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Effect of controlled atmosphere packaging on psychrotrophic growth and succession on steak surfaces

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The University of Arizona, 1985

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EFFECT OF CONTROLLED ATMOSPHERE PACKAGING ON PSYCHROTROPHIC GROWTH AND SUCCESSION ON STEAK SURFACES

by

Hamdi Abdulilah Ahmad

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ABSTRACT

Top loin steaks were used to determine the influence of packaging procedure on the microbial growth and succession on the top and bottom surfaces of steaks during a 12 day storage period. The following packaging treatments were used: (1) Gas permeable Resinite film overwrap as a control; (2) gas flush with 1% CO, 40% O_2, 59% N_2 for 2 minutes followed by film overwrap; (3) loose packaging in gas impermeable barrier bags with 100 to 150 cc ambient air; (4) 15% CO_2, 40% O_2, 45% N_2 gas atmosphere; (5) 60% CO_2, 40% O_2 gas atmosphere; and (6) 10% CO_2, 5% O_2, 85% N_2 gas atmosphere.

Total psychrotrophic counts obtained from the top and bottom surfaces did not differ significantly for most sampling periods. Similar growth patterns were observed on both steak surfaces, increasing ($P<.05$) primarily between Day 3 and Day 9 of post-treatment storage. The steaks packaged within the gas atmospheres had lower ($P<.05$) total growth than the control steaks. Comparing atmospheres, the steaks packaged in relatively low CO_2 and O_2 levels (10% and 5%, respectively) had lower ($P<.05$) microbial growth than steaks packaged in 15 to 60% CO_2 and 40% O_2 when initial contamination was low.

Pseudomonas dominated the microflora on the steak surfaces in all packaging treatments during early storage. Other aerobic organisms which survived gas atmosphere treatments include Coryneforms, Micrococcus, and Microbacterium, although they did not show large increases in growth. Coryneforms were also competitive on the bottom surface of the control steaks despite domination by the pseudomonad organisms.

Loose packaging in impermeable barrier bags allowed the growth of Serratia liquifaciens after 6 days of storage. This bacterium also tended to dominate the flora within the gas atmosphere packages, although other facultative organisms (Enterobacter aerogenes and Yersenia enterocolitica) were also identified.

When initial contamination was low, the gas atmospheres provided an effective means of prolonging the shelf-life of fresh beef steaks.

INTRODUCTION

Animals harbor organisms, which can cause food spoilage and food-borne diseases, within their gastrointestinal tract and nasal passages as well as on their skin, hooves, hair, and other outer surfaces. Futhermore, lymph nodes are considered an internal source of microorganisms. During slaughter and processing, these organisms may be readily transferred to the edible portion of the animal carcass. Therefore, humans are an important source of meat contamination throughout the meat handling system.

Meat can provide the microbial cells with the necessary energy to synthesize cellular protoplasm. Furthermore, meat can supply the required carbon, nitrogen, and other growth factors (vitamins, minerals, and water) necessary for microbial growth and development. The water activity of fresh meat (0.95 to 1.00) is in the optimum range for microbial growth on meat surfaces.

Recently consumers have become much more conscious of meat value and quality. Consumers are more sensitive to off-odors, off-flavors, discoloration, or any other indication of spoilage. Obviously, the quality of meat is the most important factor in marketing meat products.

Microbial growth is of major concern in the meat industry since meat is often aged 10 to 14 days and may be imported or shipped through centralized distribution systems. Therefore, in addition to refrigeration, it becomes necessary to develop packaging techniques which extend the shelf-life of meat products.

Because meat is perishable, it is stored at refrigerated temperatures. Psychrophilic and/or psychrotrophic organisms dominate and are primarily responsible for meat spoilage. The amount of nutrients available for the organisms may be higher on the bottom surface of meat cuts due to the accumulation of cell exudates, while available oxygen may be greater on the top surface. Unfortunately, sampling procedures for microbial growth generally require only the top surface of meat cuts to be tested when, in fact, the growth and succession may vary between the top and bottom surfaces.

