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# Microbiological, physical and chemical studies on fresh red meats packaged under different modified gas atmospheres

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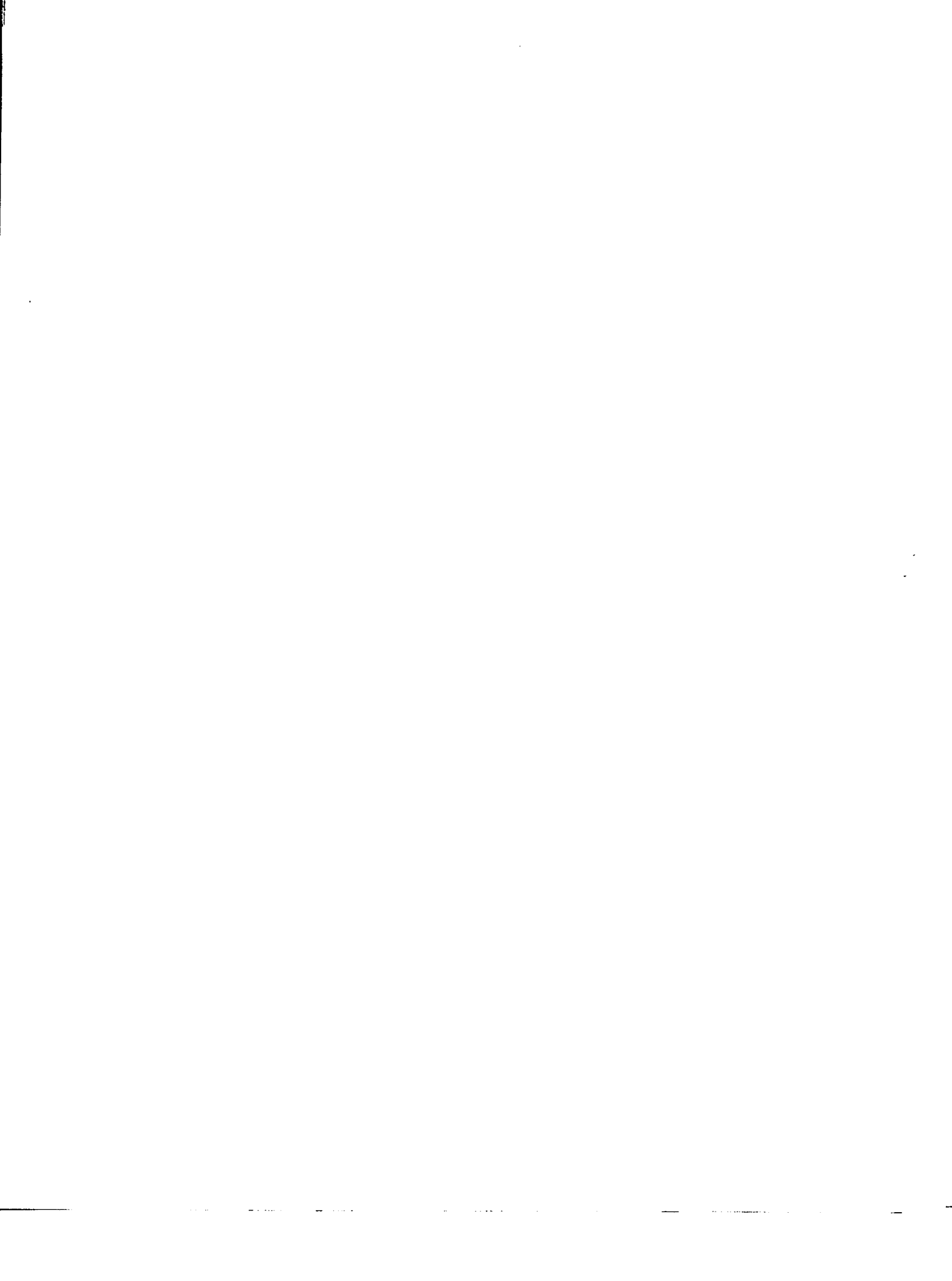
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**Microbiological, physical and chemical studies on fresh red  
meats packaged under different modified gas atmospheres**

**Manu-Tawiah, Wireko, Ph.D.**

**Iowa State University, 1991**

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**Microbiological, physical and chemical studies on fresh red meats packaged under  
different modified gas atmospheres**

**by**

**Wireko Manu-Tawiah**

**A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of the  
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### DEDICATION

To my mother, Madam Adwoa Appiah-Tawiah, whose hard work and responsibility during her youthful years has provided a future for all her children. To my late grandfather, Nana Assuming Buobae, Abenasehene (the late King) for his love and support for my mother. To my Elementary school headteacher, Mr T.M. Wiredu, for his motivation when I was growing up.

## INTRODUCTION

At present, primal cuts of fresh red meats are transported from abattoirs to retail stores. Upon arrival, the meats are cut into consumer-unit sizes, overwrapped in oxygen-permeable film on polystyrene foam trays and displayed for sale. This final manufacturing step conducted at the retail level has both economic disadvantages and microbiological safety implications. For example, expensive in-shop space and labor are required for preparing final retail packs, and lack of proper technical supervision of retail level cutters can also affect the microbiological quality of the meats (Hotchkiss and Galloway, 1989). In addition, the packaging of the consumer-unit cuts in oxygen-permeable films reduces the shelf life of the meat tremendously. Even with proper refrigeration, the meat ultimately undergoes deteriorative changes including microbial spoilage, oxidative changes in pigments and lipids, and weight loss (Urbain and Campbell, 1987). The shelf life of fresh meats (nonvacuum) is therefore, approximately 14 days (Huffman, 1974), with only 3-6 days retail life (Bartkowski et al., 1982). It has been proposed that if the U.S. meat industry wants to compete successfully in the international market, it would require at least 21 days shelf life for its air-packaged fresh meats (Lioutas, 1988). Consequently, there has been interest in recent years in technologies which would allow centralized packaging of fresh red meats in consumer-unit packages, and also in extending the microbiological and organoleptic shelf life of the fresh meat. One of these technologies which is becoming increasingly popular is modified-atmosphere packaging (MAP).

Modified-atmosphere packaging of fresh meats involves enclosing the fresh meat cut in systems in which the gaseous environment has been altered (Farber,

1991). The technology is based upon the principle that the composition of the atmosphere in which a food is kept affects the growth rate of microorganisms (Genigeorgis, 1985). MAP, when combined with proper sanitation and refrigeration, extends the shelf life and quality of fresh meats by reducing microbial growth and retarding enzymatic spoilage (Young et al., 1988). MAP also has the advantage of providing longer color stability, less surface desiccation, less product shrinkage and trim, more product utilization, better distribution of desired cuts to targeted areas, reduced freight costs by elimination of chill-pack ice weight and overall economic benefits compared to ambient air-atmosphere packaging (Brody, 1989). Retarding or preventing changes that occur in fresh meats stored in air by packaging in modified atmospheres would not only increase shelf life in the retail stores, but could provide sufficient storage life to allow meat to be prepackaged in the packing plant under proper technical supervision and distributed directly to retail stores over relatively long distances (Terilizzi et al., 1982). Today, in North America, MAP is applied to packaging fresh meat such as beef, pork, lamb and poultry for industrial and consumer uses (Young et al., 1988).

There are three techniques of MAP used for fresh meats: vacuum packaging, hypobaric storage and use of gas mixtures. The vacuum packaging technique involves evacuation of air from the package and application of a hermetic seal. It is used largely for wholesale cuts and it forms a key part of the distribution of boxed fresh meats (Urbain and Campbell, 1987). However, due to color darkening, the technique is less frequently used for packaging of retail cuts, since darkening appears undesirable to consumers (Young et al., 1988). Vacuum packaging also increases purge loss, distortion of cuts, and bone-in primal cut leaker rates (Smith et al., 1983), creates anoxic conditions in the package that may stimulate the



growth of anaerobic pathogenic microorganisms (Hotchkiss and Galloway, 1989; Young et al., 1988) and may select for the growth of some strains of a new species of pathogen in red meats at low temperatures (Hanna et al., 1976; Myers et al., 1982). Hypobaric storage system involves storing the meat in a chamber with a precisely controlled combinations of low pressure, low temperature, high humidity and ventilation (Urbain and Campbell, 1987). This procedure has the disadvantage of allowing psychrotrophic spoilage bacterial growth because of the presence of about 5% oxygen in the chamber (Genigeorgis, 1985). Therefore, it is used only for fresh meats not meant for distant shipment. Gas flush systems involve the removal of air, then back flushing with one or mixture of gases such as O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub> (Terilizzi, 1982). Proper application of gas flush packaging can optimize meat color and microbial inhibition since O<sub>2</sub> is used to maintain the oxymyoglobin or bloomed color and to inhibit growth of obligate anaerobic microorganisms, and CO<sub>2</sub> is use to inhibit the growth of aerobic spoilage bacteria (Clark and Lentz, 1969; Hotchkiss and Galloway, 1989; Taylor, 1973). Of all the MAP systems that have been considered, those employing gas mixtures have been most effective in extending the shelf life of fresh meats (Urbain and Campbell, 1987). Consequently, the name MAP has become synonymous with gas flush packaging and therefore, will be referred to hereafter as MAP.

Like any other packaging system, there are variables that influence the microbiological and organoleptic quality of MAP fresh meats. They include the initial concentrations of the gases in the atmosphere in which the meat is stored, the amount of headspace in the package, and the use of reducing substances (Hotchkiss and Galloway, 1989). The concentrations of the primary gases in the headspace influence the rate of growth of microorganisms and the type of species

of microorganisms which might be present on the surface of the fresh meat (Beebe et al., 1976; Blickstad et al., 1981; Christopher et al., 1979; Enfors et al., 1979; Gardner et al., 1967; Sutherland et al., 1977). This is because individual bacterial species present initially on the food show different responses to the O<sub>2</sub> and CO<sub>2</sub> contents of the atmosphere in which the meat is stored (Newton et al., 1977). Extensive research has been done on the effect of various combinations of gases on the growth of spoilage microorganisms in fresh meats (Genigeorgis, 1985; Lioutas, 1988; Seideman and Durland, 1984). However, only a few studies have addressed the selective growth of pathogens under modified atmosphere storage. These studies investigated the survival of existing pathogens such as *Clostridium botulinum*, *Salmonella* and *Staphylococcus aureus* under selected atmospheres (Hintlian and Hotchkiss, 1987; Silliker and Wolfe, 1980) and organisms whose growth can be inhibited with proper refrigeration at 5°C or less (Farber, 1991; Palumbo, 1986). Recently, some new species of pathogens have been found to grow at refrigeration temperatures (Palumbo, 1987). They include *Listeria monocytogenes*, *Yersinia enterocolitica* and *Aeromonas hydrophila* (Palumbo, 1987). In addition, these organisms have frequently been isolated from fresh meats in slaughter plants and in retail establishments (Barnhart et al., 1989; Buncic, 1991; O'Keef et al., 1975; Watkins and Sleath, 1981). They are facultative anaerobes and have been reported to proliferate on vacuum-packaged fresh meats and meat products (Barnes et al., 1989; Hanna et al., 1976; Myers et al., 1982). The major safety question is whether some of these psychrotrophic pathogens can be favored by a particular gas combination and proliferate while the normal organisms that serve as indicators of spoilage are inhibited (Farber, 1991; Hotchkiss and Galloway, 1989; Lioutas, 1988). *L. monocytogenes* has been found to grow on

minced raw chicken packaged in 75%CO<sub>2</sub> with or without 5%O<sub>2</sub> at 4°C (Wimpfheimer et al., 1990). Strains of *Y. enterocolitica* and *A. hydrophila* were also found to survive and grow on beef stored in 100%CO<sub>2</sub> at 0-5°C (Gill and Reichel, 1989). For fresh meats in which red color maintenance is important, O<sub>2</sub> concentrations higher than 5% are generally used so that the formation of metmyoglobin will be retarded for an extended period of time (Farber, 1991). Carbon dioxide is also generally used in concentrations ranging from 15-40%, as higher concentrations can cause bleaching or discoloration of the meat surface due to the denaturation of meat protein (Farber, 1991). For example, 50%CO<sub>2</sub> has been found to discolor fresh meats very rapidly even when 10%O<sub>2</sub> is present (Silliker et al., 1977). So far no work has been reported on the effect of various modified atmospheres containing CO<sub>2</sub> concentrations up to 40%, and O<sub>2</sub> concentrations up to 40% on growth of spoilage microorganisms and cold tolerant pathogens on fresh pork chops. Therefore, the first objective of this project was to study the influence of several gas combinations of CO<sub>2</sub> and O<sub>2</sub> in concentrations up to 40%, on the numbers of common spoilage microorganisms and to determine how these gas combinations affect the populations of some of the new species of pathogens or potential pathogens present as part of the natural flora of fresh pork; and to conduct inoculated studies to determine the survival and growth of *L. monocytogenes* or *Y. enterocolitica* on fresh pork packaged in selected atmospheres.

Modified-atmosphere packaging of fresh meat directly influences the oxidative state of the muscle myoglobin (Hotchkiss and Galloway, 1989), and may exert detrimental effects on meat color (Silliker and Wolfe, 1980). High concentrations of CO<sub>2</sub> may discolor meat or denature certain meat proteins (Clark

and Lentz, 1969; Gill, 1986); low levels of O<sub>2</sub> also accelerate the formation of brown metmyoglobin (Livingston and Brown, 1981), while high levels of O<sub>2</sub> may catalyze oxidative rancidity (Watts, 1954). Therefore, the second objective of this project was to determine the effect of different ratios of CO<sub>2</sub> and O<sub>2</sub> on the physical and chemical properties such as color, purge loss and fat oxidation in fresh pork.

The brown discoloration from metmyoglobin formation reduces consumer acceptance of prepackaged fresh meat cuts. A longer shelf life would be advantageous to the marketing of fresh meat. However, because of the dynamic changes which occur in fresh meat, surface discoloration is inevitable (Faustman and Cassens, 1991). Consequently, there has been a need to slow the formation of metmyoglobin without masking quality deterioration of fresh meat. Reductants such as sodium ascorbate or its derivatives in conjunction with phosphates and chelators such as citric acid or EDTA have been suggested for use to retard oxidative reactions (Cheng, 1987). The process also suggests the use of modified atmospheres for microbial control. The concern about this process is that ascorbic acid or its derivatives have little effect on the growth of meat spoilage organisms (Okayama et al., 1987; Shivas et al., 1984); the regulated levels of phosphates and citric acid used in conjunction with ascorbate in the fresh meats may not provide sufficient antimicrobial effect. Therefore, the process may stimulate growth of spoilage microorganisms or select for naturally occurring pathogens in the meat stored in modified atmospheres. The third objective of this project was, therefore, to evaluate the microbiological characteristics of fresh meat treated with a color maintenance mixture consisting of sodium erythorbate, tetrasodium pyrophosphate and citric acid, and packaged under modified atmosphere.

### Explanation of Dissertation Format

The alternate format was used for this dissertation. It consisted of a literature review, three papers, and a summary and references at the back of the dissertation. Figures and tables can be found at the end of each paper. The format of the Journal of Food Science was used in the preparation of the three papers. The third paper (Journal Paper J-14224 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa; Project No. 2780) has already been published in the Food Technology Journal. The first two papers will be submitted for publication to professional journals. The results of this dissertation, with the exception of the Hunter color, and TBA values, were the original research conducted by the candidate while working under the guidance of his co-major professors. The Hunter color, and TBA values were measured by the staff of the Iowa State University Meat Laboratory, Ames Iowa.

## LITERATURE REVIEW

### Preservation of fresh meats

#### Spoilage of fresh meats

Meats are highly perishable food products because of dynamic changes including microbial spoilage, various oxidation states of the myoglobin pigment, and fat oxidation that occur in fresh meats (Wolfe, 1980). Fresh meats contain an abundance of available nutrients required for the growth of microorganisms (Jay, 1986). Fresh meats such as beef, pork and lamb also have pH values within the growth range of most microorganisms. Carcasses from most healthy animals appear to have sterile tissues (Ingram and Simonsen, 1980). Most microbial contamination of muscle tissue, therefore, occurs during eviscerating on the kill floor, handling through distribution and storage or fabrication into primal, subprimal and consumer-unit cuts (Kraft, 1986). Nonspoiled meats most often have a varied flora which represents the organisms that existed in the original environment of the product in question, or contaminants picked up during processing, handling, packaging, and storage (Jay, 1986). The genera of bacteria most frequently found on prepackaged fresh meats include *Acinetobacter*, *Aeromonas*, *Moraxella*, *Pseudomonas*, *Brochotrix*, several members of the *Enterobacteriaceae* family, *Clostridium*, *Salmonella*, *Staphylococcus*, micrococci, and lactic acid-producing bacteria (Jay, 1986). While the oxidation-potential (O/R) of whole meat is low, O/R conditions at the surface tend to be higher and hence, allow strict aerobes and facultative anaerobes as well as strict anaerobes to grow suitably. Fresh meats are

normally held at refrigerated temperatures (0-5°C) where microbial spoilage begins upon prolonged storage at these temperatures.

Cuts coming from the usual channels of processing and packaging in O<sub>2</sub>-permeable films normally have retail shelf life of 3-6 days limited by an undesirable color change and microbial growth leading to spoilage (Bartkowski et al., 1982). Bacterial spoilage of refrigerator-stored fresh meats is a surface phenomenon reflective of external contaminants (Ingram and Dainty, 1971). There are fewer genera of bacteria in spoiled meats than on fresh meat, because the temperature selects for specific organisms (Jay, 1967). Spoilage of retail display tray-overwrapped fresh meat is generally from aerobic microorganisms, predominantly *Pseudomonas* and *Moraxella-Acinetobacter* species (Vanderzant et al., 1982; Vrana et al., 1985). They grow the fastest and utilize glucose at refrigerated temperatures, and hence, possess the capacity to outcompete members of other genera (Gill and Newton, 1977). *Brochotrix thermosphacta* also utilizes glucose but because of its slower growth rate, it is a poor competitor to the pseudomonads (Jay, 1986). Spoilage caused by *Pseudomonas* is essentially surface sliminess (Kraft and Ayres, 1952). The first evidence of spoilage is the development of discoloration (Kraft, 1986). Odors can also be detected when the surface bacterial count is greater than log 7.0 (Kraft, 1986).

Meat preservation involves application of measures to prevent or retard the microbiological, physical and chemical processes that make the meat unusable as a food or which reduce the quality aspect of it (Urbain and Campbell, 1987). Some of these measures include the use of appropriate packaging and storage procedures.

### Refrigeration

In the past, whole animal carcasses were shipped to retail outlets where the carcasses were fabricated into retail items by butchers in individual stores (Cole, 1986), and refrigerated. Refrigeration is still used extensively to retard spoilage and prolong the shelf life of fresh meats. Nevertheless, refrigeration alone has been found inadequate to completely prevent the growth of spoilage organisms (Palumbo, 1986). For example, there was yeast and mold growth in a wide variety of meats held at 5°C (Elliot and Michener, 1964). Refrigeration also selects for the growth of psychrotrophic aerobic bacteria that cause spoilage of meat (Johnson et al., 1982). In the mid-1950's refrigeration was also considered adequate to prevent growth and/or toxin formation by foodborne pathogens such *Salmonella*, *Staphylococcus aureus* and *Clostridium botulinum* types A and B. Luiten et al. (1982) stated that normally refrigerated beef is not a problem in respect of foodborne illness because the low temperature of storage sufficiently inhibits the growth of most pathogenic organisms. Quite recently, however, new species of psychrotrophic foodborne pathogens have been isolated. They include *C. botulinum* type E, *Yersinia enterocolitica*, enteropathogenic *Escherichia coli*, *Listeria monocytogenes*, and *Aeromonas hydrophila*; they are capable of growing at temperatures below 5°C (Palumbo, 1986). Schmidt et al. (1961) reported that *C. botulinum* type E grew and produced toxin in heat-sterilized beef stew substrate at 3.3°C. The minimum temperature for growth of *Y. enterocolitica* is 4°C (Palumbo, 1987), and that for *L. monocytogenes* is 0°C (Khan et al., 1973). Olsvik and Kapperud (1982) found that toxin-producing strains of *E. coli* were able to grow and produce heat-stable toxin at 4°C. A recent study has revealed that *A. hydrophila* is capable of growth at 4°C (Palumbo et al., 1985a). In addition, there have been



several reports cited by Witter (1961) of the growth of various members of the *Enterobacteriaceae* at 5°C

#### Modified atmosphere packaging (MAP)

In the 1930's CO<sub>2</sub> was added to the air in the ship's lockers to extend the storage life of the meat (Newton et al., 1977) because it was found that growth of some low-temperature microorganisms could be retarded or controlled by CO<sub>2</sub> atmospheric storage (Lioutas, 1988). By 1938, 26% of the beef from Australia and 60% of that from New Zealand was shipped to Britain under CO<sub>2</sub> atmospheres (Lawrie, 1985). The practice was, however, abandoned during the war and was followed by the "box beef" system. This system consisted of fabricating the carcasses into primal or sub-primal cuts, vacuum packaging, boxing and then distributing to retail outlets where they are fabricated into consumer units, overwrapped in O<sub>2</sub>-permeable films on polystyrene foam trays and displayed for sale (Cole, 1986). The box protects against environmental contamination and weight loss while vacuum packaging with impermeable film enhances shelf life (Johnson et al., 1982). Sutherland et al. (1977) and Gill and Tan (1980) observed that the multiplication of *Pseudomonas* decreased and the lag phase increased. They attributed it to increasing levels of CO<sub>2</sub> formed by respiration of the muscle tissue or the microorganisms or both in the vacuum-packaged meat. Vacuum packaging of meat selects for the growth of lactic acid-producing bacteria (Sutherland et al., 1975). Pierson et al. (1970) found that 90-95% of the total bacterial count in vacuum-packaged beef stored for 15 days at 3.3°C were lactobacilli. They also noted that although extremely high levels of lactobacilli developed, the sensory scores were about the same as those of fresh controls.

Vacuum packaging currently is a preferred means of packaging fresh meat meant for long distance transportation because it minimizes shrink loss, discoloration and microbial growth compared to the shipment of either unprotected wholesale cuts or carcasses (Seideman et al., 1980). Nevertheless, vacuum packaging increases bone-in primal cuts leaker rates, purge loss and distortion of cuts (Smith et al., 1983). Evidence also exists about the survival of certain species of pathogens in vacuum-packaged meats. In 1977, multiple outbreaks of salmonellosis caused by the ingestion of vacuum-packaged roast beef was investigated (Anon., 1977). It was observed that the raw beef used in the incriminated outbreaks was not vacuum-packaged but that the cooked product was (Johnson et al., 1982). On the other hand, Goodfellow and Brown (1978) inoculated *Salmonellae* into vacuum-packaged beef but the bacteria did not multiply below 10°C. They, therefore, concluded that vacuum packaging did not favor the growth of *Salmonella* in beef. Hanna et al. (1976) found *Yersinia enterocolitica*-like microorganisms in vacuum-packaged beef and lamb. Seelye and Yearbury (1979) recovered up to 10<sup>7</sup> *Y. enterocolitica*-like bacteria per g in the vacuum-packaged beef having a pH>6.0. Johnson et al. (1982) recovered *Y. enterocolitica*-like bacteria from 66 out of 142 beef samples to confirm that low temperature and vacuum packaging seem to select for these bacteria in beef. More recently a fatal case of listeriosis was traced to contaminated vacuum-packaged turkey franks (Barnes et al., 1989).

Recently, there has been a renewed interest in the storage of chilled meat in gas mixtures containing CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> alone and in various combinations as a means of retarding microbial spoilage and controlling the color of the meat (MacDougall and Taylor, 1975; Patterson and Gibbs, 1977; Taylor, 1973). In 1970, two separate British patents were assigned which described the process of using

gas mixtures containing O<sub>2</sub> and CO<sub>2</sub> for packaging fresh meat cuts (Georgala and Davidson, 1970; Schweisfurth and Aktiengesellschaft, 1970). These patents generated interest from the European meat industry, and today, modified gas atmosphere packaging of fresh meat is the biggest application of MAP technology in Europe (Lioutas, 1988). In 1972 the Tectrol, a patented MA system, was introduced in the U.S.A. and has been used for shipment of fresh meats such as fresh beef, pork, lamb and poultry from the continental U.S. to a variety of places including Japan and Europe (Veranth and Robe, 1979). The system was based on conventional refrigerated sea-hold or truck-box containing the product and special atmosphere of 35-75% CO<sub>2</sub>, 21-28% O<sub>2</sub> and N<sub>2</sub> as the remainder, and kept at -1 to +4°C (Genigeorgis, 1985). The system reduced rancidity and bacterial growth (Holland, 1980).

#### Effect of MAP on growth of meat spoilage microorganisms

The ability of MAP to inhibit meat spoilage organisms has been well documented. Clark and Lentz (1973) demonstrated that increasing the CO<sub>2</sub> content of the aerobic atmosphere surrounding beef from 0% to 20% reduced the growth of psychrotrophic organisms. Silliker and Wolfe (1980) compared the microbial growth on pork loins stored in air with those stored in 50% CO<sub>2</sub> for 14 days at the same temperature, and reported that the counts of the loins stored in the CO<sub>2</sub>-enriched atmosphere were a million cells lower than those of the loins stored in air; the meat also showed satisfactory color and odor. Huffman (1974) found that pork chops stored in CO<sub>2</sub> had significantly lower counts for the entire 5-week storage period than chops stored in air, O<sub>2</sub> or N<sub>2</sub>.

The three primary gases used in MAP possess different antimicrobial properties. Nitrogen has no known antimicrobial properties although it has been reported to have more antimicrobial effect than air (Cole, 1986). At 100% concentration, nitrogen showed no inhibition of aerobic bacterial flora on fresh pork (Holland, 1980; Huffman, 1974). Oxygen inhibits the growth of obligate anaerobic bacteria, although there is a very wide variation in the sensitivity of anaerobes to oxygen (Farber, 1991). Oxygen, however, stimulates the growth of aerobic spoilage microorganisms (Clark and Lentz, 1973), although, at concentrations above 30%, it inhibited the growth of certain psychrotrophic strains of *Pseudomonas* and *Moraxella -Acinetobacter* group (Clark and Burki, 1972). Brown and Hugget (1968) studied the effect of various partial pressures of O<sub>2</sub> on microbial growth and concluded that O<sub>2</sub> was toxic for many forms of life. According to Gardner et al. (1967), O<sub>2</sub> must be drastically reduced to affect aerobic bacterial growth.

The ability of CO<sub>2</sub> to inhibit bacterial growth was first observed over 100 years ago (Frankel, 1889; Kolbe, 1882). Since then, its potential to retard spoilage has been explored for a number of commodities including pork, beef and poultry (Adam and Huffman, 1972; Clark and Lentz, 1973; Ogilvy and Ayres, 1951). The overall effect of CO<sub>2</sub> on microbial growth is that it appears to increase the lag phase and generation time which delays the increase in microbial population (Finne, 1982; Holland, 1980). The inhibition of microorganisms by CO<sub>2</sub> is, however, selective. Molds, yeast, and gram-negative aerobic spoilage microorganisms on chilled meats are especially susceptible (Daniels et al., 1985; Genigeorgis, 1985). *Pseudomonas-Achromobacter* spp. and other related psychrotrophs which grow rapidly and produce off-odors and flavors in raw meats are selectively inhibited by CO<sub>2</sub> (Gardner et al., 1967; Silliker and Wolfe, 1980).

Facultative bacteria, however, may or may not be inhibited by CO<sub>2</sub>, while lactic acid bacteria, such as streptococci and lactobacilli, and anaerobes are very resistant (Enfors and Molin, 1980; Silliker and Wolfe, 1980). For example, it has been reported that CO<sub>2</sub> had little effect on the facultative anaerobe *Enterobacter-Hafnia* spp. and *B. thermosphactum* (Gardner et al., 1967). Erichsen and Molin (1981) noted that *B. thermosphacta* on beef increased at the same rate as total counts in air, vacuum, and in a 20%CO<sub>2</sub>/2%O<sub>2</sub>/78%N<sub>2</sub> mixture. According to Ledward et al. (1971), since pseudomonads are inhibited by CO<sub>2</sub> there is little competition for slower growing lactobacilli and *Brochotrix* which can grow at low concentrations of O<sub>2</sub> and high concentrations of CO<sub>2</sub>. Most gram-positive pathogens are facultative or strict anaerobes whose growth is apparently not inhibited by CO<sub>2</sub> (Gill and Tan, 1980). The predominating flora of meats stored in modified atmospheres, therefore, comprises these organisms, which develop more slowly than *Pseudomonas* and related gram-negative psychrotrophs (Silliker and Wolfe, 1980). However, Silliker and Wolfe (1980) found that counts of two strains of enterococci on ground beef packaged in MA containing 60% CO<sub>2</sub> were about 1,000 times less than those for the air-stored samples. They concluded that CO<sub>2</sub>-enriched atmospheres inhibited the growth of these gram-positive potential food poisoning bacteria. Huffman (1974) also found that anaerobic counts were reduced by both a high CO<sub>2</sub> atmosphere and a 25%CO<sub>2</sub>/5%O<sub>2</sub>/70%N<sub>2</sub> gas mixture at 14 days postmortem when compared to air. Christopher et al. (1980) compared the microbiological quality of retail pork chops packaged in 20%CO<sub>2</sub>/80%N<sub>2</sub>, and 40%CO<sub>2</sub>/60%N<sub>2</sub> atmospheres with pork chops or loins packaged in a vacuum. They reported that psychrotrophic bacterial counts and lactobacilli counts of chops stored in CO<sub>2</sub>-N<sub>2</sub> atmospheres usually were lower, though not statistically

significant, than those of comparable vacuum-packaged chops or loins. Studies by Blickstad and Molin (1983) showed that *B. thermosphacta* and *Enterobacteriaceae* were the principal spoilage organisms of vacuum- and CO<sub>2</sub>- packaged normal, skinned pork; *B. thermosphacta* caused spoilage of vacuum-packaged pork held at 4 or 0°C, and of CO<sub>2</sub>-packaged pork held at 4°C.

### Factors that affect the inhibitory effect of CO<sub>2</sub>

#### Carbon dioxide concentration

According to Valley (1928), CO<sub>2</sub> concentrations slightly above atmospheric levels can actually stimulate bacterial growth, but that in higher concentrations bacterial growth is inhibited. Ogilvy and Ayres (1951) demonstrated that an increase in the CO<sub>2</sub> concentration from 0 to 25% resulted in a two-fold increase in average generation time of bacteria at 10°C; in a 2.5 -fold increase in at 4°C; and a 3.5-fold increase at 0°C. Finne (1982) noted that increasing the CO<sub>2</sub> concentration extends the lag phase and reduces the growth rate of bacteria. The effectiveness of CO<sub>2</sub> concentrations above 25% has, however, been debated. Clark and Lentz (1969) reported little or no increase in effectiveness on meat or complex media by increasing the CO<sub>2</sub> concentration above 25%. Gill and Tan (1980) found that in aerobic systems, such as those used for display packs, increasing the CO<sub>2</sub> concentration beyond 20 to 30%CO<sub>2</sub> had little additional inhibitory effect on *Pseudomonas*-dominated spoilage flora. On the other hand, in anaerobic systems used to extend the storage life before meat is prepared for display, Gill and Penny (1986) recommended that atmospheres of 100%CO<sub>2</sub> may be used, as the inhibitory effects of CO<sub>2</sub> on both lactobacilli and *Enterobacteriaceae* components of anoxic floras increase with increasing CO<sub>2</sub> concentration. The effect of CO<sub>2</sub> on

spoilage bacteria differs with the medium. Gill and Tan (1979) found that in minimal medium the degree of inhibition of *P. fluorescens* was proportional to the CO<sub>2</sub> concentration, but in a complex medium a maximum degree of inhibition was attained at relatively low CO<sub>2</sub> concentrations. King and Nagel (1967) controlled the various growth factors for pure cultures of *P. aeruginosa*, and found a linear relationship between generation time and CO<sub>2</sub> concentrations. Blickstad et al. (1981) determined the effect of hyperbaric pressure of CO<sub>2</sub> on the microflora of pork. They reported that the time needed for the total aerobic counts to reach  $5 \times 10^6$  organisms/cm<sup>2</sup> at 4°C was about three times longer in 5 atm CO<sub>2</sub> than in 1 atm CO<sub>2</sub> and about 15 times longer than in air, and concluded that the bacteriostatic/preservative effect of CO<sub>2</sub> increased with increasing partial pressure. Hintlian and Hotchkiss (1987) have indicated that the inhibitory effect of CO<sub>2</sub> is a function of how much is available, i.e., the concentration in the mixture and the amount of headspace in the package. Gill (1988b) reported that when CO<sub>2</sub> was added in excess of that required to saturate the meat at atmospheric pressure, growth of lactobacilli, the predominant organisms in the spoilage flora of anoxically packaged meat, was retarded. The same condition also imposed an extensive lag on enterobacteria that ultimately caused putrid spoilage of high-pH red meats in CO<sub>2</sub> packaging (Gill and Penny, 1988); and could extend the storage life of chilled red meat over 24 weeks (Gill, 1986).

#### Age and initial concentration of bacteria

Carbon dioxide is more effective if applied before bacteria have adjusted to environmental conditions, because it produces an extended lag phase in addition to the reduced growth rate of the spoilage flora (Clark and Lentz, 1969). Bacteria in the exponential phase of growth are more resistant to CO<sub>2</sub> than those at the lag

phase (Finne, 1982; Holland, 1980; Ogrydziak and Brown, 1982). For example, application of a 20%CO<sub>2</sub> atmosphere to freshly dressed beef carcasses extended the lag phase of bacterial growth by 11 days at 5°C, while application of the same atmosphere 48 hr after dressing extended the lag phase by only 12 hr at 5°C (Clark and Lentz, 1969). Nortje' and Shaw (1989) observed that *Pseudomonas spp.* and *Enterobacteriaceae* grew far more extensively on meat samples aged before packaging in modified atmosphere than on those packaged immediately after slaughter. Packaging systems only maintain a product at its original quality (NFPA, 1988). Therefore, the initial organoleptic and microbiological state of the prepackaged meat also affects the shelf life of MAP products (Genigeorgis, 1985). Higher initial counts will accelerate spoilage and shorten the shelf life of the product. CO<sub>2</sub> is therefore more effective with low initial bacterial loads (Genigeorgis, 1985).

#### Storage temperature

The effectiveness of MAP increases with decreasing temperatures (Lawrie, 1985). According to Gill and Tan (1980), a decrease in the growth temperature increases the degree of inhibition at any CO<sub>2</sub> concentration. The low temperature generally slows the growth of microorganisms and also increases the solubility of CO<sub>2</sub> in the meat (Ogrydziak and Brown, 1982). Clark and Lentz (1969) noted that 20%CO<sub>2</sub> contributed to a longer shelf life at 0°C but detected no benefit at 20°C. Blickstad et al. (1981) found that the shelf life of pork in 1 atm CO<sub>2</sub> increased by a factor of four when the storage temperature was lowered from 14 to 4°C, while that of the air-stored sample was increased by a factor of three. Studies on the growth of *Pseudomonas* and *Achromobacter* in meat have shown that the nearer the temperature to 0°C, the higher the CO<sub>2</sub> concentrations and the earlier the stage of



growth at the time CO<sub>2</sub> was applied, the greater the extension of the shelf life of meat (1969). Shaw and Nicol (1969) reported that the growth of *Salmonella* and *E. coli* occurred on meat at 10°C but growth of these organisms could be completely inhibited by reducing the temperature of the chilled meat below 7°C. Kader et al (1985) have indicated that the ideal temperature for storing MAP fresh meat is 0-1°C. Barnett et al. (1971) found that the increased effect of CO<sub>2</sub> occurred at a minimum temperature of 1°C, below which no additional bactericidal effect was gained. However, Gill et al. (1988) stored MA packaged pork at -1.5 to -1.0°C, the minimum commercially practicable temperature at which packaged meat can be held indefinitely without risk of freezing, and reported that at 100%CO<sub>2</sub> a 24 week shelf life was achieved. Using the same concentration of CO<sub>2</sub> in another experiment, Gill and Harrison (1989) found that pork chops stored at 3°C had only about 5.5 week storage life.

#### Ultimate pH of the muscle

The ultimate pH of fresh meat also affects the efficiency of CO<sub>2</sub> inhibition and the shelf life of meat. Erichsen and Molin (1981) compared microbial flora of normal and dark, firm, dry (DFD) beef stored at 4°C in different gas environments, and reported that there was no difference in total counts between normal pH beef and DFD beef in air and in 100%CO<sub>2</sub>, but total counts increased at a higher rate on the DFD meat in a 20%CO<sub>2</sub>/2%O<sub>2</sub>/78%N<sub>2</sub> mixture. Gill and Penny (1988) packaged beef samples of normal pH (5.5-5.7) and of high pH (>6.0) in different volumes of CO<sub>2</sub> and reported that at all volumes, total count and numbers of *Enterobacteriaceae* were higher on the high-pH samples. Greening and putrefaction have frequently been associated with packaged DFD meat and have been attributed to microbiological generation of H<sub>2</sub>S under conditions of high pH

and low O<sub>2</sub> tension (Gill and Newton, 1979; Nicol et al., 1970). Vacuum packaging of DFD meat also results in a shorter shelf life than vacuum packaging of normal beef. Vacuum packaging together with the high pH and lack of glucose in the muscle (Gill and Newton, 1979) selectively favor increasing proportions of *Enterobacteriaceae* and of *A. putrefaciens* on the meat (Nicol et al., 1970; Patterson and Gibbs, 1977). Erichsen and Molin (1981) observed that *Pseudomonas* spp. and *B. thermosphacta* seemed to have more resistance to CO<sub>2</sub> on DFD meat, while lactic acid-producing bacteria had a slight advantage on normal-pH meat. However, reports on storage of vacuum-packaged pork indicated that pH-sensitive bacteria of high spoilage potential such as *A. putrefaciens*, *B. thermosphacta* and *Enterobacteriaceae*, grew readily on fat and skin tissues, and could cause spoilage of pork irrespective of the pH of the tissue (Blickstad and Molin, 1983; Gill and Newton, 1979; Talon and Montel, 1986). Gill and Harrison (1989) studied the effect of CO<sub>2</sub> packaging on pork samples with pH range 5.5 to 6.1, and reported that the relative wide range of muscle tissue pH introduced no gross variation among samples with regard to the course of spoilage development. Therefore, pH variability of pork muscle tissue is not likely to have a great effect on storage studies of fresh pork.

#### Mechanism of CO<sub>2</sub> inhibition

The mechanism of CO<sub>2</sub> inhibition of the growth of bacteria has often been debated. One of the first explanations for the action of CO<sub>2</sub> was that it displaced some or all of the O<sub>2</sub> available for bacterial metabolism, thus slowing bacterial growth (Daniels, 1985). However, this possibility was discounted when Frankel (1889) showed that some anaerobic bacteria were also inhibited by CO<sub>2</sub>

atmosphere. Callow (1932) also confirmed this finding when he replaced the bacterial growth atmosphere with 100% N<sub>2</sub> and observed that the degree of inhibition due to CO<sub>2</sub> was much higher. According to King and Nagel, (1975, 1967), the inhibitory effect of CO<sub>2</sub> is partly caused by lowering the pH. The surface pH is lowered as a result of the absorption of CO<sub>2</sub> on the muscle surface and subsequent ionization of the formed carbonic acid (Genigeorgis, 1985). Solubility of CO<sub>2</sub> increases as the partial pressure above a solution increases or as temperature decreases (Quinn and Jones, 1936). CO<sub>2</sub> is hydrated to carbonic acid which is quickly dissociated to form bicarbonate (HCO<sub>3</sub><sup>-</sup>) and hydrogen (H<sup>+</sup>) ions (Daniels, 1985). Kraft and Ayers (1952) showed that CO<sub>2</sub> increased shelf life by lowering the pH of meat. However, Huffman et al. (1975) detected only a 0.1 unit pH drop in CO<sub>2</sub> stored beef samples and suggested that this pH drop did not account for lowered bacterial counts alone.

It has also been suggested that when gaseous CO<sub>2</sub> is applied to a biological tissue, it first dissolves into the liquid phase of the tissue, then is absorbed as carbonic acid in the undissociated form (Barnett et al., 1971; Golding, 1945; Sears and Eisenberg, 1961). According to Turin and Warner (1977) CO<sub>2</sub> has the ability to penetrate the bacterial membranes and cause intracellular pH changes that are of greater magnitude than that caused by external acidification and cannot be buffered by the organism. Hence, Hotchkiss and Galloway (1989) have speculated that the inhibitory effect of CO<sub>2</sub> could be due to intracellular pH since microorganisms, particularly, the gram-negative ones may have the ability to concentrate carbonic acid thereby lowering their internal pH. However, earlier studies conducted by Coyne (1932) demonstrated that the observed effect of CO<sub>2</sub> was not due to internal acidification alone. He adjusted the pH of bacterial growth

media to approximately pH 5.8 then grew cultures of *Achromobacter*, *Pseudomonas* and *Bacillus* under air and under CO<sub>2</sub>. He observed that, in all trials, the CO<sub>2</sub> produced a greater degree of inhibition.

An alternative theory suggests that CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> ions may alter contact between the cell and its external environment by affecting the structure of the cell membrane (Daniels, 1985). Sears and Eisenberg (1961), using a model system, observed that the concentration of bicarbonate ions influenced the molecular arrangement at the interface between lipid droplets and water. They further reported that higher concentrations of HCO<sub>3</sub><sup>-</sup> ions caused a decrease in the interfacial tensions and increased hydration of the membrane. They also found that in the presence of CO<sub>2</sub>, there was a tendency toward dehydration of the membrane.

Another proposed theory is that once in the cell, CO<sub>2</sub> interferes with various metabolic processes. According to Finne (1982) one of the decarboxylating enzymes may be inhibited by mass action. This is because CO<sub>2</sub> is a product of that enzymatic system and too much may inhibit it (King and Nagel, 1967). These authors found different growth rates for *Pseudomonas* grown on various substrates, and postulated that CO<sub>2</sub> may interfere with formation of enzymes that break down the substrate before absorption by the bacteria. King and Nagel (1975) in a latter study investigated the effect of CO<sub>2</sub> on the metabolism of *P. aeruginosa* in a liquid-salts medium with added substrate and phosphate or bicarbonate buffers. They measured O<sub>2</sub> uptake, intermediate metabolic acid and activity of fumarase, succinate dehydrogenase, cytochrome C oxidase, oxaloacetate decarboxylase, malate dehydrogenase and isocitrate dehydrogenase. They found that there was no effect of CO<sub>2</sub> upon the efficiency of total synthesis of cell material. When the

cells were grown on glucose, acetate, fumarate, or succinate they reported that there was a longer lag time in the uptake of substrate with CO<sub>2</sub>-treated culture than the air control. Analysis for volatile neutral compounds and acids showed no accumulation of metabolic intermediates in medium with the air- or CO<sub>2</sub>- treated growth cultures. They also reported that there was essentially no difference in enzyme activity between the air and CO<sub>2</sub> treatments for cytochrome oxidase, fumarase, oxaloacetate dehydrogenase or succinate dehydrogenase; however, rate inhibition caused by CO<sub>2</sub> was observed for isocitrate dehydrogenase and malate dehydrogenase activities. They therefore, concluded that at concentrations above 20%, CO<sub>2</sub> inhibited the activity of only isocitrate dehydrogenase and malate dehydrogenase. Mitsuda et al. (1980), using a model system, demonstrated that CO<sub>2</sub> interacts with enzymes to cause a transient inactivation, particularly with many hydrolases that cause autolysis after death. Gill and Tan (1980) determined the effect of CO<sub>2</sub> on the respiration rates of some food spoilage and pathogenic organisms in a liquid medium. They reported that the rates of respiration of species of *Pseudomonas*, *Acinetobacter*, *Y. enterocolitica* and *A. putrefaciens* were reduced significantly, while CO<sub>2</sub> had no effect on the respiration of *Enterobacter* or *B. thermosphactum*. They concluded that respiration as well as growth was inhibited, whereas anaerobic growth of CO<sub>2</sub>-susceptible facultative anaerobes was unaffected. They therefore suggested that enzymes of oxidative metabolism rather than decarboxylating enzymes might be affected by CO<sub>2</sub>. They went further to state that if the inhibition was the result of mass action on decarboxylating enzymes, then a decrease in growth rate should be directly proportional to CO<sub>2</sub> concentration, but in most cases maximum inhibition was not total and occurred at comparatively low CO<sub>2</sub>.

### Effect of MAP on the growth of pathogenic bacteria

MAP when used in combination with proper sanitation and refrigeration can deliver all the benefits a packaging system can offer to fresh meats. On the other hand, these same principles are also the main cause of controversy surrounding the potential health hazards that accompany such systems (Genigeorgis, 1985; Palumbo, 1986). Most fresh meat spoilage microorganisms are aerobic and proteolytic. They normally outgrow any pathogens and cause organoleptic changes that usually warn consumers of spoilage (Farber, 1991). Thus, the major safety concern about MAP is whether or not pathogenic organisms will proliferate while the normal organisms that serve as indicators of spoilage are inhibited. The atmosphere modification, in particular, the reduction or elimination of O<sub>2</sub> from the headspace may inhibit the growth of aerobic spoilage organisms and favor growth of anaerobic pathogens which cause no organoleptic changes (Genigeorgis, 1985). The meat appears to be unspoiled which leads to greater consumer mishandling and thermal abuse (Lioutas, 1988).

*S. aureus* is a facultative anaerobe, but grows best in the presence of O<sub>2</sub> (Hintlian and Hotchkiss, 1986) even though it can grow and produce toxin under anaerobic conditions (Genigeorgis and Riemann, 1979). The organism failed to grow in ground beef stored in 60% CO<sub>2</sub>/25% O<sub>2</sub>/15% N<sub>2</sub> atmosphere (Silliker and Wolfe, 1980). Luiten et al. (1982) found that after inoculating retail beef cuts with cells of *S. aureus* there were significantly fewer staphylococci per cm<sup>2</sup> for vacuum- and gas- packaged steaks than those overwrapped in air-permeable film. They concluded that packaging systems using a 60% CO<sub>2</sub>/40% O<sub>2</sub> gas atmosphere did not promote the growth of *S. aureus*. Coyne (1932) noted that this organism was strongly inhibited by 50% or 100% CO<sub>2</sub> at 10°C. Silliker and Wolfe (1980)

inoculated ground beef with six strains of *Salmonella* and stored them in 60%CO<sub>2</sub>/25%O<sub>2</sub>/15%N<sub>2</sub> mixture at 10°C. They reported a final count at seven days of only 0.6 log greater than at time zero, whereas numbers increased steadily in air controls by more than 3 logs.

Parekh and Solberg (1970) found that elevated levels of CO<sub>2</sub> did not prevent the growth of *C. perfringens* in laboratory media at optimum temperature. On the other hand, Silliker and Wolfe (1980) found that MA containing 60%CO<sub>2</sub> was slightly inhibitory for the growth of Putrifactive Anaerobe 3549 at 10°C and 20°C when compared to air. These authors also dipped 25-g cubes of pork into a suspension containing spores of *C botulinum* type A and B strains, and exposed the inoculated meats to 60%CO<sub>2</sub> with air samples serving as the controls. Results of toxin analysis showed that the toxin was detected after 1 day of storage in the atmosphere containing 60%CO<sub>2</sub> and in air. Eklund (1982) observed that type E strains produced toxin in salmon stored in MA before the product became organoleptically unacceptable. Enfors and Molin (1978) found that high partial pressures of CO<sub>2</sub> may stimulate the germination of *Clostridium* spores. But Blickstad et al. (1981) using 1 and 5 atm CO<sub>2</sub> found that none of the atmospheres supported growth of *Clostridium* spp. on cold stored pork. Schmidt et al. (1961) reported 3.3°C as a minimum temperature for growth and toxin production by *C. botulinum* type E. The potential for contamination could, therefore, be minimized by maintaining temperatures below this level.

At first, the major safety concerns about MAP was the anaerobic pathogens, especially, the psychrotrophic-nonproteolytic clostridia (Farber, 1991). Recently however, new safety issues have been raised by the emergence of new species of pathogens that are capable of growing under refrigeration (Farber, 1991).

Furthermore, most of these species are facultative anaerobes. For example, *L. monocytogenes* is enhanced by reduced O<sub>2</sub> content but it does not grow anaerobically (Lovett, 1988). However, Gill and Reichel (1989) found *L. monocytogenes* incapable of multiplying on high-pH beef packaged in 100%CO<sub>2</sub> at 5°C or less; although the organism did grow in CO<sub>2</sub>-packaged meat stored at 10°C, as well as on vacuum-packed meat stored at 0, 2, 5 and 10°C. Wimpfheimer et al. (1990) also observed that the organism did not survive well in minced raw chicken packaged in a 75% CO<sub>2</sub>/25% N<sub>2</sub> atmosphere, but it grew when as little as 5%O<sub>2</sub> was added.

*V. parahaemolyticus* is able to grow under both aerobic and anaerobic conditions (Twedt, 1989). Eklund and Jarmund (1983) noted CO<sub>2</sub> inhibition of *Y. enterocolitica*. They reported that at 20°C, *Y. enterocolitica* numbers were 57% of those in air; at 6°C, 2% of those in air; and at 2°C no growth occurred. Gill and Tan (1980) also noted that the respiration rate of *Yersinia* decreased and the generation time increased in CO<sub>2</sub>. Zee et al (1984) observed that 10%CO<sub>2</sub> stimulated the growth of *Yersinia* as compared to growth in air, 40%CO<sub>2</sub> increased the lag phase and 100%CO<sub>2</sub> both increased the lag phase and decreased the growth rate during the logarithmic period in TSB medium. However, Gill and Reichel (1989) found *Y. enterocolitica* to be capable of growing on high-pH beef packaged in 100%CO<sub>2</sub> at both 5 and 10°C. They also observed that storage at 0, 2 or -2°C in 100%CO<sub>2</sub> inhibited the growth of *Y. enterocolitica*. On the other hand, Kleinlien and Untermann (1990) showed that *Y. enterocolitica* was incapable of growing in minced beef at 4°C in a 20%CO<sub>2</sub>/80%O<sub>2</sub>. However at 15°C, growth of *Y. enterocolitica* was equal to that of the air control, while growth at 10°C was only slightly delayed. Not much work related to growth of *A. hydrophila* in MA has been



published. Myers et al. (1982) isolated *A. hydrophila* from commercially vacuum-packaged fresh pork stored at 5°C for 7 to 21 days. Enfors et al (1979) recovered the organism at  $10^6$  CFU/cm<sup>2</sup> from 100%N<sub>2</sub>-packed cuts but not from packages with pure CO<sub>2</sub> atmosphere. Blickstad and Molin (1983) also found less prevalence of *A. hydrophila* in CO<sub>2</sub>-flushed and air-flushed pork samples as compared to N<sub>2</sub>-flushed samples. However, Molin and Ternstrom (1982) observed that in aerobically stored meat *A. hydrophila* was capable of competing and occasionally even outnumbering the fast-growing *Pseudomonas* spp. Grau (1981) examined the effect of aerobic and anaerobic incubation on the ability of isolates of *A. hydrophila* to grow at 5°C on vacuum packaged beef muscle of high and low pH. He reported that the organism grew aerobically and anaerobically at low pH (pH 5.4-5.6) on adipose tissue removed from the beef muscle, and on the muscle tissue at high pH (pH 6.0-6.3), but did not grow anaerobically or aerobically on muscle tissue at low pH.

