxygen levels and duration of CAP storage ($p > 0.05$). Week 5 had the highest level of residual O_2 at about 85 ppm. It must be noted, however, that only one package was analyzed per storage week. Pinholes could not be found on the respective master package. Incomplete sealing of the package might have been responsible for the higher than expected residual oxygen levels. In the initial meat trial (which was eventually abandoned), pinholes were found in the storage bags. In other instances, incomplete seals on the bag could be found when held up to the light. Before initiating the storage trials, all master packages were thereafter heat-sealed twice before placement in the CAP container so as to decrease incomplete sealing.

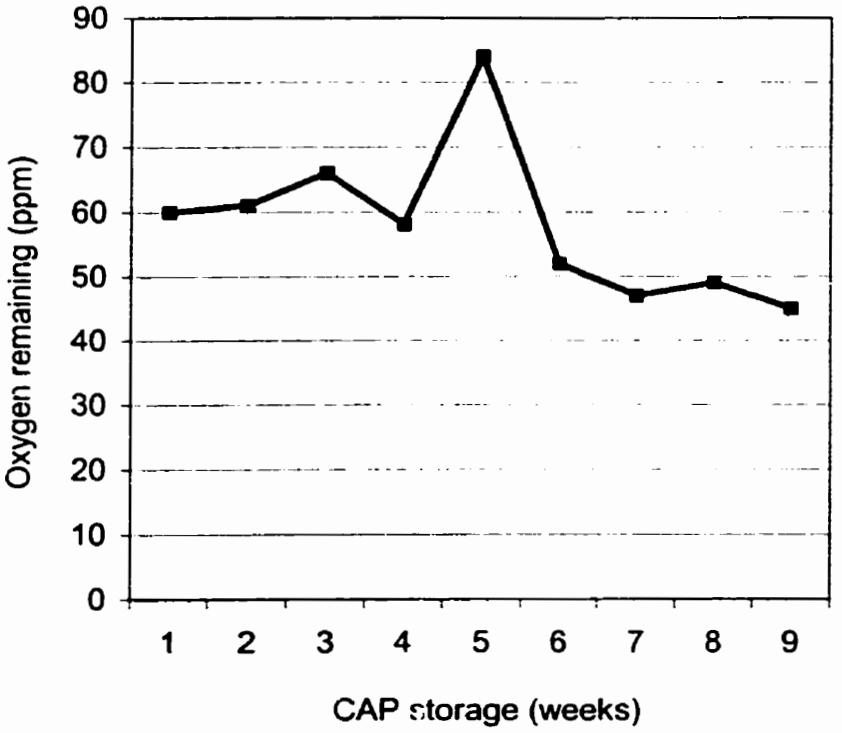

Figure 5: Amount of oxygen remaining in CAP packages after storage in CAP container (-1.5 ± 0.5 °C, 100% CO_2).

1. Sensory data

The residual oxygen in the master packs upon removal from the container negatively correlated ($p < 0.05$) with panelist scores for flavour (indicating a deterioration in flavour when O_2 levels were higher) and positively correlated with off-flavour intensity scores (indicating off-flavours became more apparent when O_2 levels were higher). Scores for overall palatability did not correlate ($p > 0.05$) with residual oxygen levels. This may indicate that only the flavour components of overall palatability are affected by residual oxygen levels and that other components - such as texture or tenderness - may not have been as sensitive since overall palatability scores did not correlate with residual oxygen levels. Residual O_2 levels strongly and positively correlated with panelist scores of off-odour intensity and odour acceptability ($p < 0.05$). That is to say: when residual oxygen levels were high within master packages, scores for off-odour intensity were higher (indicating that odours were more apparent to panelists) and scores for odour acceptability were higher (indicating chop odour was less acceptable to panelists).

2. Bacterial data

Residual oxygen levels were not high enough to allow bacterial populations to reach spoilage levels while pork was stored for up to 9 weeks in the container. Residual oxygen levels present within master packages negatively correlated with the total population of bacteria ($p < 0.05$), *Enterobacteriaceae* ($p < 0.05$) and the pseudomonads ($p < 0.05$). The highest level of residual oxygen was associated with the master package removed after five weeks of storage in the container (Figure 5). During this week,

bacterial population numbers were at their lowest (Figure 3). Population numbers on chops from CAP week 0 were significantly greater than that on chops from CAP weeks 4 ($p = 0.0031$) and 5 ($p = 0.0055$). The differences were greater than 1 log. This is surprising since it was anticipated that residual oxygen present in the master packages would facilitate growth by aerobic populations. Lower microbial numbers at CAP weeks 4 and 5 may be attributed to meat samples which had a lower initial microbial load.

PART II

H. Selective Recovery of Carnobacteria on CTSI

The inability of carnobacteria to grow on Rogosa agar (Table 3) verified the inability of this genus to grow on agar containing acetate. Although LAB and organisms occasionally or frequently found in meat environments were unable to grow on CTSI using the two-temperature aerobic incubation procedure (Table 3), ten strains of carnobacteria from five different species were able to grow. On CTAS agar, none of the carnobacteria strains tested were able to grow under the incubation conditions chosen. *Enterococcus* (En.) *faecalis* was the only interfering organism on CTAS but its growth was inhibited on CTSI. The differences between the mean recovery of *Carnobacterium* spp. on CTSI and APT (Table 4) were not statistically different ($p > 0.05$).

Table 3: Evaluation of the selectivity of CTSI and CTAS agars incubated for at 25 °C for 2 d, followed by 2 d at 8 °C.