For many years, various gases have been known to inhibit the growth of microorganisms. Carbon dioxide, oxygen, and nitrogen are most commonly used individually or in combination to increase the shelf-life of fresh meat.

Even though gas atmosphere packaging can be effective in increasing shelf-life, it does not inhibit every kind of organism found on meat. Therefore, the purpose of this study was to evaluate the effect of different controlled

atmospheres on the growth and succession of the microflora found on meat surfaces during storage at low temperature.

LITERATURE REVIEW

Sources of Contamination

With the exception of external surfaces and gastrointestinal and respiratory tracts, the tissue of normal healthy animals contain few microorganisms. According to Gardner and Carson (1967) and Gill, Penney and Nottingham (1976) a high proportion of sterile meat can be obtained from healthy animals if enough aseptic precautions have been taken. This agreed with the findings of Burn (1934) and Jensen and Hess (1941) who indicated that bacterial contamination was postmortem rather than antimortem. Mallmann (1932) and Moran (1935) reported that most of the meat spoilage problems are bacterial in nature and found on the surface. Empey and Vickery (1933) and Empey and Scott (1939) agreed that most of the initial flora found on meat surfaces had come from the hide and hair of animals. Similar findings were reported by Ayres, Ogilvy and Stewart (1950) in association with the contamination from feathers, feet, and feces. Ayres (1955) and Frazer (1978) stated that although the literature shows some conflicting reports, it is generally accepted that muscle tissue of healthy living animals contain few or no microorganisms and the principle

invasion of these occur during the various processes related to slaughtering, dressing, and handling of the carcasses.

Since the animals' defense mechanisms effectively control infectious agents in the healthy living body, the number of microorganisms within tissues are affected by the stress state of the animals; the number rising under stress and falling again after rest (Narayan and Takacs 1966). The number of organisms found on the surface of the animals at slaughter depend upon several factors such as method of transportation and temperature of the soil. The proportion of psychrotrophic bacteria is higher in soil from temperate zones than that from tropical areas (Empey and Scott 1939). Animals from feedlots may carry fewer soil organisms and more fecal bacteria than these from pasture. The nature and amount of fecal organisms are affected by the type of diet (Grau and Smith 1974).

The first major source of meat contamination starts with the slaughter process. This microbial contamination includes the normal flora of the skin (staphylococci, micrococci, yeast, and molds) as well as fecal and soil microorganisms. Ayres (1955) stated that the total number of microorganisms found on the skin may reach 10^9 cells/cm^2. He also indicated that the composition of the flora found on the meat reflect different sources of contamination. Knives, hands, clothing of the workers, and water used in washing the carcasses are also sources of contamination

during the dressing process. However, Ingram and Simpson (1980) noted that the most important source of meat contamination is from the animal itself. Washing the surface of the carcass with chlorinated hot water may reduce the total number ten-fold from the initial contamination (Kelly, Dempster and McLoughlin 1974).

Chilling is one of the factors which increases meat contamination due to psychrophilic and psychrotrophic organisms which grow well at refrigerated temperatures. According to Nottingham and Wayborn (1975), the time the carcasses are held in the chilling room may have more effect on their microbial populations than the temperature of chilling. The contamination in the chilling room is mainly air borne contamination, and it is important not only to economics, but also to air borne pathogen transfer (Heldman 1974). Heldman (1974) also reported that drying the floor of the meat plants caused significant increases in the microbial population in the air within 12-15 hours.

Cutting and boning the meat are also important sources of contamination by transferring organisms from the cutting surface to the fresh meat cut (Ingram and Simpson 1980). Furthermore, contamination that builds up on equipment through inadequate sanitization is likely to contain high proportions of psychrophilic bacteria.