More recently Gill and Reichel (1989) inoculated *A. hydrophila* onto high-pH (>6.0) beef samples packaged in 100% CO<sub>2</sub> and under vacuum, and stored at -2, 0, 2, 5 or 10°C. They found that at 10°C *A. hydrophila* grew on the vacuum-packaged samples at a rate greater than that of the spoilage flora, but at the rate as the spoilage flora on CO<sub>2</sub>-packaged samples. The lag for *A. hydrophila*, however, increased with decreasing storage temperatures. The organism grew on vacuum-packaged samples stored at -2°C but did not grow on the CO<sub>2</sub>-packaged samples stored at the same temperature.

Characteristics of some common psychrotrophic pathogenic  
bacteria found in fresh meats

Listeria monocytogenes

General characteristics

*Listeria* are gram-positive, nonsporeforming, small coccoid rods. This organism has been referred to as a coryneform bacteria (Khan et al., 1973), but it has also been categorized in a genus of uncertain affiliation (Seeliger and Jones, 1984). There are several species of *Listeria*, which include *L. monocytogenes*, *ivanovii*, *innocua*, *welshimeri*, *seeliger*, *murray*, *gray*, and *denitrificans* (Seeliger and Jones, 1984). So far only *L. monocytogenes* and *L. ivanovii* have been shown to exhibit pathogenicity towards humans (Donnelly, 1988). A few documented outbreaks of human illness have been linked to *L. ivanovii* (Seeliger, 1984).

*L. monocytogenes* is facultatively anaerobic to microaerophilic, grows in a temperature range 2.5-42°C, with the optimal growth between 30 and 37°C, and a pH range from 5.6 to 9.8 (Seeliger and Finger, 1976). However, recent studies by Connor et al. (1986) revealed that *L. monocytogenes* can grow at pH 5.0. Growth of the bacterium is enhanced under decreased oxygen concentrations and in carbon dioxide (Seeliger, 1961). Zink et al. (1951) found that isolation was better under reduced oxygen tension or at lower oxidation-reduction potential than under aerobic conditions.

### Habitat

*L. monocytogenes* is commonly found in the environment; it has been detected in the soil, on decaying plant matter such as silage, in raw and treated sewage, and in effluents of slaughter houses (Ai-Ghazali and Al-Azawi, 1986; Fenlon, 1986; Gray and Killinger, 1966; Watkins and Sleath, 1981). The common presence of *L. monocytogenes* in the soil has led some authors to believe that the organism is saprophytic in nature (Weis and Seeliger, 1975).

### Reservoirs

Livestock have most often been identified as reservoirs; they include cattle, sheep, swine, goats and fowl (Gitter, 1985). Buncic (1991) found that 45% of pigs and 29% of cattle examined harbored *L. monocytogenes*. Other animals such as horses, birds, rodents, fish, dogs and cats also harbor *Listeria* (Brackett, 1988). According to Ralovich (1984), the organism is often associated with the intestinal tract or with latent (subclinical) infections of both domestic animals and humans.

### Listeriosis

Listeriosis is the human disease caused by *L. monocytogenes* infection. Because the organism grows well at refrigerated temperatures, consumption of moderate levels of *Listeria* occurs commonly (Theno, 1991). Therefore, anyone can become infected with the organism, but many persons remain symptomless. However, some subpopulations are likely to develop disease if infected; they include pregnant women, newborns, and infants, and adults with a compromised immune system such as cancer, leukemia or AIDS patients (Marth, 1988). Mild symptoms such as fever, chills, headache, backache, discolored urine (Seeliger and Finger, 1976) occur during early infection. These may develop into more serious diseases such as inflammation of the pelvis and kidney, meningitis and

septicemia (Seeliger and Finger, 1976). Infection of pregnant women may also lead to abortion or stillbirth of the fetus (Marth, 1988).

#### Mechanism of illness

The mechanism(s) of listeriosis is poorly understood. The organism produces a series of toxins including hemolytic and lipolytic toxins, which may be involved in the disease processes (Marth, 1988). Hemolysins are markers for virulence since the loss of hemolysis has been found to lead to a loss of virulence (Gaillard et al., 1985). Ralovich (1984) has also found that the cell wall of *L. monocytogenes* is also involved in its pathogenicity. The protein and carbohydrates in the cell wall are antigenic and pyrogenic (Ralovich, 1984). *Listeria* endotoxin has all the characteristics of gram-negative endotoxin (Wexler and Oppenheim, 1979). However, the relationship of cell wall structure to mucosal attachment and invasion remains unclear (Schlech, 1988). There is also no evidence that virulence is plasmid-mediated (Schlech, 1988). It has been reported that the virulence of *L. monocytogenes* is enhanced at lower temperatures (Basher et al., 1984).

#### Epidemiology

Five well-investigated listeriosis outbreaks involving foods occurred in the past decade. In 1981, thirty-four cases of prenatal listeriosis and 7 cases of adult disease were reported in the Maritime Province of Nova Scotia. The vehicle of transmission was coleslaw prepared with a cabbage grown in a field that had been fertilized with sheep manure (Schlech et al., 1983). In 1983, forty-nine individuals in Massachusetts acquired listeriosis with a mortality rate of 29%. A specific brand of pasteurized whole or 2% milk was reported as the vehicle of transmission (Fleming, 1985). In 1985 a Mexican-style soft cheese was also implicated as the

vehicle of transmission of listeriosis in southern California (Linnan et al., 1988). These products were also strongly implicated in the remaining two outbreaks (Lovett et al., 1991).

Recently, additional cases of listeriosis have implicated meat products. For example, vacuum-packaged turkey franks contaminated with *L. monocytogenes* were associated in a fatal case of listeriosis (Barnes et al., 1989). A study of sporadic listeriosis in six states in the U.S. indicated that cases were associated with consumption of uncooked hot dogs and undercooked chicken (Kaczmarek and Jones, 1989; Schwartz et al., 1988).

#### Contamination of fresh meats

The isolation of *L. monocytogenes* from livestock makes it likely that red meat and poultry are contaminated with the bacterium. Kwantes and Isaac (1971) isolated *L. monocytogenes* from 57% of fresh poultry samples. The results of a preliminary survey in the U.S. indicated that about 70% of ground beef, 43% of pork sausage and 48% of poultry were contaminated with this bacterium (Brackett, 1988). Buncic (1991) isolated *L. monocytogenes* from 69% of minced meat (mixed pork and beef), samples tested in Yugoslavia. The presence of the organism in slaughterhouse effluents (Watkins and Sleath, 1981) is a further suggestion that contamination of meat and poultry may be common. *L. monocytogenes* has the ability to proliferate at refrigeration temperatures and this makes refrigerated foods, especially fresh meats, susceptible to higher-than-usual populations (Brackett, 1988).

Johnson et al. (1986) investigated the survival of *L. monocytogenes* in ground beef held at 4°C for 2 weeks. The ground meat was inoculated with  $10^5$  to  $10^6$  cells/g and then packaged in either oxygen-permeable or oxygen-

impermeable bags. They reported that the number of *L. monocytogenes* in the ground beef remained constant throughout the sampling period and was not affected by the oxygen permeability. Khan et al. (1975) found a significant growth of *L. monocytogenes* in the sarcoplasmic proteins of pork, but not in the sarcoplasmic proteins of lamb or beef. They, therefore, suggested that some inexplicable differences in the growth of *L. monocytogenes* may occur on different types of red meat.

#### Isolation and enumeration

Enrichment procedures The concentration of naturally occurring *L. monocytogenes* in foods is so low that isolation by direct plating has been generally unsuccessful (Lovett, 1988). Proposed methods involve enrichment in one or two stages before isolation on solid media (Lovett, 1988). Cold enrichment at 4°C was used as a means of selecting for the psychrotrophic *L. monocytogenes* while suppressing competitors that were unable to grow at 4°C (Gray et al., 1948). This method is still used as the primary or pre-enrichment step in the isolation of *L. monocytogenes*. The main disadvantage of this method is that it requires prolonged incubation; the organism has a generation time of 1.5 days at 4°C (Lovett, 1988). Doyle and Schoeni (1986) proposed an enrichment procedure that took advantage of the microaerophilic nature of the organism. However, when compared to the cold enrichment procedure, Doyle and Schoeni (1987) found that the procedure was not as effective as the cold procedure in recovering *Listeria* isolates. Antibiotics, toxic metal compounds, and dyes have been used in enrichment formulations to suppress or inhibit the growth of competitors (McBride and Girard, 1960; Ralovich et al., 1971), and to shorten the incubation period from weeks to days if enrichment is done at the optimum growth temperature (Lovett,

1988). Using these antimicrobial agents, different formulations and procedures have been developed (Loessner et al., 1988). Numerous studies have compared the ability of various new procedures to recover *L. monocytogenes* from foods (Buchanan et al., 1989; Fernandez and Genigeorgis, 1990; Hitchins, 1989; Hitchins and Tran, 1990; Lammerding and Doyle, 1989; Northolt, 1989; Slade and Collins, 1988; Tiwari and Aldenrath, 1990; Truscott and McNab, 1988). Two of the most popular formulations were developed by U.S. Food and Drug Administration (FDA) for dairy products (Lovett et al., 1987) and by the U.S Department of Agriculture (USDA) for meats (McClain and Lee, 1988). The difference between the two procedures is primarily in the selective enrichment and plating media used. The FDA selective enrichment broth is called *Listeria* enrichment broth (LEB), and consists of a trypticase soy broth (TSB) base to which yeast extract, cycloheximide, acriflavine HCl, and nalidixic acid are added; the plating medium, called modified McBride agar (MMA), contains of 35.5 g phenylethanol agar/L to which is added 10 g glycine anhydride, 0.5 g lithium chloride (LiCl) and 190 mg cycloheximide (Lovett et al., 1987). The USDA enrichment medium is called University of Vermont (UVM) broth and consists of proteose peptone and tryptone base to which meat extract, yeast extract, sodium chloride (NaCl),  $\text{KH}_2\text{PO}_4$ ,  $\text{Na}_2\text{HPO}_4$ , esculin, nalidixic acid, and acriflavin are added; the plating medium consists of phenylethanol agar base to which glycine anhydride, LiCl and moxalactam are added (McClain and Lee, 1988). The two methods have been quantitatively compared for their ability to recover *L. monocytogenes* from different food products. Lovett et al. (1991) reported that the FDA procedure isolated heated *L. monocytogenes* from seafoods at a lower level than the USDA method; however, the two methods isolated unheated cells equally well. They also found that the USDA procedure had a

greater selectivity, and hence may offer an advantage for isolating nonheat-stressed *Listeria* when the aerobic plate count of the product is high. Crawford et al. (1989) found that both methods provided comparable and excellent recovery of uninjured *L. monocytogenes* cells, but the USDA method was consistently less efficient than the FDA method in recovering heat-injured cells in bovine milk. Swaminathan et al. (1988) observed lower recovery of *Listeria* in the USDA broth than in the FDA. McClain and Lee (1988) showed that LPM agar suppressed the growth of most background microflora and allowed the growth of *Listeria* from a highly mixed culture. Hitchins and Tran (1990) described the improved recovery of *L. monocytogenes* from a wide variety of inoculated foods by the FDA procedure when the associated agar was replaced by that prescribed for the USDA method. They found that the detectability limit for 40% of the foods sampled, including ground beef and frankfurter, was < 1 CFU/g and between 1 and 10 CFU/g for an additional 51% of samples under those conditions.

However, both MMA and LPM agars were found not to be selective against the growth of staphylococci, streptococci and micrococci (Buchanan et al., 1987). Further, both media rely on subsequently detecting *Listeria* on the basis of colonies taking on a blue-gray color when illuminated with reflected light at an angle of 45° (Lachica, 1990a). This means of selection is less than ideal as certain non-*Listeria* organisms such as streptococci exhibit a similar bluish cast (Bailey et al., 1989; Hao et al., 1987; Siragusa and Johnson, 1988). Another disadvantage with the oblique transillumination technique is it is too tedious to view uncovered agar plates right side up instead of viewing covered plates bottom side up (Lachica, 1990a). Lachica (1990a) proposed a much simplified technique that increased the angle of illumination to 127°; this made viewing and counting of distinct bluish



colonies much easier. However, he observed that some non-listerial strains appeared to have a bluish hue similar to *Listeria* with this technique. Consequently, alternative diagnostic properties have been explored. Among these are dark colors resulting from the reaction of ammonium ferric citrate with the hydrolytic product of esculin (Curtis et al., 1989; Van Netten et al., 1988), and tellurite reduction (Buchanan et al., 1987). Buchanan et al. (1987) used plating media containing tellurite to isolate *Listeria* from inoculated food samples; they reported that recovery of the organism from meat samples was very effective. Fraser and Sperber (1988) developed a broth which consists of the USDA enrichment broth to which is added ferric ammonium citrate. They compared the broth to the USDA broth for the detection of *Listeria* in meat samples, and reported that all the cultures produced positive reaction in the Fraser broth while only 13% yielded *Listeria* isolates in the USDA broth. According to Cassidy et al. (1989), in addition to recovery of *L. monocytogenes*, factors that must be considered in selecting a plating medium include ease of counting the colonies of the organism in the presence of high populations of background microflora, restriction of growth of background microflora, and colony size and color. Oxford selective medium consists of an agar base that contains Columbia blood agar base, esculin, ferric ammonium citrate and lithium chloride, and an antimicrobial supplement that contains cycloheximide, colistin sulphate, acriflavine cefotetan and fosfomycin (Curtis et al., 1989). Varabiouff (1990) demonstrated that Oxford selective medium recovered more *Listeria* isolates than did McBride agar from chicken.

Confirmation of *Listeria* spp. Presumptive *Listeria* colonies are gram-positive, small rod or coccoid-shaped which are motile with tumbling activity or umbrella shape at 25°C , and which give a positive catalase reaction (Lovett, 1988). Confirmation test includes *B*-hemolysin production on sheep blood agar. Three species are hemolytic: *L. monocytogenes*, *L. seeliger* and *L. ivanovii* (Lovett, 1988). They are differentiated by the CAMP test, which uses hemolysis enhancement by metabolites diffused into the blood agar plate by *S. aureus* (a beta-hemolytic strain) and *Rhodococcus equi* (McClain and Lee, 1988). The hemolytic reactions of *L. monocytogenes* and *L. seeliger* are enhanced in the vicinity of the *S. aureus* culture but not in the vicinity of *R. equi*. *L. ivanovii* cultures are more hemolytic in the vicinity of *R. equi* but are unaffected by *S. aureus* (McClain and Lee, 1988). A negative D-xylose acidification test distinguishes *L. monocytogenes* from *L. seeliger* and other avirulent species (Schlech, 1988).

### *Yersinia enterocolitica*

#### General characteristics

The genus *Yersinia* currently belongs to the family *Enterobacteriaceae* by virtue of common antigens, and biochemical and core DNA relatedness (Brenner, 1979; Edwards and Ewing, 1972). It is made up of *Y. enterocolitica* and *Y. enterocolitica*-like organisms (Schiemann, 1989). Strains previously termed *Y. enterocolitica*-like organisms have been classified into seven separate species namely, *Y. intermedia*, *Y. frederiksenni*, *Y. kristensenii*, *Y. aldovae*, *Y. rohdeii*, *Y. mollaretti* and *Y. bercovieri* (Aleksic, et al., 1987; Brenner, 1979; Wauters et al., 1988) based upon their DNA relatedness (More and Brubaker, 1975). None of these species have been associated with human or animal disease (Kapperud,

1991). All strains capable of causing disease belong to *Y. enterocolitica* (Kapperud, 1991). This species is a facultatively anaerobic gram-negative, nonsporeforming rod; it is generally motile below 30°C and nonmotile at 37°C (Schiemann, 1989). The pH range for the survival and growth of *Y. enterocolitica* is 4.6 - 9.0 (Stem et al., 1980) with optimum range from pH 7.0 - 8.0 (Hanna et al., 1977b). Seelye and Yearbury (1979) observed that when grown at 4°C, strains of *Y. enterocolitica* grew slowly at pH values of 5.2 and 5.4 and showed heavy growth at pH 5.6-7.6. Most strains grow in the temperature range 25-39°C with the optimum at 25°C (Schiemann, 1989). The organism is one of the few enteric pathogens that can grow at refrigeration temperatures. Hanna et al (1977a) found that the numbers of *Y. enterocolitica* in experimentally inoculated raw beef or pork increased significantly in 3-4 days when stored at 7°C. Stern and Pierson (1979) reported that at temperatures of 4-7°C small numbers of this organism developed into large numbers of viable cells.

#### Reservoirs

Animals appear to be a significant reservoir of *Y. enterocolitica*. For example, swine have been implicated as a major reservoir of strains involved in human infections (Swaminathan et al., 1982). *Y. enterocolitica* 0:3 is a normal inhabitant of tonsils, tongues and feces of pigs (Christensen, 1982). Other animals that have been incriminated as potential reservoirs include cows, sheep, horses, deer, dogs and rodents such as rats (Hubbert, 1972; Wooley et al., 1980). Inoue and Kurose (1975) isolated *Y. enterocolitica* from the intestinal contents of cows. *Y. enterocolitica* has also been isolated from water, hence it has been suggested that water may also be a reservoir for this organism (Botzler et al., 1977; Caprioli et al., 1975; Kapperud, 1977; Schiemann, 1978).

### Pathogenicity

Yersiniosis is the name of the disease caused by human *Y. enterocolitica* infections. Symptoms of *Y. enterocolitica* infections vary with the strain, the dose, genetic factors, and age and physical condition of the host (Bottone, 1977; Larson, 1979). Serotypes that have been found to be human pathogens include 0:3, 0:8 and 0:9 (Schiemann et al., 1981). Serotype 0:3 is the most common type of *Y. enterocolitica* isolated from humans (Schiemann et al., 1981). In the U.S., however, serotype 0:8 has been reported more frequently from humans (Schiemann et al., 1981). Gastroenteritis and mesentric lymphadenitis are the predominant symptoms in infants, children and adolescents; acute abdominal disorders, diarrhea, and arthritis are the major manifestations among persons aged 20-60 years, and erythema nodosum is the most striking symptom in persons aged over 60 years (Winbald, 1973). There are three stages of clinical manifestations of *Y. enterocolitica* infections: the primary (acute) phase occurring within 2-3 days of contracting the infection and characterized by enteritis, lymphadenitis, appendicitis, acute terminal ileitis, tonsillitis, pneumonia, fever, septicemia or abscesses; the secondary or complication stage appearing 1-3 weeks after acute phase and characterized by inflammatory skin disorders, hemorrhaging into the skin, and connective tissue disorders; and the tertiary phase which manifest itself in the form of rheumatoid arthritis, inflammation of the cornea and muscles, and the hardening of connective tissues (Larson, 1979). Gastroenteritis in humans is the major expression of pathogenic strains. Frequently reported symptoms of *Y. enterocolitica* enteritis include: diarrhea, fever, vomiting, abdominal pain, nausea and headaches (Stern and Pierson, 1979).

Pathogenicity of strains of *Y. enterocolitica* has been reported to be plasmid-mediated (Zink et al., 1980). They found that a plasmid of about 42 megadaltons (Mdal) mediated tissue invasiveness in the Sereny test. Aulisio et al. (1983) reported that three of six strains of *Y. enterocolitica* recovered from tofu, suspected to be the vehicle of an outbreak harbored a 42-Mdal plasmid. Kay et al. (1982) also found that a larger plasmid (82 Mdal) that is usually accompanied by the 42-Mdal plasmid may be responsible for virulence of *Y. enterocolitica*. Genski et al (1980) determined the presence of a plasmid in three strains of *Y. enterocolitica* serotype 0:8 which was associated with invasiveness as determined by Sereny and mouse lethality tests. Carter et al. (1980) found that the presence of the same plasmid was related to a calcium requirement for growth at 37°C. However, some investigators have reported that some strains that harbor a 42-Mdal plasmid are not pathogenic (Aulisio et al., 1983; Kay et al., 1982). Such strains lack virulence factors such as autoagglutination at 37°C and the initiation of a lethal infection in the suckling mouse (Aulisio et al., 1983).

The mechanism of pathogenicity of *Y. enterocolitica* is invasiveness. Lee et al (1981) demonstrated that certain strains of the organism were capable of invading HeLa cells. The organism possesses the ability to invade epithelial cells in the human gut (Une, 1977). Stern and Pierson (1979) isolated several strains of *Y. enterocolitica* from pork which were invasive by the HeLa cell test for virulence. However, more recently it has been reported that invasiveness by *Y. enterocolitica* does not appear to be associated with the presence of a 42-Mdal plasmid since many strains lacking plasmids were found to be invasive (Schiemann, 1989).

*Y. enterocolitica* possess lipopolysaccharide O antigens similar to other gram-negative bacteria (Swaminathan et al., 1982). The O antigens are part of the

endotoxin produced by *Y. enterocolitica*. The organism also produced a heat-stable enterotoxin (ST) in a synthetic medium at 25°C but not at 35°C (Pal et al., 1978). De Guzman et al. (1991) found that 16 strains of *Y. enterocolitica* isolated from fresh sausages, bovine tongues, and porcine ceca and tongues produced ST in tryptose soy broth, although the association of illness with *Y. enterocolitica* ST has not been demonstrated (Aulisio et al., 1983). The pyrrozinamidase test can distinguish strains correctly into naturally occurring virulence plasmid-positive and -negative classes (De Zutter and Van Hoof, 1987). Positive autoagglutination at 37°C, calcium dependency at 37°C and latex particle agglutination are some of the other tests used to predict the potential virulence of *Y. enterocolitica* (De Zutter and Van Hoof, 1987). These authors, however, reported a number of false-positive as well as false-negative reactions with the latex particle agglutination tests. The infectious nature of the organism seem to be related to the incubation temperature. Nilehn (1973) reported that a culture grown at 25°C in a synthetic medium was more virulent than one incubated at 37°C.

### Epidemiology

*Y. enterocolitica* rivals *Salmonella* as a cause of acute gastroenteritis in many Western countries (Anon., 1991). In the U.S there have been fewer outbreaks. These outbreaks have been associated with raw milk, pasteurized milk, reconstituted powdered milk, bean curd, and bean sprout (Anon., 1991). The largest among them involved pasteurized milk which was presumed to have been contaminated by mud on the crates used to transport outdated milk to pig farms (Stanfield et al., 1985). Yersiniosis was also linked to the consumption of pork after the eating habits of young children were investigated (De Zutter and Van Hoof,

1987). Most recently, 15 cases involving children in Atlanta occurred, and were traced to contaminated chitterlings or pork intestines (Anon., 1991).

#### Contamination of fresh meats

*Y. enterocolitica* has been isolated from fresh pork, beef, lamb and poultry. De Zutter and Hoof (1987) isolated the organism from 38.7% of samples of pork head muscles. Hanna et al. (1976) isolated several strains of atypical *Y. enterocolitica* from vacuum-packaged beef and lamb cuts. Schiemann (1980a) isolated the organism from 49% of 121 raw pork samples obtained from retail stores. He noted that maximum numbers of isolations were obtained from pork tongues (65%) and ground pork (60%), and that a majority of the isolates were predominantly pathogenic serotypes 0:3 and 0:5A. Hence, he cautioned that these strains could easily be disseminated to other parts of the carcass during slaughter and processing operations. Seeley and Yearbury (1979) indicated that the counts of *Y. enterocolitica* on vacuum-packaged high pH (>6.0) meat were much higher than those of low pH (<6.0) meat held at 0-2°C for 10 days. This was confirmed by Gill and Newton (1979) when they found that *Y. enterocolitica* grew consistently in high numbers on vacuum-packaged DFD meat derived from the carcasses of stressed animals. According to Stern and Pierson (1979), fresh meats may naturally carry the pathogen or meat may be contaminated by handlers through the transfer of intestinal materials to the individual cuts.

#### Isolation and enumeration methods

Enrichment procedures Isolating *Y. enterocolitica* from food is generally more difficult than isolating it from clinical specimens because of the low numbers of the organism typically present and a greater variety of background microflora in foods (Doyle and Hugdahl, 1983). Most isolation methods involve

enrichment of the sample followed by plating onto selective media (Doyle and Hugdahl, 1983). Wauters (1973) proposed cold enrichment at 4°C using selenite cystine broth with 40g/l novobiocin or Rappaport broth (RMC) containing magnesium chloride, malachite green and carbenicillin. However, in an inoculation study, Lee et al. (1980) reported that RMC broth and the selenite broth were effective in recovering the 0:3 serotype from ground pork, but they completely inhibited and failed to recover the more sensitive 0:8 clinical strains commonly recovered in the U.S. They also found that high levels of indigenous background flora grew in samples enriched in the selenite broth. According to Stern (1982), the above enrichments are not useful for typical 0:8 serotypes indigenous to the U.S. Aulisio et al. (1980) used a combination of phosphate buffer saline (PBS pH 7.6) and potassium hydroxide (KOH) to recover *Y. enterocolitica* strains from food inoculated with 10-1000 cells per gram. After 2 days incubation at 25°C, they streaked onto MacConkey agar and incubated the plates for 48 hr at 26°C. They reported that the method increased sensitivity by 10-1000 times. Stern et al. (1980) found that *Y. enterocolitica* was tolerant of relatively alkaline conditions. Lee et al. (1980) compared PBS-KOH enrichment and the utilization of KOH treatment for the modified selenite broth. They reported that the PBS-KOH enrichment at 22°C did not favor the growth of a slow-growing sensitive strain (IP 107) and did not suppress the growth of other species of bacteria. Doyle and Hugdahl (1983) indicated that although modified selenite broth enrichment was substantially shorter than cold enrichment with PBS, the modified selenite broth was limited in that it was primarily useful for recovering the clinically important 0:3 and 0:9 serotypes only when their numbers were sufficiently large. Doyle et al. (1981) found that cold enrichment in PBS followed by treatment with dilute alkali



recovered the greatest number of 0:3 and 0:8 strains from pork tongues, while no strain was isolated by enrichment in modified RB or in modified selenite medium with or without a KOH postenrichment. They concluded that cold enrichment in PBS combined with a KOH postenrichment treatment was clearly the most successful technique for isolating virulent *Y. enterocolitica* from naturally contaminated meat. Doyle and Hugdahl (1983) further improved this procedure by enriching the samples in PBS at 25°C instead of at 4°C, and using 0.25% instead of 0.5% KOH. This cut the recovery time from about 21 days to 3 days or less, recovered as few as 10 cells of the organism per g of raw meat, and recovered the known, clinically important serotypes.

Direct plating media Various differential and/or selective enteric plating media have also been used with nonuniform success for the isolation of *Y. enterocolitica* (Stern and Pierson, 1979). They include deoxycholate citrate agar also known as Leifson agar (Wauters, 1973), bismuth sulfite (BS) agar (Hanna et al., 1977c), lysine-sucrose-urea agar (Lee, 1977a), MacConkey's agar (Feeley et al., 1976), MacConkey agar modified with Tween 80 (MT), deoxyribonuclease agar modified with Tween 80 plus sorbitol DST (Lee, 1977b), *Salmonella-Shigella* (SS) agar (Feeley et al., 1976) and the cefsulodin-irgasan-novobiocin (CIN) agar (Schiemann, 1979). Stern (1982) indicated that false positive reactions may be obtained when bismuth sulfite agar is used, hence biochemical tests are subsequently needed for differentiation. The recovery of *Y. enterocolitica* from foods on MacConkey or SS agar is difficult because colonies are indistinguishable from those of many other foodborne lactose-negative bacteria that also grow on these agars (1977b). Lee (1977b) also found that SS agar was inhibitory to many strains of *Y. enterocolitica* in meat. There are Tween-negative strains of *Y.*

*enterocolitica* as well as other gram-negative bacteria that are also Tween-positive on DST and MT, therefore, it is likely to have false-negative or false-positive reactions (Lee, 1977b). The CIN agar has had the greatest acceptance for the recovery of *Y. enterocolitica* from foods (Stern, 1982). It is a selective and differential basal medium which supports the growth of *Y. enterocolitica* (Devenish and Schiemann, 1981). Selectivity is due to the presence of bile salts, crystal violet and Irgasan which effectively inhibit the growth of gram-positive and a number of gram-negative organisms (Schiemann, 1980b). The agar is supplemented with *Yersinia* antimicrobial CN containing cefsulodin and novobiocin additives which enhance inhibition of normal enteric organisms. The differential property of the medium is based on fermentation of mannitol which produces a localized pH drop around the colony followed by absorption of the neutral red which imparts a red color to the colony. Colonies of organisms that do not metabolize mannitol to acid remain colorless and translucent. Doyle and Hugdahl (1983) compared CIN and MacConkey agars for the recovery of *Y. enterocolitica* from raw meat. They reported that the organism was more easily detected on CIN agar because of the bacterium's distinctive colonial morphology and the medium's greater selectivity, which restricted the development of many background colonies that developed on MacConkey agar.

Characterization Characterization of *Y. enterocolitica* is accomplished through biochemical, growth requirement and immunological tests. Strains of this organism differentially produce indole and are methyl red-positive. Most strains are o-nitrophenyl B-D-galactopyranoside (ONPG)-positive, but B-galactosidase-negative, except for those strains which contain the plasmid lac + (Cornelis et al., 1976). Atypical strains of *Y. enterocolitica* may be divided into four

major groups, two of which are sucrose-negative and two of which are rhamnose-positive (Swaminathan et al., 1982). One group of sucrose-negative strains exhibits all other typical biochemical reactions of *Y. enterocolitica*, while the other group of sucrose-negative strains is also negative for ornithine decarboxylase, nitrate reduction, Voges-Proskauer reaction (V-P) at 37°C, ONPG, sorbose, sorbitol and trehalose (Swaminathan et al., 1982). Among the rhamnose-positive strains, one group is more metabolically active at 22 than at 37°C and positive for raffinose, melibiose,  $\alpha$ -methyl glucoside and citrate, while the other group is negative for raffinose, melibiose,  $\alpha$ -methyl glucoside or citrate (Brenner et al., 1976). Chester and Stotzky (1976) found that rhamnose-positive strains of *Y. enterocolitica* differed from rhamnose-negative ones in their ability to ferment raffinose and lactose, to utilize citrate and in their inability to grow on Hektoen enteric, *Salmonella-Shigella* or xylose-lysine deoxycholate agars. Brenner (1979), on the basis of DNA hybridization studies on strains of *Y. enterocolitica*, concluded that the biochemically variant strains (rhamnose-positive, sucrose-negative, etc) were more closely related to *Yersinia* than any other genus in the family *Enterobacteriaceae*. Other distinguishing biochemical characteristics of *Y. enterocolitica* are positive glucose (acid +; gas -), mannitol and sorbitol fermentations, negative lactose fermentation, phenylalanine deaminase and lysine decarboxylase reactions (Stern and Pierson, 1979). The serotype 0:8 found in the U.S also is indole-positive and esculin- and salicin-negative (Stern, 1982).

The USDA has developed two new tests based on the use of crystal violet or congo red dyes for rapid detection of virulent strains of *Y. enterocolitica* (Anon., 1991). The crystal violet binds to the plasmid-bearing virulent cells, turning them purple; the nonvirulent strains do not bind the dye and remain white. The Congo

red is incorporated into a calcium-deficient agarose medium. Virulent strains absorb the dye, forming tiny red colonies which can be easily distinguished from the larger, white nonvirulent strains. The entire test can be completed in 12 hr after isolating the organism from food (Anon., 1991). The problem with these tests is that they all require an initial isolation of the organism from the food product. In addition the crystal violet test cannot be read after some time because the dye diffuses and makes the differentiation between pathogenic and nonpathogenic strains difficult with time (Anon., 1991).

#### Rapid biochemical diagnostic kits

Rapid identification systems have been developed for the speciation of members of the *Enterobacteriaceae* family. They include the API, Minitek, Micro ID, Enterotube and many others. They are reported to have high levels of generic accuracy and reproducibility (Cox et al., 1984; Fung and Cox, 1981; Hartman and Minnich, 1981). Restaino et al. (1979) found that the API 20E and Minitek systems could identify correctly 80 to 100% of 25 cultures of *Y. enterocolitica* from human and environmental sources, respectively. Guthertz and Okoluk (1978) reported that API strips were employed to identify 96.1% of *Enterobacteriaceae* isolates to the species level. Biochemical reactions in these kits are generally read after 24 hr of incubation, except the Micro ID and the API RE systems which provide results after 4 hr of incubation (Cox and Bailey, 1986). Biochemical patterns of reactivity translate into unique profile numbers that, upon comparison with a manufacturers-compiled data base, lead to a genus or species identification with a stated probability of correct identification (D'Aoust, 1989). Each system has its unique characteristics; the MicroID is rapid, the Minitek is biochemically versatile, the APT

20E has a solid data base and definitive chemical reactions, and the Enterotube II is easy to inoculate (Cox et al., 1984).

Although these rapid identification systems have not been developed specifically for the members of the *Vibrionaceae* family, the existing systems have included some members such as *A. hydrophila* in their data base.

### *Aeromonas hydrophila*

#### General characteristics

The genus *Aeromonas* is a member of the *Vibrionaceae* family and it includes two distinctly separate groups (Stelma, 1989). The first group consists of a single psychrotrophic and nonmotile species, *A. salmonicida*. The organism is highly pathogenic to fish but not to humans, and it does not grow at 37°C (Popoff, 1984). The second group consists of the motile aeromonads classified into three species; *A. hydrophila*, *A. caviae*, and *A. sobra* (Popoff, 1984; Popoff et al., 1981). Their classification is based on biochemical characteristics and similarities in DNA (Popoff et al., 1981). The motile species are facultatively anaerobic gram-negative rods, oxidase- and catalase-positive and ferment carbohydrates with the production of acid or acid and gas (Popoff, 1984). They are mesophilic with an optimum growth temperature of 28°C and a maximum growth temperature of 42°C (Popoff, 1984). Some strains have psychrotrophic characteristics and can grow at 4°C (Palumbo et al., 1985b). *Aeromonas* spp. are able to grow over a pH range of 4.0-10.0 (Hazen et al., 1978).

Among the motile species, *A. hydrophila* has been implicated as a foodborne pathogen (Agger et al., 1985; George et al., 1986; Shread et al., 1981). This organism is becoming a major source of gastroenteritis since a survey of fecal

samples from individuals suffering from gastroenteritis symptoms indicate that *A. hydrophila* is isolated at approximately the same rate as other important enteric pathogens such as *Salmonella* and *Campylobacter jejuni* (Bechtel and Campos, 1984). Also, a variety of extracellular virulence factors produced by this organism supports the epidemiological evidence that this is an enteric pathogen. Diseases caused by *A. hydrophila* include a cholera-like illness characterized by watery stool and mild fever, and a dysentery-like illness characterized by the presence of blood and mucus in the stool (Gracey et al., 1982). The organism has also been implicated as the cause of septicemia (Wolff et al., 1980) and meningitis (Ellison and Mostow, 1984). Pathogenicity of the organism is determined by the ability to produce cytotoxin and hemolysin. Turnbull et al. (1984) and Okren et al. (1987) found a strong correlation between enterotoxin production and hemolysis. Majeed and Mac Rae (1991) reported that certain strains of *A. hydrophila* produced enterotoxin and hemolysin in meat extracts at 5°C.

#### Reservoirs

*A. hydrophila* occurs in lakes, river waters, and oceans (Hazen et al., 1978; Kaper et al., 1981; Peele et al., 1981). The organism has also been isolated from chlorinated water, livestock feces and bone marrow (Burke et al., 1984; Stern et al., 1987). Gray (1984) detected *A. hydrophila* in about 12% of healthy farm animals including cows, sheep, pigs and horses.

#### Epidemiology

Reported foodborne outbreaks of *A. hydrophila* gastroenteritis have all been associated with seafoods. Raw oysters were implicated as the possible cause of gastroenteritis in 1982 and 1983 (Abeyta et al., 1986; Herrington, 1984).

### Contamination of fresh meats

Some strains of *A. hydrophila* have been found as contaminants of fresh meat from slaughter houses as well as in retail stores (Palumbo et al., 1985a). In a study of the incidence of *A. hydrophila* on raw pork, beef and chicken obtained from a slaughter house, Ternstrom and Molin (1987) reported that *A. hydrophila* was found on 32% of the beef and chicken samples, and 24% of the pork samples. Okrend et al. (1987) found *Aeromonas* spp. in chicken thigh, ground beef, and ground pork obtained from a local food store. Barnhart et al. (1989) isolated *A. hydrophila* from 98% of all broiler carcasses tested, and 92% of all chill water samples in a broiler processing plant. According to Popoff (1984), at refrigeration temperatures *A. hydrophila* grows at a sufficiently rapid rate to be competitive with other psychrotrophic species associated with refrigerated foods. Consequently, *A. hydrophila* is able to develop and occasionally actually spoil meat (Ternstrom and Molin, 1987). *A. hydrophila* contamination of fresh meats may occur during the slaughtering and/or packaging processes, especially during the evisceration step (Barnhart et al., 1989). However, Stern et al. (1987) believed that the source of contamination was chlorinated water used to wash carcasses during slaughtering and processing, and not from fecal origin, because they found that the organism was not frequently present in the feces of cattle, swine or turkey.

### Isolation and enumeration methods

Specific information on the possibility that fresh meats may be significant vectors for the transmission of enterotoxigenic *A. hydrophila* is largely unavailable, and it is partly due to the absence of selective media for the isolation of *A. hydrophila* from a food matrix (Palumbo et al., 1985a). None of the selective media developed for the isolation of the organism from medical and environmental

samples permitted quantitative recovery of the organism from a food matrix (Palumbo et al., 1985a). For example, Rimer-Sotts (RS) medium contains deoxycholate and novobiocin which inhibit gram-positive bacteria and *Vibrio* spp. The medium also inhibits *Proteus* and *Plesiomonas shigelloides*. Members of the genus *Citrobacter* are the only false positives to be anticipated, but subsequent tests for oxidase activity (*A. hydrophila* +; *Citrobacter* -) eliminate the possibility of *Citrobacter* spp (Popoff, 1984). However, Ternstrom and Molin (1987) reported that when RS was used to isolate *A. hydrophila* from fresh meats, the medium was often overgrown with other bacteria making isolation very difficult. Okrend et al. (1987) evaluated five selective differential plating media for efficiency of isolation of *Aeromonas* spp. from chicken, beef and pork samples. The five media included; Columbia blood agar containing sheep blood and ampicillin (CBA), peptone beef extract glycogen agar (PBG), starch ampicillin agar (SA), MacConkey xylose ampicillin agar (MXA); and MacConkey mannitol ampicillin agar (MMA). They found no difference among the media when pure culture of *A. hydrophila* was used, but when meat was sampled, CBA proved unsatisfactory for routine isolation because not all strains of *A. hydrophila* were hemolytic on the sheep blood agar plates. This made isolation of nonhemolytic *Aeromonas* spp. difficult. MXA agar was not suitable because many xylose-negative, non-aeromonads grew on the medium. PBG agar involved overlaying with a nonnutrient agar, and that made picking of colonies difficult. Thus, they concluded that the two most suitable media for the recovery of *A. hydrophila* from meat samples were SA agar and MMA agar. However, Molin and Ternstrom (1982) had earlier direct-plated meat samples on SA agar and MMA agar and had reported that recovery of *A. hydrophila* was better on SA agar than on MMA agar. According to von Graevenitz (1985), the poor



recovery of *A. hydrophila* on MMA agar could be due to the fact that strains of the organism include both lactose-negative and lactose-positive ones, and therefore, the usual enteric media such as MacConkey agar are unsuitable. Palumbo et al. (1985a) developed SA agar to sample red meats. The ampicillin effectively suppresses coliforms and other members of the *Enterobacteriaceae*. Starch is added to the medium because amylase activity among gram-negative bacteria is restricted to *Aeromonas* and *Vibrio* spp. Hence the possibility of amylase-negative gram-negative bacteria such as *Pseudomonas* spp. interfering with the detection of *A. hydrophila* is eliminated. *Aeromonas* colonies are honey yellow and are surrounded by a yellow zone on SA agar (Palumbo et al., 1985a). Okrend et al. (1987) sampled raw meat by direct plating on SA agar. They, however, reported that many of the background flora of the meats were not inhibited by the ampicillin. They therefore suggested that recovery on SA agar would be facilitated by enrichment procedures. They evaluated five enrichment broths for their effectiveness in isolating *A. hydrophila*. The broths included; alkaline peptone water, tryptose soy broth (TSB), TSB with ampicillin (TSBA), TSBA with 2% extra NaCl, and tryptone broth (8g/l tryptone, 5g/l NaCl, pH=7.0). Based on the percent recovery of typical colonies on SA agar and confirmed positive colonies, they reported that TSBA was the best among the broths.

Methods of enumerating *A. hydrophila* in meats have included direct plating (Palumbo et al., 1985a), cold enrichment followed by direct plating (Myers et al., 1982), rinse method with membrane filtration (Barnhart et al., 1989) and MPN followed by direct plating (Hood et al., 1984). Isolates of *A. hydrophila* have been characterized by biochemical characteristics, hemolysin production and cytotoxin enterotoxin production (Callister and Agger, 1987). Biochemically, *A. hydrophila*

gives positive reactions to esculin hydrolysis, glucose fermentation (acid + gas -), V.P. reaction, lysine decarboxylase, gluconate oxidation, oxidase and catalase reactions (Popoff, 1984).

#### Rapid methods for isolating pathogenic microorganisms in foods

The methods for isolation, identification, and enumeration of pathogenic microorganisms are tedious and time-consuming. For example, enrichment, isolation and identification takes 2 weeks or more before *L. monocytogenes* is confirmed (Lovett, 1988). The diverse physical and chemical composition of food also affects the sensitivity of culture methods (Young, 1987). Therefore, shorter procedures are being sought after and developed. The most current are immunoassays which are based on the interaction of an antibody that has been produced against a specific microbial component (antigen) and subsequent detection of that interaction (Jay, 1986). The techniques include fluorescent antibody, radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), and agglutination immunoassays (Jay, 1986). Among them, ELISA is currently the favorite technique for identification of some food pathogens (Flowers, 1985). Farber and Speirs (1986) described an ELISA for *L. monocytogenes* in raw milk, using monoclonal antibodies (MAb) directed against flagellar antigens. Mattingly et al. (1988) described an ELISA called *Listeria*-TeK which can be used directly on enrichment broth samples of meat and poultry, thereby eliminating the need for pure culture from agar plates to detect *Listeria*. They stated that the MAb-based ELISA is extremely sensitive and very specific for *Listeria*; results are obtained within 48 hr after enrichment. The problem with ELISA is that it often requires between 1000 to 1,000,000 organisms per milliliter of liquid medium for detection

and it takes 24-48 hr enrichment incubation to reach this level (Walker and Dougan, 1989). A more rapid and sensitive technique involves the use of deoxyribonucleic acid (DNA) probes. Because an organism is related to the sequence of nucleotide bases in its DNA, it not only can be identified by its DNA sequence, but even minor differences between similar organism are detectable (Berry and Peter, 1984). Other advantages of DNA probes over immunoassays include better understanding of interactions between nucleic acids than those between antibody and antigen (Pestka and Rosen, 1987) and the chemical stability of nucleic acid is much more tolerant of assay and food processing conditions (Parsons, 1988). Datta et al. (1987) developed a *L. monocytogenes* beta-hemolysis gene probe which has been approved by the FDA for detecting the organism in artificially contaminated raw milk by direct plating. A commercial DNA probe developed by Gene-Trak is also used to detect *Listeria*; it is a synthetic oligonucleotide which uses a colorimetric assay (James et al., 1985). DNA probes have also been developed to screen *L. monocytogenes* plasmids arrayed on a hydrophobic grid-membrane filter (Peterkin et al., 1989)

A DNA probe has also been developed to study the contamination of *Y. enterocolitica* in foods. The probe is directed against a plasmid region that encodes a virulence-associated calcium dependency factor (Hill et al., 1983). Jagow and Hill (1986) investigated enumeration by DNA colony hybridization of virulent *Y. enterocolitica* colonies in selected artificially contaminated foods including pork. They reported that DNA colony hybridization was effective for rapidly enumerating virulent *Y. enterocolitica* strains in a number of foods, however, identification and enumeration was affected by the level of indigenous microflora and not the type of food.

A disadvantage of the use of DNA probes for enumerating pathogenic organisms in foods is that enrichment of the organism to detectable levels is still required, and this can take days (Tenover, 1986). In addition, the use of plasmid rather than chromosomal DNA carries the risk of missing clones that have lost the characteristics during subculturing from the food (Schiemann, 1989). In general, rapid methods are expensive, require special training and skills of technicians, and often cross reactivity with the food matrix occurs (Jay, 1986). Other rapid methods that have been evaluated to enumerate *L. monocytogenes* include flow cytometry (Donnelly and Baigent, 1986) and the same-day identification scheme (Lachica, 1990b). The flow cytometry method is based on several characteristics such as morphology, nucleic acid content and surface antigenicity of the bacterial cell (Donnelly and Baigent, 1986). Cells are treated with appropriate stains and passed rapidly in suspension on a cell-by-cell basis through a laser beam. By analyzing the interactions of each cell (light scatter, fluorescence) with the beam, a representation of the distribution of the desired parameter within the population is acquired (Donnelly and Baigent, 1986). The method, however, is not practical for most laboratories due to the cost of the equipment involved (Donnelly and Baigent, 1986). The same-day identification scheme requires initial plating and incubation of samples for 40 hr at 30°C followed by a series of tests including hemolytic activity, sugar acidification, phase-contrast microscopy, catalase reaction and KOH viscosity, with each test taking 4-7 hr (Lachica, 1990). The scheme will, however, require more experimental data on its efficiency before it may be adapted for food analysis.

### Experimental protocols to study microbiological safety of MAP food products

Four main experimental protocols have been used to study the microbiological safety of MAP food products (Hotchkiss, 1988). They include: (1) inoculation studies in which a particular food is inoculated with a pathogen, and the survival and/or growth of the organism in a MAP food is followed with time; (2) organoleptic spoilage and toxigenicity studies which involve inoculating a food product with a test pathogen and determining the earliest time at which toxin can be detected and at which organoleptic spoilage occurs; (3) predictive modeling which uses mathematical models to generate regression equations, which may be used to predict the probability of microbial growth and/or toxin production in a certain food product; and (4) relative spoilage and pathogenicity which involves inoculating a food product with both a pathogen and a food spoilage microorganism and following the growth in a MA environment with time; the ratio of the log of the spoilage organisms to the log of pathogenic organisms (safety index ratio) is used as a measure of the relative safety of the test conditions (Hotchkiss, 1988).

The dominant question about organoleptic spoilage and toxigenicity studies is whether detectable spoilage precedes or follows toxigenesis (Farber, 1991). Type E strains of *C. botulinum* produced toxin in salmon stored in MA before the product became organoleptically unacceptable (Eklund, 1982; Stathan, 1984). The use of predictive modeling generally ignores the role of spoilage (Farber, 1991). For example, Lindroth and Genigeorgis (1986), and Garcia and Genigeorgis (1987) used this procedure to determine the probability of botulinum toxin formation in MA-packed fish. While their regression analysis provided statistical estimates of the likelihood of toxin formation under different storage conditions, it

did not consider the role of spoilage in the safety of such products (Wimpfheimer et al., 1990). Wimpfheimer et al. (1990) included aerobic plate count values as a measure of spoilage in the safety evaluation of fresh chicken inoculated with *L. monocytogenes*. They used linear regression to fit the linear portion of the log growth phase. This approach undercounts, however, organisms during initial lag phase, and overestimates in the asymptotic phase. The predictive model also has the inability to extrapolate from a liquid culture to a food system and also to extrapolate beyond the parameters used in the experiment (Farber, 1991). Relative spoilage and pathogenicity approach is good for cooked or sterilized food products. For fresh meats with high background microflora, inoculating with spoilage microorganisms would lead to increased competition with the pathogenic organism which could override the effect of the atmosphere (Kleinlein and Untermann, 1990). Although Wimpfheimer et al. (1990) have reported that low versus high initial poultry microflora levels had virtually no effect on *L. monocytogenes* growth, very high concentrations of background microflora could also make isolation and counting of the test pathogenic organism very difficult, since no medium is 100 percent selective. Therefore, among these protocols, inoculation studies seems to be the most applicable when raw meats are involved, especially when dealing with nonproteolytic nontoxin producers such as *Y. enterocolitica* or *L. monocytogenes*. Gill and Reichel (1989) used this protocol to follow the growth of *Y. enterocolitica*, *A. hydrophila* and *L. monocytogenes* on high-pH beef packaged under MAs. Silliker and Wolfe (1980) conducted microbiological safety experiments with MAs by inoculating strains of enterococci, *S. aureus* and *Salmonella* and following growth over time.

## Fresh meat color

### Heme pigments

The color of fresh meat is related to heme pigments, particularly myoglobin (Fox, 1966). The relative amounts of three of the most common forms of myoglobin determine the color of fresh meat (Young et al., 1988). The reduced myoglobin (deoxymyoglobin) is the predominant muscle pigment in the absence of O<sub>2</sub>. It produces the characteristic purplish-red color meat exhibits when first cut as well as the expected color of vacuum-packaged meat. When exposed to air, the purplish red pigment is oxygenated to form oxymyoglobin which is responsible for the desirable bright red color in meat. In both the deoxymyoglobin and oxymyoglobin form, the iron atom is divalent. Metmyoglobin, which is the form with an undesirable brown color, is formed from the oxidation of deoxymyoglobin or oxymyoglobin in low O<sub>2</sub> tension situations (0.5-1.0%); from the oxidation of the iron heme in the myoglobin molecule; or when meat is exposed to air for long periods of time (Seideman et al., 1984). The iron atom in metmyoglobin is in the ferric form. The rate of discoloration may be influenced by either the tendency toward myoglobin oxidation, O<sub>2</sub> consumption rate (O'Keef and Hood, 1982) or by the activity of the reducing system (Ledward, 1985). These factors vary widely according to the species, breed, sex, and age of animals (Okayama, 1987) and the muscle type (Renner and Labas, 1987). Differences between species and muscles in their relative tendency to form metmyoglobin on postmortem storage can be related to inherent differences in the concentration of mitochondria, the activity of

mitochondrial enzymes, and the content of coenzymes such as nicotinamide adenine dinucleotide (NAD) in the muscle (Hood, 1980).