Organism	Strain	Presence of growth (+/-) [a]		
		CTSI	CTAS	ROGOSA
Carnobacterium gallinarum	ATCC 49517	+	-	-
Carnobacterium mobile	ATCC 49516	+	-	-
Carnobacterium piscicola	ATCC 43224	+	-	-
Carnobacterium piscicola	ATCC 43225	+	-	-
Carnobacterium divergens	ATCC 35677	+	-	-
Carnobacterium divergens	M5S4 [c]	+	-	-
Carnobacterium divergens	M5S3 [c]	+	-	-
Carnobacterium piscicola	M5R1 [c]	+	-	-
Carnobacterium piscicola	M5S1 [c]	+	-	-
Carnobacterium piscicola	M5L1 [c]	+	-	-
E.coli O157:H7	ATCC 7268	-	-	ND [b]
Serratia grimesii	S12 [d]	-	-	ND
Weissella viridescens	ATCC 12706	-	-	ND
Salmonella typhimurium	# 98 [e]	-	-	ND
Shewanella putrefaciens	ATCC 8071	-	-	ND
Brochothrix thermosphacta	B2 [f]	-	-	ND
Pediococcus acidilactici	122P [g]	-	-	ND
Listeria monocytogenes	UMB [h]	-	-	ND
Enterococcus faecalis	ATCC 7080	-	+	ND
Staphylococcus aureus	# 427 [e]	-	-	ND
Yersinia enterocolitica	490 [i]	-	-	ND
Yersinia enterocolitica	490a [i]	-	-	ND
Yersinia enterocolitica	UG [j]	-	-	ND
Lactobacillus hilgardii	ATCC 8290	-	-	ND
Lactobacillus sakei	6 [k]	-	-	ND
Lactobacillus sakei	7 [k]	-	-	ND
Lactobacillus sakei	9 [k]	-	-	ND
Leuconostoc mesenteroides	1 [k]	-	-	ND
Leuconostoc mesenteroides	11 [k]	-	-	ND
Lactobacillus curvatus	10 [k]	-	-	ND
Lactobacillus curvatus	15 [k]	-	-	ND

[a] + growth, - no growth. [b] ND (Not Determined).
[c] From commercial vacuum packaged refrigerated pork. [d] Ward et al., 1998.
[e] Dr. F. Bartlett, Agriculture and Agri-Food Canada, Guelph, ON.
[f] Dr. G.G. Greer, Agriculture and Agri-Food Canada, Lacombe, AB.
[g] From commercial starter culture for dry fermented sausage (R.A. Holley)
[h] Dr. G. Blank, Dept. Food Science., Univ. Manitoba.
[i] Dr. P. Maeba, Dept. Microbiol., Univ. Manitoba.
[j] Dr. M.W Griffiths, Dept. Food Sci., Univ. Guelph. [k] Holley et al., 1996.

Table 4: A comparison of the mean recovery of *Carnobacterium* spp. grown on APT and CTSI agars [a]

Bacterial species	Strain	Population (log_{10} CFU/ml) [b]	
		APT	CTSI
Cb.divergens	ATCC 49517	8.52 a	8.20 a
Cb.gallinarum	ATCC 49516	8.21 a	8.19 a
Cb.piscicola	ATCC 43224	8.18 a	8.23 a
Cb.piscicola	ATCC 43225	8.18 a	8.11 a
Cb.mobile	ATCC 35677	8.72 a	8.22 a
Cb.divergens	M5S3 [c]	8.56 a	8.59 a
Cb.divergens	M5S4 [c]	8.33 a	8.25 a
Cb.piscicola	M5R1 [c]	8.59 a	8.39 a
Cb.piscicola	M5L1 [c]	8.30 a	8.36 a
Cb.piscicola	M5S1 [c]	8.26 a	8.10 a

[a] Agar plates incubated aerobically at 25 °C for 2 d, followed by 2 d at 8 °C.
[b] Values within the same row followed by the same letter are not significantly different ($p \leq 0.05$) according to Duncan's comparison of means.
[c] From wholesale vacuum packaged refrigerated pork (G. Zhang, this laboratory).

SECTION V: DISCUSSION

A. Adverse Pigment Changes

When chops were on retail display, metmyoglobin content increased linearly with display time. The aerobic conditions under which the chops were displayed allowed for the rapid oxidation of myoglobin pigment to the met-form. Zhu and Brewer (1998) previously found that chops stored aerobically at 4 °C for 4 d had a 20% metmyoglobin content in the absence of light versus a metmyoglobin content of 45% when illuminated with 3013 lux of light using a 12 h on / 12h off light cycle. For this thesis work, a standardized illumination period of 24 h/d (American Meat Science Association, 1991) was followed. An average of 33% metmyoglobin was present on control chops (no previous CAP storage) after 4 d of display at 4 °C when 1024 lux of illumination was used. Drawing from the study conducted by Zhu and Brewer (1998), it is suggested that the metmyoglobin content in chops would have been lower during retail display had the case been illuminated with an on/off cycle. This is of practical importance because display cases are only lighted during that part of the day when a store is open to customers.

During long-term storage of chops in the CAP container, there were no linear increases of metmyoglobin with increasing periods of storage. Instead, metmyoglobin content appeared to increase between weeks 0 and 3, weeks 5 and 6 and weeks 7 and 9. Low metmyoglobin content was present on pork chops between weeks 3 and 5 and between weeks 6 and 7. The erratic nature of metmyoglobin formation during the entire

experimental period (Figure 1) may have been due to the presence of different residual oxygen levels (Figure 5) within the individual CAP storage bags. Previous studies have demonstrated that metmyoglobin formation is influenced by the oxygen consumption rate (Zhu and Brewer, 1998). Different ratios of CO_2 volume/mass of meat between the various master packages could have led to differences in the oxygen consumption rate. When master packages were filled with 100% CO_2, the target was a ratio of 2 L CO_2/ kg of meat. However, the combined mass of pork chops packaged may have been less than needed to achieve that ratio. While a greater volume of CO_2 used would normally be beneficial, in some instances it may have yielded a higher than desirable residual O_2 level during the gas flush operations with respect to the actual mass of meat present in the package. As such, the residual O_2 concentration available at the surface of the pork chops (during the course of CAP storage) would be greater, thus facilitating the oxidation of myoglobin to metmyoglobin. Futhermore, erratic metmyoglobin content may have been found to be more uniform during the course of CAP storage if more than one master package had been sampled.