Types of Microorganisms

Oxidative Microorganisms

The types of organisms isolated from meat stored at refrigerated temperatures have been studied extensively. In 1901, Glage, as reviewed by Ayres (1960b), reported that the moist surfaces of meat stored at low temperature and high humidity became covered with bacterial colonies. These organisms, which he called "aromabacterien", were oval to rod shaped with rounded ends and occurred occasionally in chains. They were motile aerobes which liquified gelatin slowly and turned litmus milk alkaline. They grew well at 2 C but poorly at 37 C, and the optimum temperature was thought to be 10 to 12 C. Glage also noticed a characteristic odor which he considered pleasant. The odor accompanied the growth of the organisms on the surface of the meat which developed to form viscus or slimy coating. Haines (1937) found that the organisms responsible for the formation of the slime layer on the meat surface was identical with, or closely related to, "aromabacterien" found by Glage. Haines (1933) reported that with the exception of certain numbers of *Pseudomonas* and a few *Proteus*, the bacteria growing on lean meat stored at 4.0 C almost all belong to the *Achromobacter* group. This agreed with the findings of Empey and Scott (1939) who found that the genus *Achromobacter* may represent 90% of the total organisms isolated from meat stored at 0 C. At the same time, they

reported that the Flavobacterium form represented 3%, the Micrococcus form 7%, and the Pseudomonas form less than 1% of the total organisms. A number of workers (Stewart 1932, Moran 1935, Scott 1936, Ayres et al. 1950, and Ogilvy and Ayres 1951) reported that the formation of slime on the surfaces of fresh meat was due to organisms of Achromobacter and Pseudomonas types. Ayres et al. (1950) also reported that out of 50 representative colonies isolated from ground meat, 78% were typical spoilage bacteria (Achromobacter-Pseudomonas) while only 18% of the isolates were comprised of other typical spoilage forms.

Empey and Vickery (1933) found 95% of the initial flora of beef which grew at 30 F were members of the genus Achromobacter; the remainder were Pseudomonas and Micrococcus. They also reported that during storage the relative numbers of Achromobacter and Pseudomonas increased while those of Micrococcus decreased. Lochhead and Landerkin (1935) identified the genera Micrococcus, Flavobacterium, and Achromobacter, and found them to be the predominant bacteria which developed on poultry skin during storage. Kirsch, Berry, Baldwin and Foster (1952) reported that gram negative, non-spore forming rods which belong to Pseudomonas and/or Achromobacter genera were predominant throughout the storage of ground beef at 0 to 2 C. They also identified some of the organisms which were isolated before storage as Micrococcus, although they were overgrown during storage.

Kirsch et al. (1952) tried to demonstrate the succession of microorganisms in ground meat stored at refrigeration temperature. In this study two samples were taken and 5Ø isolates were identified from each sample. In the first sample the initially predominant organisms were Lactobacillus and non-pigmented Pseudomonas species. These pseudomonads were found to be equally divided between gelatin liquifiers and non-liquifiers. As the storage time increased, the proportion of gelatin liquifiers increased and remained high throughout the remaining storage time. In the second sample, lactobacilli represented only a small proportion of the initial isolates. Gelatin non-liquifier pseudomonads were predominant in the beginning but later they were replaced by gelatin liquifying types.

Halleck, Ball and Stier (1958a) investigated the bacterial flora of pre-packaged fresh meat during four weeks storage period at 34 to 38 F. They found that non-pigmented Achromobacter-Pseudomomas type and members of the genus Lactobacillus were predominant during the first two weeks of storage while Pseudomonas fluorscens type organisms predominated in the later storage period. Halleck et al. (1958a) used a number of packaging materials and found that packaging material did not selectively affect growth rates of the predominant genera.

In another study, Halleck, Ball and Stier (1958b) reported that the packaging material had an effect on

bacterial growth. The gas permeable films allowed rapid growth of bacteria until surface dehydration and mold growth started. The impermeable films increased the lag phase and the total bacterial count was not as high as the total count in the permeable films.