In normal meat, postmortem glycolysis reduces the pH to 5.8 or lower which impairs mitochondria O<sub>2</sub> consumption (Ashmore et al., 1972) and allows normal bloom on meat surfaces exposed to air. The O<sub>2</sub> status is affected by residual respiratory activity of the mitochondria (Lawrie, 1983). The rate of O<sub>2</sub> consumption, however, decreases as the pH falls postmortem due to the depletion of substrates and coenzymes, and to the denaturation of the enzymes involved (Bendall and Taylor, 1972). The postmortem rates of metabolism and the associated rate of pH decline are higher in porcine muscles than in bovine ones (Lundberg and Vogel, 1987). The low pH can denature proteins and cause pale soft and exudative condition known as PSE (Fortin and Raymond, 1987). PSE meat is considered undesirable because of the characteristic pale color and excessive weight lost as drip during retail display or processing (Smith and Lesser, 1982; Topel et al., 1976). pH values of less than about 6 at 45 min postmortem (pH<sub>45</sub>) are associated with PSE meat (Warris and Brown, 1987). The wet surface of PSE pork and the denatured proteins cause light scattering giving the muscle a pale color. Nevertheless, the muscle of some pigs can appear wet without being pale (Kempster et al., 1984) while those of some others appear pale but not wet. According to Barton-Grade and Olsen (1984) muscles of the latter group could represent those with a low amount of myoglobin rather than being caused by faster-than-normal rates of glycolysis immediately postmortem. Warris and Brown (1987) determined the relationship between initial pH, reflectance and exudation in pig muscle. They reported that at pH 6.0, corresponding to reflectance value of 50, the average muscle still appeared normal but showed considerable exudation; at



pH<sub>45</sub> of 6.0, the muscle started to appear pale; and at a pH<sub>45</sub> of 5.6 it appeared very pale and very exudative. They therefore concluded that below pH 6.1, lower pH<sub>45</sub> had higher reflectance values. Moss (1987) reported that meat with reflectance values greater than 50 appeared pale. A rapid pH decline during postmortem glycolysis coincides with a higher carcass temperature; there is partial denaturation of the water-binding myofibrillar proteins which consequently bind less water (Hamm, 1986). Swatland (1984) reported that light reflectance from PSE and normal pork was 40% and 30% respectively. Ledward et al. (1986) found that beef with an ultimate pH greater than 5.8 was more color-stable than similar meat with an ultimate pH of 5.6. Gill and Penny (1985) found that the myoglobin molecule was more rapidly oxidized in low-pH muscle (pH <5.4) than in normal-pH (5.6) muscle. A low pH environment also accelerates the protonation of bound oxygen and favors the release of superoxide anion (Livingston and Brown, 1981) a potential prooxidant species.

The myoglobin of muscle with a very high postmortem pH (>6.0) is protected against oxidation to brown metmyoglobin (Egbert and Cornforth, 1986). The ultimate high pH also inhibits the formation of bright-red oxymyoglobin because it enhances O<sub>2</sub> utilization by mitochondria (Egbert and Cornforth, 1986). According to Lawrie (1983), mitochondrial cytochrome oxidase is more active at pH values above 6.0. At such pH values, the proteins of the muscle are on the alkaline side of the isoelectric point, and hence they bind more water, swell and create a barrier to O<sub>2</sub> entry from the atmosphere (Lawrie, 1985). Little oxymyoglobin can thus be formed on the meat surface; a condition known as dark, firm, dry (DFD). Egbert and Cornforth (1986) noted that redness of DFD beef increased during chilling. They

speculated that chilling inhibited mitochondria respiration and allowed myoglobin to remain oxygenated.

The composition, oxidative susceptibility, and muscle content of myoglobin differs among animal species (Faustman and Cassens, 1990). Atkinson and Follet (1973) reported that beef meat had a much lower O<sub>2</sub> consumption rate and a better color stability compared to lamb meat. Myoglobin obtained from PSE porcine muscle is less stable than that from normal porcine muscle (Bembers and Satterlee, 1975). The muscle myoglobin content of meat-producing animals increases with increased red fiber content and with increased animal age (Lawrie, 1985).

#### Other factors that influence metmyoglobin formation

##### Oxygen availability

Although there is constant reversible conversions of deoxymyoglobin and oxymyoglobin to metmyoglobin on freshly-cut muscle surfaces at all O<sub>2</sub> pressures (Cole, 1986), low partial pressures of O<sub>2</sub> enhance metmyoglobin formation. Vacuum packaging removes O<sub>2</sub> from the package and as a result lowers the partial pressure on the muscle surface leading to the development of purple color (Cole, 1986). Some researchers have found a correlation between metmyoglobin concentration and the low partial pressure of O<sub>2</sub> on the muscle surface (Ledward et al., 1986; Seideman et al., 1984). Pierson et al (1970) also found that a transformation of oxymyoglobin through metmyoglobin to myoglobin occurred in 0 to 20 hr after packaging, but the characteristic purple color of the muscle was evident after approximately 8 hr. Faustman and Cassens (1991) have suggested that in order to minimize metmyoglobin formation in fresh meat, oxygen must totally

be excluded from the packaging environment or present at saturating levels. Atkinson and Follet (1973) reported that the uptake of oxygen by muscle of meat at 48 hr postmortem was species-dependent and followed the order, lamb > pork > beef. Renerre and Labas (1987) found that muscles with the poorest color stability had the highest oxidative activities. They noted highly significant correlations between myoglobin oxidation and O<sub>2</sub> consumption rates which indicated that the decreased O<sub>2</sub> present in muscle after animal death favored myoglobin autoxidation. Hence they concluded that the rate of meat discoloration was influenced by the autoxidation of myoglobin because, in aerobic storage, O<sub>2</sub> continuously associates and dissociates from the heme complex. O'Keefe and Hood (1982) found that high O<sub>2</sub> consumption resulted in a greater proportion of reduced myoglobin in the deoxy form. Deoxymyoglobin is less stable than oxymyoglobin and would be expected to oxidize to metmyoglobin more rapidly. Faustman and Cassens (1990) demonstrated that species with higher muscle NAD content discolored more rapidly. Metmyoglobin formation in ground meat is greater than in cut meat because catalysts of unknown nature are more active in ground meat than in sliced or cut meat (Bendall, 1972; Ledward et al., 1977). Mitochondria activity in postmortem muscle is enhanced by high storage temperature and pH values (Bendall and Taylor, 1972; Cornforth and Egbert, 1985).

#### Fat oxidation

Increased fat content also raises the autoxidation rate of myoglobin (Giddings, 1974), and stimulates catalysis mechanisms, by way of free radicals (Renerre and Labas, 1987). Many studies have also reported a close interrelationship between lipid oxidation and metmyoglobin formation (Govindarajan et al., 1977; Hutchins et al, 1967; Koizumi et al., 1978; Lin and

Hultin, 1977). Recently, Faustman et al. (1989) reported that TBA values and percent metmyoglobin were highly correlated in control ( $r=0.91$ ) and vitamin E-supplemented ( $r=0.72$ ) groups. The free radical intermediates from lipid oxidation can decompose heme, causing loss of color (Haurowitz et al., 1981).

### Temperature

The rate of discoloration of fresh meats increases with increasing storage temperature. Rickert et al. (1957) observed that meat samples stored at 29°C lost redness very rapidly while those stored at 1°C increased in redness during the first day of storage and thereafter gradually decreased in redness for 20 days. It has been reported that increasing the temperature of the meat causes oxymyoglobin to be more dissociated to myoglobin at constant O<sub>2</sub> pressure (Brooks, 1933; Neill and Hastings, 1925). Metmyoglobin formation is favored by myoglobin formation (Kempster et al., 1984), hence the expected increase in the rate of discoloration at higher temperatures (Rickert et al., 1957). An increased temperature also accelerates pigment oxidation rate by increasing the rate of any pro-oxidant reactions within the tissue (Faustman and Cassens, 1991). Brook (1933) showed that an increase in temperature also decreased the depth of O<sub>2</sub> penetration into the tissues. Since metmyoglobin is formed most rapidly at the inner surface of the oxymyoglobin layer, a decrease in the O<sub>2</sub> penetration allows rapid metmyoglobin formation to occur near the surface of the meat (Rickert et al., 1957). In addition, high temperatures result in an increase in O<sub>2</sub> consumption by the tissues (Bendall and Taylor, 1972; Cheah and Cheah, 1971), enhanced microbial growth (Lawrie, 1985), and accelerated lipid oxidation processes (Labuza, 1971), factors which contribute to enhanced meat discoloration.

### Bacterial growth

The influence of bacteria on fresh meat color has been well documented. Butler et al. (1953) studied the effect of bacteria on the color of prepackaged beef steaks stored under various conditions. They reported that the metmyoglobin formation rate was maximal during the log phase of bacterial growth. They also found that bacteria such as *Pseudomonas* commonly found on meat cuts caused discoloration. Butler et al. (1953) and Robach and Costilow (1961) proposed that initially, bacteria would reduce the oxygen partial pressure at the meat surface to the critical level for maximum metmyoglobin formation. Stringer et al. (1969) noted that surface discoloration was a function of the number of bacteria on the meat surface. Greig and Hoogerheide (1941) showed that the rate of oxygen consumption by *P. fluorescens* in culture was directly proportional to bacterial population. Renerre and Montel (1986) reported that redness 'a' values of beef steaks decreased as *Lactobacillus* population numbers increased. Jensen (1945) indicated that microorganisms, both living and dead, and their enzymes can cause the pigments of fresh meat to become oxidized. On the other hand, Rickert et al. (1957) found an increase in the redness of meat inoculated with *Achromobacter* as the growth of the bacteria increased. They assumed that this microorganism had an influence on the oxidation-reduction potentials in the meat. Discoloration of meat can also be due to the growth of hydrogen sulfide-producing bacteria. Hydrogen sulfide reacts with myoglobin to form sulfmyoglobin and choleglobin which cause surface greening (Fernandez and Pierson, 1985). Erichsen et al. (1981) and Hermansen (1980) obtained higher counts of hydrogen sulfide bacteria on high pH meat than on normal meat.

### Metmyoglobin reducing system(MRS)

The mitochondria enzyme systems are also reported to be responsible for the reduction of metmyoglobin which is constantly generated in muscle to the ferro-derivative form (Giddings, 1974). Reduced nicotinamide adenine dinucleotide (NADH) also plays a role in metmyoglobin reduction in meat muscles by accelerating metmyoglobin reduction (Ledward, 1970). Different muscles also possess different NADH ferrihemoglobin and ferrimyoglobin reductase activities (Ledward, 1970). These enzyme systems work in anaerobic (Stewart et al., 1965) or in aerobic conditions (Ledward, 1985). Greene (1969) noted that packaging the meat in an O<sub>2</sub>-impermeable film would produce anaerobiosis and pigment reduction. One disadvantage of this technique is that reduced myoglobin is purple, which is not the typical color the consumer associates with fresh meat (Greene, 1969). Vacuum-packaged fresh meats develop purple color, hence attempts to market vacuum-packaged consumer unit-size fresh meats have not been very successful (Young et al., 1988). Greene (1969) found that not all samples that were packaged anaerobically resulted in metmyoglobin reduction. When the metmyoglobin activity in those samples were tested, activity was barely detectable. She concluded that anaerobic packaging can only be useful if sufficient reducing activity was present in the meat. According to Giddings (1974), the loss of anaerobic metmyoglobin-reducing activity in post-rigor meat is due to factors such as coenzymes (NADH), oxidative deteriorative changes and decreasing enzymatic activities including disintegration of mitochondrial particles. Ledward (1972) noted a high negative correlation between metmyoglobin formation and metmyoglobin-reducing activity under aerobic conditions. O'Keef and Hood (1982) demonstrated both aerobic and anaerobic reduction in meat but reported that a muscle's

reducing ability was of little consequence to its color. However, Ledward (1985) maintained that a muscle's enzymatic reducing activity was the important factor determining metmyoglobin accumulation in meat.

Mitochondrial activity gradually decreases during conditioning or storage of meat, and produces a relatively low rate of O<sub>2</sub> consumption (Lawrie, 1983). Therefore, meat conditioned for more than 7 days develops a brighter red hue than fresh meat (O'Keef and Hood, 1982) because more O<sub>2</sub> is available to provide a deeper layer of oxymyoglobin on the surface exposed to the atmosphere (Lawrie, 1983).

#### Effects of MAP on fresh meat color

Oxygen in modified atmospheres exposed to the myoglobin molecule normally results in oxymyoglobin at the meat surface (Cole, 1986). Hence, the major functions of O<sub>2</sub> in modified atmospheres are to extend fresh meat color and odor shelf life, to maintain the oxygenated form of myoglobin and to prevent the irreversible conversion of myoglobin to metmyoglobin (Clark and Lentz, 1973; Walters, 1985). The depth to which O<sub>2</sub> penetrates muscle depends upon its concentration in the headspace and the respiration of the meat which consumes O<sub>2</sub> (Taylor, 1973). Storage of meat slices in atmospheres of about 80% O<sub>2</sub> prolonged color shelf life (Daun et al., 1971; Lopez et al., 1980; Okayama, 1987; Taylor and MacDougall, 1973). Marchello et al. (1974) found that beef cuts had extended color shelf life when stored in 90% O<sub>2</sub> compared to samples packaged in air. According to Faustman and Cassens (1991), O<sub>2</sub>-enriched atmospheres satisfy the oxygen demand of residual mitochondria activity without sacrificing the oxygenation and stabilization of reduced pigment. Some researchers, however,

have indicated that modified atmospheres high in O<sub>2</sub> will provide normal bloomed color for only short distribution periods after which deleterious oxidative rancidity, microbial activity and color changes reduce acceptability of the fresh meat (Bartkowski et al., 1982; Clark and Lentz, 1963; Watts, 1954). For example, Ordonez and Ledward (1977) reported that the TBA number progressed at a similar rate in fresh pork slices in either air or a gas containing 80% O<sub>2</sub>. More recently, Okayama (1987) found similar results when he compared the TBA numbers of beef samples stored in 20%CO<sub>2</sub>/80%O<sub>2</sub> atmosphere with those of samples stored in air. He obtained no significant difference between the TBA of the air sample and that of the MA sample after 6 days storage at 4°C. Newton et al. (1977) noted the development of rancid flavors in lamb chops stored for three weeks in high-O<sub>2</sub> atmospheres. Taylor (1973) observed lipid oxidation in beef after storage in O<sub>2</sub> at high pressures.

Metmyoglobin can also be formed in modified atmospheres if the concentration of CO<sub>2</sub> in the mixture is high (over 20%) (Ogilvy and Ayres, 1951; Patterson and Gibbs, 1977; Silliker and Wolfe, 1980). Thus, one of the drawbacks of fresh meat stored in a high CO<sub>2</sub> atmosphere is surface discoloration depending on the myoglobin content. Pohja (1967) reported that meat stored in 10-30%CO<sub>2</sub> and N<sub>2</sub> became grey in color after 33 days. Ledward (1970) determined metmyoglobin formation in beef stored in CO<sub>2</sub>-enriched and O<sub>2</sub>-depleted atmosphere. He found that the presence of 12%CO<sub>2</sub> had a negligible effect upon the formation of metmyoglobin, but meat stored in 60%CO<sub>2</sub> often developed a greyish tinge believed to be due to the lowering of the pH and subsequent precipitation of the sarcoplasmic proteins. Kraft and Ayers (1952) indicated that CO<sub>2</sub> can decrease muscle pH which could cause oxidation of the myoglobin



molecule, and result in a rapid discoloration of pre-packaged fresh meats. Savell et al (1981) found that 25%CO<sub>2</sub>/75%O<sub>2</sub> mixture resulted in faster discoloration than samples packaged in O<sub>2</sub>-permeable film held under simulated retail display conditions. Seideman et al. (1979a) compared beef packaged in 50%CO<sub>2</sub>/50%O<sub>2</sub> mixture to vacuum-package controls and reported that the sample packaged in the gas blend deteriorated in color much faster than the vacuum-package controls. Silliker et al. (1977) observed repeatedly that beef samples held in 50%CO<sub>2</sub> discolored rapidly, even if 10%O<sub>2</sub> was included in the mixture. On the other hand, Huffman et al. (1975) reported that CO<sub>2</sub> levels greater than 20% gave acceptable color for up to 4 weeks. In an earlier experiment, Huffman (1974) found no significant difference in the color of chops stored in 100%CO<sub>2</sub> and those stored in a 25%CO<sub>2</sub>/5%O<sub>2</sub>/70%N<sub>2</sub> mixture. Seideman et al. (1979b) packaged beef steaks in 100%O<sub>2</sub>, 100%CO<sub>2</sub>, 100%N<sub>2</sub> or in a vacuum of 29.3 in. of Hg, and stored them at 1-3°C for 28 days. They reported that steaks stored initially in 100%O<sub>2</sub> had significantly more surface discoloration than did steaks stored in any of the other packaging treatments. They also found that steaks held in atmospheres initially containing either 100%CO<sub>2</sub> or 100%N<sub>2</sub> were brown during storage but, upon exposure to air for 4-5 hr, they bloomed. They concluded that high CO<sub>2</sub> concentrations did not discolor meats, but that there could be competitive binding of CO<sub>2</sub> or N<sub>2</sub> to myoglobin which can be reversed upon subsequent exposure to O<sub>2</sub>. In the same experiment Seideman et al. (1979b) also found that the percentage of oxymyoglobin generally remained constant irrespective of packaging treatment and that steaks initially stored in 100%CO<sub>2</sub> had the highest numerical percentage of reduced myoglobin.

It has been reported that fresh red meats which are higher in myoglobin are more susceptible to discoloration by CO<sub>2</sub> atmospheres (Genigeorgis, 1985). Pork has a substantially lower concentration of myoglobin than beef (Lawrie, 1983). Silliker et al. (1977) compared visually the color of pork and beef samples stored in modified atmospheres, and reported that beef deteriorated in color much faster than the pork samples. In that experiment, however, the beef samples were stored in 60%CO<sub>2</sub>-atmosphere and the pork sample in a 50%CO<sub>2</sub> atmosphere. Therefore, the difference in the rate of color deterioration could be due to the different CO<sub>2</sub> concentrations used.

#### Other critical parameters for a successful MAP system

##### Film permeability

The permeability of the packaging film may influence the percentage of the modified gas over periods of time by allowing gaseous diffusion from the inside to the outside atmosphere or vice versa (Cole, 1986). Kraft and Ayers (1952) found that materials with high O<sub>2</sub> permeability, which preserved the bright red color of meats in early phases of storage, permitted the most rapid proliferation of microorganisms on the surface of the sample. Newton and Rigg (1979) showed that the storage life of vacuum-packaged meat, as assessed by discoloration and development of putriferous odors, was inversely related to film permeability. Oxygen transmission characteristics of packaging material are affected by factors including material composition, temperature and exposure to humidity (Eustace, 1981). For example, polyethylene has a very high O<sub>2</sub> permeability and will not maintain an anaerobic environment, but nylon or polyvinylidene chloride (PVDC) has a very high barrier to O<sub>2</sub> and is usually included in a packaging film used for

vacuum packaging of fresh meats (Eustace, 1981). Aluminum foil also has great resistance to O<sub>2</sub> transmission but it is opaque and hence, is unsuitable for packaging fresh meat (Eustace, 1981). For gases, permeability increases with temperature (Eustace, 1981). There have also been reports that marked increase in the permeability of nylon film occurs above 70% relative humidity (r.h). (Rigg, 1979). Eustace (1981) reported that increases in the r.h. from 75 to 98% increased the oxygen transmission rates (OTRs) of nylon from 3.3 to 110, 8.2 and 5.3 ml m<sup>-2</sup> 24 hr<sup>-1</sup> atm<sup>-1</sup> at 25, 3.5 and 0°C, respectively. He also found that in contrast, changes in r.h. had a negligible effect on OTRs of films in which PVDC was the gas barrier. Young et al. (1988) have stated that a packaging film with an OTR of approximately 30 cc/m<sup>2</sup> is sufficiently low to keep the color of fresh meat in the reduced myoglobin state. Lin and Sebranek (1979) recommended barrier films of < 15 cc O<sub>2</sub>/m<sup>2</sup>/24 hr OTR at about 23°C, 0% r.h. and 1 atm for minimal loss of cured-meat color and greater retention of the nitrosoheme pigment.

The rate of permeation for CO<sub>2</sub> is 2 to 4 times greater than for O<sub>2</sub> (Hotchkiss and Galloway, 1989). This means that CO<sub>2</sub> which is injected into a package will, over time, permeate out of the package. Seidman et al. (1979b) monitored the gas headspace of vacuum packaged and MA packaged beef. They placed 2 cm-thick beef steaks in laminated packaging pouches with an OTR of 32 cc/m<sup>2</sup>/24 hr and CO<sub>2</sub> permeability of 47 cc/m<sup>2</sup>/24 hr. The pouches were evacuated and filled with 100%CO<sub>2</sub>, 100%O<sub>2</sub>, 100%N<sub>2</sub> or packaged with a vacuum of 29.3 in. of Hg. The samples were stored at 1-3°C and analyzed every 7 days for headspace gas volume. They found that packages initially containing 100%O<sub>2</sub> or 100%N<sub>2</sub> showed an increase in the percentage of CO<sub>2</sub> as the storage period increased, whereas packages initially containing 100%CO<sub>2</sub> gradually decreased in the percentage of

CO<sub>2</sub>. They also found that the headspace of packages initially containing 100%O<sub>2</sub> showed a corresponding decrease in the percentage of O<sub>2</sub> from 14 to 28 days of storage. They concluded that since mitochondria respire for no longer than 136 hr postmortem (Cheah and Cheah, 1971), bacterial respiration might be responsible for the conversion of O<sub>2</sub> to CO<sub>2</sub> in packages initially containing 100% O<sub>2</sub>. They speculated that the losses in CO<sub>2</sub> and N<sub>2</sub> in packages initially injected with CO<sub>2</sub> or N<sub>2</sub> could be due to film permeation or dissolution of gases into meat tissues. According to Fruton and Simmonds (1953), CO<sub>2</sub> may also bind to meat proteins or go into solution in meat fluid since these events happen in the circulatory system. MAP application, therefore, involves high-barrier packaging films that will prevent atmosphere of the package from any gas exchange with the outside atmosphere (Lioutas, 1988).

#### Solubility of primary gases

The rate of dissolution of the gas in the meat tissue also affects the final headspace volume (Lioutas, 1988). The O<sub>2</sub> concentration in an atmosphere which is in contact with meat surfaces in a high-barrier packaging pouch decreases throughout the storage period due to gas dissolution into meat tissues and respiration by bacteria and tissue (Taylor and MacDougall, 1973). Cheah and Cheah (1971) reported that mitochondria consume 1-3 liters of O<sub>2</sub> per cm<sup>2</sup> per 24 hr and remain active up to 144 hr postmortem or as long as the pH remains above pH 5.5. The rate of O<sub>2</sub> consumption, therefore, varies with individual muscles, meat species, and with size of cut. Muscles and meat species with a higher amount of mitochondria or smaller unit cuts with larger surface areas would consume more O<sub>2</sub>.

Carbon dioxide is also very soluble in water and oils and when applied to meat in a rigid or flexible pack, it is absorbed by muscle and fat tissues until equilibrium is attained (Gill, 1988b). Gill and Penny (1988) packaged beef under CO<sub>2</sub> added at 200, 400, 700, 1000 or 2000 ml per kg of meat. They reported that all the packages, except those containing initially 2000 ml/ kg meat, were tightly applied to the meat after storage, indicating that all the added gas had been absorbed by meat. They concluded that CO<sub>2</sub> volumes  $\leq 1000$  ml/kg of meat were insufficient for the meat to be fully saturated with the gas and produced a partial pressure of CO<sub>2</sub> within the collapsed packs of a fraction of the ambient atmospheric pressure. To prevent the package from collapsing either N<sub>2</sub> is included in the gas mixture or large volumes of CO<sub>2</sub> relative to meat mass are added to maintain the pressure of one atmosphere within the package after equilibration of the package atmosphere with the meat (Gill, 1988b). The problem with large CO<sub>2</sub> volumes is that it may not be convenient for a commercial packaging system (Gill, 1988b). Hence, for most studies N<sub>2</sub> is included in the gas mixture.

Solubility of CO<sub>2</sub> in meat muscle tissue is affected by tissue pH, storage temperature, the proportion and composition of fat present and the partial pressure inside the package (Gill, 1988b). The author noted that the solubility of CO<sub>2</sub> in beef, pork or lamb muscle tissue at 0°C increased linearly with tissue pH by approximately 347 ml/kg for each pH unit rise, while solubility decreased with increasing temperature. He indicated that the relationship between CO<sub>2</sub> solubility and temperature can be assumed to be linear in the temperature range to which chilled meat would be exposed during commercial handling and storage (-1.5 to +10°C). In fat, Gill (1988b) noted that the solubility of CO<sub>2</sub> increased with

temperature at a faster rate for pork and lamb fat than for beef fat. The amount of absorbed CO<sub>2</sub> would also be reduced by the presence of endogenously generated CO<sub>2</sub> (Gill, 1988b). The rate of dissolution of CO<sub>2</sub> is also affected by the size and shape of the meat pieces, the composition of the exposed surfaces and boundary conditions that exist at those surfaces (Gill, 1988b). Thus, the rate of O<sub>2</sub> and/or CO<sub>2</sub> dissolution can differ for primal, subprimal, loin or chops. Hence, the ratios of gas volume to meat mass; gas volume to meat volume; and gas volume to surface area must be considered in a MAP system for fresh meats. Gill and Penny (1988) examined the effect on spoilage development of variations in the ratios of CO<sub>2</sub> volume to meat mass to determine if CO<sub>2</sub> applied in less than saturating quantities could be effective in extending the storage life of chilled beef. They packaged samples of 387-g cuts in 80, 160, 280, 400 or 800 ml per cut and stored them at 0.5-1.5°C. They reported that at all volumes CO<sub>2</sub> had an inhibitory effect on bacterial growth, but the bacterial numbers on cuts decreased successively in packs to which successively increased CO<sub>2</sub> volumes had been added. Hotchkiss and Galloway (1989) have indicated that higher ratios than 1:1 headspace : meat volume ratio used in Europe would be required for longer storage life.

#### Optimum gas concentrations

Mixtures of two or more gases have been used in research programs for optimum color and bacterial inhibition (Seideman and Durland, 1984). However, optimum concentrations of gases to achieve the desired color and inhibit growth of spoilage and pathogenic microorganisms in fresh meats are not well defined. Carpenter et al. (1975) suggested that gas mixtures of low O<sub>2</sub> and high CO<sub>2</sub> or high O<sub>2</sub> and low CO<sub>2</sub> would be possible mixtures for extending the storage life of

meat. One commercial process employs 35-75%CO<sub>2</sub> + 21-28%O<sub>2</sub> and balanced with N<sub>2</sub> to ship fresh meats from the continental U.S. to Japan, Hawaii, Alaska, and Europe (Urbain and Campbell, 1987). Wolfe (1980) recommended high concentrations of both O<sub>2</sub> and CO<sub>2</sub> for fresh meats, and defined high as any levels greater than 20%. Seideman et al. (1980) showed that by using 20 and 40%CO<sub>2</sub> gas mixtures without O<sub>2</sub>, as display time increased, surface discoloration of beef and pork cuts also increased. According to Walters (1975), a mixture of 60%O<sub>2</sub> and 40%CO<sub>2</sub> could maintain meat color for at least one week. But some investigators have stated that it is not advisable to use atmospheres containing in excess of 20% for storage of fresh meats, because at higher concentrations the appearance of the meat is adversely affected (Silliker et al., 1977). It has been claimed that 80%O<sub>2</sub> + 20%CO<sub>2</sub> mixture kept beef color for up to 15 days while bacterial counts were kept at an acceptable level (Georgala and Davidson, 1970). This claim was supported by Taylor and MacDougall (1973) when they reported that low CO<sub>2</sub> concentrations (10-15%) used in conjunction with high concentrations of O<sub>2</sub> (85%) were sufficient to produce optimum meat color and inhibit spoilage bacteria. They contended that O<sub>2</sub> concentrations higher than 60% would give better results because some of the O<sub>2</sub> will be consumed by meat tissue during respiration to produce CO<sub>2</sub>. According to Seideman et al. (1979b), however, gas mixtures containing high levels of O<sub>2</sub> do not create an atmosphere conducive for long-term storage of beef. Bartkowski et al. (1982) conducted a study to determine the effects of certain gas mixtures (containing low and high levels of O<sub>2</sub> and CO<sub>2</sub>) as well as vacuum packaging on color and microbiology of beef muscles in an attempt to select the best controlled atmosphere for preservation of color and extension of shelf life. They made the following observations: (1) levels of O<sub>2</sub> more than twice the ambient concentration

produced and maintained a desirable bright red color of beef cuts for a 9-day storage period at 4°C; (2) atmospheres with O<sub>2</sub> levels lower than ambient concentration did not promote or maintain desirable color for extended period of time; and (3) CO<sub>2</sub> at concentrations of 15% in atmosphere inhibited microbial growth and did not promote darkening of the meat. They therefore suggested that CO<sub>2</sub> may be used in higher concentrations when combined with 40% or more O<sub>2</sub> without deleterious effect on meat color. To inhibit the detrimental color change (metmyoglobin formation) produced by high concentrations of CO<sub>2</sub> in modified atmosphere packages and to reduce lipid oxidation caused by high concentrations of O<sub>2</sub>, Ledward (1970) recommended that the gas mixture contain 5%O<sub>2</sub> and 20%CO<sub>2</sub>. Holland (1980) also noted that 20%CO<sub>2</sub> was effective in extending the shelf life with negligible browning and fat oxidation, while Ogilvy and Ayres (1951) showed that 25%CO<sub>2</sub> was the maximal concentration that did not cause browning. Seideman et al. (1980) compared the appearance of fresh pork packaged in 20%CO<sub>2</sub>/80%N<sub>2</sub>, and 40%CO<sub>2</sub>/60%N<sub>2</sub> mixtures with that of pork packaged under vacuum. They concluded that storage of retail cuts of pork in the above atmospheres for 7, 14 and 21 days at 2°C increased surface discoloration of all the samples tested compared to retail cuts stored in vacuum packages. Recent studies with red meats by Moore and Gill (1987), however, showed that when O<sub>2</sub> in the pack atmosphere was initially reduced to very low concentrations and O<sub>2</sub> permeation during storage was prevented by using a gas-impermeable film, meat color did not deteriorate. According to Gill (1988a), a packaging system using an atmosphere of CO<sub>2</sub> alone is now in commercial use for chilled red meats that are transported by surface to distant markets.



Use of color maintenance substances to enhance color stability

Oxygen-enriched atmosphere retards metmyoglobin formation and maintains a desirable red surface color in fresh meat. CO<sub>2</sub>-enriched atmosphere inhibits microbial growth, especially that of aerobic gram-negative bacteria. However, because of the dynamic changes which occur in meats, surface discoloration is inevitable even with the use of modified atmosphere packaging. The evolution of the meat industry towards centralized packaging has therefore, prompted research interests in the use of certain substances to slow down formation of metmyoglobin in fresh meat without masking quality deterioration (Shivas et al., 1984). The approach involves delaying pigment oxidation and/or enhancing reduction of oxidized pigments (Faustman and Cassens, 1991). Harbers et al. (1981) reported that dip treatment of meat with ascorbic acid (AA) solution retarded myoglobin oxidation. Watts and Lehmann (Watts and Lehmann, 1952a) noted color brightening of AA-treated beef. Costilow et al. (1955) found steaks treated with a dip or spray of AA to discolor slower than controls. The addition of AA to ground beef was demonstrated to greatly increase the color stability of this meat product (Shivas et al., 1984). Hood (1975) administered intravenous injections of sodium ascorbate to cattle immediately prior to slaughter and reported that meat obtained from these animals was more color-stable than meat from control cattle. Several theories have been proposed for the mode of action of AA. According to Walters (1975) AA acts with naturally occurring tocopherols in restricting formation of fat peroxides or may have an O<sub>2</sub>-scavenging action. Lawrie (1985) suggested that AA reduces metmyoglobin as soon as it is formed or before the globin is denatured. The addition of AA to meat adds sufficient reducing equivalent to help delay the oxidation of myoglobin to metmyoglobin

(Hotchkiss and Galloway, 1989). Metmyoglobin is reduced by AA in the presence of substituted pyridines (Fox et al., 1975). The overall effect of ascorbic acid is that it may stabilize oxymyoglobin in meat products and may delay lipid oxidation (Shivas et al., 1984), since metmyoglobin acts as a catalyst of lipid oxidation and lipid oxidation intermediates also enhance pigment oxidation (Greene, 1969).

Rhee and Smith (1984) found that synergism of certain substances with ascorbate greatly enhanced its reductant activity. Greene et al. (1971) reported the greatest reduction of pigment oxidation and the highest raw meat color scores when AA was used with BHA or propyl gallate (PG). Buckley and Connolly (1980) found that fresh pork from pigs supplemented with vitamin E had lower TBA values than samples from control unsupplemented animals under typical storage conditions. Faustman et al. (1989) reported that during 6 days storage at 4°C, metmyoglobin accumulation and lipid oxidation (TBA) were greater in beef from control versus vitamin E-supplemented animals. Okayama (1987) compared the TBA number of retail beef cuts dip-treated with 3% AA and 0.08% DL- $\alpha$ -tocopherol, with a sample that was not treated after storage in an 80%O<sub>2</sub>/20%CO<sub>2</sub> gas atmosphere at 4°C. He reported that the dip treatment with AA and tocopherol solutions retarded lipid oxidation in beef steaks stored in the O<sub>2</sub>-enriched atmosphere.

The color-preserving effect of AA also depends on the concentration used. Shivas et al. (1984) observed that meat treated with 0.05 or 0.1% AA (meat weight) had brighter color compared to that treated with 0.01% AA. A 5% AA solution was judged more effective at preventing muscle discoloration than dipping into more dilute levels (Harbers et al., 1981). However, Watts and Lehmann (1952a, b) reported greening of hemoglobin solutions with 0.5% AA, and dulling of color of

raw ground pork containing 0.1% ascorbic acid. According to Rickert et al. (1957), relatively high concentrations of AA may have such a great demand for O<sub>2</sub> that will cause the removal of O<sub>2</sub> from the oxymyoglobin on the surface of the meat, and thereby favor metmyoglobin formation. They contended, however, that AA in higher concentrations may have beneficial effects upon ground meat because the antioxidant is uniformly distributed throughout the ground meat, instead of being concentrated at the surface as in treated cut samples. Ranken (1981) proposed that AA may perform a prooxidative role in fresh meat when administered at high levels. Erythorbic (isoascorbic) acid which, in the past, has been cheaper has similar functions and is also used in cured meat products (Mackey and Seymour, 1989).

Cheng (1987) has described a process for prepackaging fresh pork so that the meat can be maintained in an attractive condition and of acceptable and uniform quality for an extended period. The process involved dip or spray treatment of a solution of sodium ascorbate, EDTA or citric acid and sodium tetra pyrophosphate mixture. EDTA or citric acid is a chelating agent, and it stabilizes foods by forming a complex with metallic ions making them unavailable for other reactions (Fennema, 1985). Thus EDTA or citric acid acts synergistically with antioxidants or reductants to retard oxidation since they remove metal ions that catalyze oxidation (Cheng, 1987).

#### Antimicrobial properties of phosphates, ascorbic acid and citric acid

A variety of phosphates are widely used in many meat products. These compounds increase ionic strength, pH, particle binding (Knipe et al., 1985), and metal chelation (Hamm, 1960). Phosphates that are approved for use in meats include polyphosphates and pyrophosphates (Ancn., 1982); some of these compounds have been shown to possess antimicrobial properties. Molins et al.

(1986) found that sodium acid pyrophosphate inhibited growth of *C. sporogenes* in cooked, vacuum-packaged bratwurst held at 24-25°C for 24 to 48 hr. However, these authors found only a slight effect against naturally contaminated mesophilic and psychrotrophic bacteria in uncooked bratwurst-type sausage held at 5°C. The addition of 0.4% pure or blended phosphates to beef patties stored at -20°C also did not significantly reduce numbers of mesophiles, psychrotrophs, lactic acid-producing bacteria, *S. aureus* or viable anaerobic spores in the meat product (Molins et al., 1987).

The antimicrobial effect of AA has attracted a lot of research interests because it occurs naturally in many foods, is an essential nutrient, and has been generally recognized as safe (Mackey and Seymour, 1989). AA has activity against a number of different viruses (Morgan et al., 1976), however, its effects on bacteria are more complex; growth-stimulatory-inhibitory and bactericidal effects have all been described (Eddy and Ingram, 1953). Shivas et al. (1984) using different concentrations of AA found no difference in microbial numbers among treatments. Costilow et al. (1955) had earlier reported no measurable effect on microbial counts by dip- or spray-treatment of steaks with a 1% solution of AA. Okayama et al (1987) treated beef samples with AA and ethanol, and reported that there was no difference in the viable bacterial count from that of a control treated only with ethanol. Myrvik et al (1954), however, reported that AA inhibited tubercle bacilli. Fletcher et al. (1983) also found that AA at low concentrations protected *Campylobacter jejuni* from the deleterious action of H<sub>2</sub>O<sub>2</sub>, but the organism was inhibited by higher concentrations of AA. Mackey and Seymour (1989) found that thermal inactivation of several microorganisms was accelerated by the addition of sodium erythorbate to phosphate buffer heating medium but not to complex food

mixtures. They also reported that the lethal effect of erythorbate was nullified by heating under anaerobic conditions or by the addition of catalase. It has been shown that sublethal injury caused by heating or chilling *E. coli* or *Salmonella* sensitized them to mild oxidative stress (Mackey and Derrick, 1986; Mackey and Seymour, 1987). Robinson et al. (1982) observed that erythorbate enhanced the antibotulinal effects of nitrite in cured meat products. Proposed mechanisms of antimicrobial effects of ascorbic acid include: (a) autooxidation to produce hydrogen peroxide, (b) promoting the cyclic reduction of cupric ions which then combine with iron-containing sites within the cell, and (c) toxicity of dehydroascorbic acid or other oxidation products of ascorbic acid (Mackey and Seymour, 1989).

Citric acid is a commonly used food acidulant (Connor et al., 1990). It has a bacteriostatic effect on *L. monocytogenes* at a very low concentration at about pH 4.0 and the effect requires about 0.029M of acid (Connor et al., 1990). The antimicrobial activity of many organic acids is attributed to the undissociated form of the acid molecule (Lueck, 1980). Among organic acids used as food acidulants, citric acid was found to have the lowest concentrations of undissociated acid at its inhibitory pH values (Connor et al, 1990).

The use of citric acid and phosphates in conjunction with ascorbate or erythorbate would retarded oxidative reactions (Cheng, 1987) but might not inhibit microbial growth in fresh meats. Cheng (1987) has, therefore, suggested storage in MA for microbial control. However, MAP may not guarantee complete control of microbial growth (Gill and Tan, 1980) or may select for growth of some anaerobic and/or facultatively anaerobic pathogenic organisms (Genigeorgis, 1985). Hence, this process might have some microbiological safety implications, since the

maintenance of the bright red color could mask microbial spoilage and growth of pathogens favored by the atmosphere used for storage.

**PART I.    MODIFIED ATMOSPHERE PACKAGING OF FRESH PORK CHOPS:  
INFLUENCE OF DIFFERENT GAS MIXTURES ON THE  
MICROBIOLOGICAL, PHYSICAL AND CHEMICAL  
CHARACTERISTICS**

**MODIFIED ATMOSPHERE PACKAGING OF FRESH PORK CHOPS: INFLUENCE OF  
DIFFERENT GAS MIXTURES ON THE MICROBIOLOGICAL, PHYSICAL AND  
CHEMICAL CHARACTERISTICS**

by

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**Running Title: Modified gas atmosphere effects on fresh pork quality**



## ABSTRACT

Fresh pork chops packaged in air, vacuum, and eleven modified gas atmospheres and stored at 4°C were studied to determine the influence of varying the concentrations of carbon dioxide and oxygen on the microbiological, physical and chemical characteristics of the chops. The concentrations of carbon dioxide and oxygen were in the range of 0 to 40%. The headspace gaseous composition changed over time as the concentration of the carbon dioxide increased while that of the oxygen decreased during storage. Increasing the carbon dioxide concentration delayed growth of aerobic psychrotrophic and mesophilic bacteria, and the *Enterobacteriaceae*, but slightly enhanced that of lactic acid bacteria. Increasing the oxygen concentration reduced growth of facultative anaerobic and anaerobic bacteria and enhanced that of *Brochotrix thermosphacta*. In general, carbon dioxide had more influence on the microbiological storage life of the chops than oxygen. Increasing the carbon dioxide concentration also reduced the redness of the chops, increased purge losses and promoted lipid oxidation, but retarded the formation of volatile basic nitrogen (VBN). Increasing oxygen concentration also increased lipid oxidation but generally there was no interactive effect of carbon dioxide and oxygen for all the parameters tested. Modified gas atmospheres with more than 10% carbon dioxide concentration were superior to air for extending the microbiological storage life of fresh pork chops. Gas mixture containing 20%CO<sub>2</sub> without oxygen was as effective as mixtures containing 40%CO<sub>2</sub> with or without oxygen in extending the storage life of fresh pork chops. Mixtures containing 40%CO<sub>2</sub> with or without oxygen had better performance than

vacuum packaging in delaying growth of psychrotrophic and mesophilic bacteria, and *Enterobacteriaceae* during 21 days of storage. There was no marked difference in red color between modified gas atmosphere and vacuum, but the latter protected lipid from oxidation much better than modified gas atmospheres. Modified atmospheres in turn greatly reduced purge losses compared to vacuum. Modified gas atmospheres did not select for the growth of a particular psychrotrophic pathogen(s) or potential pathogens during storage. Volatile basic nitrogen increased proportionally with numbers of aerobic psychrotrophic bacteria.

## INTRODUCTION

Fresh meats are highly perishable products because of the microbiological, physical and chemical processes that continually occur in the muscle postmortem. Spoilage of fresh meat varies with the composition of the meat and spoilage microflora. Fresh pork generally has a shorter shelf life than fresh beef when overwrapped in O<sub>2</sub>-permeable film, probably because of the fat content, and the variable ultimate pH.

The consumption of pork lags behind beef in the U.S., but is increasing in many parts of the world (ABS, 1984). Hence the U.S. meat industry has been studying alternative packaging systems that would prolong the shelf life of fresh chilled pork and allow it to be shipped to distant markets. For the past decade, modified atmosphere packaging (MAP) has evolved as one of the methods that can be used for extending the storage and retail shelf life of fresh pork (Terlizzi, 1982). The method consists of packaging fresh meat cuts in an atmosphere of gases such as CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub>, either alone or as a mixture. When used in combination with refrigeration and proper sanitation, the method prevents excessive shrinkage, microbial growth and discoloration by protecting the meat from elements that accelerate the mechanisms that deteriorate the product (Terlizzi, 1982). It can provide double or triple the retail storage life, and more flexibility to the retailer in handling and moving pork (Anon., 1979). Modified atmospheres are currently used to package pork loins, pork butts, pork spare ribs, hams, shoulders, pork offals, and consumer-unit size cuts (Anon., 1979; Young et al., 1988).

Extensive research has been conducted with MA for packaging fresh pork to determine the effects of CO<sub>2</sub> and O<sub>2</sub> concentrations on the microbial growth and color stability of this perishable product. Huffman (1974) found that pork chops stored in a gas mixture containing 25%CO<sub>2</sub>/5%O<sub>2</sub>/70%N<sub>2</sub>, had significantly lower counts for an entire 5-week storage period than chops stored in air, 100%O<sub>2</sub> or 100%N<sub>2</sub>, but higher counts than samples stored in 100%CO<sub>2</sub>. Seideman et al. (1979) reported that a modified gas atmosphere of 20%CO<sub>2</sub>/80%N<sub>2</sub> was a suitable alternative to vacuum packaging. Christopher et al. (1980) compared the microbiological quality of retail pork chops packaged in atmospheres containing 20%CO<sub>2</sub>/80%N<sub>2</sub> or 40%CO<sub>2</sub>/60%N<sub>2</sub> with pork chops packaged in a vacuum. They reported that the psychrotrophic bacterial counts of chops stored in CO<sub>2</sub>-N<sub>2</sub> atmospheres usually were lower than those of comparable vacuum-packaged chops from 14 to 21 days of storage, but the counts were not significantly different on retail cuts stored in either 20%CO<sub>2</sub>/80%N<sub>2</sub> or 40%CO<sub>2</sub>/60%N<sub>2</sub> atmospheres. Gill and Harrison (1989) found that pork chops packaged in 100%CO<sub>2</sub> and stored at 3°C had about 5.5 weeks storage life. These authors also demonstrated that the relatively wide range of pork chops pH did not affect the course of spoilage development of pork packaged in modified atmospheres.

The concentrations of CO<sub>2</sub> and O<sub>2</sub> in the headspace have also been found to influence the type of species of microorganisms which might be present on the surface of the meat (Blickstad et al., 1981; Enfors et al., 1979). Because facultative and anaerobic bacteria can resist the inhibitory effect of CO<sub>2</sub> they normally predominate the microflora of fresh meat stored in atmospheres with high CO<sub>2</sub> concentrations and reduced O<sub>2</sub> levels (Hintlan and Hotchkiss, 1986; Silliker and Wolfe, 1980). Blickstad et al. (1981) found that after aerobic storage at 4°C, more

than 90% of the flora on pork consisted of *Pseudomonas* spp., but after storage in 1 atm or 5 atm CO<sub>2</sub>, *Lactobacillus* spp. constituted the total flora at 4°C. Studies by Blickstad and Molin (1983) showed that *Brochotrix thermosphacta* and facultative anaerobic *Enterobacteriaceae* were also principal spoilage organisms of CO<sub>2</sub>-packaged normal, skinned pork. The selective growth of naturally occurring facultative anaerobic and anaerobic bacteria on fresh meats packaged in high CO<sub>2</sub> atmospheres has microbiological safety implications, especially with the emergence of new species of pathogens that will grow at refrigerated temperatures (Palumbo, 1986), and have been isolated from pork. Among these are *Aeromonas hydrophila*, *Listeria monocytogenes*, *Yersinia enterocolitica* (Buncic, 1991; Mafu et al., 1989; Okrend et al., 1987) and other pathogenic members of the *Enterobacteriaceae* family. It is suspected that certain atmospheres may disturb the microflora equilibrium in favor of these pathogens by inhibiting the growth of competing aerobic spoilage organisms.

An atmosphere high in CO<sub>2</sub> is also thought to be unsuitable for use with fresh meat because of product discoloration (Farber, 1991). Silliker et al. (1977) noted that pork stored in 50% CO<sub>2</sub> deteriorated in color very rapidly. Seideman et al. (1980) compared the surface discoloration and appearance of pork stored in a vacuum to that stored in either 20%CO<sub>2</sub>/80%N<sub>2</sub> or 40%CO<sub>2</sub>/60%N<sub>2</sub>, and reported that storage of pork cuts in the two modified gas atmospheres increased surface discoloration compared to vacuum-packaged cuts, but there was no difference in the mean values for surface discoloration and appearance of cuts packaged in either 20%CO<sub>2</sub>/80%N<sub>2</sub> or 40%CO<sub>2</sub>/60%N<sub>2</sub>. However, it is reported that metmyoglobin accumulation in fresh pork packaged in 20%CO<sub>2</sub>/80%O<sub>2</sub>, was very slow (Asensio et al., 1988). Bartkowski et al. (1982) have suggested that CO<sub>2</sub> may

be used in high concentrations when combined with 40% or more O<sub>2</sub> without a deleterious effect on meat color. On the other hand, some investigators have indicated that a modified atmosphere high in O<sub>2</sub> would provide normal bloomed color for only a short distribution period after which deleterious oxidative rancidity, microbial and color changes would reduce acceptability of the fresh meat (Clark and Lentz, 1973; Watts, 1954). Ordonez and Ledward (1977) reported that lipid oxidation, as measured by TBA, progressed at a similar rate in fresh pork slices in either air or 80% O<sub>2</sub> atmosphere. However, Asensio et al. (1988) demonstrated that lipid oxidation was not a limiting factor of shelf life of pork in 20%CO<sub>2</sub>/80%O<sub>2</sub>.

Spoilage of chilled fresh meats can also be caused by breakdown of proteins during bacterial growth. Psychrotrophic *Pseudomonas* spp. cause protein degradation (Young et al., 1988), and evolution of ammonia by de-amination of amino acids under aerobic conditions (Gardner, 1965). Certain *Enterobacteriaceae* are also capable of decarboxylating amino acids and produce putrid odors (Gill and Harrison, 1989; ten Brink et al., 1990). Ordonez et al. (1991) reported increased levels of certain amines along with the *Enterobacteriaceae* in fresh pork stored in O<sub>2</sub>-enriched gas mixtures with 20%CO<sub>2</sub>. Volatile basic nitrogen (VBN) contains an appreciable amount of amine and ammonia, and it is an index of fish quality (Reineccius, 1979). In Tawian, VBN has been used to detect meat freshness for many years (Fu et al., 1991).

Previous investigations on the effect of modified gas atmospheres on the storage life of fresh pork used only one or two gas mixtures, usually containing high or low levels of CO<sub>2</sub> and O<sub>2</sub> to either extend microbial shelf life (Gill, 1988; Gill and Penny, 1988), or maintain freshness and desirable color (Lopez et al., 1980; Okayama, 1987). None of these investigations dealt with the optimization of gas

mixtures using several combinations of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> in relation to the microbiological, physical and chemical characteristics of fresh pork in terms of shelf life, selective growth of naturally occurring species of pathogens that can grow at refrigeration temperature, color, lipid oxidation and formation of nitrogenous compounds during storage.

The objective of this study was to determine the effects of eleven different gas-mixtures with moderate amounts of CO<sub>2</sub> and O<sub>2</sub> on the physical, chemical and microbiological characteristics of pork chops, i.e., how they affected numbers of spoilage microorganisms; the relative proportions of naturally occurring pathogenic and potential pathogenic members of the *Enterobacteriaceae* family, *L. monocytogenes* and *A. hydrophila*; and their effect on color, lipid oxidation and other chemical and physical properties of fresh pork.