It is well known that metmyoglobin is perceived as discolouration by consumers and largely determines overall preference (Brewer et al., 1998). In this study, there was a strong correlation between the increased metmyoglobin content in chops during retail display and the perceived percent discolouration as assessed by panelists. Overall visual acceptability of chops on retail display as assessed by panelists decreased as metmyoglobin content increased. However, chops were still acceptable at the end of the study period.

B. Sensory Changes

Because the acceptability of fresh pork is ultimately determined by the consumer, it was necessary to conduct analyses of sensory changes which occurred during CAP storage and subsequent retail display of the product. Measurements of metmyoglobin content and scores of percent discolouration on pork chops were found to correlate positively. Thus panelists were visually sensitive to the aging process. However, after 9 weeks of CAP storage, chops were still deemed visually acceptable to panelists. This was in agreement with Jeremiah et al. (1992) where a deterioration in the appearance of fresh pork chops was observed only after 18 weeks at –1.5 °C in an atmosphere of 100% CO_2. McMullen and Stiles (1994), however, found that fresh pork chops stored at –1.5 °C in 100% CO_2 were rejected after 7 weeks due to a deterioration in appearance.

It was observed during CAP storage that pork chops acquired off-flavours which increased with storage time. As well, chop flavour was scored as less desirable with increasing periods of storage. This is in agreement with the findings of Jeremiah and Gibson (1997) where the flavour and palatability of fresh pork was found to steadily deteriorate as storage time under CAP (100% CO_2, –1.5 °C) progressed. A correlation (r) of –0.60 between perceived off-flavour intensity and overall chop palatability was found. It was noted by Jeremiah and Gibson (1995) that off-flavours tended to develop between 6 to 9 weeks of storage at –1.5 °C in 100% CO_2. After 9 weeks of storage in the container, chops still remained palatable to panelists despite the growing intensity of off-flavours. This is in agreement with the results obtained by Jeremiah et al. (1992), where

the flavour acceptability of fresh pork loins stored under CAP (–1.5 °C, 100% CO_2) remained acceptable for up to 18 weeks. It is interesting that on day 4 of retail display, scores for flavour desirability and overall palatability did not vary significantly with the duration of previous CAP storage; however, off-flavour intensity scores did vary significantly. This may have resulted from panelists' perceptions of the chops after 4 d of retail display as being "old" and therefore less enjoyable in terms of flavour and overall palatability. This was unavoidable since panelists were also members of the research team. Scores for off-flavour intensity may have differed between various weeks of previous CAP storage because panelists were required to rate intensity instead of giving personal preference in terms of "desirability". Chops were still desirable in terms of palatability after 9 weeks of CAP storage with an additional 4 d of retail display.

It was interesting that off-odour intensity of pork chops did not vary significantly with the duration of previous CAP storage, whereas odour acceptability scores did. This may have resulted from panelists' perceptions that the chops were "old" as CAP storage time increased. As such, this could have affected odour acceptability scores because the test requires panelists to give a rating based on "desirability". Jeremiah et al. (1992) found that fresh pork chops stored at –1.5 °C in an atmosphere of 100% CO_2 remained acceptable in terms of odour for at least 24 weeks. When chops were scored on day 4 of retail display, however, scores for off-odour intensity did vary significantly with the duration of previous CAP storage, whereas scores for odour acceptability did not. In the present work, after 9 weeks of storage in the CAP container and additional 4 d of retail display, chops were still acceptable in terms of odour.

C. Microbial Analyses

To begin work, it was necessary to ascertain the microbial load on chops to be used in this study. Because the purpose of the storage unit was not to improve but to maintain the pork quality, it was imperative to verify that chops were handled in a highly sanitary manner before the study was initiated. Total microbial numbers were approximately 10^2 CFU per cm^2 of pork at the start of the study. The initial microbial load for fresh pork reported by McMullen and Stiles (1994) was similar (10^2 to 10^3 CFU/cm^2).

The ability of the CAP storage unit to slow microbial growth on pork chops was verified by enumerating bacterial populations during various stages of CAP storage. Total bacterial numbers on chops never exceeded 10^3 log CFU/cm^2 for the entire 9 week storage period. Jeremiah et al. (1992) observed that a dominant lactobacilli population emerged (10^3 CFU/cm^2) on fresh pork chops stored at −1.5 °C in 100% CO_2 after 6 weeks. During our CAP storage study, the emergence of a dominant LAB population became apparent. This is in agreement with previous studies where these organisms emerged to become the largest bacterial population under low temperature storage and a CO_2-saturated atmosphere (Egan, 1983; Greer et al., 1993). *Enterobacteriaceae* and pseudomonads were present in very small numbers and did not grow significantly during the entire 9 week storage period. This result verified their inability to proliferate at −1.5 °C in an atmosphere of 100% CO_2.

After 9 weeks of storage in the container, carnobacteria started to emerge though not as prevalent as LAB. This profile was in disagreement with McMullen and Stiles (1994) where it was noted that *Carnobacterium* spp. dominated the microflora on fresh pork chops stored under 100% CO_2 at −1.5 °C. An investigation by Nadon (1998) found that carnobacteria were at levels between 10^6 and 10^7 after 9 weeks at −1.5 °C in 100% CO_2. The discrepancy was most likely due to our use of CTSI which was devised for the selective enumeration of carnobacteria. The previous investigators (McMullen and Stiles, 1994; Nadon, 1998) used the differential counting method to enumerate carnobacteria populations on fresh pork. This method, however, was not as reliable as CTSI because the differential counting procedures used presume that carnobacteria are represented by the difference in counts obtained on a non-selective agar (like APT agar) and an acetate containing agar (like MRS agar). There was less uncertainty when CTSI was being used because it was designed to only isolate carnobacteria.