Brown and Weidemann (1958) studied the taxonomical characteristics of psychrophilic meat spoilage bacteria. They isolated 189 psychrophilic bacteria from beef stored at chilled temperatures and from associated sources. Of these, 182 colonies were gram negative and 7 colonies were gram positive. Out of the total gram negative, 128 isolates were classified typical Pseudomonas because of the following characteristics: All had one or more polar flagella, all produce acid oxidatively from glucose, and all were resistant to penicillin. In addition to these characteristics, 56 pseudomonad isolates produced green pigments. There were 52 isolates that were non-motile and classified as Pseudomonas based on their oxidative production of acid from glucose and their resistance to penicillin.

Ayres (1960b) tried to identify the meat spoilage organisms of refrigerated beef at different temperatures. He found that at 10 C and below, the bacteria responsible for slime layer formation were almost without exception pseudomonads. At 15 C or above, there was an equal proportion between Micrococcus and Pseudomonas.

The effect of temperature and packaging materials on the storage life and type of bacteria found in ground beef was studied by Jaye, Kittaka and Ordal (1962). They studied two types of packaging material, Cellophane (gas permeable) and Saran (gas impermeable), at 30 and 38 F and found that total plate counts of ground beef packaged in cellophane at 38 F increased more rapidly than that stored at 30 F. They also noted the total plate count of ground beef stored in Saran at 38 F did not increase in the first week of storage, but did increase with storage time. This subsequent increase in total plate count was attributed to the increase in the lactic acid bacteria, which was found to form 50% of the total plate count of that sample. This study also showed an increase in lactic acid bacteria in the cellophane packaged sample stored at 38 F, but this increase represented only a fraction of the total plate count of the sample.

Shewan, Hobbs, and Hodgkiss (1960) found the most important organisms which predominate on pork stored aerobically at chill temperature were Pseudomonas-Achromobacter groups, the majority being classified as Pseudomonas. They represented 96% of the total flora at 2 C storage and 49% at 16 C storage. Gardner (1965) studied the change in the aerobic flora of meats stored at 15, 9, and 4 C. He stated that in all cases the proportion of the total flora able to grow at 37 C decreased as the storage time increased. He

also found that micrococci did not grow well on minced meat at storage temperatures below 15 C, and observed that molds and yeasts were present in detectable numbers only at 15 C. In general, he found that at all storage temperatures used, the predominant flora in the later storage time were the Pseudomonas-Achromobacter group. Gardner, Carson, and Patton (1967) isolated the following flora from fresh, prepackaged pork: Pseudomonas-Achromobacter species, Microbacterium thermosphactum, Enterobacter, Hafnia species, Flavobacterium, Coryneforms, Micrococcus, and Lactobacillus. The researchers used six samples and not one of the nine isolated bacteria was common to all samples.

Stringer, Bilskie, and Nauman (1969) found that transportation of meat caused considerable increases in the total bacterial plate counts. They found the logarithm of the mean counts per square inch from the area sampled was 4.70 after slaughter, 4.78 prior to shipment from plant, and 5.94 on arrival at the retail store. They also found that the predominant microorganisms present on carcasses at the packing plant were Pseudomonas fragi, Ps. geniculata, and Micrococcus luteus. However, Ps. fragi and Ps. geniculata were the predominant organisms on carcasses, loins, and steaks at the retail store. Ingram and Dainty (1971) and McMeeking (1975) reported that the aerobic spoilage floras of fresh meat stored at chill temperature is usually dominated by species of Pseudomonas, although other

organisms such as Acinetobacter, Moraxella, Enterobacter, and Microbacterium thermosphactum may be present. Gill and Newton (1977) studied the growth rates of fluorescent and non-fluorescent species of Pseudomonas, Enterobacter, Acinetobacter and Microbacterium thermosphactum isolated from meat. Pure culture of meat slices were inoculated with each of the above organisms and incubated at different temperatures. Fluorescent and non-fluorescent pseudomonads grew faster than the other bacteria at all temperatures and the differences in the growth rates increased with the decreasing temperature. At the same time, they reported that the substrate exhausting at the meat surfaces was not the limiting factor for the aerobic spoilage of the meat but that oxygen was the limiting factor for that spoilage.