## MATERIALS AND METHODS

### Meat sample preparation

Bone-in pork loins were obtained directly from the processing line of a commercial plant and transported to the Meat Laboratory of Iowa State University. The loins were deboned and cut into 2.5 cm (1 in) thick chops. Three chops were placed on one styrofoam tray, weighed, placed in high-barrier packaging material ( $O_2$  permeability of  $< 2.5 \text{ cm}^3/645 \text{ cm}^2/24 \text{ hr}$  at  $23^\circ\text{C}$  and  $0\% \text{ R.H.}$ , and water vapor transmission  $< 1.0 \text{ g}/645 \text{ cm}^2/24 \text{ hr}$  at  $38^\circ\text{C}$  and  $90\% \text{ R.H.}$ , Curlon™ 863 Saran, Curwood Inc. New London, WI, USA), and randomly assigned to 11 different gas-mixtures (Table 1). Food-grade gasses were obtained from Liquid Carbonic (Glenellyn, IL, USA) and mixed with a three-way gas blender (Model 180 SCFH, Smith Equipment, Watertown, SD, USA). The samples were packaged by evacuating the air in the pouches followed by double flushing with the gas mixture to obtain a headspace:meat volume ratio of about 2:1 and heat sealing the bag using a CVP machine (model A300, CVP Systems, Inc., Downers Grove, IL, USA). Control samples were vacuum-packaged in the Curlon™ 863 Saran pouch to 722 mm Hg and heat-sealed or air-packaged by overwrapping the trays with  $O_2$ -permeable polyethylene film (Stretch meat film, Cat # 37016, Hantover, Kansas City, MO, USA). The packages were placed in cardboard boxes and stored at  $4^\circ\text{C}$ . All samples were analyzed at day 0, i.e., the day of packaging, followed by sampling of air packages every 2 days and the others, every week.



### Headspace analyses

The O<sub>2</sub> concentrations in the headspace of the modified atmosphere packages, except that of vacuum, were measured on each day of sampling with a digital O<sub>2</sub> analyzer (Model IL 307<sup>TM</sup> Ingold<sup>TM</sup>, Instrumentation Laboratory Inc., Wilmington, MA, USA) by inserting a needle attached to the instrument through a septum fixed to the outer surface of the package. Oxygen in the sample diffuses through a membrane in the analyzer and is reduced within a sensor to OH<sup>-</sup> ion producing a signal that is directly proportional to the O<sub>2</sub> concentration in the sample. Carbon dioxide was measured using an Infrared Spectrometer (AccuLab<sup>TM</sup> 2 Beckman Instruments, Inc., Palo Alto, CA, USA) with a silver chloride crystal cell. A 50-ml sample of headspace gas was drawn into a syringe, injected into the cell through a septum and scanned at 700-600  $\mu$  (Pomeranz and Meloan, 1987). The volume of the CO<sub>2</sub> was calculated from the standard curves of samples of known composition.

### Microbiological analyses

On sampling days, the packages were aseptically opened and two 12.5 cm<sup>2</sup> surface circles of 3-mm thick were obtained from each of the three chops. This allowed removal of 75 cm<sup>2</sup> of sample surface which was macerated for 1 min in a sterile bag containing 150 ml 0.1% sterile peptone water using a Stomacher 400 lab blender (Tekmar<sup>TM</sup> Co., Cincinnati, OH, USA). Serial dilutions were prepared according to the recommended microbiological procedure (Speck, 1984). Total aerobic counts of psychrotrophic and mesophilic bacteria were obtained using All-purpose tween agar (APT, BBL, Cockeysville, MD, USA) incubated at 7°C for 10 days and 30°C for 48 hr, respectively. Anaerobic and facultatively anaerobic

bacteria were also enumerated in APT incubated at 30°C for 48 hr in anaerobic systems (GasPak™, BBL). *Enterobacteriaceae* were recovered in Trypticase soy agar (TSA, BBL) which was overlaid with double-strength violet red bile agar (VRB, DIFCO, Detroit, MI, USA) and incubated at 37°C for 24 hr (Hartman et al., 1975). Counts for lactobacilli were obtained by using lactobacilli selective agar (LBS, BBL) to which 0.017% acetic acid was added and incubating at 30°C for 72 hr. *Brochotrix* were enumerated on streptomycin sulfate-thallos acetate-actidione (STAA) agar incubated at 25°C for 48 hr (Gardner, 1966). Plates were counted with a laser bacterial colony counter (Spiral System Instruments, Inc., Bethesda, MD, USA), except for those corresponding to the *Enterobacteriaceae* which were counted with a Darkfield Quebec colony counter (American Optical Co., Buffalo, NY, USA).

Naturally occurring *Listeria* spp. and *Aeromonas* spp. were enumerated using the most-probable-number (MPN) method. *Listeria* spp. were enumerated by a three-tube MPN procedure done with 25-, 2.5-, and 0.25 mL (Glass and Doyle, 1989) of blended solution in *Listeria* enrichment broth (LEB, BBL) and incubated at 30°C for 48 hr (Lovett et al., 1991). Tubes of highest dilution that showed growth (turbidity) were direct plated onto *Listeria* selective agar base (Oxoid, Basingstoke, England) to which had been added *Listeria* selective antimicrobial supplement (Oxoid, Curtis et al., 1989), and incubated at 37°C for 48 hr. Typical black colonies were scored as presumptive *Listeria* spp. and confirmed by the procedure described below. *Aeromonas* spp. were also enumerated by a three-tube MPN procedure done with 25-, 2.5-, and 0.25 mL of blended solution in trypticase soy broth (TSB, BBL) to which had been added 10mg of ampicillin/ml (Okrend et al., 1987). The tubes of highest dilution that showed growth (turbidity) were plated on

starch-ampicillin agar, which consisted of phenol agar base (DIFCO), soluble starch (10g/L), and ampicillin (10mg/L), and incubated at 28°C for 24-48 hr (Palumbo et al., 1985). Typical colonies having a clear zone surrounding them were scored as presumptive *A. hydrophila* and confirmed by the procedure described in the next section.

A random sample of 10 chops taken after cutting and before packaging were sampled to determine the initial psychrotrophic counts for each replication. All microbiological counts were reported as  $\log_{10}$  CFU/cm<sup>2</sup>.

#### Identification of cultures

Ten representative colonies were randomly picked from each countable *Enterobacteriaceae* plate, streaked on TSA and incubated at 37°C for 24 hr. The Gram-negative organisms were identified as to genera according to the scheme of Harrison et al. (1981). Further identification of the Gram-negative organisms as to species was accomplished by the use of the Minitek *Enterobacteriaceae* differentiating system (BBL, Lebepe et al., 1990). The uncertain identifications of the gram-negative organisms were further assisted by criteria from Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984). Lactic acid bacteria were identified using the method and genus/species characteristics of Shaw and Harding (1984). The general key by Sneath and Jones (1976) was used to identify *B. thermosphacta*.

Presumptive *Listeria* isolates were transferred into Brain Heart Infusion (BHI) broth (DIFCO), incubated at 37°C for 24 hr and confirmed by using the following protocols, tests, and media: gram stain, catalase reaction, umbrella motility in Motility Test Medium (DIFCO), and beta-hemolysis using Columbia CNA agar (BBL) to

which had been added 5% defibrinated sheep blood (BBL) (Glass and Doyle, 1989; McClain and Lee, 1988). *L. monocytogenes* species were identified by the CAMP test using the *Staphylococcus aureus* factor in commercially prepared B-lysin discs (REMEL, Lenexa, KS, USA), and carbohydrate fermentation (dextrose, mannitol, xylose and rhamnose) tests (Glass and Doyle, 1989; McClain and Lee, 1988).

Presumptive *A. hydrophila* isolates were transferred onto TSA plates, incubated at 30°C for 24 hr and confirmed using gram, oxidase, catalase and DNase reactions (Palumbo et al., 1985), and the Minitek Nonfermenter differentiating systems (Lebepe et al., 1990).

#### Physical and chemical analyses

The surface pH of the remaining pieces of the chops was measured by the use of a flat surface combination electrode (Fisher Scientific, Pittsburg, PA, USA) attached to a pH meter (Zeromatic SS-33, Beckman Instruments, Inc., Palo Alto, CA, USA). Purge loss was measured by the difference between the weight of the packaged chops before the opening of the bag and that of the bag and liquid exudate remaining after the chops were withdrawn. Purge was expressed as percent meat weight.

Color was measured after repackaging the chops in O<sub>2</sub>-permeable film and storing in a display case at 5°C overnight to allow red color development. The Hunterlab Labscan spectrophotometer (Hunter Associate Laboratory, Inc., Reston, VA) was used to determine Hunter L, a, b color values (DeMan, 1976). Lipid oxidation was determined by the reaction of malonaldehyde with 2-thiobarbituric

acid (TBA ) as described by Tarladgis et al. (1960). Volatile basic nitrogen (VBN) was determined by using the micro-diffusion method of Conway (1958).

### Statistical analyses

Mean values were determined from the results of the three replicates. Data were analyzed by analysis of variance using the General Linear Model procedure (SAS, 1986) to determine main effects and interaction between O<sub>2</sub> and CO<sub>2</sub>. Because the gas combination 10/0 was not used, there were eleven combinations. Therefore, nine combinations, to give a 3X3 factorial, were used to determine main and interaction effects of CO<sub>2</sub> and O<sub>2</sub>. The remaining two combinations: 20/0 and 40/0 (Table 1) were considered as controls. Since they did not contain O<sub>2</sub> it was assumed that samples packaged in them would have storage life similar to those in vacuum. Paired contrasts were used to determine significance among treatment means, and least square difference was used to compare the means.

## RESULTS AND DISCUSSION

### Headspace analysis

Complete removal of air before flushing with the gas mixtures was never achieved. There was always about 1.5 -2.5% O<sub>2</sub> in the headspace of the packages after flushing and heat sealing. For example, at day 0, i.e., the day of packaging, the 20/0 (CO<sub>2</sub>/O<sub>2</sub>) and 40/0 mixtures which were supposed to contain no O<sub>2</sub>, had about 2.0%O<sub>2</sub>. A general increase in CO<sub>2</sub> concentration (Fig. 1) and simultaneous decrease in O<sub>2</sub> concentration (Fig. 2) with storage time was observed. This might be due to the use of O<sub>2</sub> for respiration by aerobic microorganisms and the muscle tissue postmortem, and the production of carbon dioxide by respiratory activities (Taylor and MacDougall, 1973). It has been reported that mitochondria in meat (Baltzer, 1969) together with bacteria (Johnson, 1974) respire and convert O<sub>2</sub> to CO<sub>2</sub>. Mitochondria consume 1-3 liters of O<sub>2</sub> per cm per 24 hr and remain active up to 144 hr postmortem or as long as the pH remains above 5.5 (Cheah and Cheah, 1971). Since the pH values of the prepackaged chops were above 5.5, it was assumed that the initial decrease in headspace O<sub>2</sub> concentration was due to muscle respiration, but microbial respiration took over with storage time. Changes in the concentrations of CO<sub>2</sub> and O<sub>2</sub> were influenced by their initial concentrations in the gas mixtures. Mixtures with 10% or 20% initial CO<sub>2</sub> concentration consistently increased in CO<sub>2</sub> concentration with time of storage. On the other hand, mixtures initially containing 40%CO<sub>2</sub> decreased in headspace CO<sub>2</sub> concentration during the first 7 days of storage at 4°C (Fig. 1). The decrease, which was about 7%, was not influenced by the initial O<sub>2</sub> concentration (Fig. 1). A

decrease in headspace CO<sub>2</sub> concentration of the packages at high CO<sub>2</sub> concentrations has also been observed by other investigators (Fu et al., 1991; Seidman et al., 1979), and has been attributed to dissolution of CO<sub>2</sub> in the meat fluid and subsequent formation of carbonic acid (Mitsuda et al., 1980), or to permeation of the packaging film (Seidman et al., 1979). The contribution by the latter was very minimal since pouches filled with 100%CO<sub>2</sub> and stored at 4°C and at 23°C remained the same (98%) over a 6-week period, and there was no difference between the CO<sub>2</sub> concentrations at the two temperatures. Hence, reduction in the headspace CO<sub>2</sub> concentration was more likely due to dissolution in meat fluid. Gill (1988) stated that CO<sub>2</sub> is very soluble in water and oils and when applied to meats, it is absorbed by the muscle and fat tissues until equilibrium is attained. According to Fruton and Simmonds (1953) a decrease in headspace CO<sub>2</sub> could also be due to binding of the gas to meat proteins.

Packages with less than 40% initial O<sub>2</sub> concentration produced anaerobic atmospheres i.e., less than 0.5%O<sub>2</sub> in the headspace (Lioutas, 1988) after 35 days of storage at 4°C (Fig. 2). Those that initially contained 40%O<sub>2</sub> remained aerobic throughout the study period.

#### Surface pH

In general, variation in CO<sub>2</sub> or O<sub>2</sub> concentrations did not significantly influence the surface pH ( $P > 0.05$ ). However, the results showed a slight decrease in pH with increasing CO<sub>2</sub> concentration. The pH values of the chops packaged in mixtures initially containing 40%CO<sub>2</sub> were in most cases about 0.1 to 0.2 pH units lower than those of the chops packaged in either the 20%CO<sub>2</sub> or 10%CO<sub>2</sub> mixtures (Table 2). The lower pH values were the result of absorption of CO<sub>2</sub> on

the muscle surface and subsequent ionization of the formed carbonic acid (Genigeorgis, 1985). The surface pH values of the air- and vacuum-packaged chops were similar to those of the chops packaged in the 40%CO<sub>2</sub> mixtures.

Pork chops used for this study did not have much variability in the initial pH values. There were no significant differences in pH values on day 0 for all the atmospheres used ( $P>0.05$ ). Changes in pH values were also not affected by storage time. There was a slight but insignificant increase in pH ( $P>0.05$ ) with storage time for all the atmospheres except values of the 10/10, 10/20 and 20/20 mixtures ( $P<0.05$ ), which might be due to the effect of microbial activity such as putrefaction (Okayama, 1987) in the meat during storage at 4°C.

### Psychrotrophs

The initial microbial counts of the pork chops before packaging were low; the psychrotrophic numbers were about 1.5-2.0 logs CFU/cm<sup>2</sup> and indicated that the carcass processing was properly controlled at the packing plant (Nortje et al., 1990). Increase in the CO<sub>2</sub> concentration significantly inhibited the growth of psychrotrophic bacteria ( $P<0.05$ ). Numbers of bacteria in the 10%CO<sub>2</sub> mixtures were higher than those in the 20%CO<sub>2</sub> mixtures which were also higher than those in the 40%CO<sub>2</sub> mixtures at all levels of O<sub>2</sub> studied (Fig. 3). The 40%CO<sub>2</sub> mixtures produced a 7-day lag period in increase of bacterial numbers at 10% and 20%O<sub>2</sub> levels, and a decrease in the overall psychrotrophic bacterial numbers at 40%O<sub>2</sub> during storage.

When the microbiological shelf life in terms of the psychrotrophic numbers in the chops stored in the various atmospheres were compared, it was evident that at 10%CO<sub>2</sub>, there was a significant additive effect of 40%O<sub>2</sub>. The order of numbers of



psychrotrophs in the mixtures was 10/10 (CO<sub>2</sub>/O<sub>2</sub>) > 10/20 > 10/40 (Fig. 4). The 10/40 mixture produced a 7-day lag period for increase of psychrotrophs and reached spoilage levels (numbers > 7 log cfu/cm<sup>2</sup>, Kraft and Ayres, 1952) in 21 days compared to 14 days by 10/20 or 10/10. The enhanced inhibitory effect of 40%O<sub>2</sub> with 10%CO<sub>2</sub> is consistent with the observation by Clark and Burki (1972) that O<sub>2</sub> concentrations above 30% markedly inhibited the growth of certain psychrotrophic strains of *Pseudomonas* and *Moraxella-Acinetobacter* group. Psychrotrophic numbers in the air-packaged chops were more than 2 log CFU/cm<sup>2</sup> higher than those in the chops packaged in the 10/40 mixture, about 1.4 log CFU/cm<sup>2</sup> higher than those in the 10/20 mixture, and not significantly different (P>0.05) from those in the 10/10 mixture at day 7 (Fig. 4). Chops packaged in air reached spoilage levels in 12 days.

For the 20%CO<sub>2</sub> mixtures, the 20/20 mixture produced a lag period of 7-days on psychrotrophs (Fig. 4) but was not sustained. All the mixtures, except the 20/0, reached spoilage levels at day 28. The 20/0 mixture had lower numbers than any of the other 20%CO<sub>2</sub> mixtures (P<0.01) from day 14. Samples packaged in this mixture did not reach spoilage levels throughout the period of study (Fig. 4). At 40%CO<sub>2</sub>, there was a lag period of about 7 days on psychrotrophs by the 40/10 mixture and a decrease in numbers of bacteria in the 40/40 mixture during 7 days of storage. However, no significant differences in psychrotrophic numbers were found among the 40%CO<sub>2</sub> mixtures during the first 28 days of storage. Numbers of psychrotrophs in the chops packaged in the 40/20 and 40/40 mixtures reached spoilage levels within 42 days, while those in the chops packaged in the 40/0 and 40/10 mixtures remained below 7 log CFU/cm<sup>2</sup> throughout the 42 days of storage, with numbers in the chops packaged in the 40/40 mixture more than 1 log

CFU/cm<sup>2</sup> higher than those in the chops packaged in the 40/10 or 40/20 mixture on day 42. There was no additive effect of O<sub>2</sub> at CO<sub>2</sub> concentrations higher than 10% which may suggest that at higher CO<sub>2</sub> concentrations, the inhibition of CO<sub>2</sub> overrides that of high O<sub>2</sub> concentrations on growth of psychrotrophic spoilage bacteria that are susceptible to high O<sub>2</sub> concentrations.

Numbers of psychrotrophs in the chops packaged in the 40%CO<sub>2</sub> mixtures were lower than those in the vacuum-packaged chops from day 7 to day 21 of storage. For example, numbers of bacteria in the chops packaged in the 40/0 or in 40/10 mixture were more than 1 log CFU/cm<sup>2</sup> lower than those in the chops packaged under vacuum. A similar observation was made by Christopher et al. (1980) when they compared psychrotrophic bacterial numbers of chops stored in 40%CO<sub>2</sub>/0%O<sub>2</sub>/60%N<sub>2</sub> and in vacuum. The high numbers of psychrotrophs in the vacuum-packaged chops could be attributed to residual air trapped around or in the meat maintaining aerobic bacterial growth during the first 28-days of storage at 4°C (Hodges et al., 1974). However, because of difficulty in obtaining headspace in the vacuum packages, changes in the O<sub>2</sub> concentrations of the packages over storage time were not monitored. Seidman et al. (1980) have reported that complete evacuation of atmospheric air from the headspace of packages is difficult. No difference in numbers of psychrotrophs was found between vacuum-packaged chops and those packaged in the 40/0 or 40/10 mixture after day 21 probably because most of trapped air had already been utilized. Comparatively, the psychrotrophic numbers in the chops packaged in the 20/0 mixture were not significantly different from those in the chops packaged in the 40/0, 40/10 or 40/20 mixture throughout the storage period, but lower than those in the chops packaged in the 40/40 mixture from day 35. Compared to vacuum, chops packaged in the

20/10 mixture had slightly lower numbers during the first 21 days of storage, but the difference was not significant throughout the storage period ( $P>0.05$ ).

### Mesophiles

Similar effects of increasing CO<sub>2</sub> concentration were found on growth of mesophiles (Fig. 5). That is, increasing the CO<sub>2</sub> concentration significantly reduced the growth of aerobic mesophilic bacteria on the pork chops. Fig. 6 shows that numbers in the air-packaged chops were higher than those in the chops packaged in the 10/40(CO<sub>2</sub>/O<sub>2</sub>) mixture ( $P<0.05$ ) or those packaged in the 10/10 or 10/20 mixture ( $P>0.05$ ). No difference in the numbers of mesophiles were found between chops packaged in the 10/10 mixture and those packaged in the 10/20 mixture ( $P>0.05$ ). Numbers in the chops packaged in the 10/40 mixture were the lowest among the 10%CO<sub>2</sub> mixtures which indicates that 40%O<sub>2</sub> also had an additive effect at 10%CO<sub>2</sub> on growth of mesophiles.

The mesophilic numbers in the chops packaged in the 20/0 mixtures were the lowest ( $P<0.05$ ) among those in the 20%CO<sub>2</sub> mixtures after day 7 (Fig. 6). No significant differences were found among numbers in the chops packaged in the 20/10, 20/20, and 20/40 mixtures. Numbers of mesophiles in the vacuum-packaged chops were higher than those in the chops packaged in any of the 40%CO<sub>2</sub> mixture from day 7 to day 21 (Fig. 6). A lag period of 7 days was produced by the 40/10 and 40/20 mixtures on mesophiles but no difference ( $P>0.05$ ) was found among numbers of bacteria in the chops packaged in the 40%CO<sub>2</sub> mixture during the first 28 days of storage. Thereafter, numbers in the chops packaged in the 40/40 mixture increased rapidly and were highest ( $P<0.05$ ) on day 42. Growth in

the chops packaged in the 20/0 mixture was not significantly different from that in chops packaged in the 40/0 or 40/10 mixture.

Although there was a significant increase in growth of the psychrotrophs and mesophiles with storage time ( $P < 0.01$ ) for all the atmospheres tested, there was evidence that the addition of 20-40%CO<sub>2</sub> significantly inhibited the growth of psychrotrophs and mesophiles compared to aerobic storage. For example, mixtures containing 20%CO<sub>2</sub> increased the storage life by 14 days when they contained O<sub>2</sub> and by 30 days when no O<sub>2</sub> was present. Those that contained 40%CO<sub>2</sub>, with or without O<sub>2</sub>, also increased the storage life by 30 days. This is consistent with the observation by Finne (1982) that increasing the CO<sub>2</sub> concentration reduces the growth rate of spoilage bacteria. Gill and Tan (1980) found that in aerobic systems increasing the CO<sub>2</sub> concentration beyond 20% to 30% had little or no additional inhibitory effect on spoilage flora. Their finding may be true only when the O<sub>2</sub> concentration in the atmosphere is less than 10%. This is because at 10%O<sub>2</sub>, 20%CO<sub>2</sub> spoiled at a much faster rate than 40%CO<sub>2</sub>, but at 0-2%O<sub>2</sub>, both 20%CO<sub>2</sub> and 40%CO<sub>2</sub> packages never reached spoilage levels in 42 days.

### *Enterobacteriaceae*

The growth of *Enterobacteriaceae* was influenced by the concentrations of CO<sub>2</sub> in the mixtures ( $P < 0.05$ ). The highest numbers were obtained in the 10%CO<sub>2</sub> mixtures followed by the 20%CO<sub>2</sub> mixtures and the lowest numbers in the 40%CO<sub>2</sub> (Fig. 7). CO<sub>2</sub> at the concentrations tested did not produce a lag period. Numbers of bacteria at day 0 were about 1 log CFU/cm<sup>2</sup> higher in the 10%CO<sub>2</sub> mixture than in either the 20%CO<sub>2</sub> or the 40%CO<sub>2</sub> mixtures. It has been reported

that CO<sub>2</sub> concentrations slightly above atmospheric levels are stimulatory to bacterial growth (Valley, 1928) and might explain the high numbers on day 0 in the 10%CO<sub>2</sub> mixture. At 10%CO<sub>2</sub> an additive effect of 40%O<sub>2</sub> was observed after day 7; numbers of bacteria in the chops packaged in the 10/40(CO<sub>2</sub>/O<sub>2</sub>)mixture were about 1.5 log CFU/cm<sup>2</sup> lower than those in the chops packaged in the 10/20 mixture and about 2.5 log CFU/cm<sup>2</sup> lower than those in the chops packaged in the 10/10 mixture (Fig. 8). Numbers in the chops packaged in air and in the chops packaged in the 10/10 or 10/20 mixture were not significantly different ( $P>0.05$ ).

At 20%CO<sub>2</sub> there were no significant differences in numbers of bacteria among the mixtures during the first 7 days of storage. The numbers in the chops packaged in the 20/0 mixture decreased from day 7 to day 14 and remained the lowest ( $P<0.05$ ) through to day 21 (Fig. 8). At day 28 numbers in the chops packaged in the 20/40 mixture were slightly lower than those in the chops packaged in the 20/10 or 20/20 mixture but not significantly different ( $P>0.05$ , Fig. 8). At 40%CO<sub>2</sub>, no growth of *Enterobacteriaceae* was observed on day 0 of the study, and numbers of bacteria from day 7 were not significantly different ( $P>0.05$ ) among the mixtures even though the trend was 40/0 < 40/10 < 40/20 < 40/40 mixture (Fig. 8). Numbers of bacteria in the vacuum-packaged chops were significantly higher ( $P<0.05$ ) than those in chops packaged in the 40/0 mixture during the first 28 days but not different from those in chops packaged in the other 40%CO<sub>2</sub> mixtures. Numbers of bacteria in the chops packaged in the 20/0 mixture were not significantly different ( $P>0.05$ ) compared to those in the chops packaged in the 40%CO<sub>2</sub> mixtures, except that those in the 40/40 mixture were higher ( $P<0.05$ ) on day 42.

*Enterobacteriaceae*, if present in high proportions of the total microflora, may contribute to the spoilage of meat (Blickstad et al., 1981), since the bacteria can develop a characteristic putrid flavor (Gill and Harrison, 1989). Growth increased significantly with storage time for all the atmospheres tested. Nonetheless, no characteristic off-odors or flavors were detected in either the vacuum-packaged chops or those packaged in the 40%CO<sub>2</sub> mixtures, except on day 42 in the chops packaged in the 40/40 mixture.

The slightly better performance of some of the 40%CO<sub>2</sub> mixtures than vacuum in inhibiting psychrotrophs, mesophiles and *Enterobacteriaceae* is an indication that modified gas atmospheres packaging can replace vacuum packaging for long distance distribution of fresh pork chops.

#### Facultative anaerobes and anaerobes

The growth of facultative anaerobes or strict anaerobes were not significantly influenced by variations in either CO<sub>2</sub> or O<sub>2</sub> concentrations ( $P>0.05$ ). According to Farber (1991) there is a wide variation in the sensitivity of anaerobes to O<sub>2</sub>. Nevertheless, there was a trend of the numbers of the organisms decreasing with increasing O<sub>2</sub> concentrations at all the CO<sub>2</sub> levels tested. Numbers of bacteria were higher in the 10%O<sub>2</sub> mixtures than in the 20%O<sub>2</sub> mixtures which were higher than those in the 40%O<sub>2</sub> mixtures (Fig. 9). At 10%CO<sub>2</sub>, there was a decrease in the numbers of organisms at 20%O<sub>2</sub> but it was not sustained and growth increased rapidly thereafter. The 40%O<sub>2</sub> mixtures produced a lag period of 7 days at 20%CO<sub>2</sub>, and 14 days at 40%CO<sub>2</sub> (Fig. 9), while the 20%O<sub>2</sub> produced a 7-day lag period at 40%CO<sub>2</sub>. The generally higher numbers of bacteria in chops packaged in mixtures initially containing lower O<sub>2</sub> concentrations might be due to the

development of partial anaerobic conditions in the package that might have enhanced anaerobic and facultative anaerobic microbial growth (Hodges et al., 1974).

Numbers of organisms in the chops packaged in the 0%O<sub>2</sub> mixtures and those in the chops packaged under vacuum were compared (Fig. 10). For the first 14 days, numbers in the chops packaged in the 20/0(CO<sub>2</sub>/O<sub>2</sub>) mixture were significantly higher than those in the chops packaged in the 40/0 mixture ( $P < 0.05$ ). Numbers of bacteria in the vacuum-packaged chops were higher than but not significantly different ( $P > 0.05$ ) from those in the chops packaged in the 40/0 mixture. Among the atmospheres that initially contained 20%O<sub>2</sub>, numbers of bacteria in the air-packaged (O<sub>2</sub> concentration of air = 20.9%) chops were not significantly different from those in the chops packaged in the 10/20 or 20/20 mixture ( $P > 0.05$ ) but were higher than those in the chops packaged in the 40/20 mixture (Fig. 10). No difference was found between numbers of bacteria in the chops packaged in the 10/20 mixture and those in the chops packaged in the 40/20 mixture during the first 14 days of storage ( $P > 0.05$ ). But thereafter numbers of bacteria in the chops packaged in the 40/20 mixture were significantly lower ( $P < 0.05$ ) than those in the chops packaged in the 10/20 mixture. For atmospheres containing 40%O<sub>2</sub>, chops packaged in the 40/40 mixture had significantly lower numbers of bacteria than those packaged in the 10/40 or 20/40 mixture on day 14 (Fig. 10). From day 21, however, no differences were found among the numbers of bacteria in the chops packaged in the three mixtures.

The numbers of facultative and strict anaerobes in the chops packaged in 40%CO<sub>2</sub> mixtures were about 70 to 100% of those of psychrotrophs or mesophiles from day 21. Thus, the former organisms developed more slowly than

*Pseudomonas* and related gram-negative psychrotrophs (Silliker and Wolfe, 1980). However, they increased in numbers in these atmospheres because their growth was apparently not inhibited by CO<sub>2</sub> (Gill and Tan, 1980). The selective growth of facultative and strict anaerobes in modified atmospheres has microbiological safety implications since many important pathogens or potential pathogens belong to this group (Gill and Tan, 1980).

#### Lactic acid bacteria

The growth of lactic acid bacteria was not significantly influenced by variations in CO<sub>2</sub> and O<sub>2</sub> concentrations ( $P > 0.05$ ), but results suggested that numbers of bacteria increased slightly with increasing CO<sub>2</sub> concentrations (Fig. 11), an indication that growth of lactic acid bacteria might be stimulated by high CO<sub>2</sub> (Blickstad et al., 1981). A 7-day lag period was produced by the 10%CO<sub>2</sub> mixtures at 10%O<sub>2</sub>, by the 10%CO<sub>2</sub> and the 20%CO<sub>2</sub> mixtures at 20%O<sub>2</sub> and by all the CO<sub>2</sub> mixtures at 40%O<sub>2</sub> (Fig. 11)

Fig. 12 compares the numbers of lactic acid bacteria in all the atmospheres tested. At 10%CO<sub>2</sub>, growth was not observed until day 14. At day 21 numbers in the chops packaged in the 10/10(CO<sub>2</sub>/O<sub>2</sub>) mixture were about 1 log CFU/cm<sup>2</sup> higher than those in the chops packaged in the 10/40 mixture, but not significantly different from those in the chops packaged in the 10/20 mixture ( $P > 0.05$ ). The sudden increase in the numbers of lactic acid bacteria in the chops packaged in the 10/10 mixture after day 14 could be attributed to the relatively low O<sub>2</sub> or high CO<sub>2</sub> concentration in the headspace of the package compared to those of other mixtures containing 10%CO<sub>2</sub> (Fig. 1 and 2). There was no growth of lactic acid bacteria in the air-packaged chops over a 14 day storage period (Fig. 12).



The numbers of lactic acid bacteria in the chops packaged in the 20/0 mixture were highest among the 20%CO<sub>2</sub> mixtures for the first 21 days ( $P < 0.05$ ). There was no difference in the numbers of bacteria in the chops packaged in the other 20%CO<sub>2</sub> mixtures during the first 21 days of storage. Thereafter, growth in the chops packaged in the 20/10 and 20/20 mixtures increased and were significantly higher than those in the chops packaged in the 20/40 mixture. At 40%CO<sub>2</sub>, bacterial numbers were not different among mixtures from day 0 to day 14 ( $P > 0.05$ ), but thereafter numbers of bacteria in the chops packaged in the 40/0 and 40/10 mixtures were much higher than those in the chops packaged in the 40/40 mixture ( $P < 0.05$ ). Numbers of bacteria in the vacuum-packaged chops were less than those in the chops packaged in the 20/0 or 40/0 mixture for the first 21 days. Thereafter growth in the vacuum-packaged chops increased rapidly and by day 42 the numbers of bacteria were not significantly different from those in the chops packaged in the 40/0 mixture ( $P > 0.05$ ), which were lower than those in the chops packaged in the 20/0 mixture. Fig. 2 shows that the 20/0 mixture reached anaerobic conditions ( $O_2 < 0.5\%$ ) in 21 days compared to 35 days taken by the 40/0 mixture. This might explain why the latter mixture had lower numbers of lactic acid bacteria than the former during the first 28 days of storage. In addition, even though the pH values of the chops in the two mixtures were not significantly different, the pH values of the chops packaged in the 20/0 mixture were slightly lower than those of the chops packaged in the 40/0 mixture (Table 2) making the chops in the former mixture more conducive for growth of acid-tolerant lactic acid bacteria. Gill and Penny (1988) reported that pH less than 5.8 enhanced growth of lactic acid bacteria in vacuum- or high CO<sub>2</sub>-packaged meat.

The growth of lactic acid bacteria increased with storage time for all the atmospheres tested, except for air, but numbers never reached spoilage levels of 8 log CFU/cm<sup>2</sup> (Ayres, 1980). The results generally indicated that even though lactic acid bacteria are facultative anaerobes, high CO<sub>2</sub> concentrations might enhance their growth. Brock (1970) stated that all lactic acid bacteria grow anaerobically, although most of them are not sensitive to O<sub>2</sub> and can grow in the presence or absence of O<sub>2</sub>. The results also indicate that CO<sub>2</sub> atmospheres that may be used to inhibit growth of aerobic psychrotrophic spoilage flora and *Enterobacteriaceae* on fresh pork chops, may not affect the growth of lactic acid bacteria.

#### *Brochotrix thermosphacta*

This is a meat spoilage organism found among the initial microflora of especially high-pH meat. The organism ferments glucose and ribose and simultaneously metabolizes amino acids (Edwards and Dainty, 1987; Grau, 1988) to compounds that cause early spoilage of chilled meats. Growth of *B. thermosphacta* did not seem to be affected by variations in O<sub>2</sub> or CO<sub>2</sub> concentrations ( $P>0.05$ ). However, the data showed that numbers of the organism were highest in mixtures containing 40%O<sub>2</sub>, followed by the 20%O<sub>2</sub> mixtures and lowest in those containing 10%O<sub>2</sub> at the three CO<sub>2</sub> concentrations tested (Fig. 13). There was a 7-day lag period for 10%O<sub>2</sub> and 20%O<sub>2</sub> at 10%CO<sub>2</sub>. The effect of O<sub>2</sub> on the growth of *Brochotrix* was most pronounced at 40%CO<sub>2</sub> where significant differences ( $P<0.05$ ) in the numbers of the organism were observed among the O<sub>2</sub> levels tested after day 7.

Fig. 14 shows the comparison of the numbers of the organism in chops packaged in different atmospheres. No difference was found among the numbers

of the bacteria in the chops packaged in the mixtures initially containing 0%O<sub>2</sub> or 10%O<sub>2</sub>, hence only numbers in chops packaged in the 20/0(CO<sub>2</sub>/O<sub>2</sub>), 40/0 and 40/10 mixtures were compared with those in the vacuum-packaged chops (Fig. 14). On day 0, numbers of the bacteria in chops packaged in the 20/0 mixture were not different from those in the vacuum-packaged chops but were significantly higher ( $P < 0.05$ ) than those in the chops packaged in the 40/0 or 40/10 mixture. From day 7 no significant differences in the numbers of the bacteria were found among the atmospheres. The absence of growth of this organism on day 14 could be due to competitive inhibition by lactic acid bacteria on day 14 (Roth and Clark, 1975). Growth of lactic acid bacteria in these atmospheres began to increase after 7 days of storage.

At 20%O<sub>2</sub> the 10/20 mixture produced a 7-day lag period but the numbers of the organism in the chops increased rapidly and by day 28, no significant differences ( $P > 0.05$ ) in the numbers of the organism were found among the atmospheres including air. At 40%O<sub>2</sub>, growth of the organism during the first 7 days was not different among the mixtures. Thereafter the numbers of the organism in the chops packaged in the 40/40 mixture became higher ( $P < 0.05$ ) than those in the chops packaged in the 20/40 mixture which was not different from the numbers of the organism in the chops packaged in the 10/40 mixture (Fig. 14). The enhanced growth of this organism in the chops packaged in the 40/40 mixture may be due to the high CO<sub>2</sub> concentration inhibiting the growth of the fast-growing competing *Pseudomonas* and the high O<sub>2</sub> concentration stimulating the growth of *B. thermosphacta* (Ledward et al., 1971). Because of its slower growth rate at refrigerated temperatures, *B. thermosphacta* is a poor competitor to the fast-growing *Pseudomonas* (Jay, 1986). At 10%O<sub>2</sub> or 20%O<sub>2</sub>, no additive effect was

found with 40%CO<sub>2</sub>. Besides, other investigators have reported that *B. thermosphacta* is insensitive to CO<sub>2</sub> (Newton et al., 1977; Roth and Clark, 1975). Therefore, atmospheres containing more than 20%O<sub>2</sub> with low or high CO<sub>2</sub> concentration may stimulate the growth of *B. thermosphacta*.

Blickstad and Molin (1983) observed that *B. thermosphacta* was among the principal spoilage organisms on the CO<sub>2</sub>-packaged normal pork stored at 3.3°C. In this study, the numbers of the organism in the chops packaged in the 40%CO<sub>2</sub> mixtures were about 70% of those of aerobic psychrotrophic or mesophilic flora from day 21. Erichsen and Molin (1981) reported that *B. thermosphacta* increased at the same rate as the total numbers in gas atmospheres containing 5-90%CO<sub>2</sub> and within 21 days of storage the organism was almost equal to the total numbers in air and in the gas mixture.

#### Composition of microflora

The initial microflora of pork before packaging consisted of *Pseudomonas* spp, *Acinetobacter* spp. *Enterobacter* spp. *Y. enterocolitica*. and *B. thermosphacta*. No *Listeria* or *Aeromonas* were isolated from cultures of the prepackaged chops. These pathogens were also not found in any chops from any of the atmospheres studied. Pathogens such as *Y. enterocolitica*, *Vibrio* spp., *Shigella* spp. and *E.coli* were isolated from some packages (Table 3). However, their proportions were so small that there was no apparent specificity for any particular atmosphere. The proportion of *Hafnia alvei*, a potential pathogen, was very high on day 21 in the chops packaged in the 10/40(CO<sub>2</sub>/O<sub>2</sub>), 20/0, 40/10 and 40/40 mixtures, and in the vacuum. The proportion of the organism increased with storage time in the vacuum-packaged chops. This organism has been reported to replace

*Pseudomonas* spp when relatively O<sub>2</sub>-impermeable films are used to package meats (Ingram and Dainty, 1971). Vanderzant et al. (1986) also found that numbers of *H. alvei* on refrigerated pork fat were usually greater than on the lean chops. However, there is no explanation for the sudden increase in the growth of this organism on day 21 in the above atmospheres.

The most predominant gram-negative organisms on the plates used to enumerate *Enterobacteriaceae* were *Pseudomonas* spp. and *Serratia* spp. *Pseudomonas* spp. were most predominant in the chops packaged in air (data not shown) followed by mixtures initially containing 40%O<sub>2</sub> or 10%CO<sub>2</sub> (Table 3). The proportion of *Pseudomonas* spp. in a package also decreased with storage time, probably because of a decrease in the O<sub>2</sub> concentration and an increase in CO<sub>2</sub> concentration. The low proportion of *Pseudomonas* spp. in the 40%CO<sub>2</sub> mixtures might explain the low psychrotrophic and mesophilic numbers obtained in these mixtures throughout the study. The inhibition of *Pseudomonas* spp. by CO<sub>2</sub> in MA packages is important for shelf life extension of fresh meats (Nortje and Shaw, 1989).

*Serratia* spp. on the other hand, were most predominant in packages initially containing 40%CO<sub>2</sub> and/or low O<sub>2</sub> concentration followed by 20%CO<sub>2</sub> and were lowest in the 10%CO<sub>2</sub> mixture (Table 3). Among the 10%CO<sub>2</sub>, it was also observed that the proportion of *Serratia* spp. was highest in the 10/10 mixture on day 14. These findings are in accordance with the observation by Blicsktad et al. (1981) that the presence of *Serratia* on an aerobic-stored meat might be due to the gradual increase in the CO<sub>2</sub> concentration in the package. It has been reported that some psychrotrophic *Enterobacteriaceae* are capable of growing on normal pH muscle under strictly anaerobic conditions (Gill and Penny, 1988).

Nevertheless, the vacuum-packaged chops had much smaller proportion of *Serratia* spp.

The composition of the facultative anaerobic and aerobic microflora of the chops packaged under 40%CO<sub>2</sub> atmospheres was mostly lactic acid bacteria and *Enterobacteriaceae* with small proportions of *B. thermosphacta* especially in the 40/20 and 40/40 mixtures. Considering that the identity of the *Enterobacteriaceae* isolates showed no selection for particular pathogens or potential pathogens, the high numbers of the facultative anaerobic and anaerobic flora in those chops might not produce any microbiological safety risks.

#### Hunter "L, a, b" values

These values were used to measure the effect of the various atmospheres on the color of the fresh pork. The Hunter "L" value measures lightness, the "a" value measures redness, while the "b" value measures the yellowness of meats. High "L" value and low "a" value would be associated with less bright color of the meat.

Variations in CO<sub>2</sub> or O<sub>2</sub> concentrations did not significantly influence the "L" values ( $P>0.05$ ). However, there was a general trend of increasing "L" values as the CO<sub>2</sub> concentration increased at all levels of O<sub>2</sub> tested, with 40%CO<sub>2</sub> mixtures having the highest "L" values and 10%CO<sub>2</sub> mixtures having the lowest (Fig. 15). In addition, "L" values increased slightly but not significantly with storage time.

Because no significant effect of CO<sub>2</sub> and O<sub>2</sub> concentrations or storage time on "L" values were found, only mean values for selected gas mixtures were compared with those for air or vacuum (Fig. 16). No significance differences in mean "L" values were found among air and mixtures initially containing 20%O<sub>2</sub>

(10/20, 20/20, 40/20) or 40%O<sub>2</sub> (10/40, 20/40, 40/40). There was also no difference in the mean "L" values of chops packaged under vacuum and those packaged in the 20/0(CO<sub>2</sub>/O<sub>2</sub>) or 40/0 mixture (P>0.05, Fig. 16).

In general, low "L" values corresponded with high surface pH values and vice versa. This might partly explain the high "L" values obtained for the 40%CO<sub>2</sub> mixtures since the surface pH values of the chops packaged in the 40%CO<sub>2</sub> mixtures were relatively low (Table 2). Low pH denatures proteins which consequently bind less water (Hamm, 1986). The nonbound moisture increases the reflectance of light from freshly-cut meat surface and results in a lighter appearance (Swatland, 1984).

The Hunter "a" values obtained on day 0 were not significantly different for all chops, but the values were generally very low compared to values of pork samples that have been used for previous work (Ammann, 1989). Low redness value of fresh meat may be due to intrinsically low myoglobin contents or pale, soft, exudative (PSE) qualities (Barton-Grade, 1981). There was a strong influence of increasing CO<sub>2</sub> concentration on Hunter "a" values (P=0.06). The results indicated that the 40%CO<sub>2</sub> mixtures had the lowest "a" values, followed by the 20%CO<sub>2</sub> mixtures, while the 10%CO<sub>2</sub> mixtures had the highest values (Fig. 17). Values for the 20%CO<sub>2</sub> mixtures and the 40%CO<sub>2</sub> mixtures decreased with storage time but those for the 10%CO<sub>2</sub> mixtures remained fairly constant or even increased when the O<sub>2</sub> concentration was 40% (Fig. 17). It was also observed that at the same CO<sub>2</sub> concentration, increasing the O<sub>2</sub> concentration had a slight but not significant increase in "a" values. For example, at 20%CO<sub>2</sub>, the order of "a" values was 20/10 < 20/20 < 20/40; differences were, however, not greater than 1.0 unit.

Values of the air-packaged chops were not significantly different from those of the 20%O<sub>2</sub> mixtures or the 40%O<sub>2</sub> mixtures (Fig. 18). Since O<sub>2</sub> had only a slight effect on "a" values, the mean values of chops packaged in the 20/0 and 40/0 mixtures were not significantly different ( $P>0.05$ ) than those of their corresponding mixtures. Hence, they were selected for comparison with those of the vacuum-packaged chops (Fig. 18). No significant difference was found between the "a" values of the vacuum-packaged chops and those of chops packaged in the 20/0 or 40/0 mixture. Fairly constant "a" values were obtained in the chops packaged in the 20/0, 40/0 mixture and in the vacuum during storage. This might be due to the myoglobin remaining in the reduced form under those atmospheric conditions as observed by Asensio et al. (1988).

Some investigators using CO<sub>2</sub> concentrations of 20 to 40% found rapid discoloration of fresh pork chops (Seidman et al., 1980; Silliker et al., 1950; Walters, 1975). However, others found no significant difference in the color of chops stored in high CO<sub>2</sub> concentrations (Huffman et al., 1975; Moore and Gill, 1987). The results of this study showed that the redness of the chops depended more on the CO<sub>2</sub> concentration than on the O<sub>2</sub> concentration of the gas mixture. It has been suggested that high CO<sub>2</sub> concentrations do not discolor fresh meat but that a competitive binding of CO<sub>2</sub> to myoglobin, which can be reversed upon subsequent exposure to O<sub>2</sub>, occurs (Seidman et al., 1979). However, after 24 hr of retail display there were still slight differences in the appearance of the chops used for this study.

The mean Hunter "b" values averaged over time are presented in Table 4. No difference was found among atmospheres, and values did not change significantly with storage time ( $P>0.05$ )



### Lipid oxidation

Thiobarbituric acid (TBA) numbers were used to indicate the amount of fat oxidation in the chops packaged under various atmospheres. No significant effect of CO<sub>2</sub> or O<sub>2</sub> concentration on TBA values was observed ( $P > 0.05$ ). On the other hand, at a constant CO<sub>2</sub> concentration, variation in the O<sub>2</sub> concentration produced a strong effect on the TBA values ( $P = 0.059$ ). There was an increase in TBA values with increasing O<sub>2</sub> concentration (Table 5). Also at 0%O<sub>2</sub>, 10%O<sub>2</sub> or 20%O<sub>2</sub>, but not 40%O<sub>2</sub>, increasing the CO<sub>2</sub> concentration increased the TBA values. For example, at 20%O<sub>2</sub> the order of TBA values in the packages was 40/20(CO<sub>2</sub>/O<sub>2</sub>) > 20/20 > 10/20 (Table 5). Similar effects of CO<sub>2</sub> on TBA values of poultry and beef were made by Uebersex et al. (1978) and Fu et al. (1991), respectively. This might be due to the relatively low pH produced by high CO<sub>2</sub> concentrations. A low pH environment accelerates the protonation of bound O<sub>2</sub> and favors the release of superoxide anions, which are potential prooxidant species (Livingston and Brown, 1981). The high TBA values of 40%CO<sub>2</sub> could also help explain the relatively light appearance of chops packaged in these mixtures since there is high correlation between TBA values and percent metmyoglobin (Faustman et al., 1989). However, it appeared that the effect of CO<sub>2</sub> on lipid oxidation is surpassed by the effect of high O<sub>2</sub> concentrations since at 40%O<sub>2</sub> no significant influence on TBA was found with increasing CO<sub>2</sub> concentrations.

TBA values also generally increased with storage time for all atmospheres except in a few cases where TBA increased to a point and then began to decrease. This was common with the air and vacuum chops. Air-packaged chops had relatively low TBA values compared to those for the gas mixtures. This was contrary to the observation by Okayama (1987) that there was a rapid increase in TBA

values of chops stored in air at 4°C. Although lipid oxidation occurred in chops packaged in the gas atmospheres, the TBA values never reached 5, a number at which rancid odors become apparent in pork (Ordonez and Ledward, 1977). However, neither the chops used for this study nor those for the work referred to (Ordonez and Ledman, 1977) were cooked. Some investigators have found that oxidized flavor can be detected in cooked meats with TBA values of 0.5 to 2.0 (Greene and Cumuze, 1981; Tarladgis et al. 1964). The TBA values of chops packaged under vacuum were significantly lower ( $P < 0.05$ ) than those of chops packaged in the gas mixtures (Table 5).

#### Volatile basic nitrogen (VBN)

Variation in CO<sub>2</sub> concentration had a very significant influence on the VBN of the pork chops ( $P < 0.01$ ). VBN decreased with increasing CO<sub>2</sub> concentration, i.e., the 40%CO<sub>2</sub> mixtures had the lowest values while the 10%CO<sub>2</sub> mixtures had the highest values at the same O<sub>2</sub> concentrations (Fig. 19). There was also an increase in VBN with storage time. No significant difference in VBN at day 0 was observed ( $P > 0.05$ ), which indicated that the initial VBN values for all chops were fairly equal.

Comparison of the VBN values of selected gas mixtures with those of air or vacuum showed that the values of the air-packaged chops were higher ( $P < 0.05$ ) than those of any of the gas mixtures containing 20%O<sub>2</sub> (10/20, 20/20, 40/20) (Fig. 20). No difference in values was found among chops packaged under vacuum and in the 20/0, 40/0 or 40/10 mixture ( $P > 0.05$ ), except on day 35 when values of the chops packaged in the 20/0 mixture were significantly higher than those of the chops packaged in the other atmospheres ( $P < 0.05$ ). It was observed that packages

that rapidly exceeded the spoilage level of 20-25 mg/100g meat (Oka et al., 1989) also had simultaneous rapid increase in the numbers of aerobic psychrotrophic flora. Fig. 21 shows that there is a strong correlation between VBN values and the numbers of psychrotrophs in pork chops stored at 4°C ( $R^2 = 0.918$ ), and that  $\log_{10} 7.0 \text{ cfu/cm}^2$  corresponded with VBN of about 18 mg/100g of meat, which is quite close to the spoilage level for fish (Oka et al., 1989). The high correlation of VBN with numbers of psychrotrophic flora may make VBN a good indicator of the freshness of pork. This is contrary to the findings of Fu et al. (1991) for beef. Therefore other species of meat may be tested to ascertain the possible use of VBN as an indicator of raw meat freshness.

#### Purge (Exudate)

Variations in  $\text{CO}_2$  and  $\text{O}_2$  concentrations had no significant effect on the purge loss of fresh pork ( $P > 0.05$ ). However, there was a slight increase in purge loss with increasing  $\text{CO}_2$  concentration. In general, there was more purge loss in the 40% $\text{CO}_2$  packages than in the 20% $\text{CO}_2$  ones which also had more purge loss than the 10% $\text{CO}_2$  packages (Fig. 22). At 10% $\text{O}_2$  or 20% $\text{O}_2$  a greater difference in purge loss between the 20% $\text{CO}_2$  and the 40% $\text{CO}_2$  packages was observed ( $P < 0.05$ ) from day 28. The increase in purge loss with increasing  $\text{CO}_2$  concentration may be attributed to the relatively low pH which might have denatured proteins and caused exudative conditions in the form of drip during storage (Fortin and Raymond, 1987). According to Hamm (1986) even partial denaturation of the water-binding myofibrillar proteins can cause a decrease in water-binding capacity. It was also observed that at the same  $\text{CO}_2$  concentration, there was a general decrease in purge loss with increasing  $\text{O}_2$  concentration (Fig.