When chops from various periods of previous CAP storage were displayed aerobically at 4 °C, the total populations were higher and ranged between 10^4 and 10^6 log CFU/cm^2. When chops without previous CAP storage (controls) were put on aerobic retail display at 4 °C for 4 d, the flora was comprised mainly of LAB and pseudomonads. This was surprising since pseudomonads typically dominate LAB populations on fresh meats stored aerobically under refrigerated conditions (Borch et al., 1996). When previously CAP-stored pork chops were subsequently stored for 4 d under retail display conditions, LAB continued to dominate. This is probably due to their established presence during prior CAP storage. After 9 weeks of CAP storage, pseudomonads were still not able to

dominate the LAB during aerobic retail display. This was in agreement with Greer et al. (1993) where LAB formed the dominant bacterial population (10^5–10^6 CFU/cm^2), followed by the pseudomonads (10^4 CFU/cm^2). A large pseudomonad population was, however, present after 9 weeks CAP storage and 4 d retail display, probably owing to the aerobic conditions of retail display (McMullen and Stiles, 1991). Numbers of carnobacteria on chops without previous CAP storage were low (10^2/cm^2) after 4 d of retail display. When chops were previously CAP-stored for 9 weeks, numbers of carnobacteria were about 10^4/cm^2 after 4 d of retail display. This shift in the flora after 4 d of retail display can be attributed to the previous establishment of a carnobacteria population during CAP storage which subsequently allowed them to grow to larger numbers during subsequent retail display. Numbers of *Enterobacteriaceae* after 4 d at retail display appeared to be unaffected by the duration of previous CAP storage, since their numbers were always around 10^3/cm^2 pork.

Changes in specific bacterial populations did not correlate strongly with sensory changes. This was probably due to the large decrease in most bacterial populations seen around 4 and 5 weeks of CAP storage, with a subsequent increase in numbers as storage continued. Also, bacterial numbers never reached levels characteristic of spoilage; therefore, relationships based on linear correlations would be difficult to determine. The drop in microbial populations seen around 4 and 5 weeks of CAP storage, was reflected by results obtained at day 0 and day 4 of retail storage. Chops removed from the CAP storage bags (i.e. bags removed at week 4 and week 5), were affected in some way during

CAP storage, resulting in the inhibition of microbial growth on the chop surfaces. This may have been due to a lower volume of gaseous atmosphere in the bag.

Bacterial populations never reached spoilage levels throughout the entire study. Because residual O_2 levels were quite low (< 100 ppm), growth of *Enterobacteriaceae* and pseudomonads on pork did not reach 10^2 CFU/cm^2 for up to 9 weeks in the container. Growth of LAB and carnobacteria (Figure 3) on pork chops during CAP storage did occur due to their CO_2-tolerant nature. Growth, however, was not as rapid as under the warmer temperatures of retail display (Figure 4).

D. Residual Oxygen Levels

The optimal volume of CO_2 is 2 L / kg of meat. In this study, slightly more gas (2.2 L/ kg of meat) was used in order to get a pillow effect in the master packages. As such, there was an increased risk of the presence of residual O_2. Because the CO_2 tank would invariably contain residual amounts of O_2, the addition of greater volumes of CO_2 to the master package would likewise cause an increase in the residual O_2 levels. In a study of residual oxygen levels, Tewari (2000) found that for fresh pork chops stored in 100% CO_2 at 2 °C, the use of scavengers could decrease oxygen concentration to 0 ppm within 1 week. The placement of scavengers within CAP packages would function to remove residual oxygen at a rate faster than possible by the meat itself (Gill, 1996). The presence of residual O_2 in the master packages was, however, not problematic throughout the

entire 9 week study since pork chops remained acceptable to panelists and bacterial populations never reached spoilage levels.

E. Shelf Life of CAP-Stored Pork and Significance of Results

The importance of temperature and low initial microbial numbers cannot be overemphasized. The function of the CAP container is not to improve fresh pork quality but to maintain it. If at any point between slaughter, processing and packaging of into CAP bags, there is temperature abuse or bacterial contamination, microbial activity and biochemical deterioration will be promoted. When preparing the samples for this study, pork loins were transferred on bagged ice from the slaughtering plant to the research abbatoir. Initial microbial numbers verified that pork with a low microbial load was being used for this study on shelf life.

Sensory scores of pork chops stored in the container (as analyzed on day 0 of retail display) never reached levels considered to be "slightly unacceptable" (Appendix 3). It is evident from the data that fresh pork chops can be stored for up to 9 weeks without significant deterioration to sensory characteristics. When pork chops were analyzed on day 4 of retail display, however, overall palatability of pork chops was the limiting factor for the display life of previously CAP stored pork chops because scores became "slightly undesirable" at the end of the week (Appendix 4). This, however, does not mean that if chops were eaten on the day before (day 3 retail display) they would have also have been

unacceptable. The results only indicate that a full 4 days of retail display cannot be attained with 9 weeks of storage in the CAP container.

The deviation in the sensory ratings during the fourth week of storage in the container is not expected to discourage retailers from selling fresh pork that has been stored in excess of 4 weeks in the CAP container. Even though scores deteriorated during the fourth week, they did not become unacceptable. As can be seen from Figure 2, the scores after 9 weeks of storage in the container were very much like those from pork with only one week of storage. In fact, scores were still acceptable after 9 weeks storage. In terms of the duration of retail display, sensory scores as analyzed on day 4 (Appendix 4) of retail display were considerably more variable than sensory scores as analyzed on day 0 (Appendix 3) of retail display. Consequently, retailers would be advised to use caution when displaying previously CAP-stored chops for 4 days, since the variability observed in this study may suggest that not all consumers would be satisfied with a product of this age.

The results obtained from this study were significant because it was previously thought that carnobacteria initially dominate typical LAB on fresh meats stored at –1.5 °C in an atmosphere of 100% CO_2. In this study, the use of the newly-designed CTSI agar revealed that LAB prevailed over carnobacteria at all times under CAP storage. This adds a new dimension when trying to understand relationships among spoilage organisms. Because these spoilage organisms would first have to compete for essential nutrients, the deterioration of fresh meat would be delayed. It is therefore suggested that

CTSI be used to investigate whether carnobacteria need an excess of 9 weeks under CAP storage in order to become the dominant population on fresh pork. The possibility that this might happen was supported by the observation that bacterial populations never reached spoilage levels while in the container.