Cold-tolerant bacteria have been divided into psychrophilic and psychrotrophic organisms (Eddy 1960). The psychrophilic optimum growth temperature is 15 C or less and found only in permenantly cold environments (Morita 1975) and not usually found in spoilage floras. Psychrotrophs are found on the hides of the animals (Gill and Newton 1978) and hides appear to be the major source of organisms deposited on carcasses during the dressing process (Kitchell and Ingram 1967 and Grau 1974). The variation in the seasonal temperature affects the number and composition introduced to the carcasses from the work surfaces (Blaise and Armstrong

1973 and Jones 1973). Although many species of psychrotrophic bacteria had been reported, relatively few were found as major components of meat spoilage floras. Strains of Pseudomonas, Moraxella, Acinetobacter, Lactobacillus, Microbacterium thermosphactum, and certain genera of the family Enterobactereaceae were the most common bacterial types found on spoiling meat. Strains of Flavobacterium, Alcaligenes, Vibrio, Aeromonas, and Arthrobacter were reported less frequently (Sutherland, Patterson and Murray 1975a and Hanna, Vanderzant, Carpenter and Smith 1977a). Gill and Newton (1978) concluded that at chill temperatures, the spoilage floras of meat were composed of psychrotrophs originating mainly from the hides of slaughtered animals. They also reported that under humid conditions, aerobic floras were dominated by pseudomonads while the anaerobic floras were dominated by lactobacilli.

The microflora on the hide or fleece are derived mainly from the soil, water, and vegetation as well as fecal material. Psychrotrophic bacteria, the group containing potential spoilage bacteria for chilled meat, are common in soil, water, and vegetation (Stockes and Redmond 1966, Warskow and Juni 1972, and Newton, Harrison and Wauters 1978). Newton et al. (1978) studied the sources and types of psychrotrophic bacteria which gain access to the meat at the abattoir. They found that structural and work surfaces may be as important as hides as sources of bacterial

contaminants on meat. Gram negative psychrotrophs were recovered from the hides, from structural and work surfaces within the abattoir, and from carcasses and meat at all stages of processing. Microbacterium thermosphactum was the only one which was found at all sites of sampling. Newton et al. (1978) also found that the psychrotrophic counts on meat varies with the seasons. The counts correlated positively with rainfall and negatively with temperature.

Fermentative Microorganisms

It has been found that some of the gram negative and fermentative microorganisms can tolerate cold temperature even though they are considered a mesophilic in nature. Haines (1933) isolated Proteus from a slime layer developed on meat surfaces stored at Ø to 5 C after one week of storage. However, he indicated that Proteus grew on agar plates incubated at 2Ø C, while no Proteus were recovered on a plates incubated at 4 C. Jensen and Hess (1941) isolated Proteus and Serratia from soured ham, and noted their growth at 1.1 and 3.3 C. Kirsch et al. (1952) isolated Proteus in large numbers from putrid hamburger stored at Ø to 2 C for two weeks. Kitchell and Ingram (1956) stated that organisms classified as Enterobacter-Hafnia species were found on the aerobically stored pork meat at 16 C and none were found at 2 C. At the same time, they found Aerobacter species were predominant in the slime layer of pork stored at 15 C.

Three years later, Eddy and Kitchell (1959) isolated Aerobacter and Hafnia from beef, whale, and poultry stored at 3 C. Hechelman, Bem and Leistner (1974) reported that psychrotrophic strains of the family Enterobacteriaceae from meat and meat products appeared to belong mainly in the tribe Klebsielleae. On the other hand, Sutherland et al. (1975a) and Hanna et al. (1977a) noted that strains of Vibrio can be found occasionally on spoiling meat. Hanna, Zink, Carpenter and Vanderzant (1976) isolated strains resembling Yersenia from meat.

Eddy and Kitchell (1959) reported that some of the gram negative and fermentative organisms possess an unusual degree of tolerance to cold, resembling more closely characteristics of psychrophiles than of mesophiles. Eddy and Kitchell (1959) isolated 28 strains of coli-aerogenes bacteria and one Aeromonas from various chilled meats. They reported that these strains grew well at +1.5 C and some at -1.5 C even though their optimum growth temperature was near 37 C.