22). This might be due to less CO<sub>2</sub> being dissolved because of the presence of high concentrations of O<sub>2</sub>, even though the latter is less soluble than CO<sub>2</sub>. The high percentage purge loss in 40%CO<sub>2</sub> packages might have caused loss of color since myoglobin loss in the exudate can remove pink tones of muscle tissue (Gill and Harrison, 1989).

Because purge loss was greatest in the 40%CO<sub>2</sub> packages, values of chops packaged in the 40%CO<sub>2</sub> mixtures were compared with those of vacuum. Fig. 23 shows that purge loss in the vacuum packages was much greater than that in the 40%CO<sub>2</sub> packages.

## CONCLUSIONS

The gaseous composition of the headspace of the modified gas atmosphere packages changed during refrigerated storage, producing in some cases anaerobic conditions when the initial oxygen concentrations were low.

In general, the microbiological shelf life of the fresh pork chops stored in the modified gas atmospheres seemed to depend more on the carbon dioxide concentration than on the oxygen concentration in the headspace, even though high oxygen concentrations favored growth of *Brochotrix thermosphacta*.

Modified gas atmospheres must contain more than 10%CO<sub>2</sub> to be more effective than air in prolonging the shelf life of fresh pork chops stored at 4°C. 40%CO<sub>2</sub> is equal to and in some cases better than vacuum in controlling microbial growth, especially psychrotrophs and mesophiles, on fresh pork chops at 4°C.

The absence of naturally occurring *Listeria* or *Aeromonas* contaminants and the insignificant populations of *Y. enterocolitica* and other gram-negative pathogens or potential pathogens that have psychrotrophic characteristics indicate that there is no apparent specificity of any of these organisms for gas mixtures containing carbon dioxide or oxygen from 0 to 40%.

The redness of the fresh pork chops packaged under modified gas atmospheres depended more on the carbon dioxide concentration than the oxygen concentration in the headspace of the package, but carbon dioxide at 40% had only a slight effect on the lightness of the fresh pork chops.

Vacuum packaging reduced lipid oxidation much better than modified gas atmospheres. On the other hand, the latter could be used to reduce purge loss which was usually high in the vacuum packages.

Modified gas atmosphere packaging resulted in chops with comparatively low volatile basic nitrogen (VBN). More tests will be required to determine the usefulness of VBN as an indicator of raw meat freshness.

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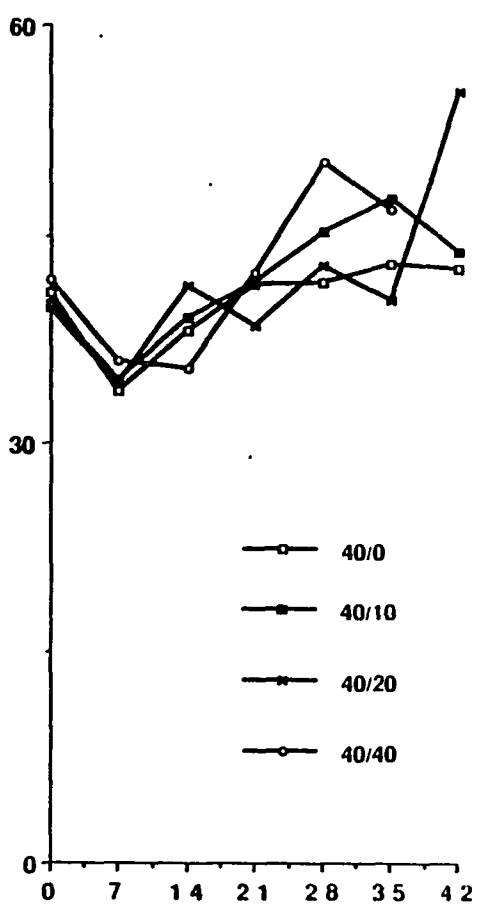
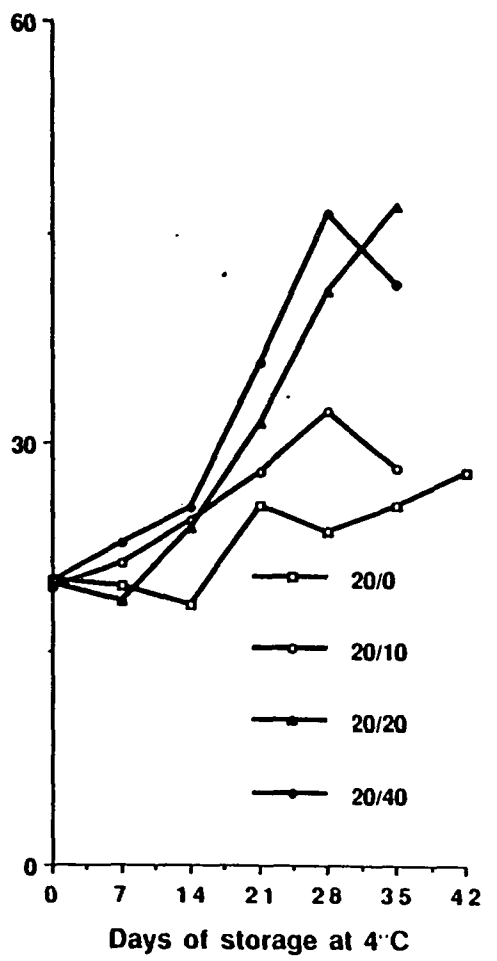
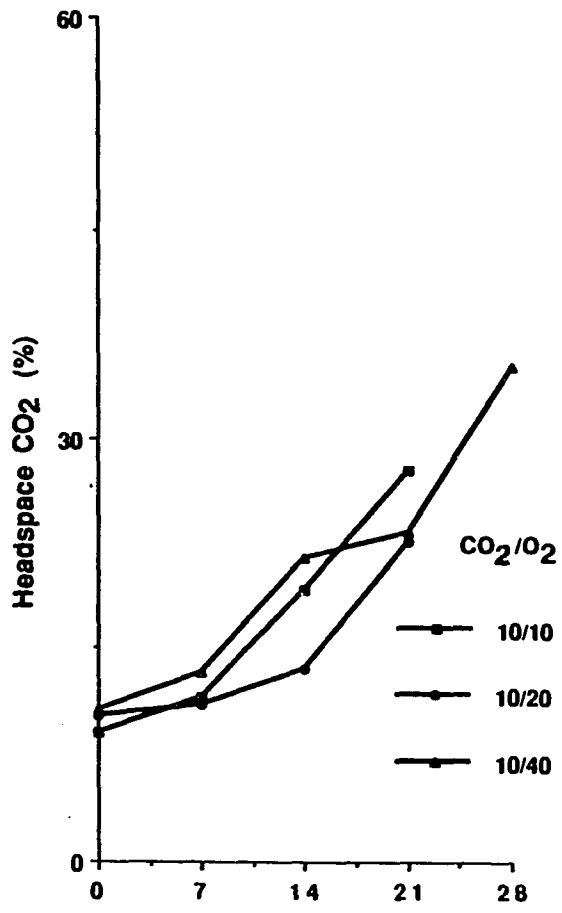
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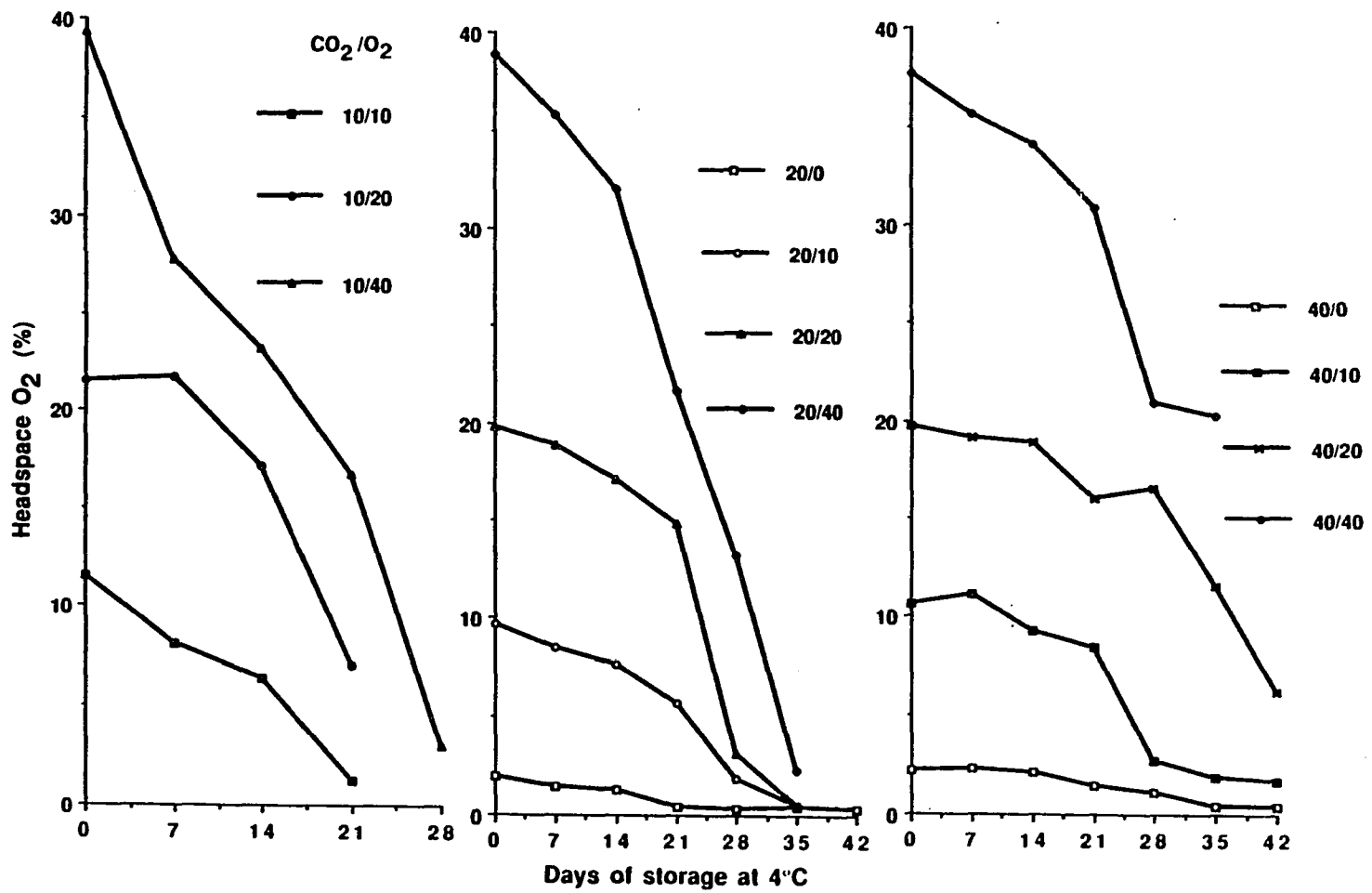
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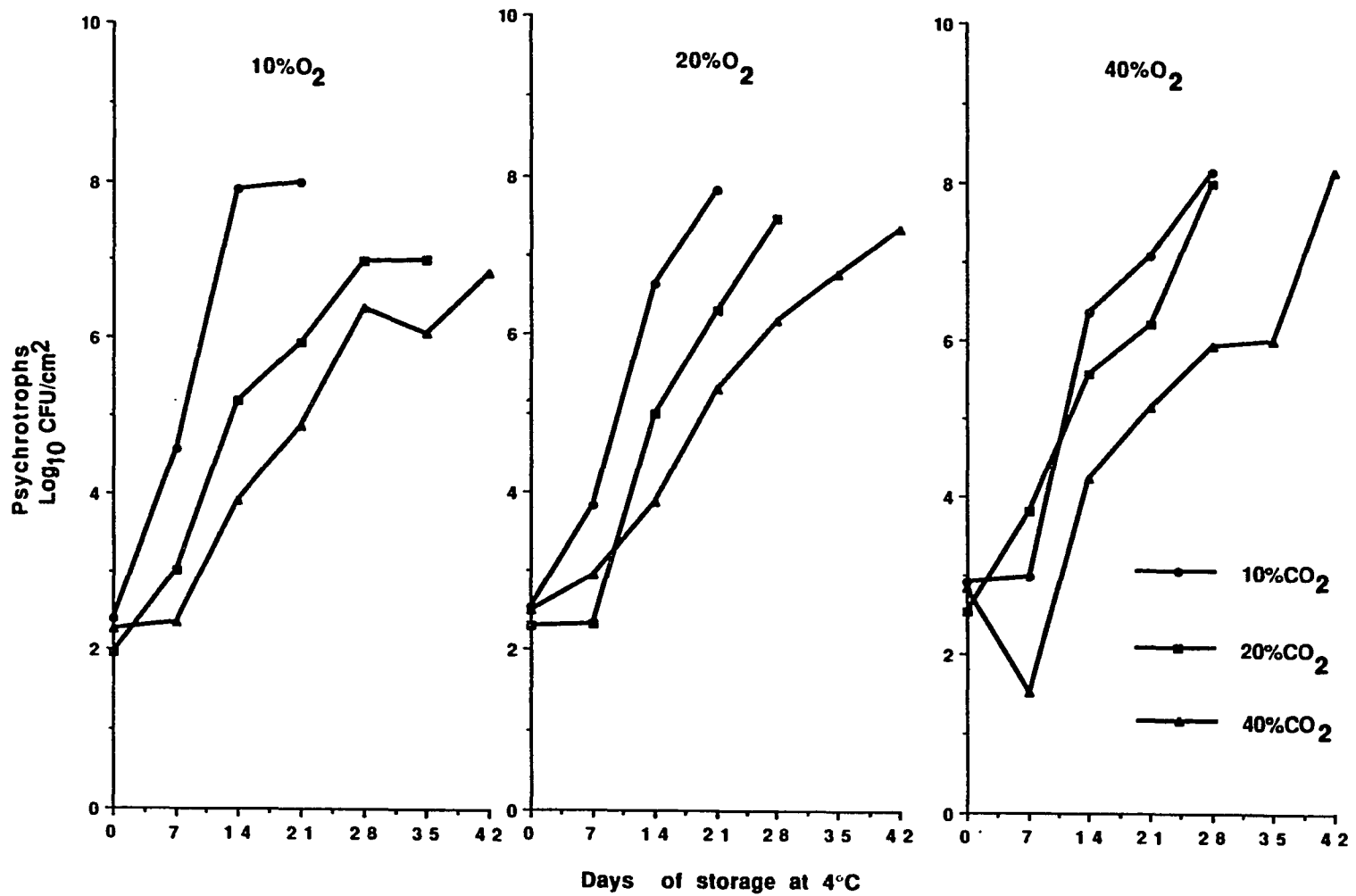
**Fig. 1. Changes in the headspace carbon dioxide concentration of modified gas atmospheres for chops during storage at 4°C**



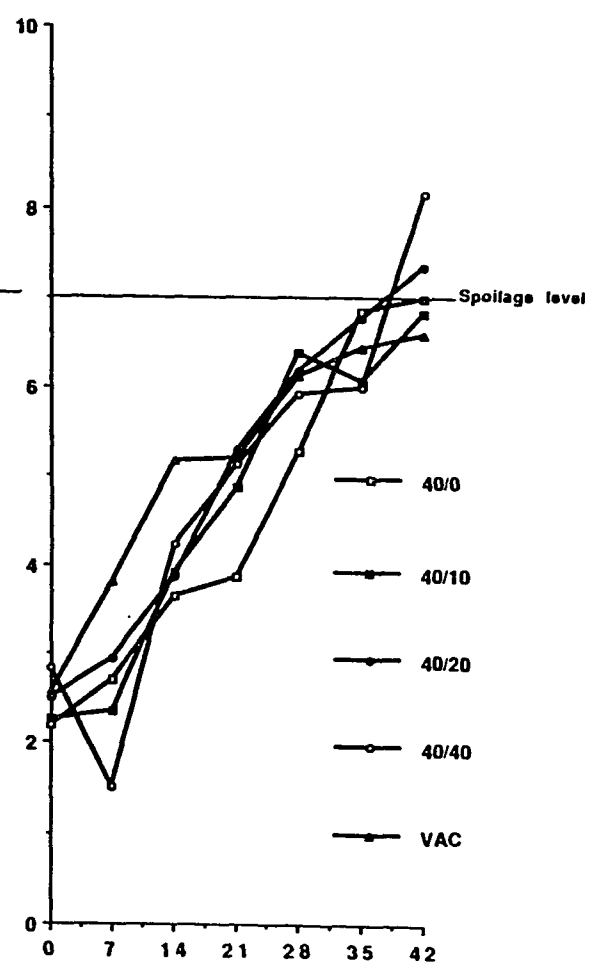
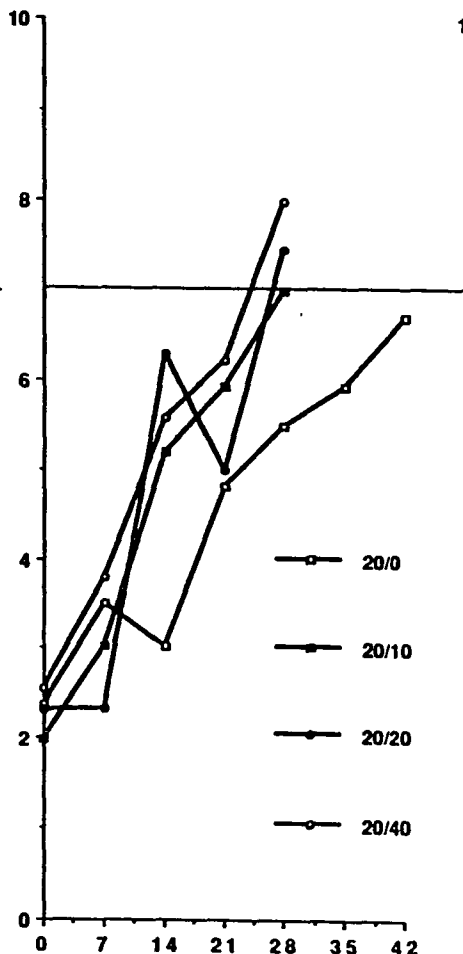
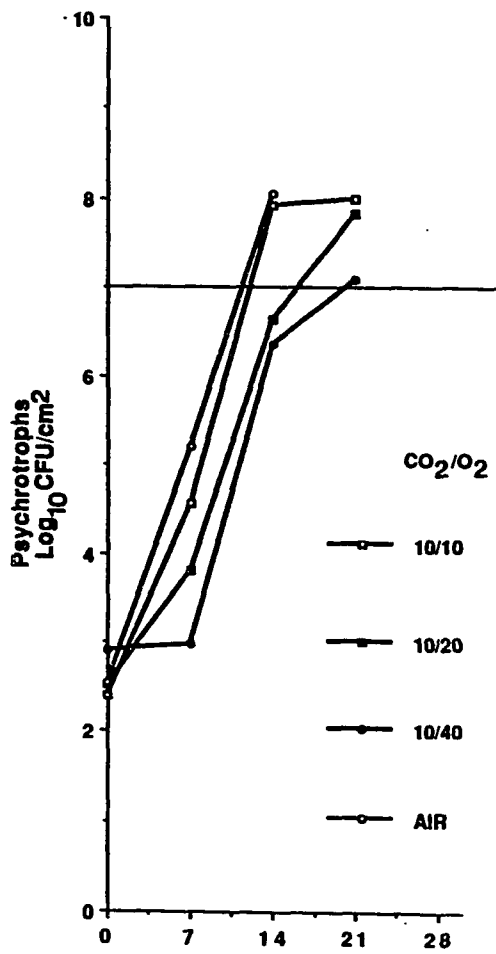
**Fig. 2. Changes in the headspace oxygen concentration of modified gas atmospheres for chops during storage at 4°C**



**Fig. 3.** Influence of concentration of CO<sub>2</sub> and O<sub>2</sub> on numbers of psychrotrophic bacteria in chops stored at 4°C.



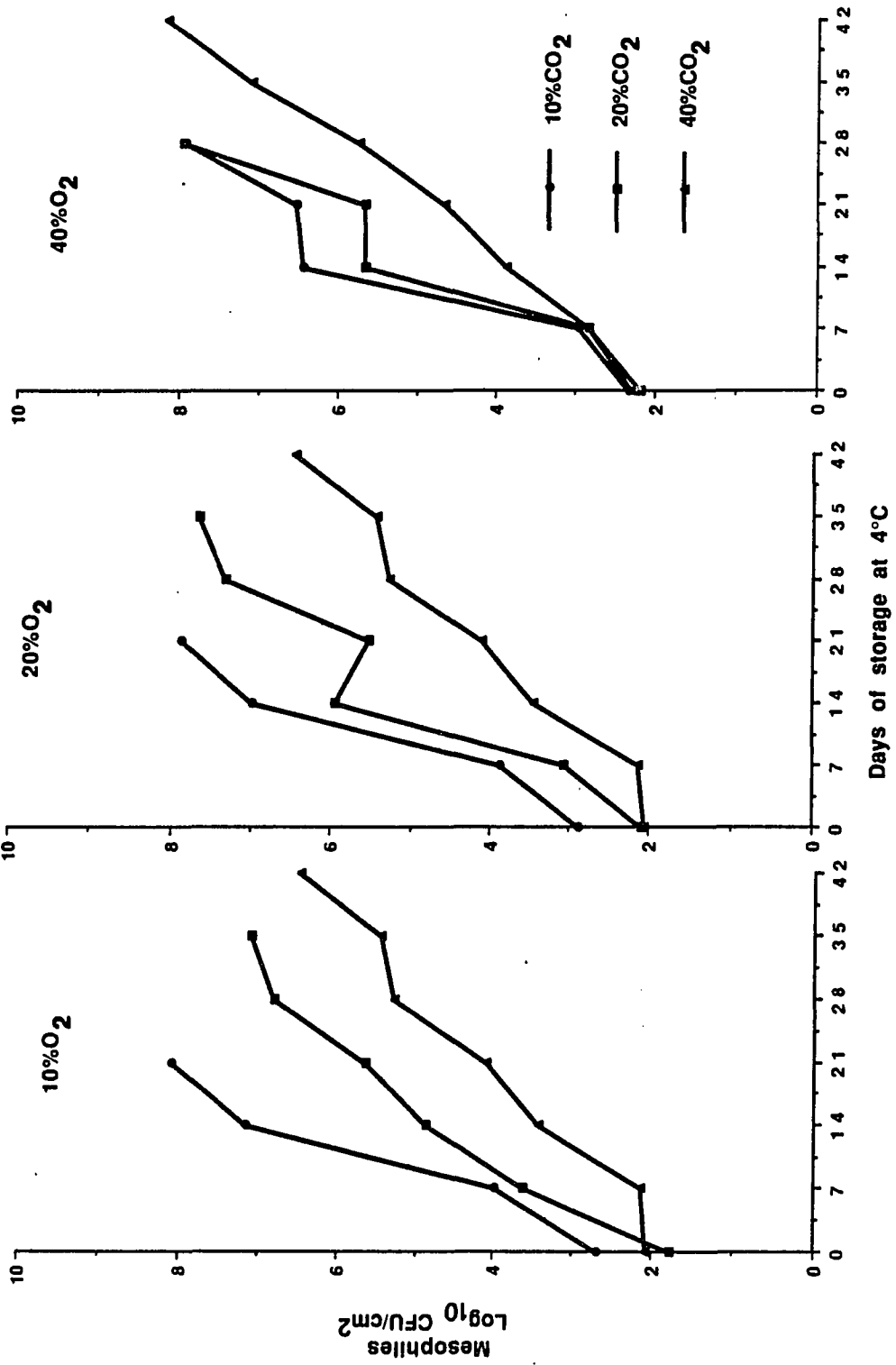
**Fig. 4. Psychrotrophic bacterial populations in chops packaged under different atmospheres during storage at 4°C**



Days of storage at 4°C



**Fig. 5. Influence of concentration of CO<sub>2</sub> and O<sub>2</sub> on numbers of mesophilic bacteria in chops stored at 4°C**



**Fig. 6. Mesophilic bacterial populations in chops packaged under different atmospheres during storage at 4°C**

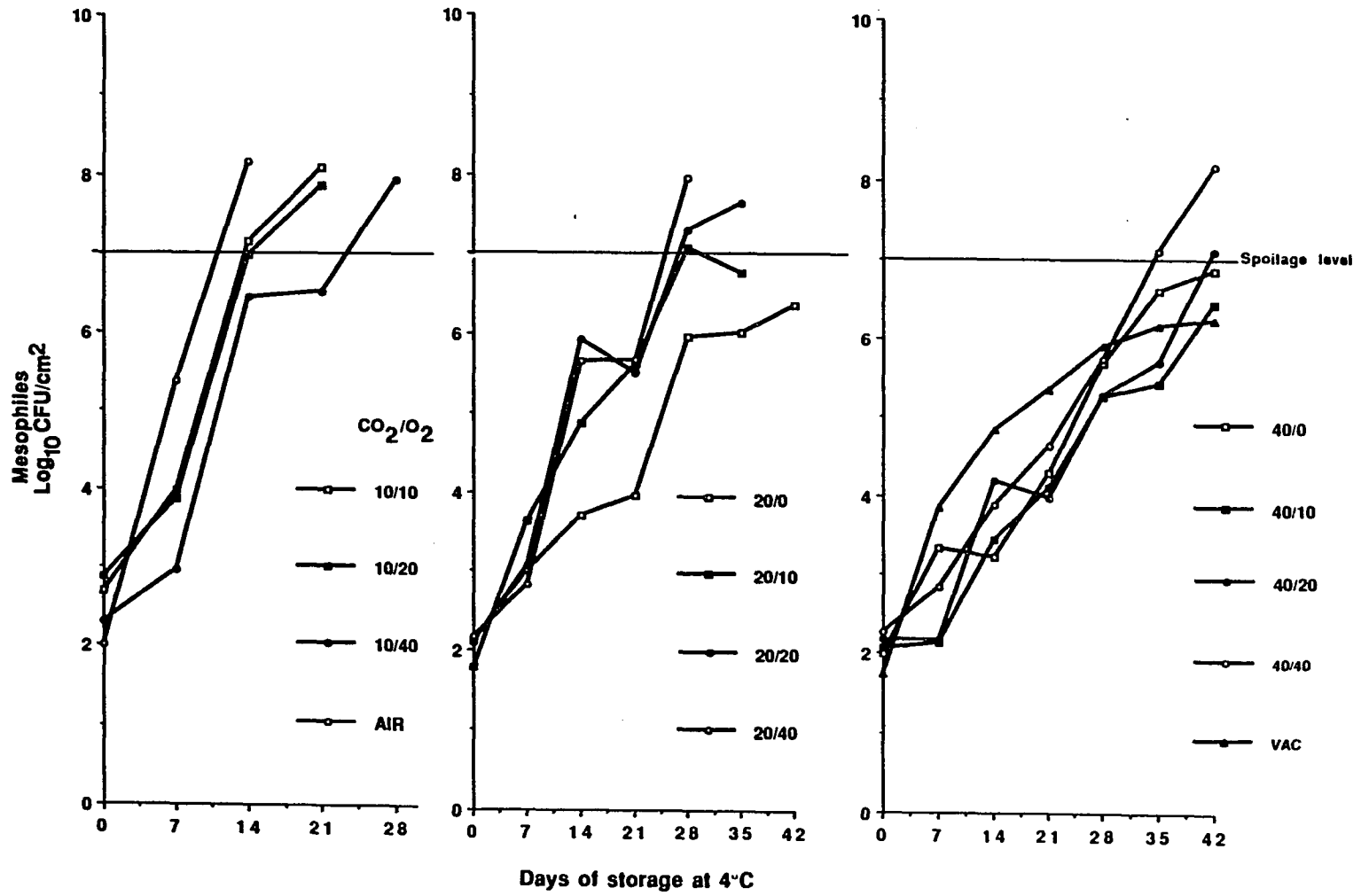


Fig. 7. Influence of concentration of CO<sub>2</sub> and O<sub>2</sub> on numbers of *Enterobacteriaceae* in chops stored at 4°C

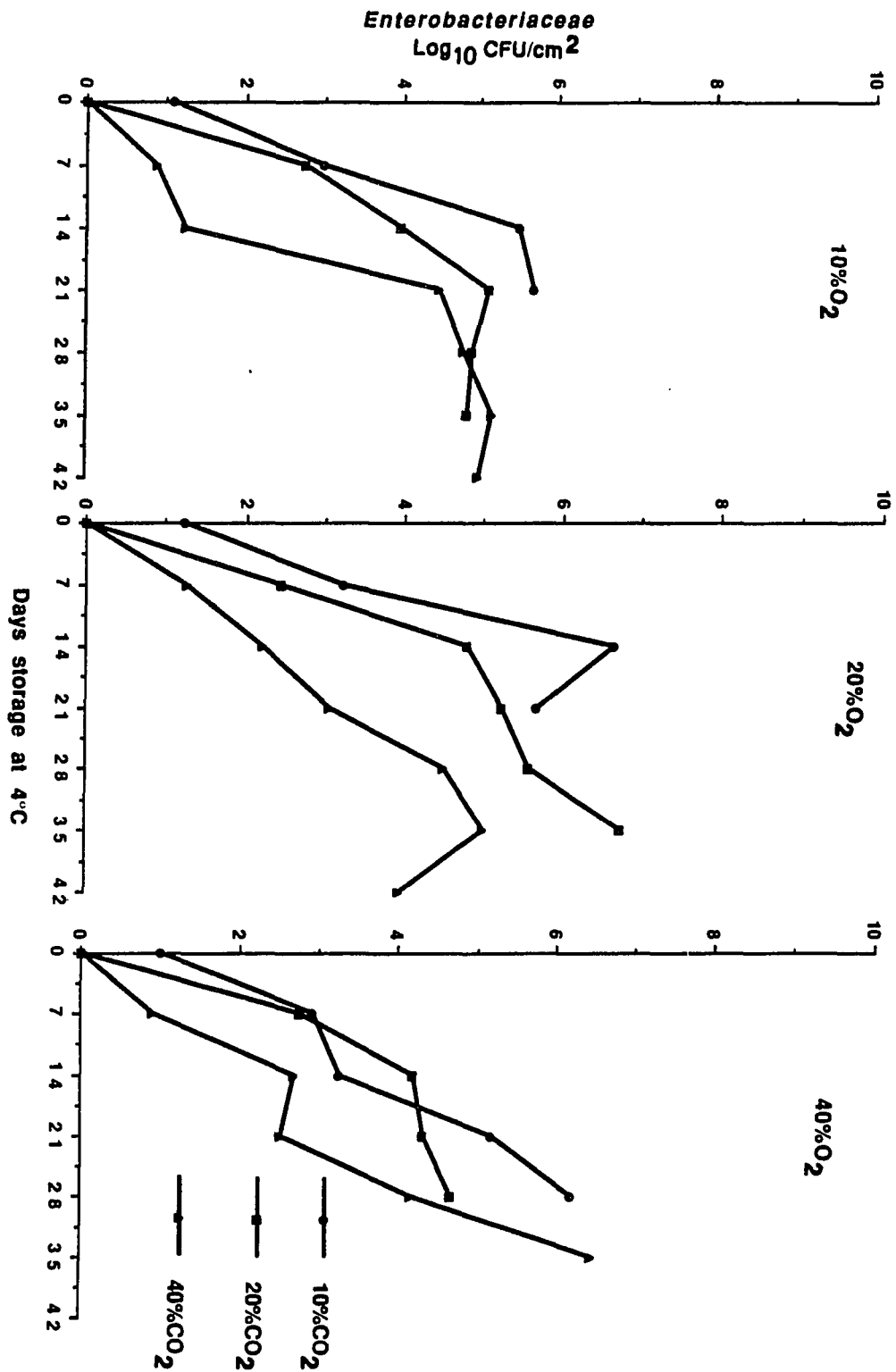
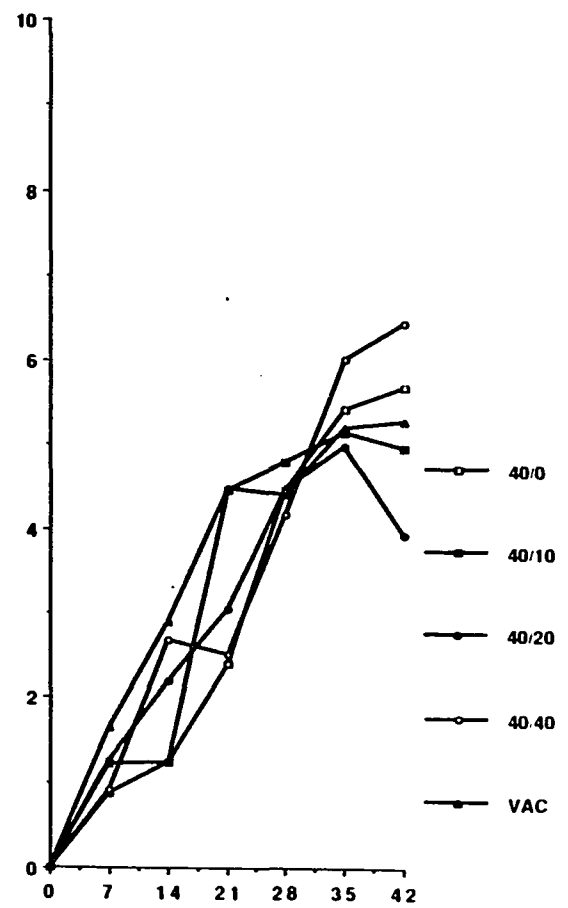
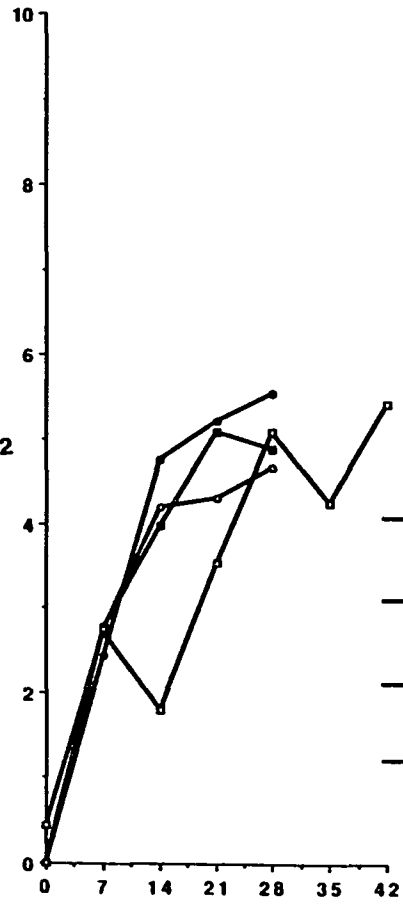
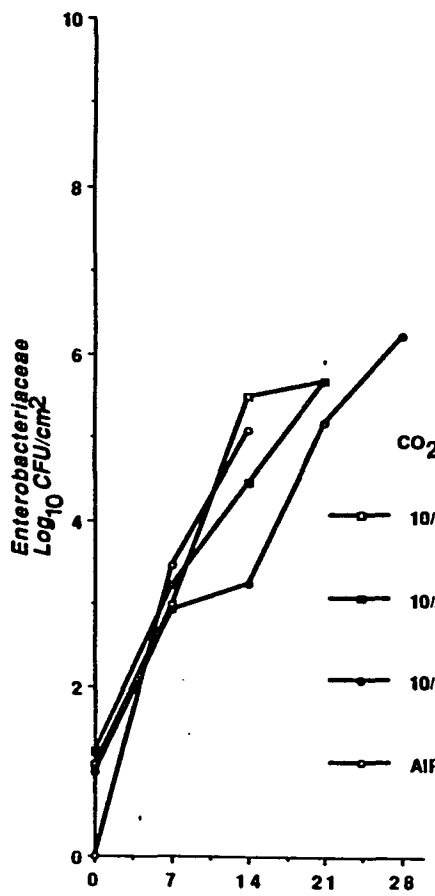


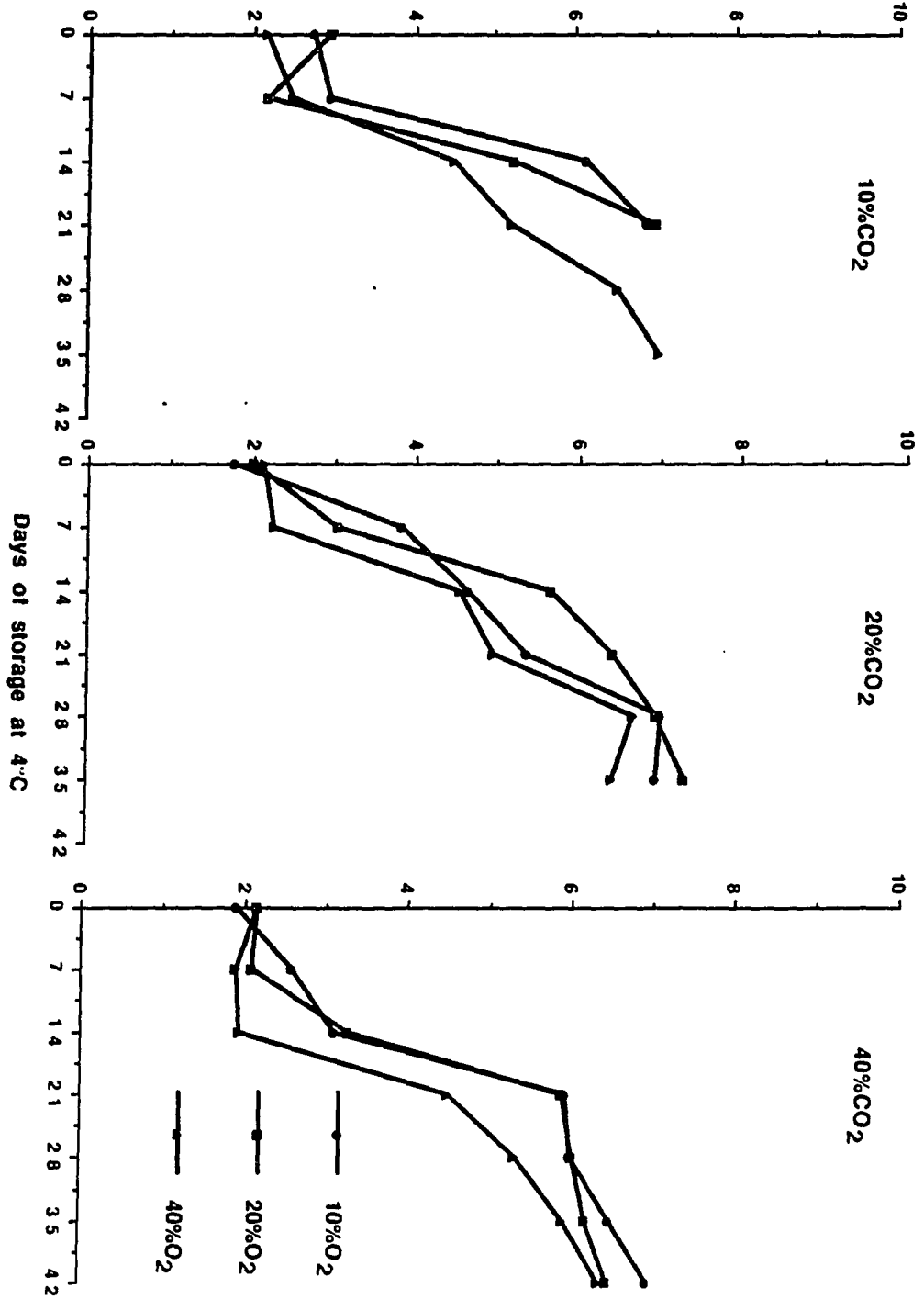
Fig. 8. Numbers of *Enterobacteriaceae* in chops packaged under different atmospheres during storage at 4°C



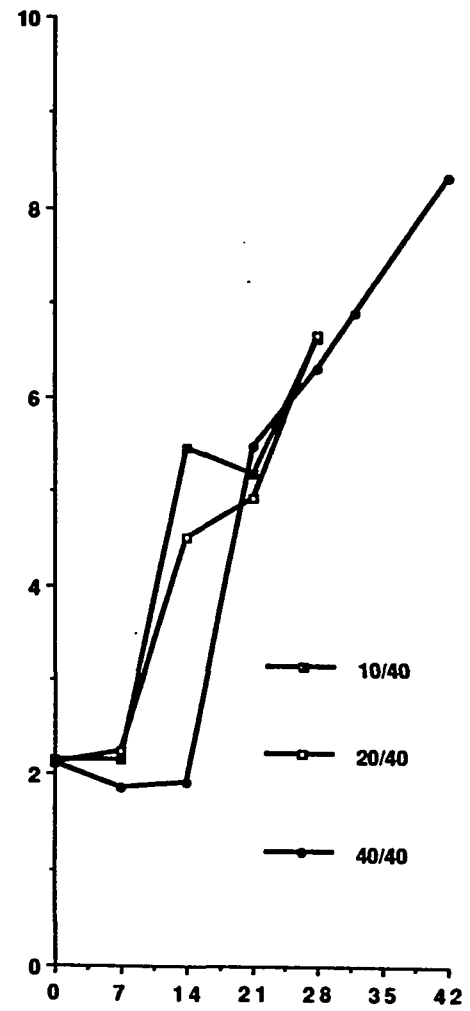
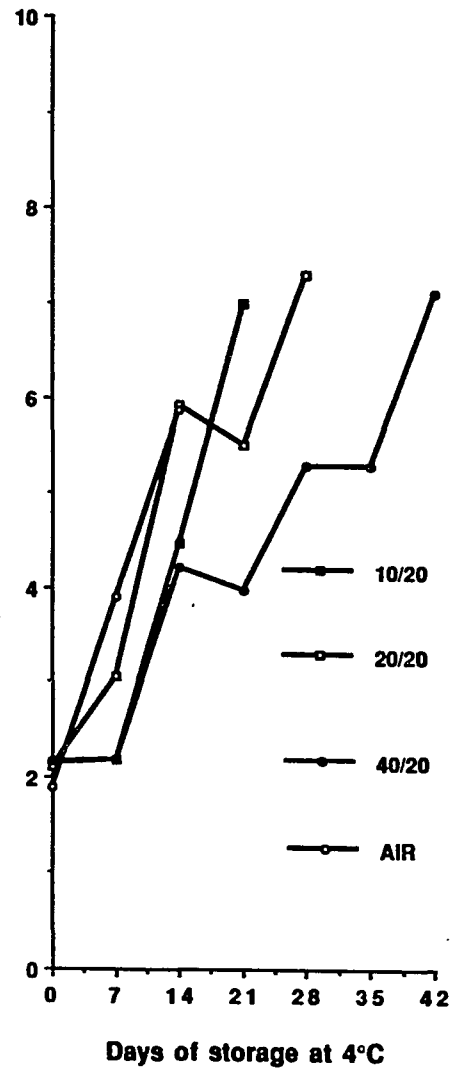
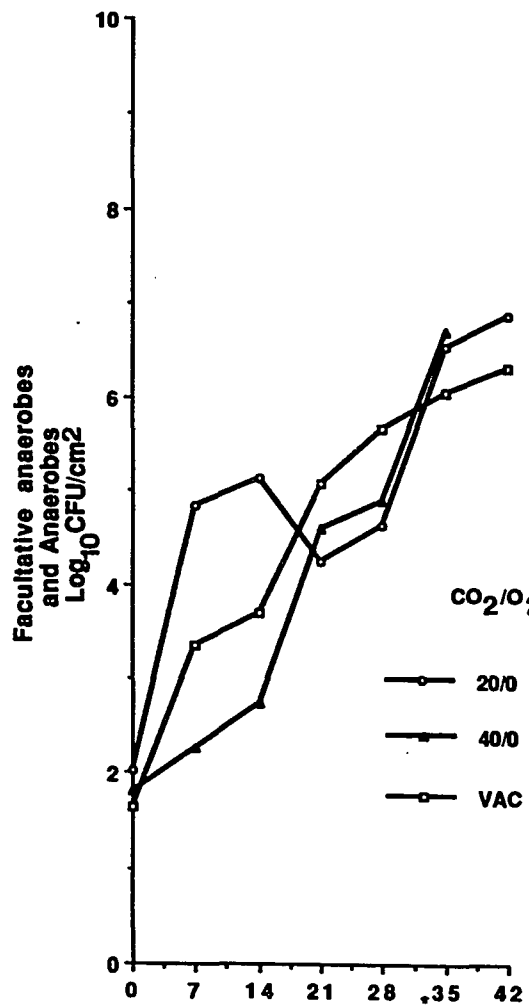


**Fig. 9. Influence of concentration of CO<sub>2</sub> and O<sub>2</sub> on numbers of facultative anaerobic and anaerobic bacteria in chops stored at 4°C**

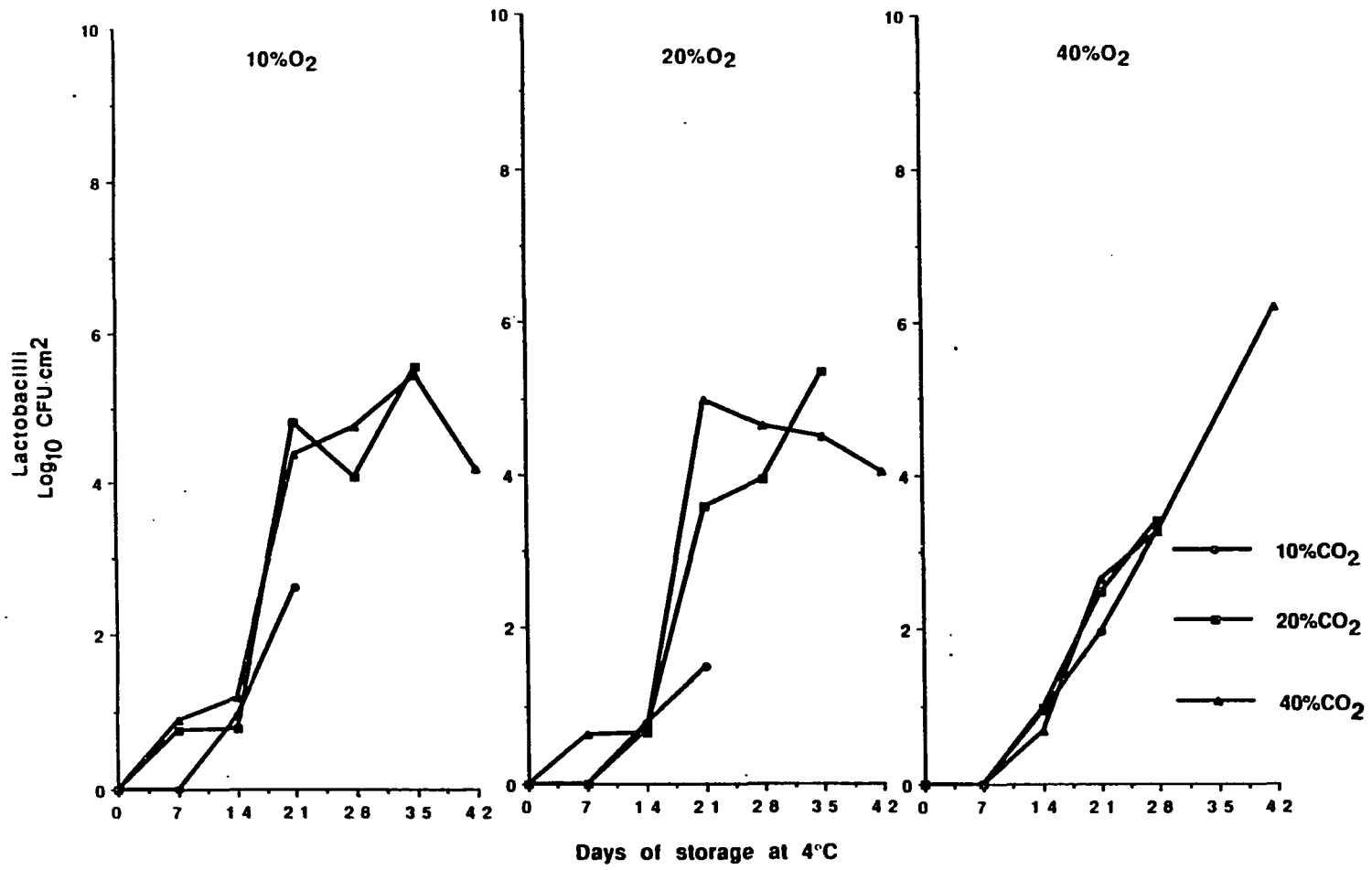
Facultative anaerobes  
and Anaerobes  
Log<sub>10</sub> CFU/cm<sup>2</sup>



**Fig. 10. Facultative anaerobic and anaerobic bacterial populations in chops packaged under different atmospheres during storage at 4°C**



**Fig. 11. Influence of concentration of CO<sub>2</sub> and O<sub>2</sub> on numbers of lactic acid bacteria in chops stored at 4°C**