It was puzzling to observe the erratic formation of metmyoglobin on chops which were stored in the newly-designed CAP container. Chops, however, were stored in different master packages. Slight variations of the total weight of the meat within the packages could have affected the overall amount of residual O_2 that was initially absorbed by the meat tissue. Repetition of this experiment would clarify results.

PART II

F. Growth of Carnobacteria on CTSI Agar

1. Growth Components in CTAS

Growth of carnobacteria was supported by several components in the original formulation of CTAS agar. Peptone was added as a necessary source of nitrogen while yeast extract provided essential amino acids and vitamins. The addition of Tween 80 provided fatty acids, which are necessary to the formation of cellular membranes during bacterial reproduction (Goldberg and Eschar, 1977). The major $C_{18:1}$ isomer is provided by Tween 80, which has oleic acid as its primary constituent (Sigma, St.Louis, MO). The major $C_{18:1}$ isomer in Lactobacillus is cis-vaccenic acid, whereas in carnobacteria, it is oleic acid.

2. Modification of Growth Components in CTAS

Although many components required for bacterial growth were present, modifications to CTAS agar were required in order to increase recovery of carnobacteria. The original sucrose concentration was decreased 2-fold and an equal amount of inulin was added in order to improve the recovery of *Cb. mobile* and *Cb. piscicola*. These species metabolize inulin (Collins et al., 1987; Holzapfel, 1992). The original manganese content was increased 2-fold in order to improve recovery of all carnobacteria (Baird et al., 1989). In LAB, catalysis by certain enzymes either requires or is stimulated by the presence of Mn (II) (Archibald, 1986). Such might also be the case for carnobacteria. Finally,

recovery of carnobacteria was made comparable to that on non-selective APT agar when thiamine hydrochloride was added.

3. Selective Components in CTAS

Several components in the original CTAS recipe were added to improve selectivity. The highly alkaline pH of CTAS agar is adequately buffered by di-potassium hydrogen phosphate. The alkalinity of pH 8-9 functions to inhibit the growth of most other organisms (including LAB) and promote growth of carnobacteria (Holzapfel and Long, 1984 unpublished, cited in Holzapfel, 1992). When used in combination, thallium and nalidixic acid effectively inhibit the growth of *Listeria*, some leuconostocs and all Gram-negative bacteria with the exception of enterococci (Holzapfel, 1992). The relatively high concentration of sodium citrate functions to improve selectivity of CTAS (Holzapfel, 1992).

4. Modification of Selective Components in CTAS

In the modification of CTAS, the thallium acetate concentration was decreased two fold to increase recovery of carnobacteria (Holzapfel, 1992). The addition of vancomycin was necessary to prevent growth of enterobacteria (Giraffa and Sisto, 1997). Interference by LAB was successfully eliminated by the inclusion of nisin (Gill, 2000) in CTSI agar. These results are surprising though because inactivation of nisin occurs at high pH (Hurst, 1981). Such should have been the case on CTSI agar, which has a pH of 9.1.

Interestingly, *Cb. divergens* has been reported to be nisin-sensitive (Rose et al. 1999) yet growth was not inhibited by the nisin-containing CTSI agar.

5. Differential Components in CTAS

Characteristic growth of carnobacteria on CTAS agar was enabled by differential components. CTAS agar was red to purple in colour and this was attributed to its alkalinity in the presence of cresol red. This pH indicator functions within a range of 7.2 to 8.8. Cresol red assumes a yellow colour when acidic and a red colour when alkaline. When carnobacteria grow, the lactic acid secreted acidifies the medium immediately surrounding the colonies. As a result, the periphery of *Carnobacterium* colonies was often characterized by a yellow colour, in stark contrast to the red-purple colour of the agar. Further differentiation was obtained by the addition of TTC - a redox indicator readily reduced by carnobacteria (Mauguin and Novel, 1994). A red "button" in the centre of colonies, which appear "thread-like" upon closer examination, characterizes the reduction of TTC. These characteristics of CTAS were retained in CTSI.

6. Differentiation Among *Carnobacterium* spp. on CTSI

Differentiation between the various *Carnobacterium* spp. was clearly apparent. *Cb. gallinarum* tended to produce dark pink colonies, whereas colonies of *Cb. piscicola* were a light pink. Dark gray colonies were characteristic of *Cb. mobile*. Yellowing of the growth medium was observed for all species except *Cb. mobile*. Colonies with a gold metallic sheen were associated with *Cb. gallinarum*. All colonies on CTSI were shiny

and pulvinate except for *Cb. mobile,* which produced dull, flat colonies. In some instances, colonies had a red centre which appeared to be "thread-like" upon closer examination. This was most commonly associated with the growth of *Cb. piscicola.* Yellowing of the medium around the periphery of colonies sometimes occurred and was usually accompanied by a clearing of the medium. This may be due to the fact that under aerobic, high pH conditions, manganese oxides are insoluble and unavailable for growth. Production of lactic acid by carnobacteria may allow for the reduction of manganese oxides to soluble manganous ions and so explain the clearing of medium around the carnobacterial colonies. The solubilization of manganese can occur with the concomitant release of organic and inorganic nutrients (Bratina et al., 1998), which may further support growth of carnobacteria. The use of CTSI for the selective enumeration and speciation of carnobacteria from unknown mixtures of bacteria cannot be recommended. Enumeration of carnobacteria from fresh meats stored under 100% CO_2 did not produce colonies which could be identified to the species level on CTSI, as was the case in laboratory testing where pure cultures were examined. When fresh pork from retail-ready packages was stored under 100% CO_2 at -1.5 ± 0.5 °C for up to nine weeks, carnobacteria were found on CTSI at levels $\leq 10^5$ CFU/cm^2, however identification at the species level was not possible from colony appearance only.