Geopfert (1976) studied the aerobic plate counts of coliforms and Escherichia coli of raw ground beef at the retail level. The study involved 956 samples taken from supermarkets throughout the United States. Two incubation temperatures (20 and 30 C) were used to determine the aerobic plate counts. The results were compared with the existing standard for E. coli in raw meat in New York and

Oregon. A comparison of the aerobic plate counts between the two incubation temperatures demonstrated an average ten-fold difference with the 20 C temperature always higher. It was also found that 36% of the samples were considered adulterated compared to the Oregon standard of 50 E. coli/g of meat. Even more alarming is that New York State would have considered 62% of the samples adulterated because the E. coli counts exceeded the arbitrarily chosen limit of 10 colonies/g of meat. Norberg (1981) studied the possibility of finding enteric pathogens in frozen chicken. He examined 82 samples of frozen chicken from retail stored for the presence of Campylobacter, Yersenia enterocolitica, and Salmonella. He found Campylobacter fetus subspecies jejuni in 22% of the samples, Y. enterocolitica in 24.5% of the samples, and Salmonella typhimurium in 1.2% of the samples. Ingram and Simonsen (1980) reported that Salmonella can be found on red meat carcasses but with variable frequency. In order to isolate these Salmonella from red meat, large samples (100 grams or more) must be taken. However, highly contaminated areas may be found. Ingram and Simonsen (1980) in their surveys suggested that Salmonella can be found in the lymph nodes and in some organ meats, such as liver in pigs and spleen in calves. They also found that the proportion of carcasses carrying Salmonella just after slaughter are usually two-fold or greater than the proportion of animals carrying Salmonella just before slaughter. This

indicated that at least 50% of the final contamination was a cross-contamination from initially contaminated animals.

Psychrotrophic Spoilage in Meat

The earlier interest of the meat microbiologist concentrated more on the nature of the microorganisms which caused meat spoilage than on the biochemical changes which they caused. However, in the last 15 years some research has been conducted to explain this. Thoronely (1967) reported that some psychrotrophic strains such as Alcaligenes and Acinetobacter were not proteolytic and did not attack amino acids easily, unlike Pseudomonas, but reacted similarly with sugars. Ingram and Dainty (1971) stated that meat spoilage attributed mainly to the breakdown of protein and their derivitives by Pseudomonas strains. Bryan (1969) noted that putrid meat under low temperature was not as dangerous as putrid meat under high temperature. However, unidentified bacterial poisoning materials might be associated with decomposition of food by massive Pseudomonas populations.

Ingram and Dainty (1971) reported that as the microbial population continued to increase, several tissue constituents were altered, such as carbohydrates, H^+ ions, amino acids, amides, and nucleotides.

Gardner (1965) found that in meat stored at 4, 9, and 15 C, the total carbohydrate concentration (anthrone-

value) fluctuated with an overall tendency to increase until the total bacterial counts exceeded 10^8/g, which was followed by a rapid decrease. Although there was a normal increase in the meat lactic acid concentration due to the anaerobic glycolysis, Ingram and Dainty (1971) reported a decrease in its concentration as the microbial population increased to 10^9-10^{10}/g.

The proteolytic activity of spoilage organisms were reported not to occur unless the microbial population exceeded 10^8/g or cm^2, when the meat has already been considered organoleptically spoiled (Ingram and Dainty 1971).

Sarcoplasmic proteins were the muscle protein succeptible to microbial attack at chill temperatures (Jay 1966). Jay (1972) reported that microbial spoilage of beef stored between 5 and 7 C did not result in significant protein degradation or proteolysis. He also stated that the production of ammonia, hydrogen sulfide, indol, skatol, and amines by spoilage organisms were responsible for the off-odor in spoiled meat, as a result of amino acid utilization. However, Jay (1972) also reported that some strains of Pseudomonas and Achromobacter which grew at refrigeration temperature were sometimes able to hydrolyze gelatin, although these organisms were not able to degrade natural protein such as purified myofibrilar proteins.