**Fig. 12. Numbers of lactic acid bacteria in chops packaged under different atmospheres during storage at 4°C**

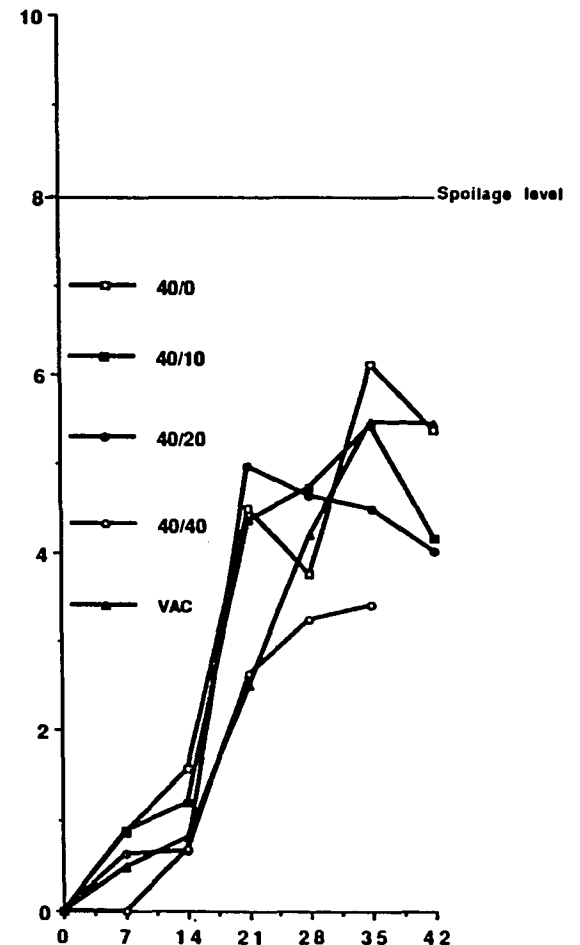
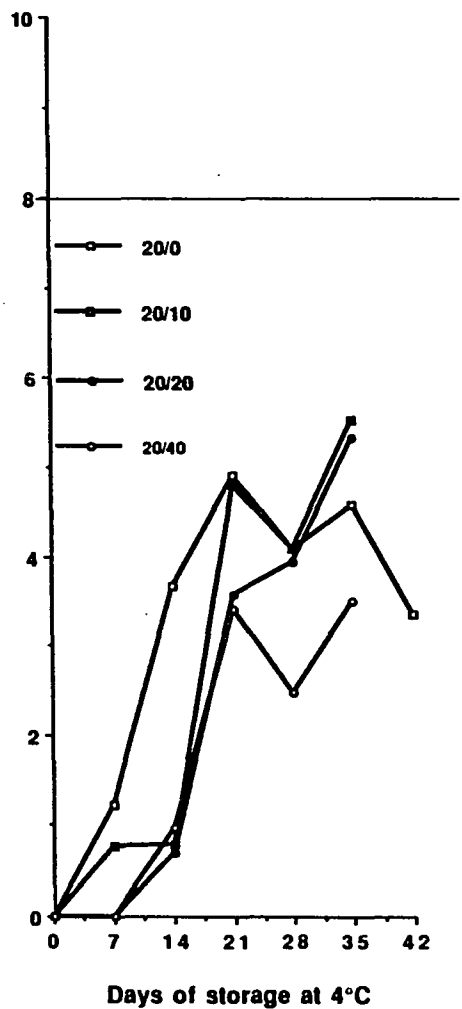
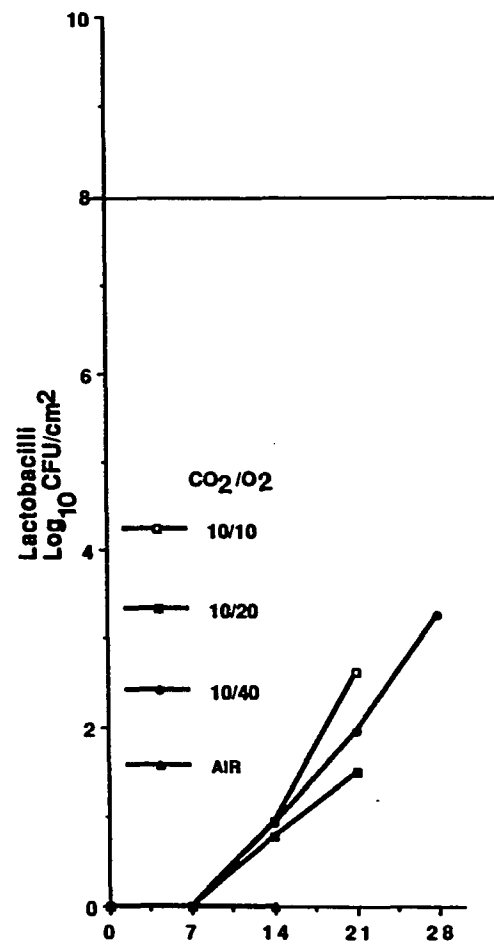




Fig. 13. Influence of concentration of CO<sub>2</sub> and O<sub>2</sub> on numbers of *Brochotrix thermosphacta* in chops stored at 4°C

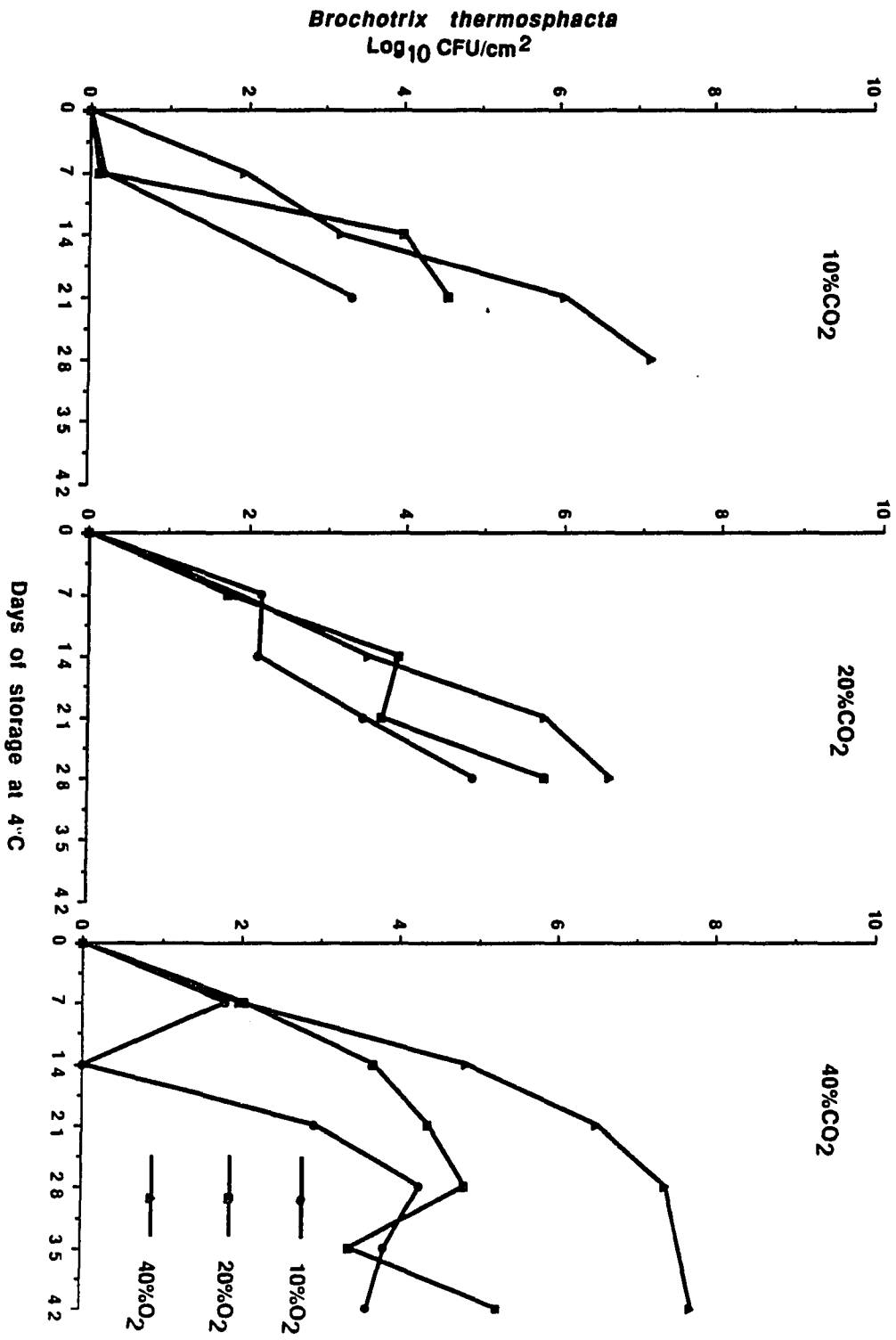
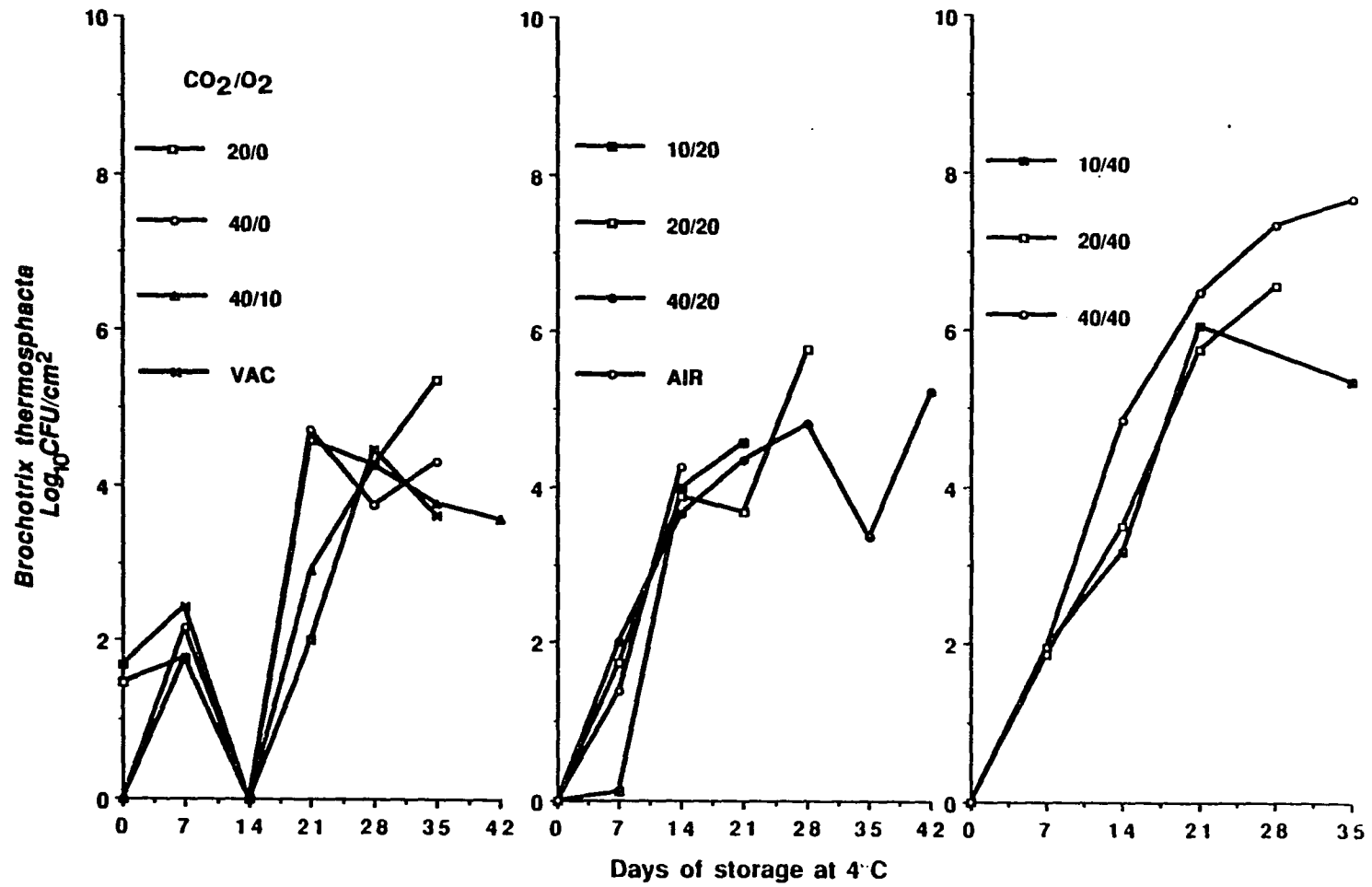
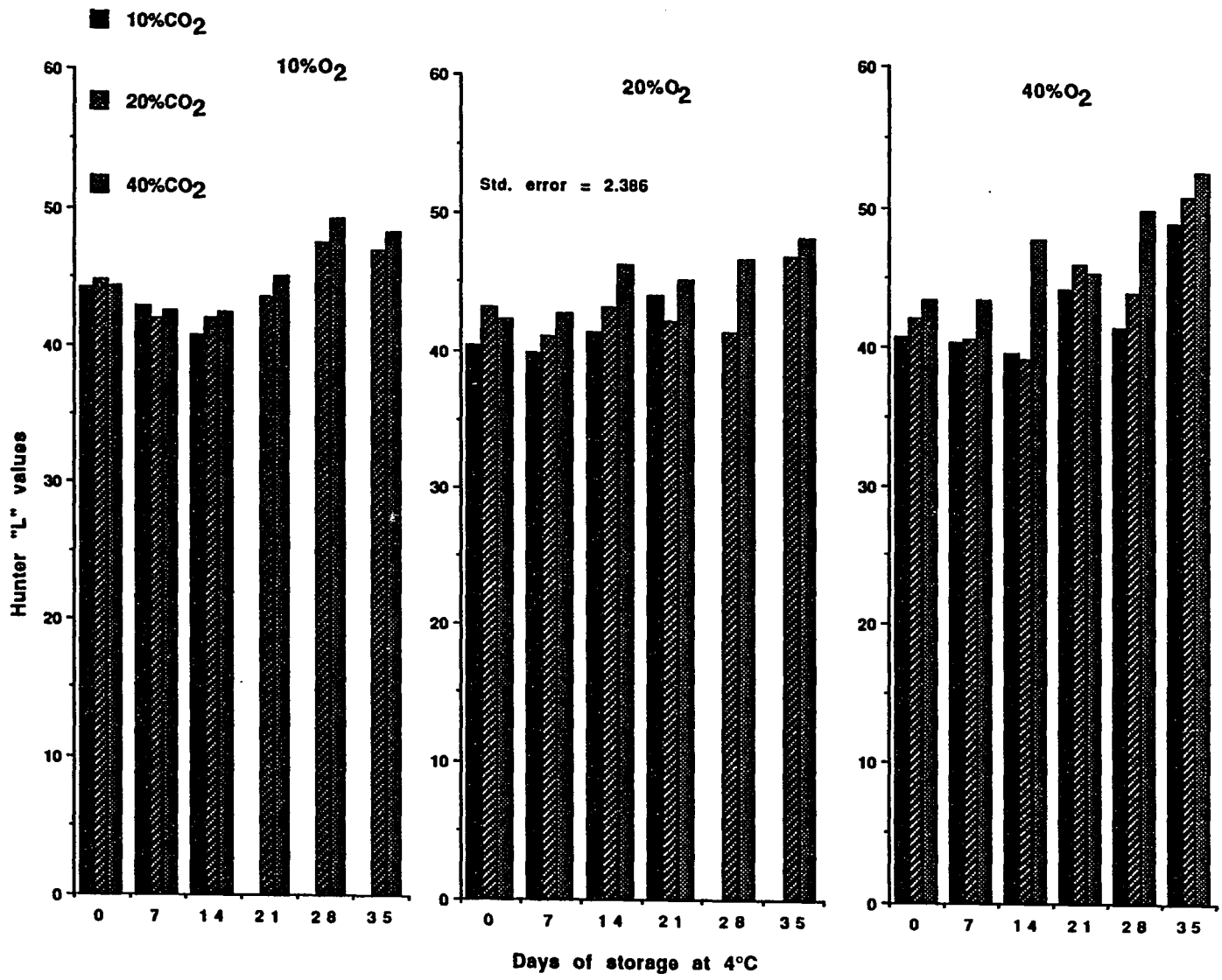


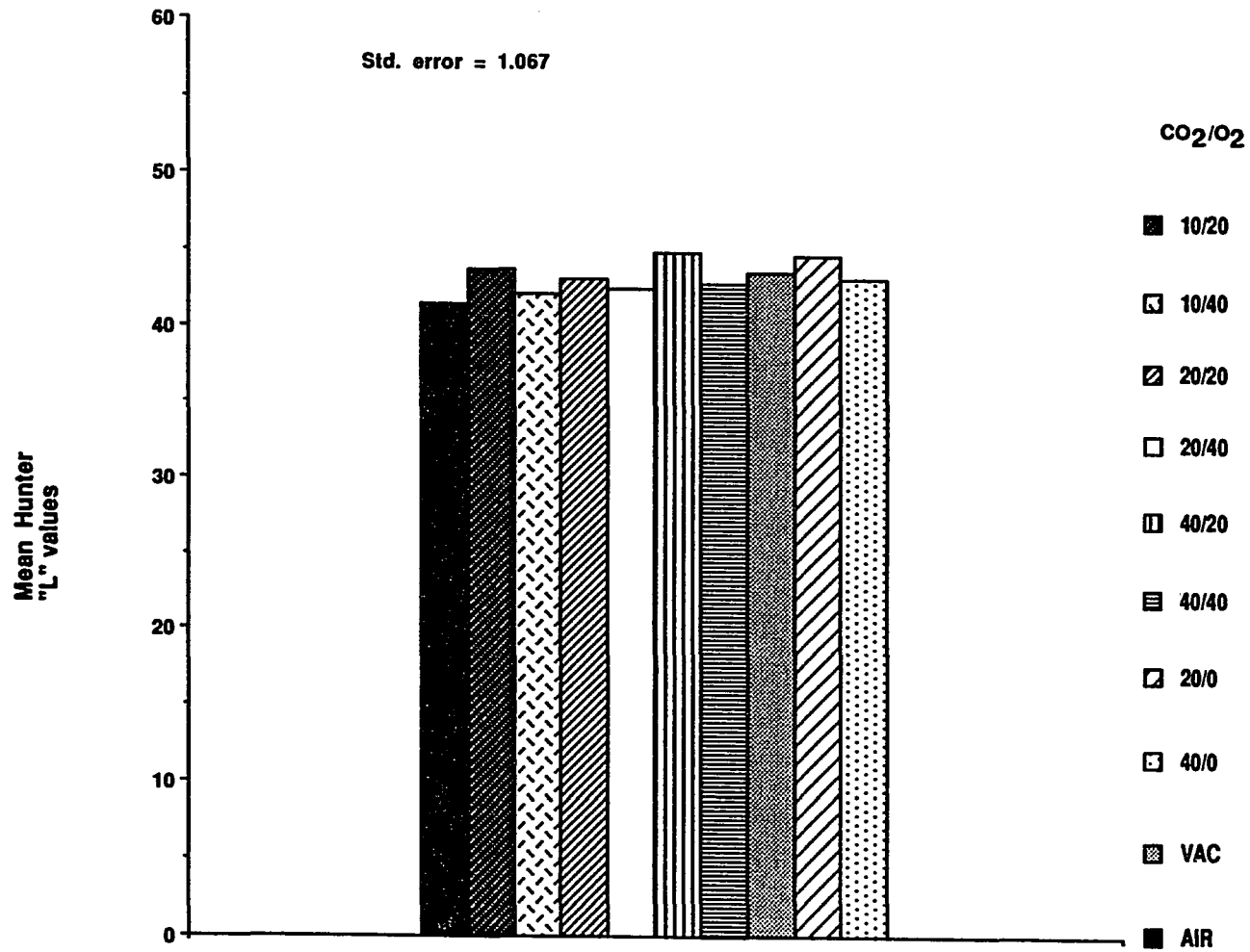
Fig. 14. Numbers of *Brochotrix thermosphacta* in chops packaged under different atmospheres during storage at 4°C



**Fig. 15. Influence of concentration of CO<sub>2</sub> and O<sub>2</sub> on Hunter "L" values of chops stored at 4°C**

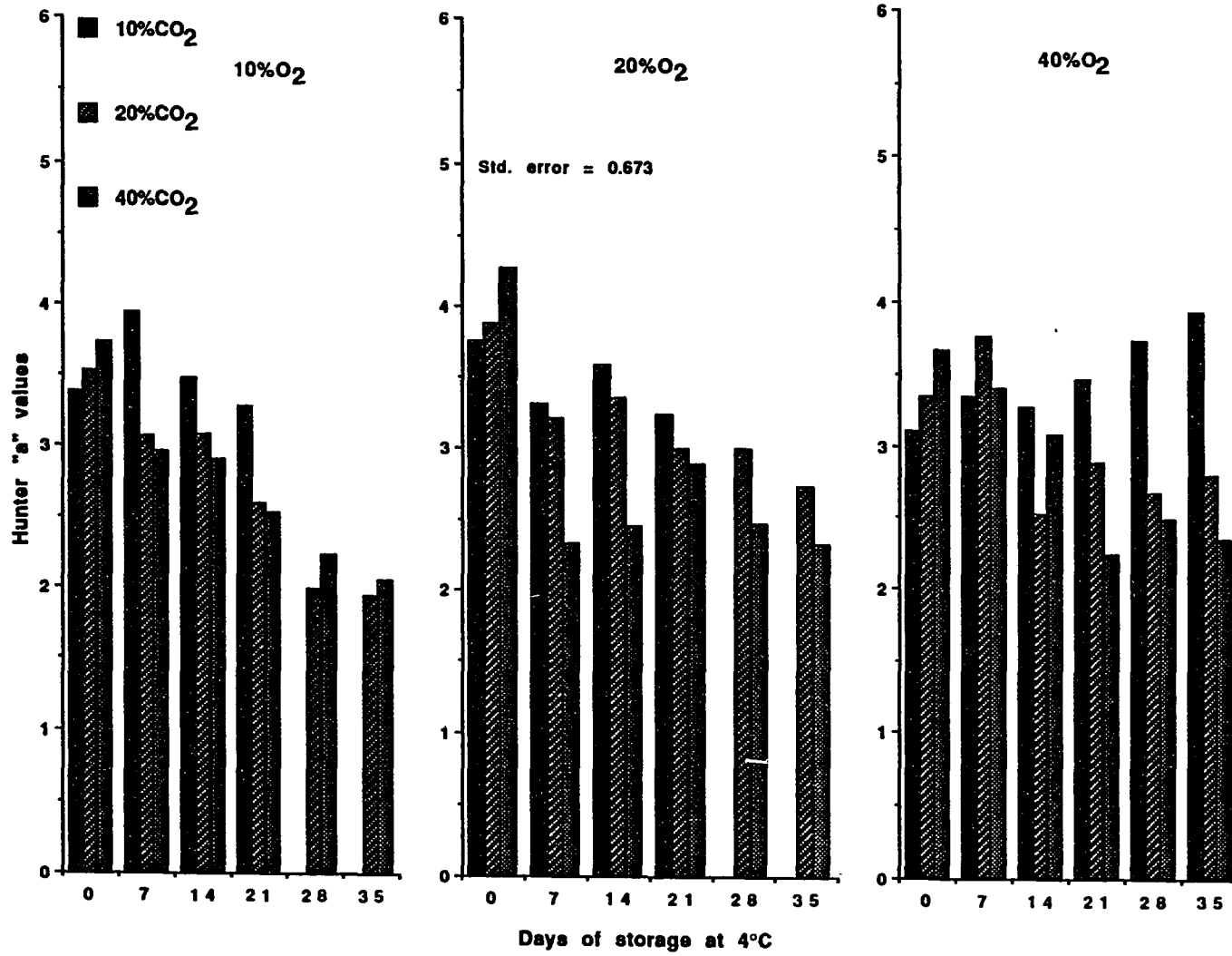


**Fig. 16. Mean Hunter "L" values of chops packaged under selected atmospheres during storage at 4°C**

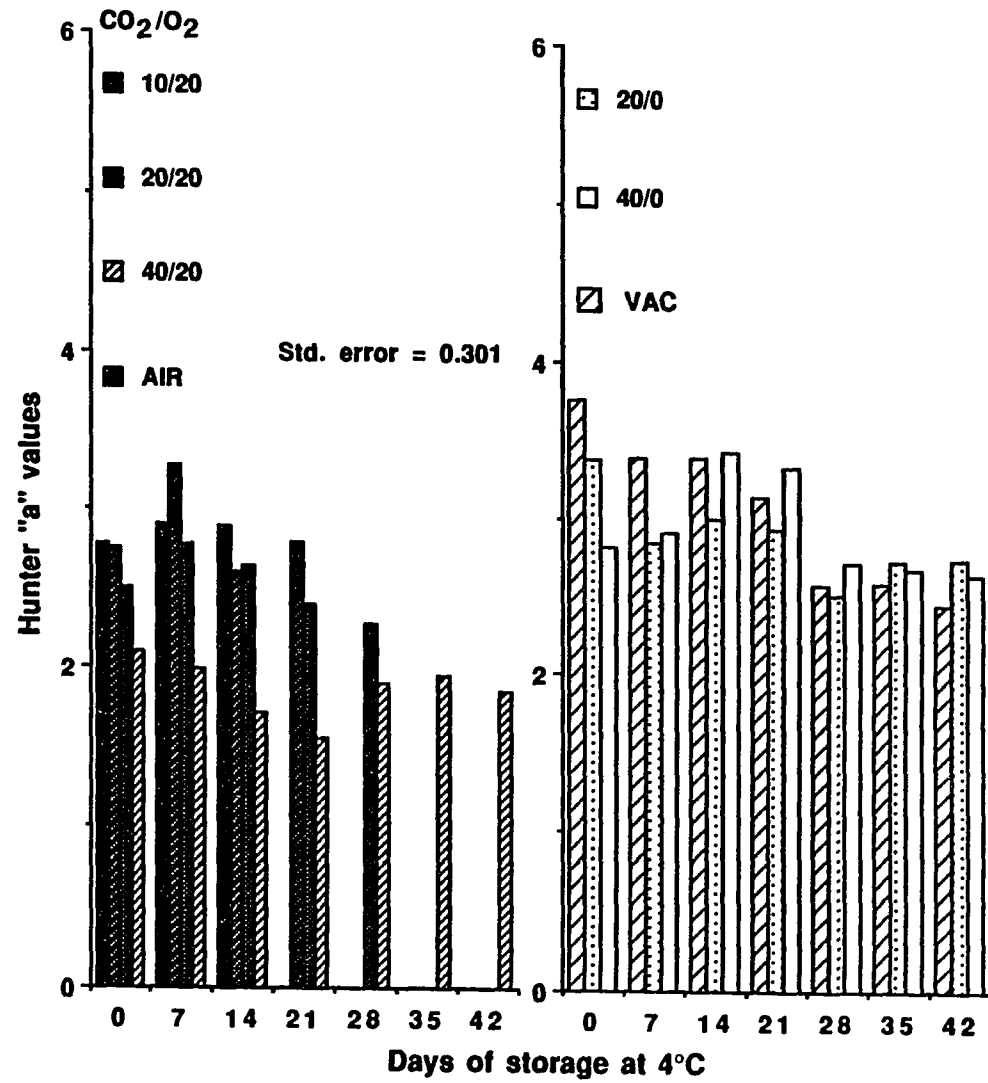




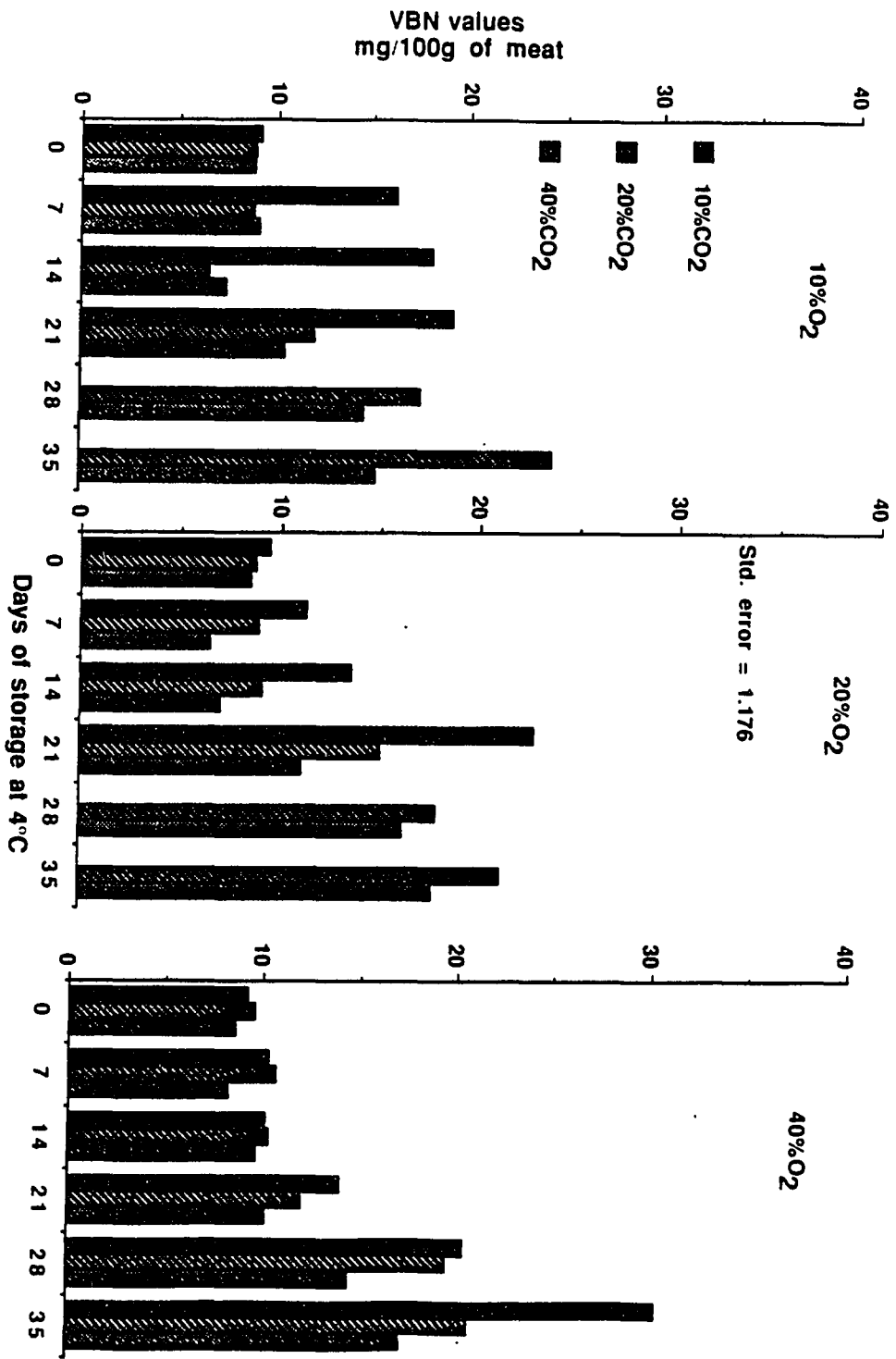
**Fig 17. Influence of concentration of CO<sub>2</sub> and O<sub>2</sub> on Hunter "a" values of chops stored at 4°C**



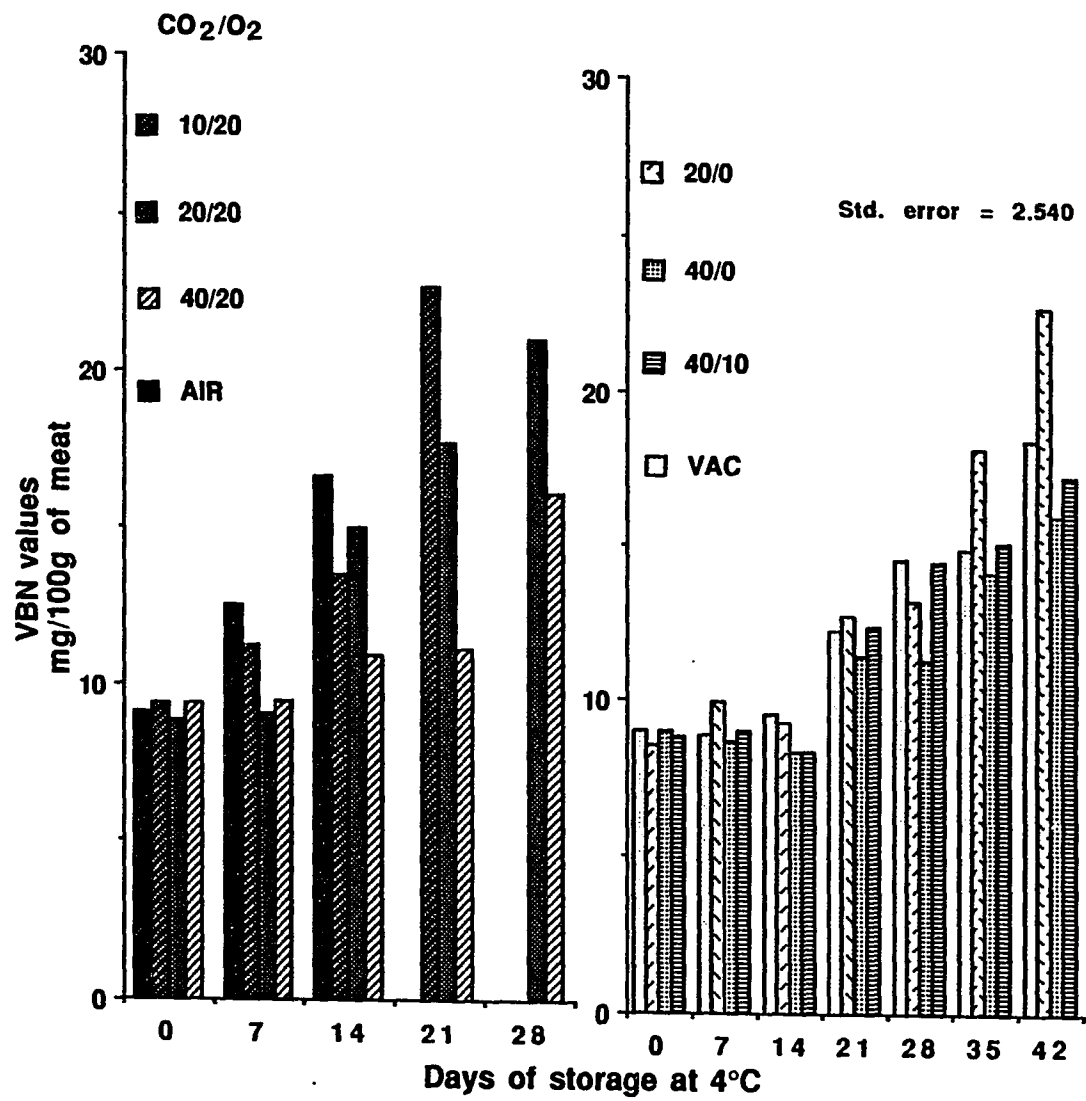
**Fig. 18. Mean Hunter "a" values of chops packaged under selected atmospheres during storage at 4°C**



**Fig. 19. Influence of concentration of CO<sub>2</sub> and O<sub>2</sub> on volatile basic nitrogen (VBN) values of chops stored at 4°C**



**Fig. 20. Volatile basic nitrogen (VBN) measured in chops packaged under selected atmospheres during storage at 4°C**





**Fig. 21. Relationship between  $\log_{10}$  cfu/cm<sup>2</sup> numbers of psychrotrophic bacteria and volatile basic nitrogen (VBN) in chops stored at 4°C**

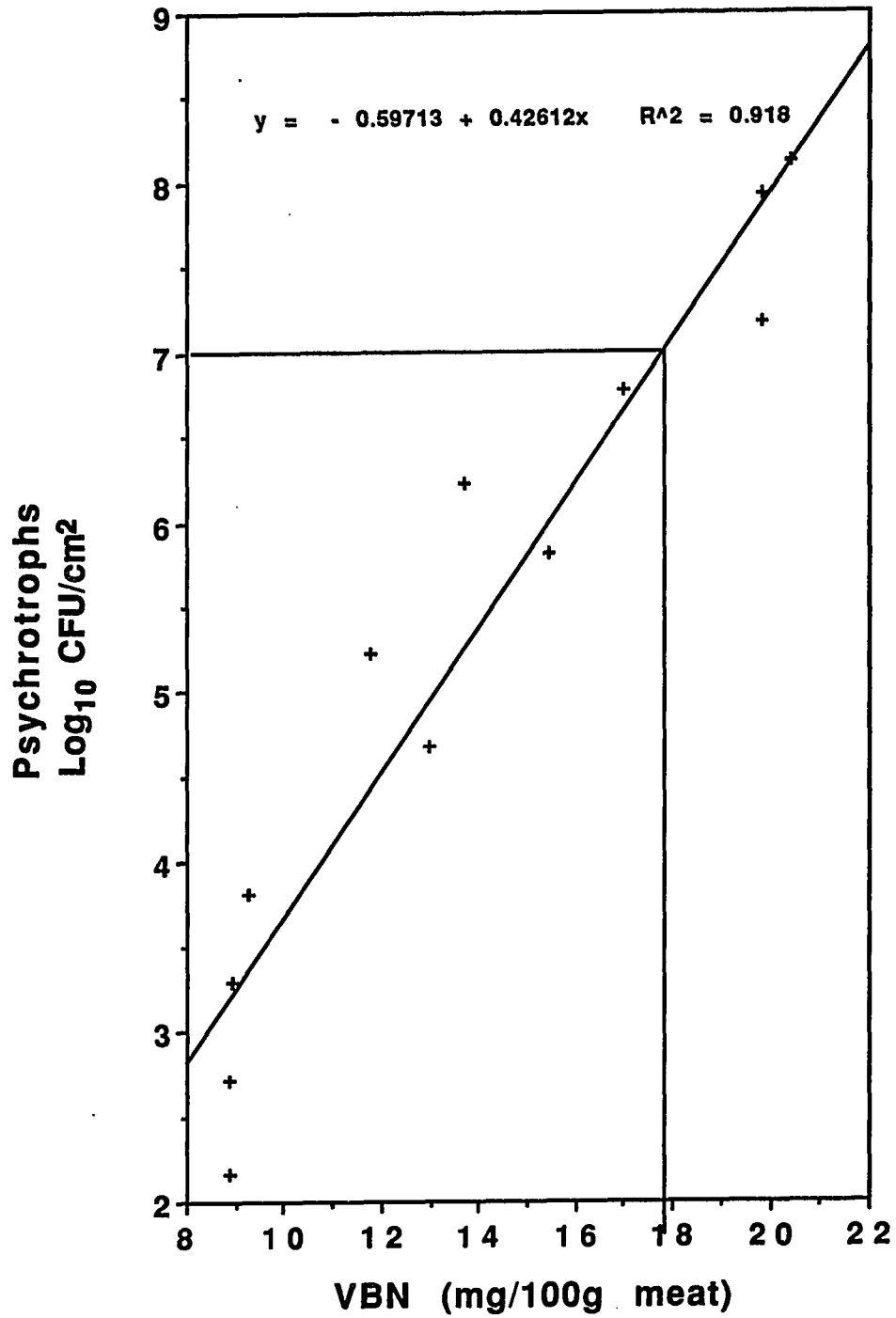


Fig. 22. Influence of concentration of CO<sub>2</sub> and O<sub>2</sub> on percent purge for chops stored at 4°C

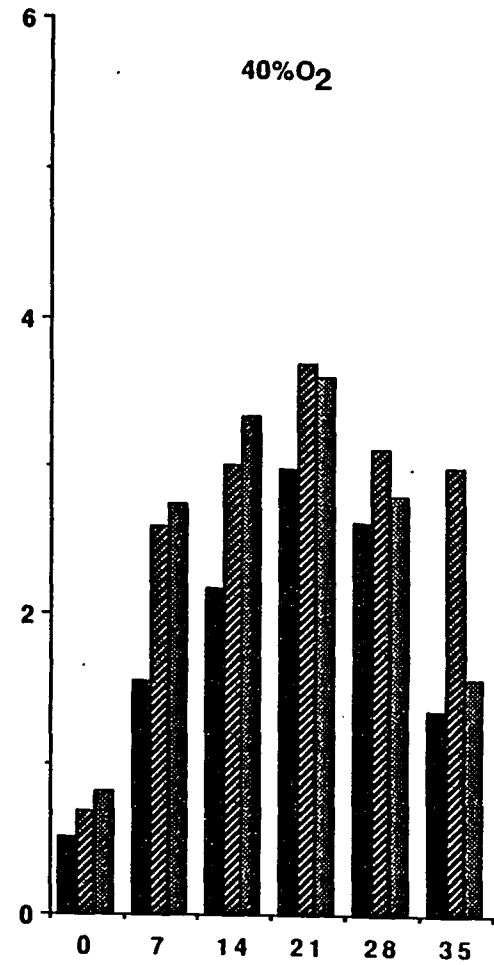
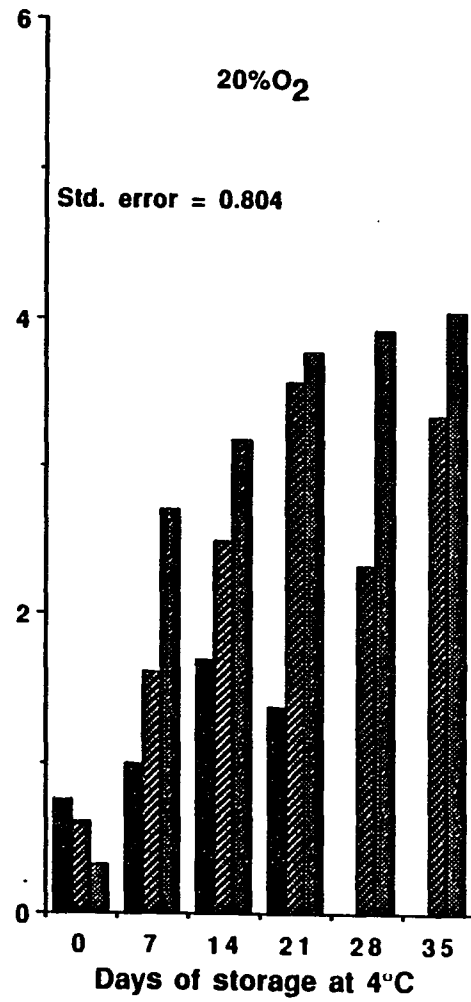
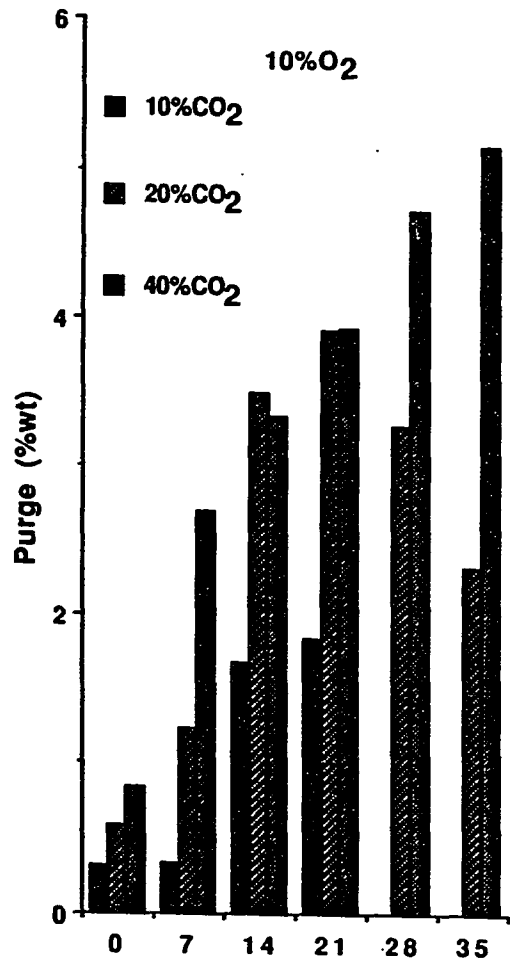


Fig. 23. Mean percent purge for chops packaged under vacuum and selected gas atmospheres during storage at 4°C

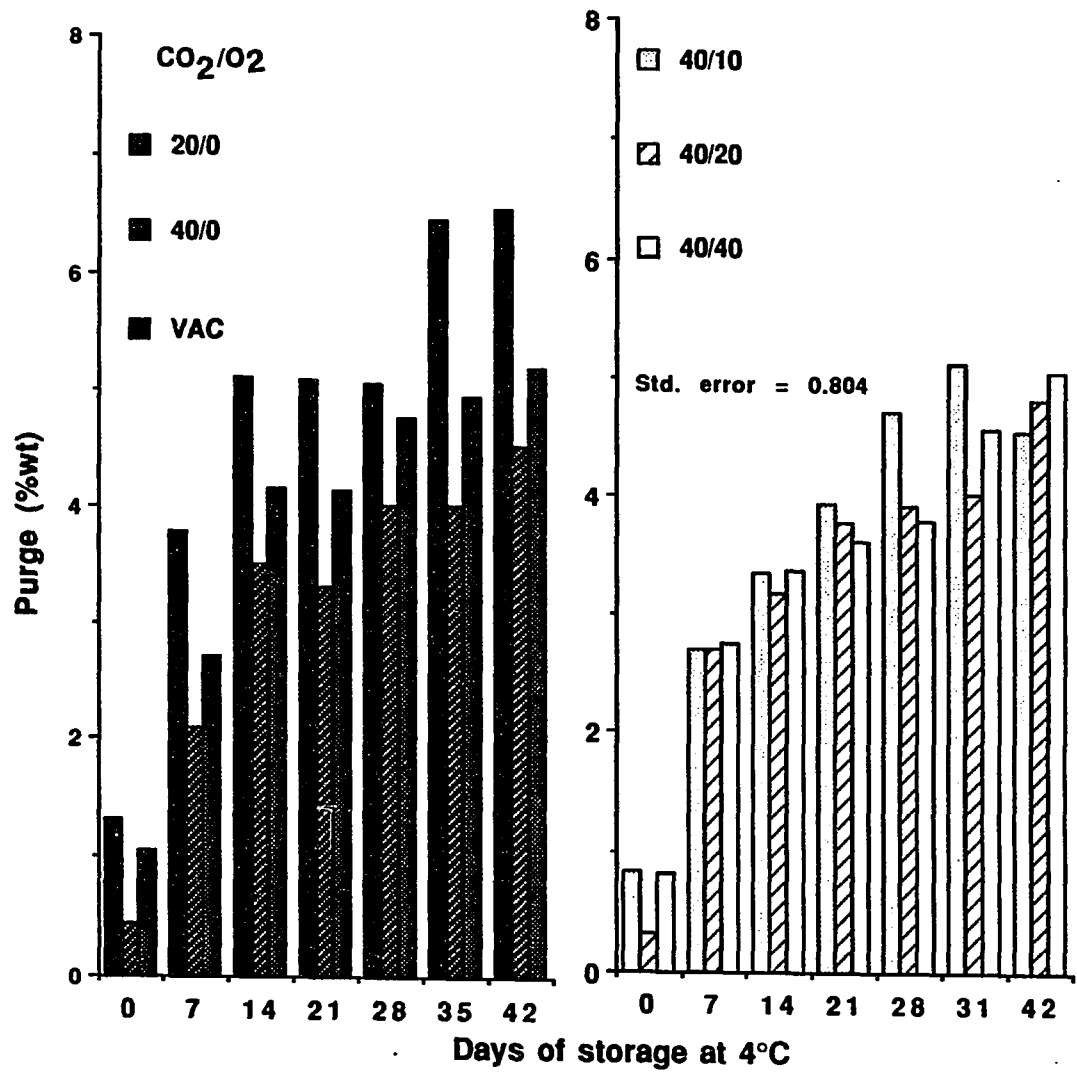


Table 1. Various combinations of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> used to package the pork chops

TRT <sup>a</sup>	10/10	10/20	10/40	20/0	20/10	20/20	20/40	40/0	40/10	40/20	40/40
Gas (%)											
CO <sub>2</sub>	10	10	10	20	20	20	20	40	40	40	40
O <sub>2</sub>	10	20	40	0	10	20	40	0	10	20	40
N <sub>2</sub>	80	70	50	80	70	60	40	60	50	40	20

<sup>a</sup>TRT = Treatments (first value is CO<sub>2</sub> concentration; second value is O<sub>2</sub> concentration, balanced with nitrogen)

Table 2. Mean<sup>a</sup> surface pH values for fresh pork packaged in different atmospheres and stored at 4°C

Atmospheres (CO <sub>2</sub> /O <sub>2</sub> ) <sup>b</sup>	Surface pH
10/10	6.09 <sup>Y</sup>
10/20	6.02 <sup>Y</sup>
10/40	5.87 <sup>XY</sup>
20/0	5.79 <sup>X</sup>
20/10	5.82 <sup>X</sup>
20/20	6.03 <sup>Y</sup>
20/40	5.90 <sup>XY</sup>
40/0	5.83 <sup>X</sup>
40/10	5.79 <sup>X</sup>
40/20	5.79 <sup>X</sup>
40/40	5.87 <sup>X</sup>
Vacuum	5.76 <sup>X</sup>
Air	5.74 <sup>X</sup>
Standard. error	0.056

<sup>a</sup>Means of three replications averaged through time; mean initial surface pH value = 5.76.

<sup>b</sup>Balanced with nitrogen.

<sup>XY</sup>Treatments with the same superscript are not significantly different at P=0.05.



Table 3. Identity of selected isolates from plates used to enumerate *Enterobacteriaceae* from pork chops packaged under various atmospheres and held at 4°C

Atmospheres (CO <sub>2</sub> /O <sub>2</sub> ) <sup>a</sup>	Days <sup>c</sup>	Genera <sup>b</sup> and number of isolates												
		AC	E	EC	HA	K	P	PS	SE	SH	YE	V	U	T
10/10	7	2	7	0	0	0	9	0	2	0	2	0	8	30
	14	1	1	0	3	0	9	0	11	0	2	0	3	30
10/20	7	2	6	0	0	0	8	0	1	6	2	0	5	30
	14	3	0	0	6	0	9	0	5	0	1	0	6	30
10/40	7	6	7	0	0	0	11	0	0	0	2	0	4	30
	14	8	3	0	1	3	14	0	0	0	1	0	3	30
	21	1	1	0	10	0	8	0	0	0	1	0	9	30
20/0	7	1	2	1	1	1	3	0	10	0	2	0	4	25 <sup>d</sup>
	14	1		0	4	0	2	0	3	0	2	1	2	20 <sup>d</sup>
	21	4	0	0	9	0	2	0	4	0	2	1	3	25 <sup>d</sup>
	28	1	2	0	1	0	1	0	6	1	1	1	6	20 <sup>d</sup>
20/10	7	1	5	0	2	0	2	0	11	0	2	0	7	30
	14	1	1	0	2	0	6	0	9	0	1	7	3	30
	21	1	1	0	4	0	11	0	4	0	2	0	7	30
	28	1	0	0	2	0	4	0	1	2	1	0	4	15 <sup>d</sup>
20/20	7	1	4	0	2	0	1	0	13	0	1	0	3	25 <sup>d</sup>
	14	1	0	0	3	0	5	0	9	9	1	1	1	30
	21	1	1	0	2	0	8	0	5	0	2	0	1	20 <sup>d</sup>
	28	1	0	0	1	0	10	0	6	0	1	4	2	25 <sup>d</sup>
20/40	7	1	2	0	3	0	8	0	4	0	2	0	0	20 <sup>d</sup>
	14	1	2	0	0	0	11	0	2	0	4	1	4	25 <sup>d</sup>
	21	1	0	0	5	0	10	0	6	0	1	1	1	25 <sup>d</sup>
	28	1	0	0	4	0	10	1	5	0	1	0	3	25 <sup>d</sup>

<sup>a</sup>Balanced with nitrogen; <sup>b</sup>Genus (and species) of isolates; <sup>c</sup>Days of storage at 2-4°C  
AC=Acinetobacter, E=Enterobacter, EC=Escherichia coli; HA=Hafnia alvei; K=Klebsiella; P=Pseudomonas spp.; PS=Plesiomonas shigelloides; SE=Serratia spp. S=Shigella spp.; YE=Yersinia enterocolitica; V=Vibrio spp, U=Number of unidentified isolates ; T=Total number of isolates from three replications; <sup>d</sup>Less than 10 colonies per replication were present for isolation or cultures did not grow upon transfer.