7. Modification of Incubation Step

In order to obtain plates free from interference from *Weissella viridescens, Listeria monocytogenes* and lactobacilli, plates were incubated aerobically. Refrigerated storage

of inoculated plates took advantage of the fortuitous observation that good growth of carnobacteria could be obtained using brief exposure of CTAS to low temperature after room temperature incubation. Modifications were made to the original procedure by including a two-step incubation procedure in which plates were initially stored at room temperature (25 °C for 2 d) followed by low temperature incubation (8 °C for 2 d).

G. Construction of CTSI

In the development of CTSI for the selective enumeration of carnobacteria, various changes to the chemical composition of CTAS agar (Baird et al., 1989) were needed in order to improve performance. Phase 1 consisted of increasing the recovery of *Carnobacterium* spp. on CTAS while Phase 2 consisted of decreasing growth of interfering genera.

1. Phase 1: Increasing Recovery of Carnobacteria

Various published suggestions on how to increase recovery of carnobacteria were used. For example, Baird et al (1989) suggested increasing the concentration of manganese in CTAS by 2-fold in order to improve recovery of carnobacteria. After these suggestions were taken into account, the medium needed further development to allow for better recovery. Good growth of carnobacteria was observed to occur on non-selective APT agar; therefore, stepwise changes to the nutrient levels of CTAS were made to resemble those levels in APT agar. In some instances, a specific nutrient in CTAS was increased

to meet the level of that in APT. In other instances, a specific nutrient was decreased. The lowering and elevating of nutrient levels, however, did not produce the needed results. The addition of thiamine hydrochloride to CTAS (at levels present in APT), however, did produce good growth of carnobacteria which was comparable to that on APT (results not shown). However, only one *Carnobacterium* strain (ATCC 49517) was used in these preliminary experiments. If all available carnobacteria strains were used in these tests, growth might have been optimized for all strains. In the end, this was not a problem, since all *Carnobacterium* strains grew well on the finally modified medium.

2. Phase 2: Decreasing Microbial Interference

In Phase 2 of the experiments, the elimination of interfering genera was of concern. To accomplish their elimination, inhibitors (vancomycin and nisin) were added. Vancomycin was added to prevent growth of *En.faecalis*, an organism which proved to be resistant to the high pH of the medium. The addition of nisin was required to prevent growth of other organisms after changes were made to the nutrient makeup of CTAS agar. For example, *Weissella viridescens*, *Lc.mesenteroides* #1, *Lb.curvatus* #15 did not grow on CTAS agar. When, however, the concentrations of thallium acetate and manganese were modified and thiamine hydrochloride was added, all three were able to grow weakly on the plates. The addition of nisin, prevented their interference.

3. Incubation Procedure

The two-step incubation was included to allow for better growth and differentiation of *Carnobacterium* colonies on CTSI agar. It was developed after observation of colony growth on previously incubated (25 °C) plates being stored in a refrigerator. It was evident that this exposure to refrigeration temperature allowed for good development of carnobacteria colonies. Experiments have shown that carnobacteria indeed grow well under low temperatures (McMullen and Stiles, 1994), however, an initial room temperature incubation step was included to accelerate colony growth. This method of incubation may seem inconvenient under some circumstances. An alternative might be to incubate the plates at 8 °C for 5-6 days, although it may take a longer period to observe growth on plates and other genera may establish visible colonies.

SECTION VI: CONCLUSIONS AND RECOMMENDATIONS

PART I: Controlled Atmospheric Packaging of Fresh Pork Chops

From this thesis work, it was determined that fresh pork chops could be stored at −1.5 °C in 100% CO_2 for up to 9 weeks and yield an additional 4 d of retail display without significant sensory changes. It is clear that the CAP system has the potential for offering fresh pork at lower costs to the consumer.

From the results obtained in this thesis work, it cannot be included that retail-ready pork chops will consistently have a storage life of 9 weeks while under CAP storage. For this study, chops were directly packaged in a 100% CO_2 atmosphere without retail wrapping. Retail-ready pork chops would have an internal retail package atmosphere which has to be considered in addition to the external 100% CO_2 atmosphere of the master package. Also, previous investigations have shown that carnobacteria tend to dominate the surfaces of fresh meats stored at 100% CO_2 at -1.5°C (McMullen and Stiles, 1994; Nadon, 1998). In contrast, a dominant carnobacteria population was not observed in this study, which lasted 9 weeks. Nadon (1998) observed that a carnobacteria population was dominant between week 6 and 8 of storage in 100% CO_2 at −1.5 °C and thereafter steadily decreased as LAB became dominant. Further research on fresh pork storage in the CAP container beyond 9 weeks may allow for a better understanding of the role carnobacteria play in offsetting spoilage.

Throughout the study, residual oxygen levels were not high enough to cause significant changes to chop colour, odour, palatability or to the bacterial growth on the surface of the chops. Furthermore, analysis seemed to indicate a possible toxic effect of residual oxygen on bacterial growth during storage in the container. A study conducted by Tewari (2000) found that the use of scavengers in master packaged pork (at –1.5 °C in 100% CO_2) could reduce the residual O_2 concentration from 200 ppm to 0 ppm within one week. Further study should assess whether the removal of residual oxygen at concentrations < 100 ppm is detrimental to the maximum storage life of master packaged pork. This may be of no practical importance, however, due to the requirement by most scavengers of at least 100 ppm of O_2 for efficient operation (Gill, 1996).

It is recommended that the thesis work concerning the microbiological and pigment changes in CAP-stored retail-ready pork be repeated in order to provide more conclusive results. If the experiments were repeated and sufficient replicates were done, the variability which was seen at weeks 4 and 5 of CAP storage might be resolved. It would also be advisable to remove more than one master package per storage week so as to eliminate the effect of the master packages themselves. Residual oxygen levels averaged from a number of master packages at any particular storage week would most likely be more uniform in their decreases as CAP storage time progressed.