Sutherland, Patterson, Gibbs, and Murray (1975b) studied some metabolic and biochemical activities which lead to the spoilage of vacuum packaged beef. Both fermentative and non-fermentative gram negative organisms were tested in this study. The authors found that little degradation of meat proteins and fats might occur by pseudomonads and related species (non-fermentative organisms) at 4 C in vacuum packaged beef since the organisms appeared not to have proteolytic and lipolytic activity under low oxygen tension. They also reported that high extract-release volume (ERV) and low pH in meat matured in vacuum were strong indications for the low proteolytic activity. They further stated that proteolytic activity occurred in vacuum packaged meat when the contaminant contained gram negative organisms which were able to attack protein anaerobically.

Extract-release volume (ERV) was used to measure the hydration capacity of meat protein. By this technique fresh meat released large volumes of aqueous extracts (low hydration capacity) while little or no extract was released after microbial spoilage took place, indicating increased hydration capacity (Jay 1964). He also found that changes in hydration capacity was somewhat linear between freshness and spoilage. Jay (1964) suggested that ground beef failing to produce 25 to 30 ml should be regarded as spoiled and he found that this value corresponded to a bacterial number of about $10^{8.5}$/g. Both Jay (1964) and Borton, Webb and

Bratzler (1968) concluded that the ERV phenomenon was not due to presence of large numbers of bacteria in the meat but rather bacterial action during growth. Riedel, Burke and Nordin (1967) also concluded that ERV values of spoiled meat was affected not only by the type of microflora found, but also by changes in pH associated with the storage conditions. Even though the basis of the ERV procedure is not clear, it may depend on the alteration of the water holding capacity of the meat as a result of bacterial damage to the sarcolemma membranes which control the permeability of the muscle fibers (Jay 1964).

The changes in low molecular weight nitrogen-containing compounds have been studied by several investigators. Saffle, May, Hamid and Irby (1961) found that the intensity of the color formed due to the reaction of ninhydrin with alpha-amino group decreased six-fold when the microbial population increased from 10^6 to 10^9/g. Similar final decrease in the ninhydrin values of meat extracts were found by Jay (1964) and Jay and Kontou (1964) in a ground beef stored at refrigeration temperatures, although there was an initial increase in alpha-amino group. However, the changes in concentration were insignificant until very high bacterial populations had developed. According to Gardner (1965) and Gardner and Stewart (1966a) there was 2- to 5-fold decrease in glutathion and glutamine in beef stored at 4, 9, and 15 C with a concomitant 5- to 8-fold increase in

glutamic acid. Gardner and Stewart (1966ab) further reported that changes in glutamine and glutamic acid were mainly due to bacterial glutaminase and not to meat enzymes. Ockerman, Cahill, Weiser, Davis, and Siefker (1969) compared the non-protein nitrogen of sterile and inoculated meat at a storage temperature below 5 C. They found that only after prolonged incubation, the inoculated samples had significantly higher non-protein nitrogen content. Jay and Kontou (1967) followed the changes in the nucleotide content of meat stored at 7 C. There was no detectable changes in the meat spoiled by its own natural contaminants, but 15% decreased in concentration was found when 10^6 cells/g were collected from another cut of meat and used as inoculum. A 43% decrease was noticed when 10^7 cells/g of both Pseudomonas and Achromobacter were used. Gardner and Stewart (1966a) also examined the effect of meat spoilage microorganisms on urea concentration in meat stored at 4, 9, and 15 C. They noticed a small increase in urea concentration in the beginning which they believed was due to meat arginase activity. However, there was a decrease in urea concentration at the logarithmic phase of bacterial growth.