Table 3 (continued)

Atmospheres (CO <sub>2</sub> /O <sub>2</sub> ) <sup>a</sup>	Days <sup>d</sup>	AC	E	EC	HA	K	P	PS	SE	SH	YE	V	U	T
40/0	7	1	0	0	0	0	0	0	10	0	1	0	3	15 <sup>d</sup>
	14	1	1	0	1	0	6	0	5	0	3	4	4	25 <sup>d</sup>
	21	1	4	0	3	1	4	0	6	0	2	0	4	25 <sup>d</sup>
	28	1	8	0	3	0	0	0	9	0	1	0	3	25 <sup>d</sup>
40/10	7	1	0	0	3	0	3	0	5	0	1	0	2	15 <sup>d</sup>
	14	1	0	0	1	0	5	0	7	0	1	0	0	15 <sup>d</sup>
	21	1	3	1	11	1	2	0	1	1	1	0	4	25 <sup>d</sup>
	28	1	1	4	3	0	1	0	6	1	7	0	1	25 <sup>d</sup>
40/20	7	2	0	0	0	0	5	0	4	0	2	0	2	15 <sup>d</sup>
	14	3	2	0	2	0	3	0	9	0	1	0	5	25 <sup>d</sup>
	21	1	0	1	6	0	6	0	9	0	1	0	7	25 <sup>d</sup>
	28	1	1	0	4	0	1	0	12	0	2	0	4	25 <sup>d</sup>
40/40	7	5	0	0	0	0	3	0	0	0	2	0	5	15 <sup>d</sup>
	14	3	1	0	4	0	6	0	2	0	2	1	6	25 <sup>d</sup>
	21	6	4	0	9	0	5	0	1	0	2	1	2	30
	28	2	4	0	2	0	4	0	5	0	1	0	2	20 <sup>d</sup>
Vacuum	7	1	3	0	0	0	6	0	2	1	1	0	1	15 <sup>d</sup>
	14	1	0	0	3	0	7	0	1	0	1	0	2	15 <sup>d</sup>
	21	0	2	0	11	0	1	0	4	0	3	0	4	25 <sup>d</sup>
	28	0	3	0	13	0	2	0	1	1	2	0	3	25 <sup>d</sup>

<sup>a</sup>Balanced with nitrogen; <sup>b</sup>Genus (and species) of isolates; <sup>c</sup>Days of storage at 2-4°C  
AC=Acinetobacter, E=Enterobacter, EC=Escherichia coli; HA=Hafnia alvei; K=Klebsiella; P=Pseudomonas spp.; PS=Plesiomonas  
shigelloides; SE=Serratia spp. S=Shigella spp.; YE=Yersinia enterocolitica; V=Vibrio spp, U=Number of unidentified isolates ; T=Total number  
of isolates from three replications; <sup>d</sup>Less than 10 colonies per replication were present for isolation or cultures did not grow upon transfer.

Table 4. Mean<sup>a</sup> Hunter "b" values of chops stored in different atmospheres at 4°C

Atmospheres CO <sub>2</sub> /O <sub>2</sub> <sup>b</sup>	Hunter "b" values
10/10	7.44 <sup>X</sup>
10/20	7.05 <sup>X</sup>
10/40	6.81 <sup>X</sup>
20/0	6.78 <sup>X</sup>
20/10	7.11 <sup>X</sup>
20/20	6.62 <sup>X</sup>
20/40	6.84 <sup>X</sup>
40/0	6.86 <sup>X</sup>
40/10	6.78 <sup>X</sup>
40/20	7.00 <sup>X</sup>
40/40	7.03 <sup>X</sup>
Vacuum	6.63 <sup>X</sup>
Air	6.61 <sup>X</sup>
Standard error	0.298

<sup>a</sup>Means of three replications averaged through time; mean initial Hunter "b" value = 6.81.

<sup>b</sup>Balanced with nitrogen.

<sup>X</sup>Treatment means with the same superscript are not significantly different at P=0.05.

Table 5. Mean TBA values<sup>a</sup> of pork chops packaged under different atmospheres and stored at 4°C

Atmospheres (CO <sub>2</sub> /O <sub>2</sub> ) <sup>b</sup>	Days of storage at 4°C						
	0	7	14	21	28	35	42
10/10	0.09 <sup>V</sup>	0.15 <sup>V</sup>	0.50 <sup>V</sup>	0.34 <sup>V</sup>	-	-	-
10/20	0.12 <sup>V</sup>	0.11 <sup>V</sup>	1.04 <sup>V</sup>	0.67 <sup>V</sup>	-	-	-
10/40	0.13 <sup>V</sup>	0.29 <sup>V</sup>	0.93 <sup>V</sup>	2.26 <sup>X</sup>	1.01 <sup>V</sup>	1.47 <sup>VX</sup>	-
20/0	0.16 <sup>V</sup>	0.33 <sup>V</sup>	0.83 <sup>V</sup>	0.60 <sup>V</sup>	0.34 <sup>V</sup>	1.79 <sup>VX</sup>	2.39 <sup>VX</sup>
20/10	0.21 <sup>V</sup>	0.52 <sup>V</sup>	0.46 <sup>V</sup>	0.62 <sup>V</sup>	0.99 <sup>V</sup>	4.01 <sup>Z</sup>	-
20/20	0.17 <sup>V</sup>	0.37 <sup>V</sup>	0.32 <sup>V</sup>	0.83 <sup>V</sup>	0.63 <sup>V</sup>	0.42 <sup>V</sup>	-
20/40	0.16 <sup>V</sup>	0.32 <sup>V</sup>	0.38 <sup>V</sup>	1.83 <sup>VX</sup>	1.40 <sup>VX</sup>	1.19 <sup>V</sup>	-
40/0	0.20 <sup>V</sup>	0.17 <sup>V</sup>	0.22 <sup>V</sup>	1.08 <sup>V</sup>	0.92 <sup>V</sup>	2.31 <sup>X</sup>	2.93 <sup>XY</sup>
40/10	0.18 <sup>V</sup>	0.23 <sup>V</sup>	0.62 <sup>V</sup>	1.23 <sup>V</sup>	1.40 <sup>V</sup>	4.34 <sup>Z</sup>	3.62 <sup>YZ</sup>
40/20	0.16 <sup>V</sup>	0.89 <sup>V</sup>	1.12 <sup>V</sup>	1.43 <sup>V</sup>	1.98 <sup>VX</sup>	3.82 <sup>YZ</sup>	3.18 <sup>Y</sup>
40/40	0.20 <sup>V</sup>	0.27 <sup>V</sup>	0.50 <sup>V</sup>	1.74 <sup>VX</sup>	1.00 <sup>V</sup>	1.63 <sup>VX</sup>	1.00 <sup>V</sup>
Vacuum	0.15 <sup>V</sup>	0.20 <sup>V</sup>	0.53 <sup>V</sup>	0.59 <sup>V</sup>	0.50 <sup>V</sup>	0.18 <sup>V</sup>	0.19 <sup>V</sup>
Air	0.16 <sup>V</sup>	0.25 <sup>V</sup>	0.17 <sup>V</sup>	-	-	-	-
Standard. error	0.464						

<sup>a</sup>Mean of three replications; mean initial TBA, 0.201 mg malonaldehyde/100g of meat

<sup>b</sup>Balanced with nitrogen

<sup>vxyz</sup>Mean values with the same superscript are not significantly different at P=0.05.

**PART II: SURVIVAL AND GROWTH OF *Listeria monocytogenes* and *Yersinia enterocolitica* IN FRESH PORK CHOPS PACKAGED UNDER MODIFIED GAS ATMOSPHERES**

**SURVIVAL AND GROWTH OF *Listeria monocytogenes* AND *Yersinia enterocolitica*  
IN PORK CHOPS PACKAGED UNDER MODIFIED GAS ATMOSPHERES**

by

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Departments of Food Science and Human Nutrition, and  
Animal Science, Iowa State University, Ames, IA 50011**

**Running Title: Growth of *Listeria* and *Yersinia* in MA-packaged fresh pork chops**

## ABSTRACT

The growth of *Listeria monocytogenes* Scott A, serotype 4, and *Yersinia enterocolitica* inoculated onto fresh pork chops and stored in different atmospheres at 4°C for 35 days was examined. The atmospheres consisted of three modified gas mixtures (20/0(CO<sub>2</sub>/O<sub>2</sub>); 40/0; 40/10, balanced with nitrogen), vacuum and air. In the air package *L. monocytogenes* or *Y. enterocolitica* grew at rates slower than those of the psychrotrophic spoilage flora. In the gas atmosphere packages *L. monocytogenes* grew at a slower rate than, but *Y. enterocolitica* grew at the same rate as, the psychrotrophic spoilage flora. Doubling the CO<sub>2</sub> concentration in the gas mixtures reduced the growth rate of *L. monocytogenes* and increased that of *Y. enterocolitica*, but not significantly. Increasing the O<sub>2</sub> concentration in the 40%CO<sub>2</sub> gas mixture to 10% reduced the growth rates of *L. monocytogenes* and *Y. enterocolitica*. Vacuum packaging appeared to be more effective than the gas mixtures in slowing down the growth of *L. monocytogenes* or *Y. enterocolitica* in fresh pork chops. Growth of *L. monocytogenes* was slower than that of the psychrotrophic flora, but growth of *Y. enterocolitica* was not significantly different from that of the psychrotrophic flora in the vacuum package.

## INTRODUCTION

To meet consumer demands for fresh meats with extended shelf life, the North American meat industry has for the past decade shown increasing interest in modified atmosphere packaging of fresh meats. This technique involves the utilization of gas mixtures including oxygen to maintain the desired bloomed color of fresh meats and to inhibit growth of strict anaerobes, and carbon dioxide to inhibit the growth of aerobic spoilage bacteria (Hotchkiss and Galloway, 1989). Many modified atmospheres contain moderate to high levels of carbon dioxide (Farber, 1991), which together with proper sanitation and refrigeration, extends the shelf life of fresh meats by reducing microbial growth and retarding enzymatic spoilage (Young et al., 1988).

Recently, it has been reported that some strains of pathogenic *Listeria monocytogenes* and *Yersinia enterocolitica* are capable of growing at refrigeration temperatures (Palumbo, 1986), and that these organisms are frequent contaminants of fresh meats (Buncic, 1991; Lee and McClain, 1987; Schieman, 1980; Stern and Pierson, 1979). Studies have also indicated that foodborne *L. monocytogenes* or *Y. enterocolitica* infections in humans can be transmitted by the consumption of raw, undercooked or recontaminated cooked meats (Aulisio et al., 1983; Kaczmarek and Jones, 1989; De Zutter and Van Hoof, 1987). Since individual bacterial species present initially on meat surfaces show different responses to the CO<sub>2</sub> and O<sub>2</sub> contents of the atmosphere in which the meat is stored (Newton et al., 1977), many safety issues have been raised concerning the concentrations of these gases in the various modified atmospheres used to



package fresh meats, and their influence on the growth rate of these pathogens over an extended shelf life period at refrigeration temperatures. It has been reported that the gaseous environment in a vacuum-packaged fresh meat contains about 20 to 80% CO<sub>2</sub> (Smith et al., 1983; Taylor, 1973) formed by respiration of the muscle tissue, the microorganisms or both (Gill and Tan, 1980; Sutherland et al. 1977), yet high numbers of *Y. enterocolitica*-like organisms (Hanna et al., 1976; Johnson et al., 1982; Seelye and Yearburg, 1979) and *L. monocytogenes* (Grau and Vanderlinde, 1990) have been found to grow rapidly on vacuum-packaged beef stored at refrigeration temperatures. Gill and Reichel (1989), however, found that strains of cold-tolerant *Y. enterocolitica* grew sometimes at the same rate as spoilage microflora on high pH (>6.0) beef packaged under 100% CO<sub>2</sub> and stored at 5°C; *L. monocytogenes* did not grow under the same conditions. Wimpfheimer et al. (1990) observed that *L. monocytogenes* failed to grow on raw chicken stored in anaerobic CO<sub>2</sub> atmosphere but proliferated on samples stored in aerobic CO<sub>2</sub> atmosphere at 4°C.

Pork muscles generally have variable ultimate pH values, and growth of certain psychrotrophic pathogens in sarcoplasmic proteins varies with meat species (Khan et al., 1975). In addition, carbon dioxide is generally used in concentrations ranging from 15-40% for commercial packaging of fresh red meats, since higher concentrations can accelerate discoloration of the meat surface (Farber, 1991). Hence the findings of Gill and Reichel (1989) and those of Wimpfheimer et al. (1990) cannot be extended to pork. Preliminary studies have shown that fresh pork chops in consumer-unit sizes packaged in 20%CO<sub>2</sub> without O<sub>2</sub> or 40% CO<sub>2</sub> with or without O<sub>2</sub> and stored at 4°C attained microbial shelf life of

6 weeks, which was comparable to the 5.5 weeks obtained by Gill and Harrison (1989) who used 100% CO<sub>2</sub> at 3°C.

The objective of this work was to determine if modified atmospheres containing CO<sub>2</sub> concentrations in the range of 20-40% with or without O<sub>2</sub> would selectively enhance growth of *L. monocytogenes* or *Y. enterocolitica*, while inhibiting other psychrotrophic flora on fresh pork chops stored at 4°C.

## MATERIALS AND METHODS

### Organisms

The cultures used were *L. monocytogenes* scott A serotype 4 obtained from the National Animal Disease Center (NADC), Ames, Iowa and a strain of *Y. enterocolitica* isolated from vacuum packaged pork chops. The organisms were grown in Brain Heart Infusion broth (BHI, Difco, Detroit, MI, USA) at 30°C for 24 hr to a stationary phase (Gill and Reichel, 1989). At specific time intervals a sample of the cell suspension was concentrated by centrifugation and then resuspended in 0.01M phosphate buffered saline (PBS). The optical density of the resuspended cells was read at 640 nm using a Bausch and Lomb Spectronic 20 against a blank PBS (Wimpfheimer et al., 1990). Further tenfold dilutions of each culture were prepared, spiral-platted onto All Purpose Tween (APT, BBL, Cockeysville, MD, USA) agar, and incubated at 37°C for *L. monocytogenes* or 25°C for *Y. enterocolitica*. Stationary phase of *L. monocytogenes* cells was reached at  $10^9$  CFU/ml and that of *Y. enterocolitica* cells at  $10^8$  CFU/ml.

### Sample preparation

Boneless pork loins were obtained directly from the processing line of a commercial meat packing plant. The loins were cut into 2.5 cm (1 in) thick chops. The surface areas of a representative number of chops were determined according to the method of Huffman (1974). It involved tracing the outline of the chops on acetate paper, and using a planimeter to measure the surface area.

The remaining chops were divided into two groups; the top surfaces of one group were inoculated with 0.1 ml PBS containing  $10^5$  cells of each culture. The inoculum was spread over the surface of the chop using a sterile glass rod (Gill and Reichel, 1989) to obtain an initial cell concentration of about  $\log 3.5$  cfu per  $\text{cm}^2$  of each inoculated chop surface. Three inoculated chops were placed on one 2S styrofoam tray with the inoculated surface upward. Each tray was overwrapped with gas permeable polyethylene film (Stretch meat film, Cat # 37016, Hantover, Kansas City, MO, USA). Some of the overwrapped trays were set aside and considered as air-packaged inoculated samples, while others were placed in high-barrier packaging pouches having an  $\text{O}_2$  permeability of  $<2.5 \text{ cm}^3/645 \text{ cm}^2/24 \text{ hr}$  at  $23^\circ\text{C}$  and 0% R.H., and water vapor transmission  $< 1.0 \text{ g}/645 \text{ cm}^2/24 \text{ hr}$  at  $38^\circ\text{C}$  and 90% R.H. (Curlon<sup>TM</sup> 863 Saran, Curwood Inc. New London, WI, USA).

The top surfaces of the second group of pork chops were spread with 0.1 ml of 0.01M sterile PBS containing no inoculum, and packaged in the same films as those used for the inoculated samples. Equal numbers of inoculated and uninoculated samples were backflushed with about 4 liters of one of the following atmospheres: 20/0( $\text{CO}_2/\text{O}_2$ ), 40/0, and 40/10, to obtain a headspace: meat volume ratio of 2:1; or packaged under vacuum and heat sealed using a CVP machine (model A 300, CVP Systems, Inc., Downers Grove, IL, USA). The packages were placed in cardboard boxes and stored at  $4^\circ\text{C}$ . All samples were analyzed at day 0, i.e., the day of packaging, and every 7 days for 35 days.

### Headspace and Surface pH analyses

On each sampling day, the O<sub>2</sub> concentrations in the headspace of the packages except the air and vacuum packages were measured with a digital O<sub>2</sub> analyzer (Model IL 307 Ingold™, Instrumentation Laboratory Inc., Wilmington, MA, USA). The CO<sub>2</sub> concentrations were measured using an infrared Spectrometer (AccuLab™ 2 Beckman Instruments, Inc., Palo Alto, CA, USA).

The surface pH of samples was measured with a flat surface combination electrode (Fisher Scientific, Pittsburg, PA).after a piece was aseptically cut for microbiological analyses.

### Microbiological sampling

Each package was aseptically opened on sampling days and 25 cm<sup>2</sup> of the top surface of each of the three chops in a package were removed with a sterile circular stainless steel borer. This allowed removal of a total of 75 cm<sup>2</sup> of sample surface which was blended for 1 min in a sterile bag containing 150 ml 0.01M sterile PBS using a Stomacher lab blender (Model 400 Tekmar™ Co., Cincinnati, OH, USA). Serial dilutions were prepared according to recommended microbiological procedure (Speck, 1984). Portions of suitable dilutions were spiral plated onto APT agar, and also onto *Listeria* selective agar (OXOID, Basingstoke, England) when the meat sample had been inoculated with *L. monocytogenes* (Curtis et al., 1989) or onto Cefselodin-Irgasan-Novobiocin (CIN, DIFCO) agar when the meat sample had been inoculated with *Y. enterocolitica* (Gill and Reichel, 1989). The APT plates were incubated aerobically at 7°C for 10 days; the *Listeria* agar plates at 37°C for 48 hr; and the CIN-agar at 25°C for 48 hr.

The numbers of *L. monocytogenes* and *Y. enterocolitica* were determined from counts on respective selective agar plates, after identification of representative, presumptive colonies. Colonies on *Listeria* agar that appeared dark were assumed to be *L. monocytogenes*. They were confirmed using the following tests: Gram, catalase, and oxidase reactions, umbrella motility at 25°C, CAMP B-hemolysis (Lee and McClain, 1987), nitrate reduction, and fermentation of glucose, rhamnose, esculin, mannitol, and xylose. Small to medium colonies on CIN-agar with a dark red center surrounded by transparent borders were presumed to be *Y. enterocolitica* (Gill and Reichel, 1989). Confirmatory tests were Gram, catalase and oxidase reactions (Schieman, 1980), and biochemical reactions using the Minitek *Enterobacteriaceae* differentiating system (Newton et al., 1977). The numbers of psychrotrophic flora other than *Listeria* or *Yersinia* were determined from APT plates of uninoculated samples. The composition of spoilage flora was determined by picking three to five colonies on APT plates of inoculated samples and identifying them to the generic level by the criteria of Harrison et al. (1981). Gram-negative organisms were further classified to species level using the Minitek *Enterobacteriaceae* and Nonfermenter differentiating systems.

### Statistical analyses

Mean values were obtained from four replications and the data were analyzed by analysis of variance using the General Linear Model procedure (SAS, 1986). Paired contrasts were used in the procedure to determine significance among means, and least square difference was used to compare the means. Linear regression equations were computed for each atmosphere over the linear portion of the log growth phase of the curves in order to predict the growth rates for

*L. monocytogenes*, *Y. enterocolitica* and other psychrotrophic colony forming organisms (Wimpfheimer et al., 1990).

## RESULTS AND DISCUSSION

The CO<sub>2</sub> concentrations of all the gas mixtures decreased during the first 7 days of storage, and thereafter began to increase (Fig. 1). No significant differences in the concentrations of CO<sub>2</sub> were found between inoculated and uninoculated chops, or between chops inoculated with *Listeria* and those inoculated with *Yersinia* ( $P>0.05$ ), hence only one set of data is presented. From day 7 to day 21 the CO<sub>2</sub> concentration of the 40/10(CO<sub>2</sub>/O<sub>2</sub>) mixture containing the inoculated sample was higher ( $P<0.05$ ) than that of the uninoculated package in the identical gas mixture, probably because more O<sub>2</sub> was converted to CO<sub>2</sub> in the former package by the respiring inoculated cell.

The initial headspace O<sub>2</sub> concentration of the 20/0 and the 40/0 mixtures were about 2.3% for both inoculated chops and uninoculated chops and for the two organisms tested. The O<sub>2</sub> concentrations in all the packages decreased with storage time (Fig. 2), reaching anaerobic condition of 0.5% (Lioutas, 1988) in the 20/0 mixture in 28 days and in the 40/0 mixture in 35 days. The 40/10 mixture remained aerobic throughout the storage period, i.e, the O<sub>2</sub> concentration never reached 0.5%. The O<sub>2</sub> concentration of the uninoculated chops packaged in the 40/10 mixture was higher ( $P<0.05$ ) than that of the inoculated chops packaged in the identical gas mixture (Fig. 2). No significant difference in O<sub>2</sub> concentrations was found between inoculated and uninoculated chops packaged in either the 20/0 or the 40/0 mixture, or between the two organisms tested ( $P>0.05$ ).

Fig. 3 shows the mean surface pH of inoculated and uninoculated chops. The mean initial pH of the chops before packaging was 5.8, which was sufficiently high to allow uninhibited growth of the test organisms during storage (Grau and



Vanderlinde, 1988; Seelye and Yearburg, 1979). Values at day 0 were not significantly different among atmospheres ( $P>0.05$ ). There were also no differences in pH between inoculated and uninoculated chops, among gas mixtures, between gas mixtures and vacuum or between the two organisms tested ( $P>0.05$ ), hence Fig. 3 represents values for one set of data. With the exception of the air-packaged chops, the pH of the chops also did not vary significantly with storage time ( $P>0.05$ ). The pH values of the air-packaged chops increased with storage time ( $P<0.05$ ) for both inoculated and uninoculated chops and for both organisms tested.

Changes in the numbers of *L. monocytogenes* on fresh pork packaged under different atmospheres are presented in Fig. 4. There appeared to be very little growth during the first 14 days of storage. Growth in the air sample decreased between day 14 and day 21 probably because of the competition from high numbers of other psychrotrophic flora. After day 7, numbers of *L. monocytogenes* in the chops packaged in the 20/0( $\text{CO}_2/\text{O}_2$ ) mixture were higher than but not significantly different from the numbers in the chops packaged in the 40/0 mixture. No difference in numbers of *Listeria* was found between chops packaged in the 40/0 mixture and those packaged in the 40/10. Thus, increasing the  $\text{O}_2$  concentration in the package did not seem to influence the numbers of *Listeria* on chops stored at 4°C. This is contrary to the findings of Wimpfheimer et al. (1990) who obtained a rapid proliferation of *L. monocytogenes* on raw chicken by increasing the  $\text{O}_2$  concentration of anaerobic  $\text{CO}_2$  atmosphere to 5%. Generally, the numbers of *L. monocytogenes* in the air- or vacuum-packaged chops were lower than, but not significantly different from those packaged in the gas atmospheres ( $P>0.05$ ).

Before packaging, the population of psychrotrophs other than *Listeria* on the pork was about  $\log 1.5$  CFU/cm<sup>2</sup>. The psychrotrophic flora grew very rapidly in both inoculated and uninoculated air-packaged chops, but numbers were much higher in uninoculated air-packaged chops than on inoculated air-packaged ones. Growth of the psychrotrophic flora in both inoculated and uninoculated chops packaged under vacuum or in the gas atmospheres increased at a much slower rate ( $P < 0.05$ ) than that on the air-packaged chops (Fig. 4). Numbers of psychrotrophs in the chops packaged under vacuum or packaged in the gas atmospheres did not reach  $\log 7.0$  cfu/cm<sup>2</sup>, the level at which meat is considered spoiled (Kraft, 1986) throughout the storage period, and were significantly lower than the numbers in the air-packaged chops ( $P < 0.05$ ).

Although the numbers of *L. monocytogenes* in the inoculated chops packaged in the different atmospheres did not vary significantly, the growth rates of the organism in the inoculated chops as determined by regression coefficient varied with the atmosphere (Table 1). Increasing the CO<sub>2</sub> concentration from 20% to 40% decreased the growth rate of *L. monocytogenes* but not significantly. However, when the O<sub>2</sub> concentration of the 40%CO<sub>2</sub> mixture was increased to 10%, there was a significant decrease in growth rate compared to growth at 20%. But no difference in growth rates of the organism was found between 40/0 and 40/10 mixture, even though there was a slight decrease in growth rate in the 40/10 mixture. *L. monocytogenes* has the ability to grow at reduced O<sub>2</sub> tensions in some foods (Brackett, 1988; Doyle, 1988) which might explain the slightly higher growth rate in the chops packaged in the 40/0 mixture than in the 40/10 mixture. Vacuum packaging seemed to have imposed more inhibitory effect on *Listeria* than the gas atmosphere packaging. Growth rate of the organism in the vacuum package was

lower than that in any of the gas mixtures. The slower growth rate of *Listeria* in the vacuum-packaged chops was in agreement with the findings of Gill and Reichel (1989) but was contrary to those of Grau and Vanderlinde (1990) who observed rapid growth of *L. monocytogenes* on inoculated vacuum-packaged beef stored at refrigeration temperatures. The growth rate of *Listeria* in the inoculated air-packaged chops was the slowest ( $P < 0.05$ ) among the atmospheres tested (Table 1). Comparatively, the growth rates of other psychrotrophic flora on uninoculated chops were higher than those of *L. monocytogenes* in the inoculated chops packaged in identical atmospheres ( $P < 0.05$ , Table 1). The highest growth rate of the psychrotrophs were obtained in the air-packaged chops ( $P < 0.05$ ). No significant differences in growth rates of the psychrotrophs were found among the remaining atmospheres. Thus, the slow growth rate of *Listeria* in the inoculated air-packaged chops could be attributed to competition from the high numbers of other psychrotrophic bacteria that also grew on the chops. The higher growth rates of other psychrotrophic spoilage flora than *Listeria* in the modified gas atmospheres is desirable in that the packaging can extend the storage life of pork chops by restricting the possibilities for *Listeria* to grow to high numbers before the product is spoiled. However, it must be noted that *Listeria* grew even though at a slower rate, and considering the relatively low infectious dose of this pathogen for humans (about  $10^2$  to  $10^3$  cells; Griffith, 1989), particularly for immunocompromized persons (Varabiouff, 1990), the risk of human infection may not be reduced by modified atmosphere packaging of fresh pork chops. The higher growth rates of *Listeria* in the gas atmosphere packages than in the vacuum package is also an indication that fresh meats packaged in MA may be susceptible to growth of *Listeria* if the package is subjected to moderate thermal abuse.

*Y. enterocolitica* had no lag period and grew very rapidly in all the atmospheres, except air (Fig. 7). Gill and Reichel (1989) reported that *Y. enterocolitica* grew on beef packaged in 100%CO<sub>2</sub> and stored at 2 or 5°C only after a prolonged lag period. The absence of a lag period at 40%CO<sub>2</sub> may be due to the organisms being sensitive to CO<sub>2</sub> at this concentration. No differences in numbers of *Y. enterocolitica* were found among the gas mixtures or between gas mixtures and vacuum ( $P>0.05$ ), even though numbers on sample packaged in the 20/0(CO<sub>2</sub>/O<sub>2</sub>) mixture were highest from day 14 to day 28. Numbers of this organism in the air-packaged chops increased only slightly from day 0 to day 14 reaching a high of about log 4.5 CFU/cm<sup>2</sup> from an initial level of log 3.1 CFU/cm<sup>2</sup> and then began to decrease. On the other hand, numbers of other psychrotrophic bacteria in both the inoculated and uninoculated chops packaged under vacuum or in the gas mixtures were lower than those of the air-packaged chops (Fig. 7). Table 2 shows the slopes of the regression plots of the growth of *Y. enterocolitica* and psychrotrophic spoilage flora in chops packaged in different atmospheres. No significant difference in growth rates was found among the gas atmospheres ( $P>0.05$ ) even though the order was 40/0 > 40/10 > 20/0. Thus doubling the CO<sub>2</sub> concentration slightly increased the growth rate while increasing the O<sub>2</sub> concentration slightly decreased the growth rate of this organism. The growth rate of *Y. enterocolitica* in the vacuum-packaged chops was significantly lower ( $P<0.05$ ) than those in the chops packaged in the gas atmospheres. Again vacuum packaging appeared to be more effective than the gas atmospheres in reducing the growth rate of *Yersinia*. Comparatively, the growth rate of *Y. enterocolitica* in the inoculated air-packaged was the lowest among the atmospheres tested ( $P<0.05$ ). The growth rates of other psychrotrophic flora on uninoculated chops packaged in

identical atmospheres are also presented on Table 2. No significant difference in growth rates of the psychrotrophs was found among the gas atmospheres, although the rate was highest in the chops packaged in the 40/10 mixture, followed by that in the chops packaged in 20/0 mixture and then the rate in those packaged in the 40/0 mixture. The growth rate of the psychrotrophic flora in the vacuum-packaged chops was significantly lower ( $P < 0.05$ ) than that in the chops packaged in the 40/10 mixture. The highest growth rate of the psychrotrophic flora was obtained in the chops packaged in air (Table 2). No significant difference was found in the growth rates of *Y. enterocolitica* in the inoculated chops and those of psychrotrophs in the uninoculated chops packaged in identical gas atmospheres or under vacuum. However, the growth rates of *Y. enterocolitica* in the inoculated air-packaged chops were significantly lower ( $P < 0.05$ ) than the growth rates of psychrotrophs in uninoculated chops packaged in air or vacuum (Table 2). The results therefore indicate *Y. enterocolitica* had the ability to grow in competition with other psychrotrophs in chops packaged in the gas atmospheres or under vacuum, but were outgrown by the populations of psychrotrophs in the chops packaged in air. This makes packaging of fresh pork chops in modified atmospheres containing up to 40%CO<sub>2</sub> vulnerable with respect to the microbiological safety of the product. However, it was observed that when the numbers of *Yersinia* reached log 6.0 CFU/cm<sup>2</sup> surface discoloration of the chops packaged in the gas atmospheres occurred. The change in the organoleptic characteristics of the meat may help in reducing the hazards of gas atmosphere packaging with respect to *Yersinia*, since consumers and retailers will be warned of spoilage before the numbers reach the infectious dose of 10<sup>9</sup> cells as reported by Swaminathan et al. (1982).

*Listeria* cells were never isolated from uninoculated chops packaged in the atmospheres tested, but *Yersinia* cells were isolated from uninoculated packages for all the atmospheres tested. However, the proportions of *Yersinia* isolates were too low to affect the numbers of other psychrotrophic flora. The composition of the psychrotrophic flora of the inoculated chops when maximum numbers were reached varied with the atmosphere in which the chops were stored, but not with the type of organism used to inoculate the chops. Air-packaged chops were dominated by *Pseudomonas* (50%), followed by *Enterobacteriaceae* (30%) and the *Brochotrix thermosphacta* (15%). The vacuum-packaged chops were equally dominated by lactic acid bacteria (50%) and *Enterobacteriaceae* (50%). There were no differences in the composition of the psychrotrophic flora of the inoculated chops packaged in the gas mixtures. The samples were dominated by lactic acid bacteria (60%), followed by *Enterobacteriaceae* (30%) and then *B. thermosphacta* (5%). *Pseudomonas* cells were also isolated from chops packaged in the 40/10 mixture but their proportion was very small.

## CONCLUSIONS

Modified gas atmospheres containing CO<sub>2</sub> concentrations normally used for commercial packaging of fresh red meats are unlikely to reduce the risk factor for listeriosis when uncooked or undercooked fresh pork chops or processed pork products are consumed. The atmospheres slowed down but did not stop the growth of *L. monocytogenes*. Instead the atmospheres inhibited the growth of other psychrotrophic spoilage flora to the extent that no organoleptic changes that will serve as indicators of spoilage were observed.

The atmospheres may also lead to development of health hazards from *Y. enterocolitica*. The organism was able to grow in competition with psychrotrophs in fresh pork chops packaged in the gas atmospheres. However, changes in the appearance of the meat with increasing numbers of the organism are likely to warn retailers and consumers of spoilage.

Vacuum packaging was more effective than modified gas atmosphere in reducing the growth rates of *Listeria* and *Yersinia* in chilled fresh pork chops, but the system may not guarantee the safety of fresh pork contaminated with either of these two psychrotrophic pathogens.

It should be noted that only uncooked or undercooked meats or meat products have been implicated in confirmed cases of listeriosis or yersiniosis traced to meat products. Proper cooking procedures can also reduce or eliminate the health hazards from these pathogens.

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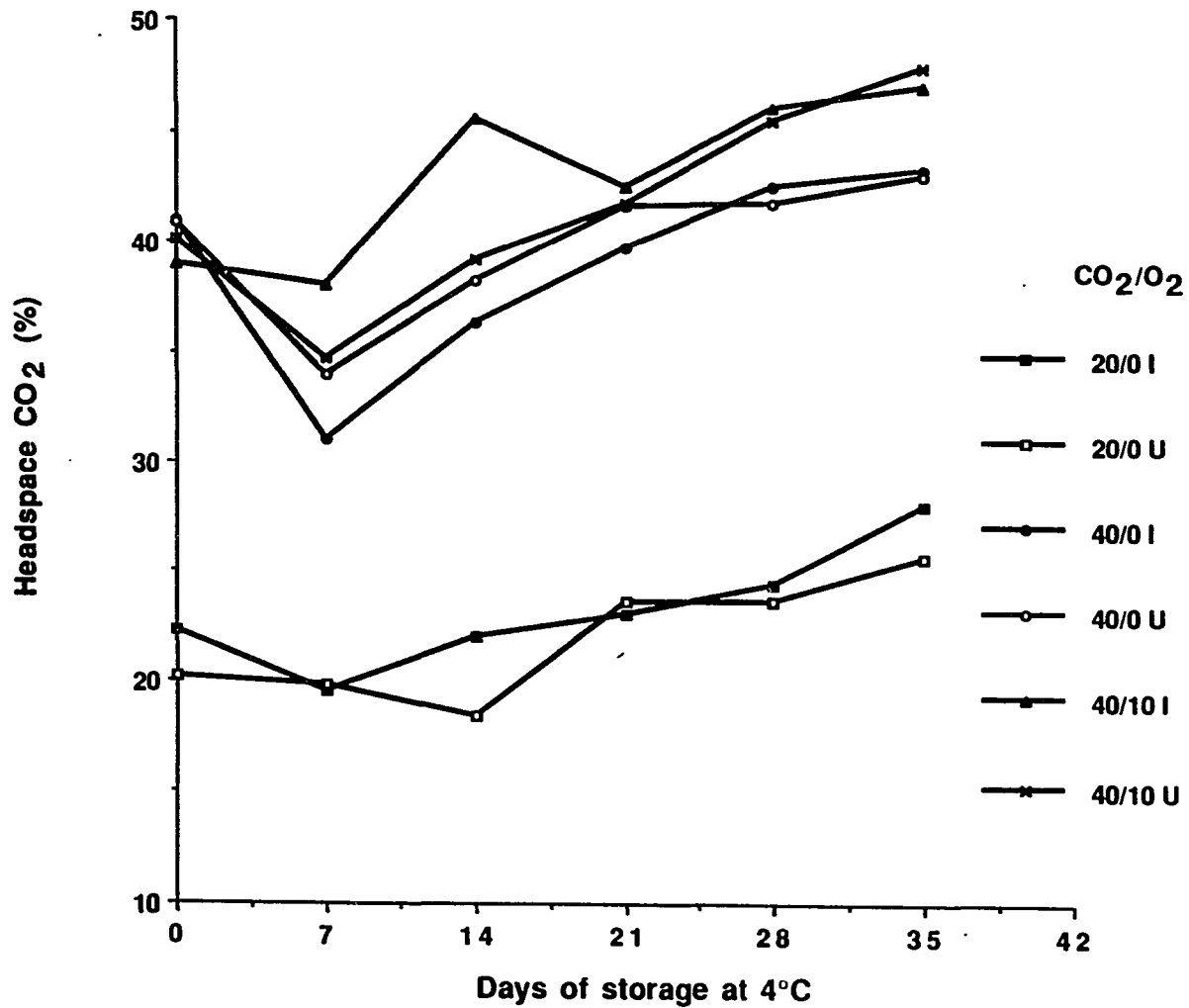


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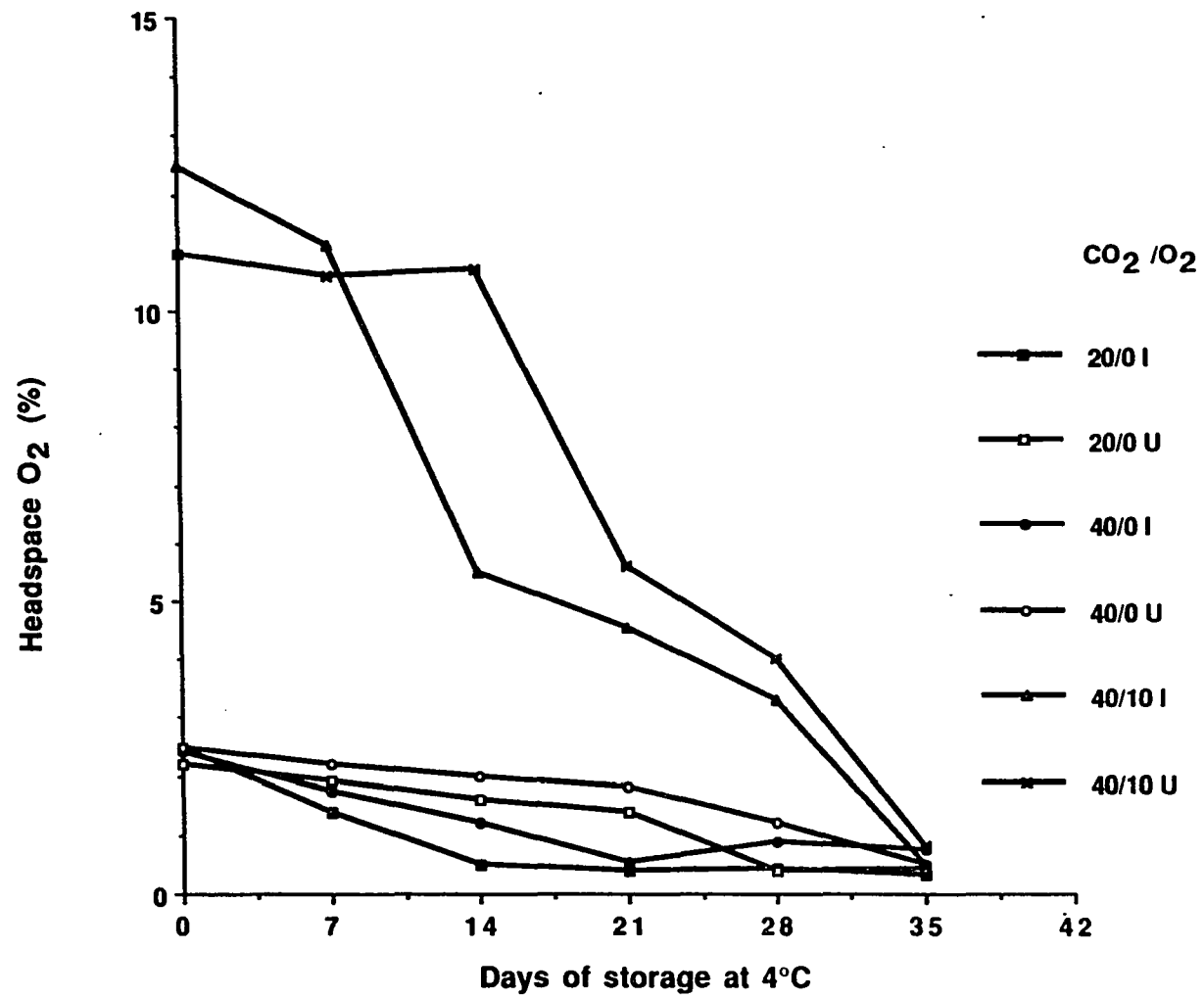
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**Figure 1. Change in the headspace CO<sub>2</sub> concentration of the gas mixtures for inoculated (I) and uninoculated (U) fresh pork chops stored at 4°C.**

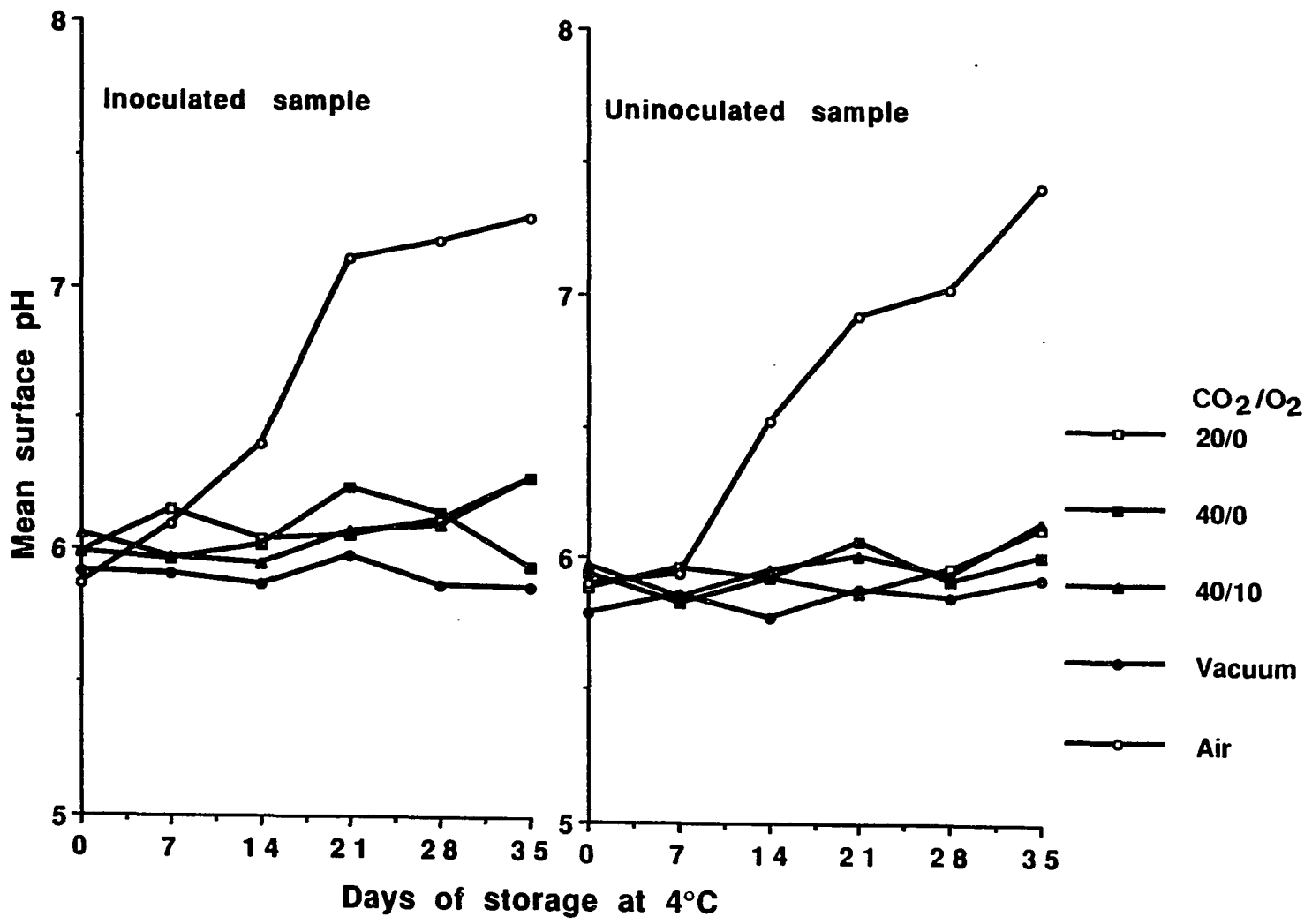


**Figure 2. Change in the headspace O<sub>2</sub> concentration of the gas mixtures for inoculated (I) and uninoculated (U) fresh pork chops stored at 4°C.**

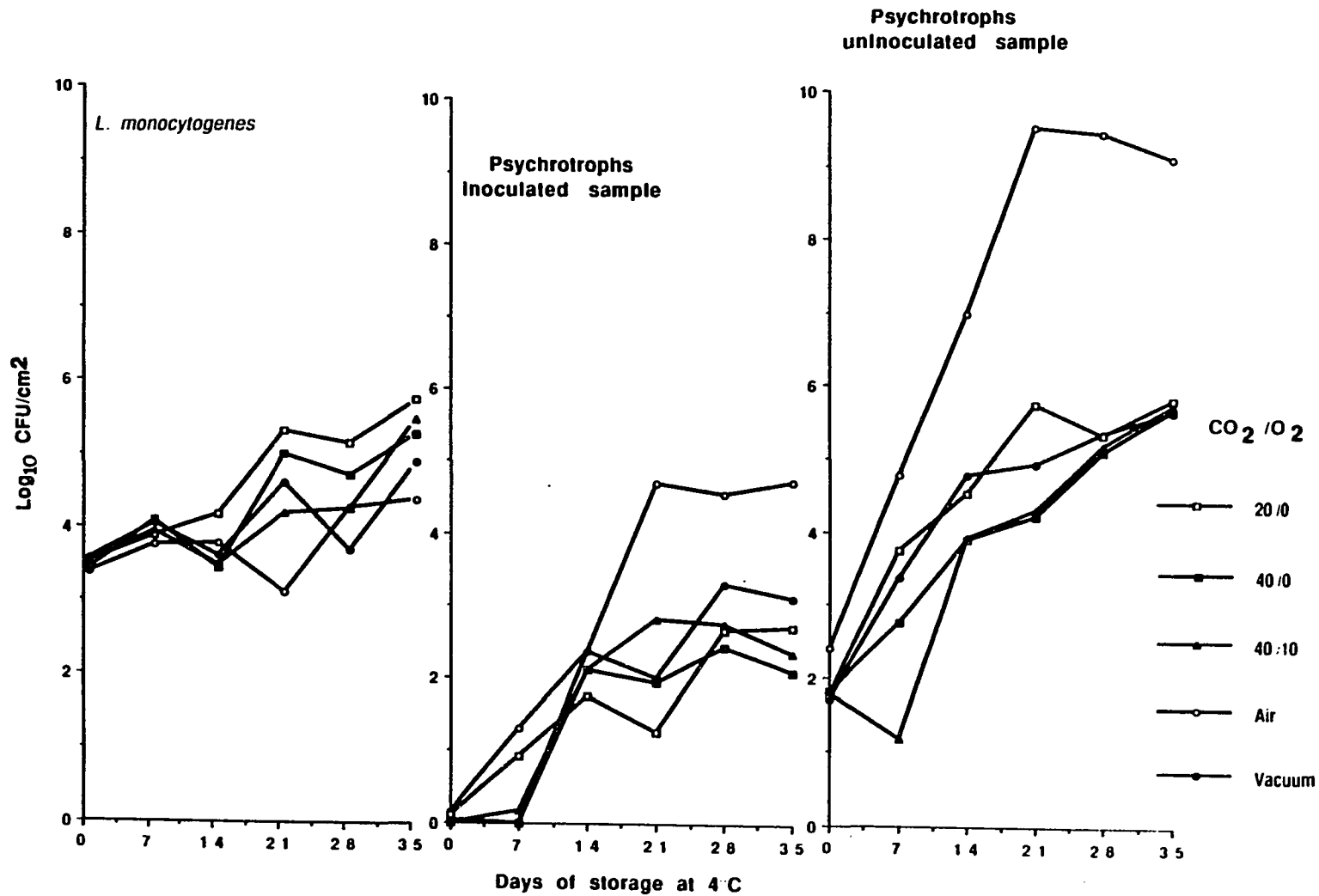


**Figure 3. Mean surface pH of inoculated and uninoculated fresh pork chops stored in different atmospheres at 4°C.**





**Figure 4.** Numbers of *L. monocytogenes* and aerobic psychrotrophic bacteria on fresh pork chops packaged in air, under vacuum or selected gas mixtures during storage at 4°C.