PART II: CTSI Agar for the Selective Recovery of Carnobacteria

A new medium, CTSI, was developed and found suitable for the recovery of all species of carnobacteria likely to be found in food. The medium did not permit growth of four major species of LAB normally found in similar environments. *B.thermosphacta*, *L.monocytogenes*, *En.faecalis* and *Y.enterocolitica* (which is favoured in highly alkaline media), as well as bacteria from six other genera known to have spoilage or pathogenic potential did not interfere. The improved selectivity of CTSI agar may prove beneficial in studies to determine the role of carnobacteria in the shelf life extension of fresh meat and other food products. The use of CTSI may aid environmental studies of pathogenic forms of carnobacteria which cause disease in some types of fish (Baya et al., 1991). Furthermore, CTSI could also be used to study antagonistic *Carnobacterium* spp. that produce bacteriocins (Duffes et al., 1999; Schobitz et al., 1999) active against pathogenic and spoilage microorganisms in food products.

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SECTION VIII: APPENDICES

Appendix 1: Composition of CTAS agar[a,b].

Component	g/L
Peptone	10.0
Yeast extract	10.0
Sucrose	20.0
Tween 80	1.0
Sodium citrate	15.0
$MnSO_4 \cdot 4H_2O$	4.0
Di-potassium hydrogen phosphate	2.0
Thallium acetate	1.0
Nalidixic acid	0.04
Cresol red	0.004
TTC	0.01
Agar	15.0
Distilled water [c]	1000ml

[a] From Baird et al., 1989.

[b] Plates were incubated aerobically at 25 °C for 2 d, followed by 2 d at 8 °C.

[c] All components (except TTC) were added to distilled water. The solution was brought to boiling to completely dissolve components. After cooling the solution to 55 °C, the pH was adjusted to 9.1 using 1N NaOH. The solution was then autoclaved followed by addition of TTC by filter sterilzation.

Appendix 2: Sensory scores used by panelists to rate fresh pork chops.

Colour:	0=completely discoloured, 1=extremely pale, 3=normal, 4=dark, 5=extremely dark
Surface discoluration:	1=0%, 2=1-10%, 3=11-25%, 4=26-50%, 5=51-75%, 6=76-99%, 7=100%
Retail acceptance:	1=extremely undesirable, 2=undesirable, 3=slightly undesirable, 4=neither desirable/undesirable, 5=slightly desirable, 6=desirable, 7=extremely desirable
Off-odour intensity:	1=no off-odour, 2=slight off-odour, 3=moderate off-odour, 4=prevalent off-odour
Odour acceptability:	1=acceptable, 2=slightly acceptable, 3=neither acceptable/unacceptable, 4=slightly unacceptable, 5=unacceptable
Flavour:	1= undesirable, 2=moderately undesirable, 3=slightly undesirable, 4=slightly desirable, 5=moderately desirable, 6=desirable
Off-flavour intensity:	1=no-off flavour, 2=slight, 3=moderate, 4=prevalent
Overall palatability:	1=undesirable, 2=moderately undesirable, 3=slightly undesirable, 4=slightly desirable, 5=moderately desirable, 6=desirable

Appendix 3: Sensory scores of pork chops with previous CAP storage (-1.5 °C, 100% CO_2).

Sensory attribute	Average sensory score (CAP storage week)* ± SD									
	0	1	2	3	4	5	6	7	8	9
Colour	3.1 ± 0.4	3.1 ± 0.5	2.7 ± 0.4	3.1 ± 0.3	2.9 ± 0.2	3.0 ± 0.4	2.9 ± 0.1	3.0	3.0 ± 0.2	3.0
Percent discolouration	1.0 ±	1.2 ± 0.4	1.0 ± 0.2	1.0 ± 0.1	1.2 ± 0.6	1.0 ± 0.2	1.0 ± 0.2	1.3 ± 0.5	1.0 ± 0.4	1.2 ± 0.4
Overall visual acceptance	6.9 ± 0.1	5.7 ± 0.9	6.4 ± 0.1	6.6 ± 0.5	6.4 ± 0.9	6.8 ± 0.3	6.8 ± 0.3	7.0	6.5 ± 0.8	6.2 ± 2.0
Off-odour intensity	1.0	1.0 ± 0.1	1.0	1.2 ± 0.4	1.2 ± 0.4	1.0 ± 0.2	1.0 ± 0.2	1.2 ± 0.5	1.0	1.5 ± 0.7
Overall odour acceptance	1.0	1.0	1.0	1.1 ± 0.3	1.1 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.2 ± 0.7	1.0	1.5 ± 0.8
Flavour	5.7 ± 0.7	5.5 ± 0.5	5.5 ± 0.8	5.6 ± 0.7	5.6 ± 0.5	5.3 ± 0.8	4.8 ± 0.9	4.8 ± 0.9	5.3 ± 0.8	4.5 ± 1.0
Off-flavour intensity	1.2 ± 0.7	1.5 ± 0.5	1.5 ± 0.8	1.1 ± 0.3	1.1 ± 0.4	1.1 ± 0.4	1.5 ± 0.8	2.3 ± 0.8	1.6 ± 0.8	2.5 ± 1.0
Overall flavour acceptance	5.8 ± 0.8	5.2 ± 0.7	5.5 ± 0.8	5.6 ± 0.7	5.6 ± 0.5	5.3 ± 0.8	4.3 ± 1.8	4.3 ± 1.8	5.3 ± 0.8	5.0 ± 2.0

*Scores were averaged from 5 panelists (n=5), except for flavour, off-flavour intensity and overall flavour acceptability where scores were averaged from four panelists (n=4).
SD Standard deviation. When no SD appears after a score, panelists were unanimous in the rating.

Appendix 4: Sensory scores of pork chops with previous CAP storage (-1.5 °C, 100% CO_2) and 4 d subsequent retail display (4 °C, atmospheric air).