**Figure 5. Numbers of *Y. enterocolitica* and aerobic psychrotrophic bacteria on fresh pork chops packaged in air, under vacuum or selected gas mixtures during storage at 4°C.**

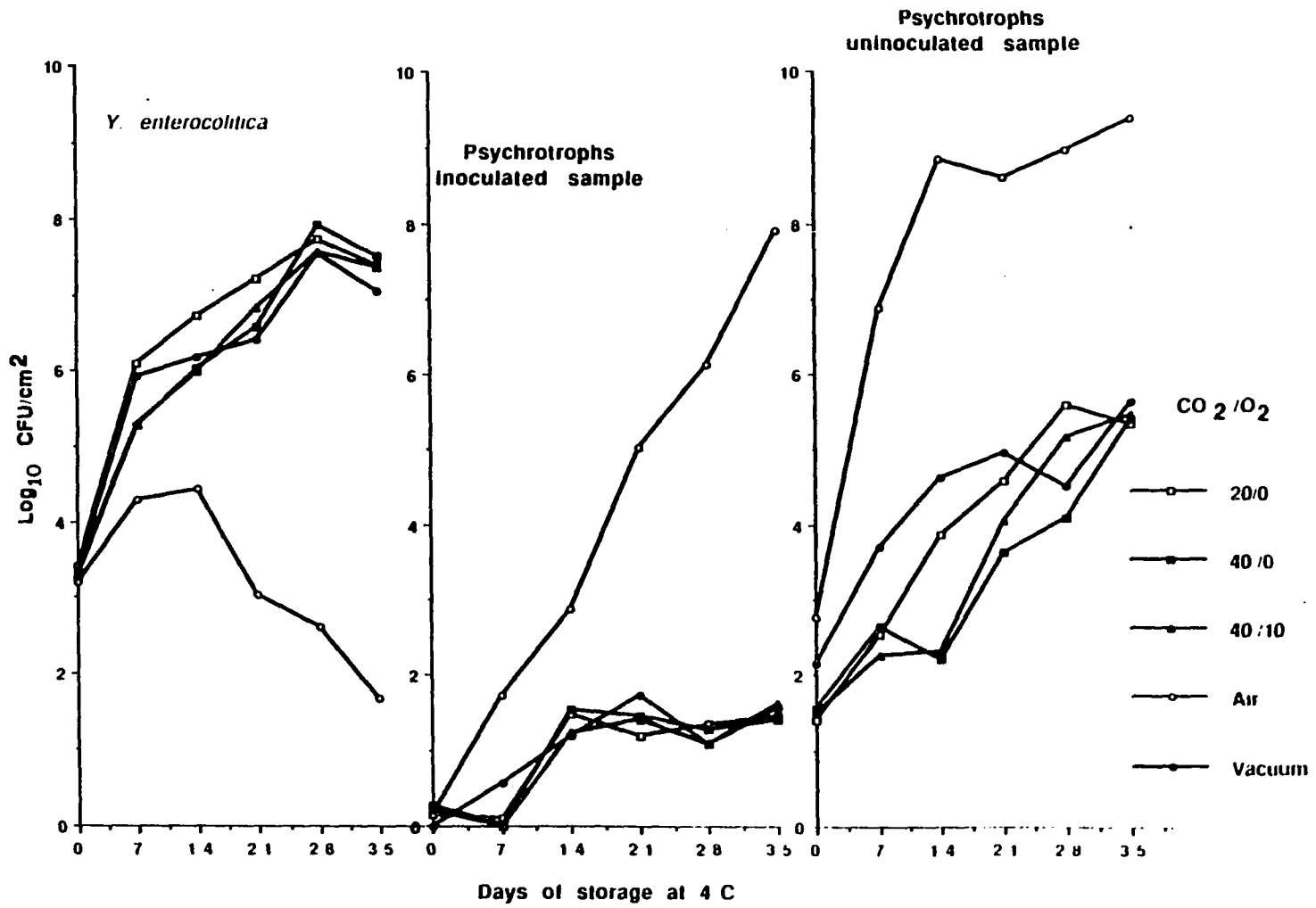


Table 1. Growth rates (regression coefficients<sup>a</sup>) of *L. monocytogenes* in inoculated fresh pork chops and aerobic psychrotrophic spoilage flora in uninoculated fresh pork chops packaged in different atmospheres and stored at 4°C

Atmospheres (CO <sub>2</sub> /O <sub>2</sub> ) <sup>b</sup>	<i>L. monocytogenes</i>	Aerobic psychrotrophic spoilage flora
20/0	0.07 <sup>x</sup>	0.11 <sup>Y</sup>
40/0	0.05 <sup>vx</sup>	0.11 <sup>Y</sup>
40/10	0.04 <sup>uv</sup>	0.11 <sup>Y</sup>
Vacuum	0.04 <sup>uv</sup>	0.11 <sup>Y</sup>
Air	0.03 <sup>u</sup>	0.34 <sup>z</sup>

<sup>a</sup>Regression coefficients for log<sub>10</sub> growth phase.

<sup>b</sup>Balanced with nitrogen.

<sup>uvxyz</sup>Values with the same superscripts are not significantly different at P=0.05.

Table 2. Growth rates (regression coefficients<sup>a</sup>) of *Y. enterocolitica* in inoculated fresh pork chops and aerobic psychrotrophic spoilage flora in uninoculated fresh pork chops packaged in different atmospheres and stored at 4°C

Atmospheres (CO <sub>2</sub> /O <sub>2</sub> ) <sup>b</sup>	<i>Y. enterocolitica</i>	Aerobic psychrotrophic spoilage flora
20/0	0.14 <sup>XY</sup>	0.15 <sup>Y</sup>
40/0	0.15 <sup>Y</sup>	0.14 <sup>XY</sup>
40/10	0.14 <sup>XY</sup>	0.15 <sup>Y</sup>
Vacuum	0.12 <sup>X</sup>	0.14 <sup>X</sup>
Air	0.09 <sup>V</sup>	0.44 <sup>Z</sup>

<sup>a</sup>Regression coefficients for log growth phase.

<sup>b</sup>Balanced with nitrogen.

<sup>vwxYZ</sup>Values with the same superscripts are not significantly different at P=0.05

**PART III. MICROBIOLOGICAL CHARACTERISTICS OF FRESH MEATS TREATED  
WITH COLOR MAINTENANCE SUBSTANCES (SODIUM  
ERYTHORBATE, TETRASODIUM PYROPHOSPHATE AND CITRIC  
ACID)**



**MICROBIOLOGICAL CHARACTERISTICS OF FRESH MEATS TREATED WITH  
COLOR MAINTENANCE SUBSTANCES (SODIUM ERYTHORBATE, CITRIC ACID,  
TETRASODIUM PYROPHOSPHATE)**

by

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**Running Title: Fresh meats color maintenance substances and microbiological  
safety**

## ABSTRACT

The effect of color maintenance substances in combination with modified atmosphere on the microbiological characteristics of fresh meats was examined. The meat samples, pork chops, beef steaks and ground beef, were treated with a mixture of sodium erythorbate, citric acid and tetrasodium pyrophosphate, packaged under modified gas atmosphere containing 50%CO<sub>2</sub>/15%O<sub>2</sub>/35%N<sub>2</sub>, and stored at 2°C under conditions simulating "wholesale" distribution, followed by retail display at 2°C. Mesophilic and psychrotrophic bacterial numbers were higher for the untreated pork chops, but there were no differences in the microbial numbers in the beef steaks or in the ground beef during "wholesale" distribution storage. There were also no differences in the microbial numbers in the pork chops or in the ground beef during retail display but the treated beef steaks were higher in the numbers of *Enterobacteriaceae* than the untreated sample. Modified atmosphere extended the shelf life of all the meat samples tested. The chemical treatment and the modified atmosphere, however, did not affect the populations of specific pathogenic microorganisms (*Yersinia enterocolitica*, *Hafnia alvei*, *Listeria monocytogenes*) isolated and identified in the pork chops and beef steaks.

## INTRODUCTION

Appearance, mainly color, is the most important factor in consumer acceptance of fresh meats. The "fresh" bright red color of meats is due to the oxygenated meat pigment, oxymyoglobin, with iron of the heme group in its ferrous state. Oxidative changes of the heme pigment produce metmyoglobin with the heme iron in its ferric form, which leads to the less desirable brown discoloration of fresh meat. The oxidative changes of the heme pigment (metmyoglobin formation) may be caused by bacterial, enzymatic or lipolytic action or by any process that leads to lower partial pressure of oxygen (Shivas et al., 1984).

Brown discoloration of fresh meat reduces consumer acceptance of prepackaged fresh meat cuts and ground meat. Hence, a longer color shelf life is advantageous to the marketing of fresh meat cuts and ground meat. The need to slow down formation of metmyoglobin in fresh meat without masking quality deterioration has been a continuing concern of the meat industry.

Work done over the past decade has indicated that reductants such as ascorbic acid or its derivatives, if used in proper amounts, are effective for maintaining fresh meat color for a long period of time, and may also delay lipid oxidation (Greene et al., 1971; Harbers et al., 1981; Shivas et al., 1984). This work has resulted in a very recent patent (Cheng, 1987) on the use of ascorbic or erythorbic acid or their salts in conjunction with phosphates and or sequestering agent (citric acid, tartaric acid or EDTA) for the extension of color shelf life in fresh meat. These three active constituents are supposed to retain the heme iron in a reduced (ferrous) state, and retard or preclude oxidation reactions by both the

reducing activity and sequestering oxidation promoting metallic ions. This patent combines chemical treatment for color preservation with a package that contains an atmosphere with elevated carbon dioxide levels. The modified atmosphere package is included to control microbial growth during storage and distribution of the product. It has been reported, however, that modified atmosphere conditions may not guarantee complete control of microbial growth (Gill and Tan, 1980). The conditions may even be suitable for growth of some anaerobic and/or facultatively anaerobic pathogenic organisms (Genigeorgis, 1985). In addition, ascorbic acid has been reported to have no effect on the growth of meat spoilage bacteria (Greene et al., 1971; Shivas et al., 1984). The treatment of fresh meat with these color maintenance substances and subsequent storage under a modified atmosphere at refrigeration temperatures may, therefore, mask spoilage of fresh meat by microorganisms or provide the right conditions for optimum growth of certain pathogenic organisms which have psychrotrophic characteristics. Consequently, there have been some concerns that the approved use of these compounds by the USDA to extend the color shelf life of pork has not been adequately evaluated for microbiological safety.

The objective of this work, therefore, was to evaluate the microbiological characteristics and safety of fresh meat (pork chops, beef steak and ground beef) which contained tetrasodium pyrophosphates, erythorbate and citric acid as color-maintenance substances; and to determine the combined effect of these compounds and modified atmosphere storage on the growth of selected, naturally occurring psychrotrophic pathogens such as *Yersinia enterocolitica*, *Hafnia alvei* and *Listeria monocytogenes*.

## MATERIALS AND METHODS

### Meat sample

Boneless pork loins, beef ribeye and coarsely ground beef (9.5mm) were obtained from a commercial source. Center cut pork chops and rib steaks were each cut 1.27 cm thick. An adequate number of meat samples was obtained for three replications.

### Color maintenance solution

A solution of the color maintenance substances was prepared containing 1.72% sodium erythorbate, 3.40% citric acid and 10.3% tetrasodium pyrophosphate resulting in the product concentrations of the compounds as permitted by USDA (MPR, 1987).

### Treatment of meat samples

The pork chops and beef steaks were hand dipped into the solution for 30 sec, removed, allowed to drip for 15 sec to give product concentrations of the compounds as permitted by USDA (MPR, 1987). They were retail packaged by placing on styrofoam trays. Each individual tray was overwrapped with oxygen-permeable commercial packaging film (Stretch Meat film, Cat # 37016, Hantover, Kansas City, MO, USA). The ground beef was mixed with 0.05% sodium erythorbate, 0.025% citric acid, and 0.30% tetrasodium pyrophosphate in a Leland mixer for 5 min. The mixture was then finely ground through a 3.2 mm plate in a Biro grinder (1). One-pound quantities of the ground beef were placed on styrofoam trays using a Risco stuffer. Equal numbers of wrapped trays of chops,

steaks and ground beef that were not treated with the color maintenance substances were also prepared.

#### Packaging and storage

Twenty-four each of the retail packages of the chops and steaks, and 30 of the ground beef packages were placed in one 75x47 cm master package with oxygen permeability of  $<1$  cc/645 cm/24 hr at 23°C and 0% R.H. and water vapor transmission  $<1.0$  g/645 cm/24 hr at 38°C and 90% R.H (Curlon 892, Curwood Inc., New London, WI, USA). Each master package contained 12 or 15 treated samples and 12 or 15 untreated samples of cuts and ground beef, respectively. There were a total of five master packages for each of the samples. Four master packages of each sample were flushed with a modified gas atmosphere containing 50%CO<sub>2</sub>/15%O<sub>2</sub>/35%N<sub>2</sub> and heat-sealed using a multivac packaging machine. The design provided two sets of control sample: one set that was not treated with the color maintenance substances but was flushed with the modified gas atmosphere, and one set that was treated with the color maintenance substances but was packaged in air (not flushed with the modified gas atmosphere).

The master packages were placed in cardboard boxes and held at 2°C (34°F) to simulate wholesale distribution and storage conditions for 0, 7, 14, 21 and 28 days before being opened. After the master packages were opened, the overwrapped packages were placed on display case shelves at 2°C (34°F) under a fluorescent light to simulate retail display condition, and to determine the residual effect of the treatments on the microbial flora of the meat samples. The samples were analyzed on the day of packaging, on each day a master package was

opened and every 2 days in retail display until the psychrotrophic bacterial counts reached  $10^7$  CFU/cm<sup>2</sup> (Kraft, 1986).

#### Microbiological sampling

On sampling days retail packages were aseptically opened and circular cuts of 50 cm<sup>2</sup> total surface area were obtained using a stainless circular borer (3.8 cm id). The circular cuts were macerated for 1 min in a sterile bag containing 100 ml 0.1% sterile peptone water using a Stomocher lab blender (Model 400 Tekmar™ Co. Cincinnati, OH, USA). This procedure made possible a minimum detection limit of 2 bacterial cells/cm<sup>2</sup> of sample surface.

Twenty-five grams of the ground beef were also macerated for 1 min in 225 ml of 0.1% sterile peptone water to obtain an initial dilution of  $10^{-1}$ . Serial dilutions were prepared from the macerate according to recommended procedures (Speck, 1984). All-purpose tween (APT, BBL, Cockeysville, MD, USA) agar was used to enumerate mesophilic and psychrotrophic bacterial counts; plates were incubated aerobically at 30°C for 48 hr and 7°C for 10 days, respectively. *Enterobacteriaceae* were enumerated in trypticase soy agar (TSA, BBL) overlaid with double-strength violet red bile (VRB, DIFCO, Detroit, MI, USA) agar and incubated at 37°C for 24 hr (Hartman et al., 1975).

The FDA enrichment procedure was used to isolate *L. monocytogenes*. It involved adding 10 ml macerate to 90 ml *Listeria* enrichment broth (LEB, BBL) and incubating at 30°C for 7 days. It was then surface plated onto modified McBride agar (MMA, BBL) and incubated at 35°C for 24 to 48 hr (Crawford et al., 1989). Blue colonies under 45° transmission light were presumed to be *Listeria* spp. (McClain and Lee, 1988).

### Microbiological identification

After counting of colonies, 10 colonies were randomly removed from each countable mesophilic and *Enterobacteriaceae* plates. The selected colonies from the mesophilic plates were streaked onto APT agar and incubated at 30°C, while the colonies selected from the *Enterobacteriaceae* plates were streaked on TSA agar and incubated at 37°C for 24 hr. Cultures were first identified by gram reactions, catalase and oxidase tests (Grau and Vanderlinde, 1990). The gram-positive isolates were further identified using the scheme of Harrison et al. (1981). The gram-negative cultures were identified to genus/species levels by using the Minitek Nonfermenter or *Enterobacteriaceae* differentiation system (BBL). The uncertain identifications of gram-negative organisms were further assisted by criteria from Bergy's Manual of Systematic Bacteriology (Kreig and Holt, 1984).

Presumptive *Listeria* colonies were transferred into sterile trypticase soy broth plus 5% yeast extract (TSBYE) and incubated at 30°C for 24 to 40 hr. Cultures were confirmed by the gram reaction, catalase, hanging drop and umbrella motility tests and also by biochemical tests (Golden et al., 1988).

### Statistical Analysis

The experiments were replicated three times. Microbial count data were transformed into logarithms and analyzed by analysis of variance using the GLM procedure (SAS, 1984). Paired contrasts were used in the procedure to determine significance among means, and least square difference was used to compare the means.



## RESULTS AND DISCUSSION

Wholesale storage

The numbers of naturally occurring mesophiles, psychrotrophs and *Enterobacteriaceae* of pork chops, beef steaks and ground beef stored in modified atmosphere under wholesale distribution conditions are presented in Fig. 1. There was an increase ( $P < 0.05$ ) in microbial populations with wholesale storage time for both treated and control samples and for all the samples tested. For pork chops, the numbers of mesophilic bacteria were higher ( $P < 0.05$ ) on the control than on the treated chops during the first two weeks of wholesale storage. Numbers of the treated sample, however, were higher than but not significantly different ( $P > 0.05$ ) from those of the control from the third week. No difference in numbers of psychrotrophic bacteria ( $P > 0.05$ ) was found for the treated and control pork chops, even though the numbers in the treated chops reached spoilage levels of  $\log 7.0$  CFU/cm<sup>2</sup> (Kraft, 1986) within four weeks of storage. The *Enterobacteriaceae* numbers in the chops were about the same during the first week of storage but thereafter those in the control chops increased and were about 0.5 to 0.9 log higher than those in the treated chops up to week three. At week four the *Enterobacteriaceae* numbers of the treated chops were higher than but not significantly different from those in the control.

For the beef steaks, no difference ( $P > 0.05$ ) in the numbers of mesophilic bacteria was found between the treated and the control samples throughout the study period, even though the numbers of the treated steaks reached spoilage levels within four weeks. On the other hand, the numbers of psychrotrophic bacteria were much higher ( $P < 0.05$ ) in the control than in the treated steaks during the first

two weeks of wholesale storage (Fig. 1), and at week four the psychrotrophic bacterial numbers in the control exceeded the spoilage level. Numbers of *Enterobacteriaceae* were not significantly different between the treated steaks and the control ( $P>0.05$ ) throughout the study period.

The initial microbial population of the ground beef sample was higher than that of the beef steaks ( $P<0.05$ ). At day 0, the mesophilic bacterial numbers in the ground beef were about log 5.0 in both treated and control samples (Fig. 1). No difference in numbers of mesophilic bacteria were found between treated ground beef and control throughout the wholesale storage period. The psychrotrophic bacterial numbers in the control were about log 1.0 higher than those of the treated sample. Nevertheless the psychrotrophs on the treated sample grew very rapidly and at week 2 were higher than but not significantly different ( $P>0.05$ ) from those in the control. The initial *Enterobacteriaceae* numbers in the control were also about 1.0 log higher than those in the treated ground beef (Fig. 1). However, the numbers in the control began to decrease after week 1 and were slightly lower than those in the treated sample at week 2.

#### Retail storage

Two sets of samples were stored in a retail display case: the air samples (i.e. those that were not packaged under modified atmosphere) and those that had been taken out of the MA packages. Fig. 2 shows the mesophilic bacterial numbers in the meat samples tested. For pork chops, no significant difference ( $P>0.05$ ) in mesophilic numbers between treated and control samples packaged in air were found. It was also observed that the retail life of samples packaged in air was longer than 8 days. On the other hand, the retail life of samples packaged under

MA depended on the wholesale storage period. The longer the samples remained in "wholesale" distribution storage, the shorter their retail life. For example, the retail life of both treated and control chops that had been stored in MA for 1 week was 8 days; for 2 weeks was 6 days, for 3 weeks was between 5 and 4 days, and for 4 weeks was 2 days. Mesophilic bacterial numbers in both treated and control chops increased very rapidly during retail storage and no difference in numbers ( $P>0.05$ ) was found between the two samples. A similar pattern of mesophilic bacterial growth was found for the beef steaks except that treated samples kept in wholesale distribution storage for 4 weeks did not have any retail life since numbers of mesophiles had exceeded spoilage levels when the MA package was opened (Fig. 2). For ground beef, the mesophilic bacterial numbers in the treated air packaged sample increased rapidly and reached spoilage levels within two days of retail display, while the control had 6 days retail life. Treated ground beef samples that were kept in wholesale storage for 2 weeks reached mesophilic bacterial spoilage levels within 4 days of retail display while those of the control took 6 days. However, no significant difference in the numbers of bacteria was found between the treated ground beef and the control ( $P<0.05$ )

Similar results were obtained for the psychrotrophic bacterial numbers (Fig. 3). That is, in general no difference in numbers of psychrotrophic bacteria were found between treated and control samples. Numbers of treated pork chops and beef steaks that had been kept in the wholesale storage for 4 weeks, however, had exceeded spoilage levels when the packages were opened while the control samples remained for two days in retail display without spoiling. Both treated and control chops and steaks kept in the wholesale storage for 2 and 3 weeks had retail life of 8 and 6 days, respectively. The retail lives of pork chops and beef steaks kept

for 1 week in the wholesale storage and those that were packaged in air were longer than 8 days for both treated and control samples. Nevertheless the psychrotrophic numbers of samples kept in wholesale storage for 1 week under MA were higher ( $P < 0.05$ ) than those of the air-packaged samples. For the ground beef no difference in psychrotrophic bacterial numbers in the treated and the control samples was found during retail storage. Both modified-atmosphere- and air-packaged samples reached or exceeded spoilage levels within 4 days of retail display. On the day the modified atmosphere packages were opened, microbial numbers of modified-atmosphere packages that had been kept for 1 week in the wholesale storage were not significantly different from those of the air-packaged samples ( $P > 0.05$ ).

Numbers of *Enterobacteriaceae* in the meat samples tested are presented on Fig. 4. For the pork chops there were generally no significant differences in numbers between treated samples and the control ( $P > 0.05$ ) with the exception of day 4 when the numbers of the treated sample that had been kept for 3 weeks in the wholesale storage under MA were about 1.2 log CFU/cm<sup>2</sup> higher than those of its control counterpart. Numbers in the air-packaged chops were very low on day 0 but increased very rapidly and within 8 days of retail storage were not significantly different from those in the chops that had been kept for 1 week in the wholesale storage under MA. For the beef steaks, treated samples that came out of the MA packages had significantly higher numbers ( $P < 0.05$ ) than their control counterparts during retail storage. No difference in numbers, however, was found between treated and control steaks that had been packaged in air. Numbers in the air-packaged samples increased only slightly with retail storage time. For the ground beef, there was no significant difference in the numbers between treated and

control samples during retail storage for air-packaged or for samples that had been kept in wholesale storage under MA. No difference was found also between numbers in air-packaged samples and those in samples originally packaged under MA throughout the retail storage time. In general MA achieved a considerable extension of shelf life since meat samples packaged under MA could be stored for additional days in retail display in some cases with similar retail life as those samples placed in retail display initially.

#### Composition of microflora

Gram-negative bacteria seemed to be more predominant than gram-positive bacteria among the mesophilic microflora of both pork chops and beef steaks stored under wholesale distribution conditions. Table 1 shows that the most predominant gram-negative mesophilic bacteria in pork chops were *Pseudomonas* and *Serratia*., followed by *Aeromonas*. *Micrococcus* were the most predominant among the gram-positive bacteria. *Listeria* spp. were not isolated from any of the chops during the study. Among the isolates from the *Enterobacteriaceae* plates, *Serratia* was the most predominant (Table 2). The organisms of concern that were also isolated from the *Enterobacteriaceae* plates were *Hafnia*, *Yersinia*, *Shigella* and *Klebsiella*. However, the proportions of these pathogens and those of *Aeromonas* were too small to present any health hazards. Generally no difference in the populations of these organisms was found between the treated sample and the control, and their proportions did not increase with storage time.

The most predominant gram-negative bacteria among the mesophilic microflora of the beef steaks were *Pseudomonas* followed by *Aeromonas* (Table 3). No *Listeria* spp. were isolated from the samples throughout the study. The most

predominant gram-positive bacteria were *Micrococcus* and *Lactobacillus*. The former appeared early during storage while the latter appeared only after 21 days of storage. Among the isolates from the *Enterobacteriaceae* plates, *Serratia* was the most predominant (Table 4). Foodborne pathogens that were isolated were *Hafnia*, *Yersinia*, *Shigella* and *Klebsiella*. Again no differences in the populations of isolates from either the mesophilic or *Enterobacteriaceae* plates were found between treated steaks and control. The proportions of foodborne pathogens or potential pathogens isolated from the beef steaks were also not high enough to cause any potential health risks. Because microbial numbers were higher initially in ground beef and increased more rapidly, isolates from ground beef were not studied for identification.

The results of a study by Ammann (1989) showed that the treatment with these color maintenance substances produced a darker and redder product surface for all the meat samples tested, but was more effective in extending the color stability of the ground beef than that of the beef steak which was much better than the pork chops. According to the author the treatment extended the retail color shelf life of the ground beef by about 1 to 3 days. However, the microbial numbers of the product during this period were close to spoilage levels. Thus, this observation, together with the significantly higher numbers of *Enterobacteriaceae* in the treated beef steaks than the control during retail display, may be potential for masking microbial problems with longer color shelf life, even though no difference in the population proportions of pathogens and potential pathogens was found in the beef steak. Hence the microbiological characteristics of the treated beef steaks point to the necessity of a very careful microbiological evaluation for any further increase in color shelf life.

## CONCLUSIONS

Microbial growth is unaffected by color treatment in all the samples during wholesale distribution storage, but modified atmosphere is effective for increasing the shelf life of all products regardless of treatment.

Color treatment in combination modified atmosphere storage does not select for growth of pathogens or potential pathogens of concern during wholesale distribution storage.

Previous studies have shown that the color treatment is effective in extending the color shelf life of fresh meats, especially ground beef. The uncontrolled growth of bacteria in ground beef and the increase in the numbers of *Enterobacteriaceae* in the beef steaks during retail display may therefore have the potential for masking microbial problems with extended color stability.

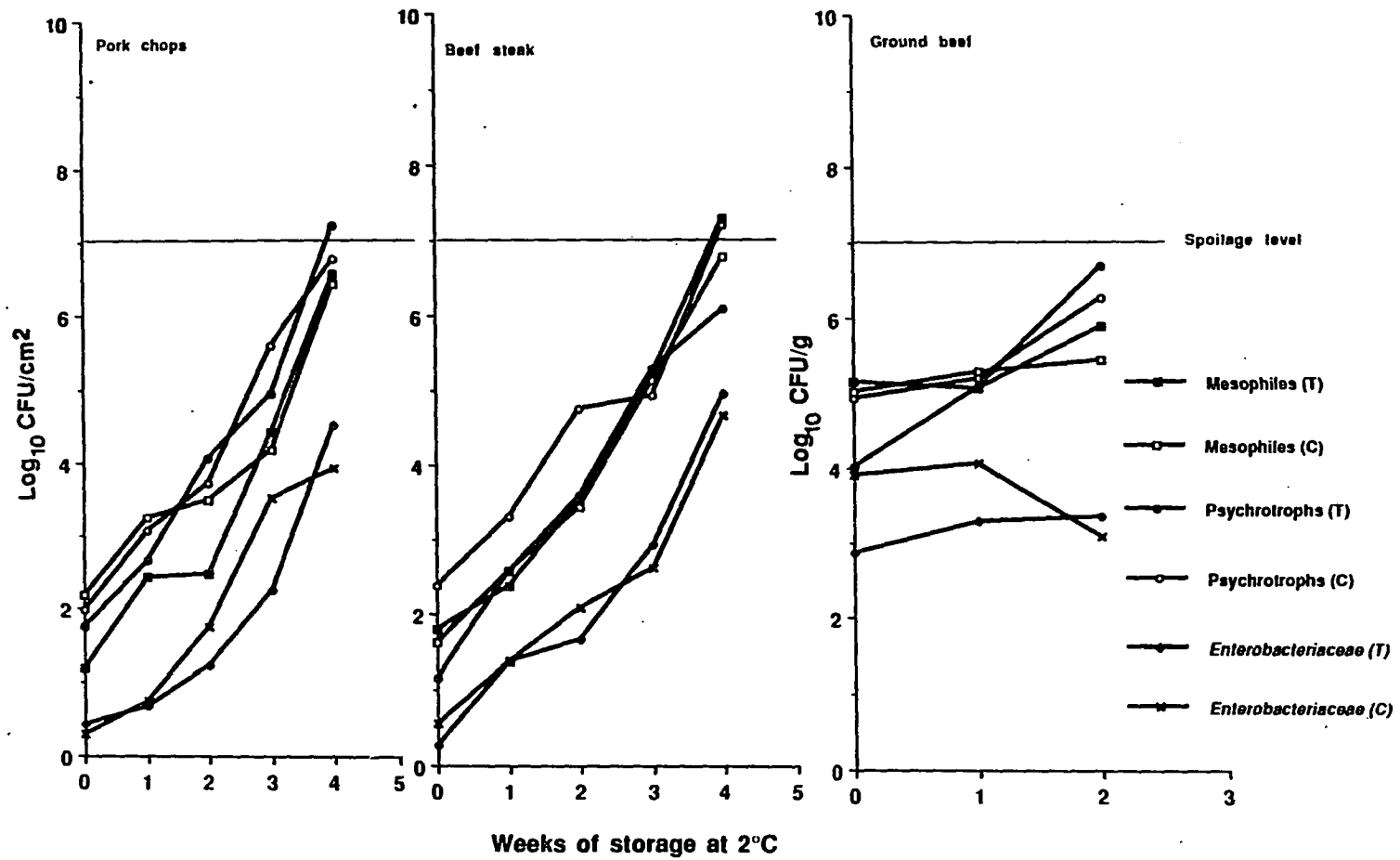
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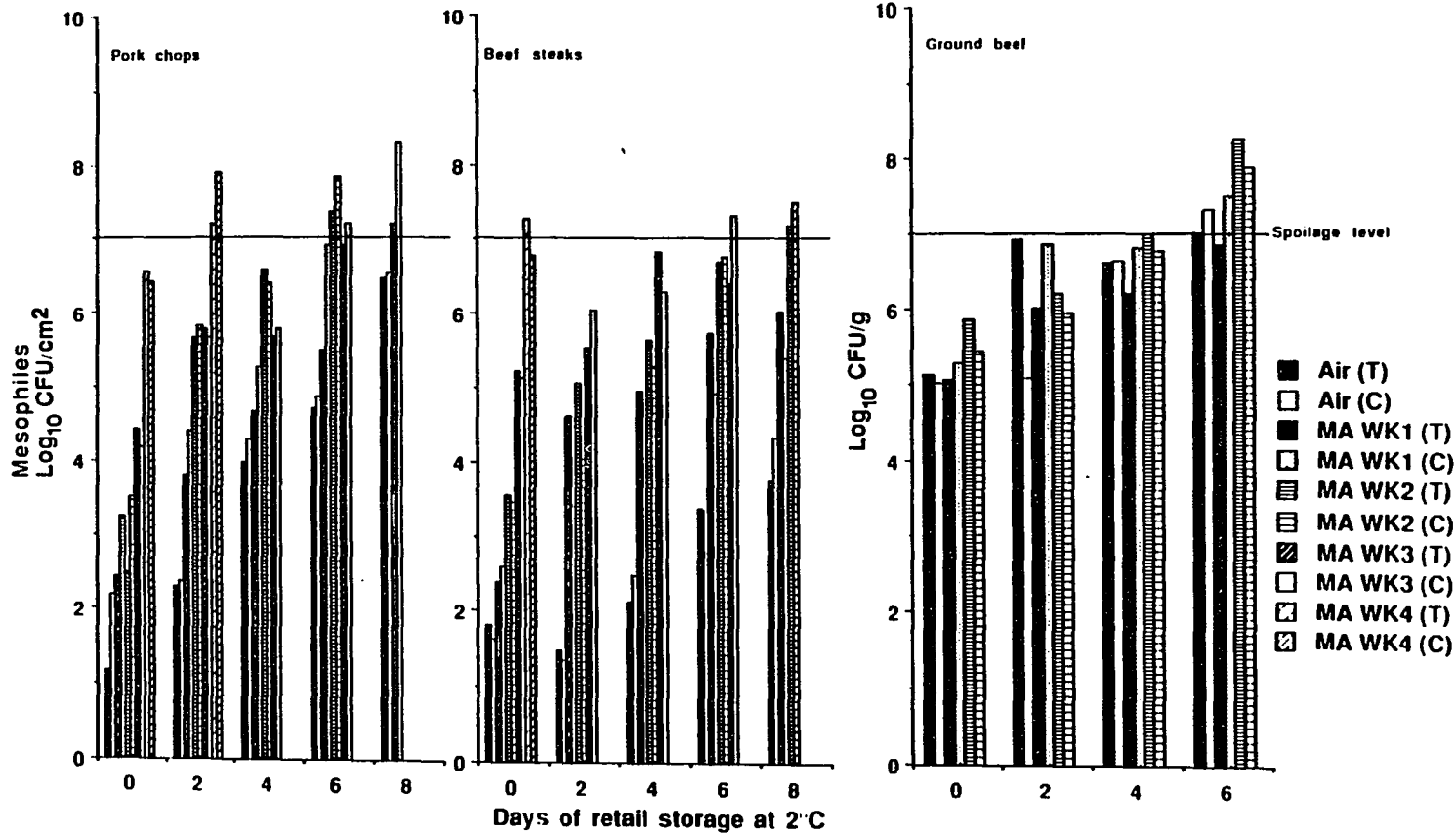


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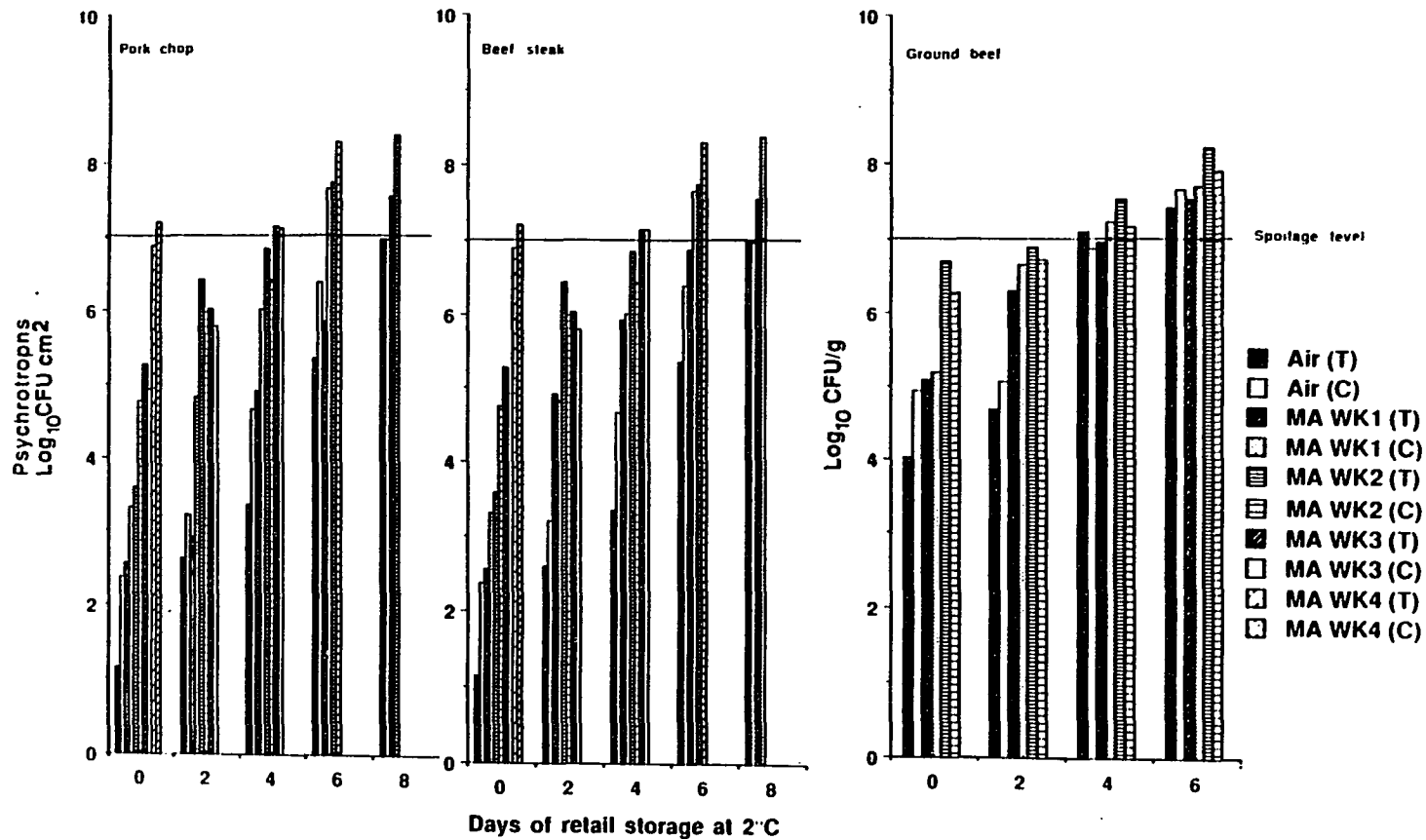
**Fig. 1. Mesophilic, psychrotrophic and *Enterobacteriaceae* numbers in treated (T) and control (C) meat samples stored in a modified atmosphere (50%CO<sub>2</sub>/15%O<sub>2</sub>/35%N<sub>2</sub>) at 2°C under "wholesale" distribution conditions.**



**Fig. 2. Numbers of mesophilic bacteria in treated (T) and control (C) meat samples initially packaged in modified atmosphere (MA) or in air and placed in retail display at 2°C.**



**Fig. 3. Numbers of psychrotrophic bacteria in treated (T) and control (C) meat samples initially packaged in modified atmosphere (MA) or in air and placed in retail display at 2°C.**



**Fig. 4.** Numbers of *Enterobacteriaceae* in treated (T) and control (C) meat samples initially packaged in modified atmosphere (MA) or in air and placed in retail display at 2°C.



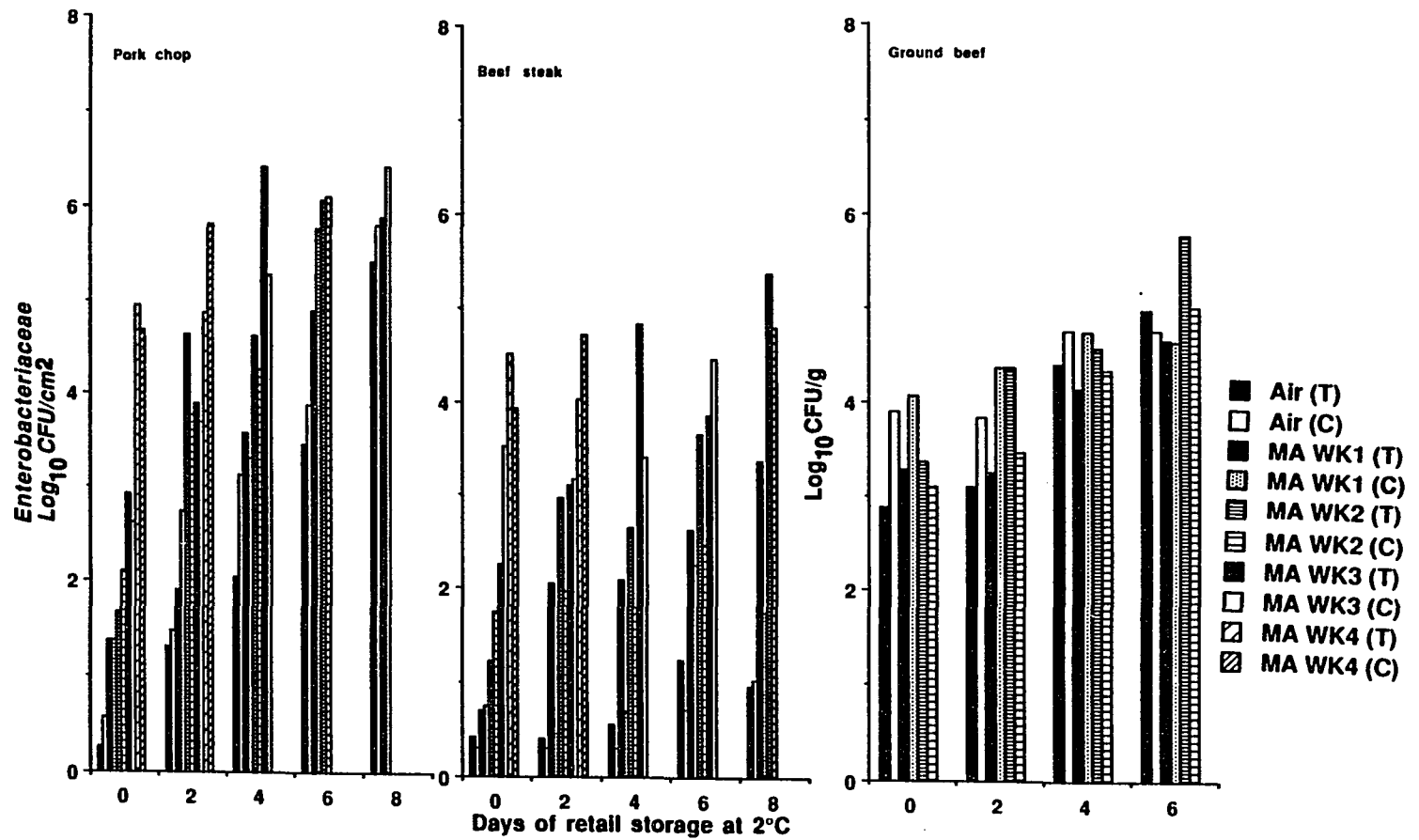


Table 1. Identity of isolates from plates used to enumerate mesophiles from pork chops stored under wholesale distribution conditions at 2°C

Sampling day and number of isolates from three replications										
	Days at 2°C									
	0		7		14		21		28	
	I	C	I	C	I	C	I	C	I	C
<b>Gram-negatives</b>										
<i>Pseudomonas</i>	9	19	12	21	12	19	11	18	17	28
<i>Aeromonas</i>	0	0	5	6	8	5	5	1	1	3
<i>Acinetobacter</i>	0	1	3	0	1	9	5	0	0	0
<i>Serratia</i>	5	4	20	19	10	18	13	17	23	22
<i>Enterobacter</i>	1	0	0	0	0	0	0	0	0	0
<b>Gram-positives</b>										
<i>Listeria</i>	0	0	0	0	0	0	0	0	0	0
<i>Coryneform</i>	0	0	5	2	1	7	3	0	0	0
<i>Micrococcus</i>	5	7	4	7	1	2	0	6	1	1
<i>Bacillus</i>	0	0	0	0	0	0	1	3	4	2
<i>Lactobacillus</i>	0	0	0	0	0	0	3	9	9	4
Yeast	0	0	3	2	0	0	1	3	0	0
No growth on transfer	10	6	5	3	15	0	22	5	4	0
Total	30 <sup>a</sup>	37 <sup>a</sup>	60	60	60	60	60	60	60	60

<sup>a</sup>Less than 20 colonies per replication were present for isolation; T=treated sample; C=control.

Table 2. Identity of isolates from plates used to enumerate *Enterobacteriaceae* from pork chops stored under wholesale distribution conditions at 2°C

Sampling day and number of isolates from three replications										
	Days at 2°C									
	0		7		14		21		28	
	I	C	I	C	I	C	I	C	I	C
<i>Hafnia</i>	2	3	1	0	0	0	0	0	0	0
<i>Serratia</i>	8	1	14	1	23	10	1	13	12	17
<i>Yersinia</i>	0	3	4	0	3	5	2	8	2	0
<i>Enterobacter</i>	4	1	0	0	0	0	3	11	4	2
<i>Shigella</i>	0	0	0	0	1	0	0	0	0	1
<i>Acinetobacter</i>	0	4	1	1	0	1	0	1	0	0
<i>Klebsiella</i>	2	1	2	0	0	0	3	0	6	0
Nonfermentative gram-negative rods	2	2	3	0	30	38	11	22	23	38
Unidentified <sup>a</sup>	2	1	5	3	3	6	2	5	3	2
Total	20 <sup>b</sup>	16 <sup>b</sup>	30 <sup>b</sup>	5 <sup>b</sup>	60	60	22 <sup>b</sup>	60	50 <sup>b</sup>	60

<sup>a</sup>Unidentified isolates.

<sup>b</sup>Less than 20 colonies per replication were present for isolation.

T=treated sample; C=control.

Table 3. Identity of isolates from plates used to enumerate mesophiles from beef steaks stored under wholesale distribution conditions at 2°C

Sampling day and number of isolates from three replications										
	Days at 2°C									
	0		7		14		21		28	
	I	C	I	C	I	C	I	C	I	C
<b>Gram-negatives</b>										
<i>Pseudomonas</i>	9	19	12	20	26	40	23	12	17	18
<i>Aeromonas</i>	0	0	17	6	1	1	5	10	10	15
<i>Acinetobacter</i>	0	6	6	8	3	3	4	0	0	0
<i>Enterobacter</i>	3	3	5	0	3	0	1	0	0	0
<b>Gram-positives</b>										
<i>Listeria</i>	0	0	0	0	0	0	0	0	0	0
<i>Coryneform</i>	0	0	4	1	6	1	5	0	6	0
<i>Micrococcus</i>	8	10	3	8	1	3	0	0	0	0
<i>Bacillus</i>	0	0	1	0	1	0	6	6	0	0
<i>Lactobacillus</i>	0	0	0	0	1	0	9	10	22	15
Yeast	0	0	0	6	5	9	3	3	0	0
No growth on transfer	15	10	9	11	13	14	16	19	5	12
Total	34 <sup>a</sup>	48 <sup>a</sup>	60	60	60	60	60	60	60	60

<sup>a</sup>Less than 20 colonies per replication were present for isolation.  
T=treated sample; C=control.

Table 4. Identity of isolates from plates used to enumerate *Enterobacteriaceae* from beef steaks stored under wholesale distribution conditions at 2°C

Sampling day and number of isolates from three replications										
	Days at 2°C									
	0		7		14		21		28	
	I	C	I	C	I	C	I	C	I	C
<i>Hafnia</i>	1	5	4	0	9	6	9	3	10	8
<i>Serratia</i>	6	12	13	14	28	23	21	11	15	22
<i>Yersinia</i>	4	4	6	5	5	10	8	15	11	9
<i>Citrobacter</i>	0	2	0	0	0	0	0	0	0	0
<i>Enterobacter</i>	10	7	0	0	3	3	2	16	5	3
<i>Shigella</i>	1	2	0	0	3	1	1	0	3	3
<i>Klebsiella</i>	1	0	1	0	3	0	4	2	1	3
<i>Acinetobacter</i>	6	3	0	1	4	5	3	6	2	2
<i>Pseudomonas</i>	5	0	0	0	3	6	2	1	1	1
Unidentified <sup>a</sup>	5	3	6	2	2	6	6	4	8	7
Total	39 <sup>b</sup>	36 <sup>b</sup>	22 <sup>b</sup>	60	60	60	60	60	60	58 <sup>b</sup>

<sup>a</sup>Unidentified isolates.

<sup>b</sup>Less than 20 colonies per replication were present for isolation.

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## SUMMARY

The influence of modified gas atmospheres containing various levels of carbon dioxide (10 to 50%) and oxygen (0 to 40%) on the microbiological, physical and chemical characteristics of fresh red meats (pork chops, beef steaks and ground beef) was examined in this study. In the first experiment, increasing the carbon dioxide concentration delayed the growth of psychrotrophs, mesophiles and *Enterobacteriaceae* in pork chops stored at 4°C. Increasing the oxygen concentration slowed down the growth of facultative anaerobic and anaerobic bacteria, but increased that of *Brochothrix thermosphacta*. In contrast the effect of increasing carbon dioxide and oxygen concentrations on growth of lactic acid bacteria was less pronounced. Growth of lactic acid bacteria increased only slightly with increasing carbon dioxide concentration. When the storage life of the pork chops was considered in terms of aerobic psychrotrophic microorganisms, it was found that increasing the carbon dioxide concentrations to 10, 20 and 40% extended the storage life by 7, 14 and 28 days, respectively, compared to air, and that 40%CO<sub>2</sub> with or without oxygen was more effective than vacuum in delaying growth of bacteria on pork chops during 21 days storage at 4°C. Inherent psychrotrophic pathogens such as *Yersinia enterocolitica*, *Hafnia alvei*, *Escherichia coli*, *Shigella*, *Klebsiella* and *Vibrio* spp., but not *Listeria*, were isolated from packages but their proportions were too small to show specificity for any gas combination.

Results obtained from the physical and chemical measurements indicated that there were no significant ( $P < 0.05$ ) differences in surface pH values among gas mixtures or among atmospheres. However, increasing the carbon dioxide

concentration slightly increased Hunter "L" values, reduced significantly ( $P < 0.05$ ) Hunter "a" values of the chops, increased purge losses, and at oxygen concentrations less than 40%, promoted lipid oxidation, but retarded the formation of volatile basic nitrogen (VBN) which increased with increasing psychrotrophic bacterial population. Increasing the oxygen concentration also increased lipid oxidation in the pork chops. No differences were found in the Hunter "a" values of pork chops packaged under gas atmosphere or under vacuum, but the latter had significantly lower ( $P < 0.05$ ) TBA values than the gas atmosphere-packaged chops. On the other hand, the gas atmosphere packages contained less exudate than the vacuum packages.

Results from the second part of the study showed that *L. monocytogenes* or *Y. enterocolitica* grew on inoculated fresh pork chops packaged in atmospheres consisting of three modified gas mixtures (20%CO<sub>2</sub>/0%O<sub>2</sub>/80%N<sub>2</sub>; 40%CO<sub>2</sub>/0%O<sub>2</sub>/60%N<sub>2</sub>; 40%CO<sub>2</sub>/10%O<sub>2</sub>/50%N<sub>2</sub>), vacuum and air. The growth rates of *L. monocytogenes* in the various atmospheres as determined by regression coefficients was in the following order: 20%CO<sub>2</sub>/0%O<sub>2</sub>/80%N<sub>2</sub> > 40%CO<sub>2</sub>/0%O<sub>2</sub>/60%N<sub>2</sub> > 40%CO<sub>2</sub>/10%O<sub>2</sub>/50%N<sub>2</sub> > Vacuum > Air. For *Y. enterocolitica* the order was 40%CO<sub>2</sub>/0%O<sub>2</sub>/60%N<sub>2</sub> > 40%CO<sub>2</sub>/10%O<sub>2</sub>/50%N<sub>2</sub> > 20%CO<sub>2</sub>/0%O<sub>2</sub>/80%N<sub>2</sub> > Vacuum > Air. Thus, doubling the CO<sub>2</sub> concentration reduced the growth rate of *L. monocytogenes* but increased that of *Y. enterocolitica*, and increasing the O<sub>2</sub> concentration reduced the growth rates of both *L. monocytogenes* and *Y. enterocolitica*. In the air packages, *L. monocytogenes* or *Y. enterocolitica* grew at rates slower than those of the psychrotrophic spoilage flora. In the vacuum and gas atmosphere packages, *L. monocytogenes* grew at a slower rate, but *Y. enterocolitica* grew at about the same



rate as the spoilage flora. The growth of these psychrotrophic pathogens on pork chops packaged under the various atmospheres support the need for proper sanitation during handling to reduce contamination of these pathogens that have frequently been isolated from fresh meats.

Results from the third part of this investigation demonstrated that microbial growth in fresh meats was unaffected by treatment with a mixture of color maintenance substances (sodium erythorbate, citric acid and tetrasodium pyrophosphate) and storage in 50%CO<sub>2</sub>/15%O<sub>2</sub>/35%N<sub>2</sub> gas atmosphere at 2°C under wholesale distribution conditions. Mesophilic and psychrotrophic bacterial numbers were higher for the untreated pork chops, but there were no differences in the microbial numbers in the beef steaks or in the ground beef samples. When the modified atmosphere packages were opened and the samples were placed in retail display, no differences were found in the microbial numbers in the pork chops or in the ground beef, but the treated beef steaks were higher than the untreated ones in the numbers of *Enterobacteriaceae*. Since the color maintenance substances have been found to be effective in extending the color stability of beef steaks, the increase in the numbers of *Enterobacteriaceae* in the treated beef steaks during retail display may have the potential to mask microbial problems with extended color stability even though the chemical treatment and modified atmosphere packaging did not affect the inherent populations of specific pathogenic microorganisms. *Listeria* was not isolated from any sample but *Y. enterocolitica*, *Hafnia alvei* and *Aeromonas* spp. were isolated from both treated and untreated samples during wholesale distribution storage. Their proportions, however, were not high enough to warrant any significant health hazard.

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