Sensory attribute	Average sensory score (CAP storage week)* ± SD									
	0	1	2	3	4	5	6	7	8	9
Colour	3.1 ± 0.3	3.3 ± 0.7	2.8 ± 0.5	3.1 ± 0.6	2.7 ± 1.2	2.8 ± 0.6	3.0 ± 0.4	2.7 ± 0.4	2.8 ± 0.4	2.6 ± 0.5
Percent discolouration	2.7 ± 0.5	2.3 ± 1.4	3.3 ± 1.8	3.0 ± 1.8	3.5 ± 2.0	2.6 ± 0.6	4.0 ± 1.7	2.7 ± 1.8	2.8 ± 1.9	3.2 ± 1.9
Overall visual acceptance	4.5 ± 0.6	4.6 ± 1.5	4.9 ± 1.8	4.5 ± 1.9	3.8 ± 2.0	4.8 ± 1.1	3.4 ± 1.7	4.9 ± 1.7	4.8 ± 2.0	4.0 ± 1.9
Off-odour intensity	1.3 ± 0.4	1.0	1.0 ± 0.2	1.1 ± 0.4	1.6 ± 0.6	1.1 ± 0.4	1.7 ± 0.4	1.5 ± 0.6	1.2 ± 0.4	1.9 ± 0.8
Overall odour acceptance	1.3 ± 0.6	1.0	1.0	1.0 ± 0.2	1.5 ± 0.9	1.2 ± 0.5	1.8 ± 0.8	1.5 ± 0.8	1.3 ± 0.6	1.5 ± 0.6
Flavour	4.0 ± 1.5	5.6 ± 0.7	5.1 ± 1.4	4.8 ± 1.6	4.1 ± 1.3	5.0 ± 1.2	4.2 ± 1.2	4.8 ± 1.6	4.3 ± 1.5	2.3 ± 0.8
Off-flavour intensity	1.8 ± 0.8	1.1 ± 0.5	1.5 ± 0.7	1.3 ± 0.8	2.0 ± 1.0	1.5 ± 0.8	2.3 ± 0.7	1.8 ± 0.9	2.1 ± 0.9	3.1 ± 0.4
Overall flavour acceptance	4.0 ± 1.5	5.3 ± 1.0	5.0 ± 1.4	4.8 ± 1.6	3.5 ± 1.7	5.1 ± 1.1	4.3 ± 1.4	4.8 ± 1.6	4.3 ± 1.5	4.5 ± 0.6

*Scores were averaged from 5 panelists (n=5), except for flavour, off-flavour intensity and overall flavour acceptability where scores were averaged from four panelists (n=4).
SD Standard deviation. When no SD appears after a score, panelists were unanimous in the ratings.

Appendix 5: Microbial growth on fresh pork during CAP storage (100 % CO_2, -1.5 ± 0.5 °C).

CAP	Bacterial counts (log_{10} CFU / cm^2 pork) ± SD				
(week)	Total bacterial numbers	LAB	carnobacteria	Enterobacteriaceae	pseudomonads
0	2.15 ± 0.16	1.08 ± 0.12	1	1	1.42 ± 0.41
1	2.18 ± 0.18	1.36 ± 0.21	1.15 ± 0.16	1.1 ± 0.17	1.20 ± 0.21
2	2.08 ± 0.15	1.86 ± 0.17	1.12 ± 0.13	1.12 ± 0.21	1
3	2.16 ± 0.11	1.88 ± 0.30	1.15 ± 0.26	1.12 ± 0.13	1.20 ± 0.25
4	1.27 ± 0.37	1.1 ± 0.17	1	1	1.08 ± 0.13
5	1.33 ± 0.34	1.05 ± 0.08	1	1	1.18 ± 0.22
6	1.69 ± 0.35	1.49 ± 0.48	1.17 ± 0.20	1	1.09 ± 0.09
7	2.06 ± 0.36	1.91 ± 0.57	1.52 ± 0.44	1.14 ± 0.23	1.05 ± 0.08
8	2.39 ± 0.40	2.49 ± 0.31	1.33 ± 0.22	1.09 ± 0.09	1.08 ± 0.13
9	2.16 ± 0.60	2.01 ± 0.61	1.64 ± 0.37	1.17 ± 0.20	1.20 ± 0.21

SD Standard deviation

Appendix 6: Microbial growth on fresh pork chops after CAP storage (100 % CO_2, -1.5 ± 0.5 °C) for 0 to 9 weeks with subsequent aerobic retail display (4 °C) for 4 d.

CAP	Bacterial counts (log_{10} CFU / cm^2 pork) ± SD				
(week)	Total bacterial numbers	LAB	carnobacteria	Enterobacteriaceae	pseudomonads
0	4.76 ± 0.03	3.73 ± 0.54	2.51 ± 0.41	3.18 ± 0.39	3.40 ± 0.35
1	5.13 ± 0.37	4.30 ± 0.44	2.69 ± 0.09	2.97 ± 0.27	4.16 ± 0.60
2	6.23 ± 0.48	5.76 ± 0.20	5.19 ± 0.12	3.70 ± 0.99	5.59 ± 0.86
3	4.98 ± 0.52	4.58 ± 0.68	3.80 ± 0.43	2.70 ± 0.29	4.48 ± 0.63
4	4.58 ± 0.29	3.44 ± 0.24	3.62 ± 0.35	2.88 ± 0.60	4.25 ± 0.36
5	4.59 ± 0.30	3.94 ± 0.64	2.95 ± 0.59	2.62 ± 0.14	3.39 ± 0.44
6	5.89 ± 0.19	5.55 ± 0.13	3.44 ± 0.41	2.75 ± 0.34	5.21 ± 0.67
7	6.32 ± 0.46	5.93 ± 0.39	4.13 ± 0.42	2.58 ± 0.28	4.93 ± 0.87
8	6.13 ± 0.43	5.37 ± 0.30	3.98 ± 0.38	3.09 ± 0.26	4.97 ± 1.12
9	6.28 ± 0.54	6.16 ± 0.52	4.11 ± 0.23	2.81 ± 0.40	5.12 ± 0.35

SD Standard deviation