Jay (1972) further reported that some other changes might occur in spoiled meat, such as increasing pH due to microbial production of alkaline compounds, microbial alterations of metal ion balance on the surface of protein molecules, denaturation of the surface proteins with the

release of water-binding sites, and the production of amino sugar complexes. Shelef and Jay (1969) reported that amino sugar complexes increased meat spoilage in about the same proportion as increasing numbers of spoilage organisms when meats were allowed to spoil in the conventional manner. The same researchers further stated that glucosamine or amino sugars were not produced by the flora developed on the fresh meat which were packaged in gas-impermeable films at 5 C.

Bala, Marshall, Stringer and Naumann (1977) found that Pseudomonas fragi was one of the most important organisms in meat spoilage. It caused an increase in the pH of the meat surface from 5.5 to 6.6 when its total counts increased from log 2.2 to log 7.2 per cm^2. According to Tarrante, Jenkins, Pearson and Dutson (1973), the increased pH might accelerate proteolytic spoilage of meat because the extra-cellular proteases from Pseudomonas fragi have optimum pH activities between 6.5 and 7.5. It was also reported by Nashif and Nelson (1953) that increased pH accelerates the lipolytic acitivities of extra-cellular lipases from Ps. fragi which have an optimum pH of about 7.Ø. Increased pH above 6.Ø resulted in increasing the production of hydrogen sulfide and sulfomyoglobin by bacteria especially under low oxygen tension (Nicol, Shaw and Ledward 197Ø). This finding was supported by Bala et al. (1977) who reported a negative correlation between the degree of redness and the pH and metmyoglobin on meat surfaces.

Mencher and Alford (1967) found that Ps. fragi produced extra-cellular lipases which, in turn, increased the free fatty acids on the meat surfaces. Bala et al. (1977) supported this by demonstrating significant positive correlations between the growth of Ps. fragi and increased free fatty acids. They also reported a three-fold difference in the amounts of free fatty acids between sterile and inoculated meat samples with Ps. fragi after 20 days of storage. Furthermore, lipolysis caused discoloration in fresh ground beef as shown by application of extracellular lipases (Govindarajan 1973).

Gas Atmosphere Packaging

Each year about 33 billion pounds of meat are produced in the United States, half of which is distributed as prepackaged fresh meat (Daun, Solberg, Frank and Gilbert 1971). For this reason, extending the shelf-life of fresh meat has been the most important goal in the meat industry in recent years.

The most important factor which reduces fresh meat shelf-life is the growth of microorganisms. The microorganisms that can be found on or in fresh meat can be divided on the basis of their oxygen requirement: Aerobic organisms require oxygen to grow; anaerobes do not grow in the presence of oxygen; and facultative organisms grow in the presence or absence of oxygen (Lechowich 1971). The use

of gas atmospheres in packaging of fresh meat to extend the shelf-life has been investigated for several years. The gases most commonly used are oxygen, carbon dioxide, carbon monoxide, nitrogen, and/or mixtures of these gases.

Oxygen

The bright cherry-red color of meat is due to the binding of oxygen with the myoglobin to form oxymyoglobin. According to Daun et al. (1971), the desired color begins to change immediately and continuously after its formation to the brown color of metmyoglobin. Under normal supermarket storage conditions, the prepackaged meat cuts become undesirable and are rejected by customers after 3 days. George and Stratmann (1952) reported that when oxygen is used in a concentration higher than that of air, the rate of isolated myoglobin oxidation did not increase. In addition, Fellers, Wahba, Caldans and Ball (1963) and Baush (1966) claimed that higher oxygen concentrations improved the color retention of fresh meat significantly. Taylor and MacDougall (1973) reported that the reactions between oxygen and muscle myoglobin resulted in the formation of either oxymyoglobin or metmyoglobin, depending on the partial pressure of oxygen. However, they also reported that the desired red color of the meat can be maintained longer by maintaining high oxygen pressure and low temperature during storage. Futhermore, Daun et al. (1971) compared the effects of high

oxygen (90%) and normal atmospheric oxygen on the shelf-life of fresh meat color. Packaged samples were kept at 4 C and chromatographic analysis for oxygen, carbon dioxide, and nitrogen were done. They suggested that metmyoglobin reached unacceptable concentrations (70 to 80%) in about 6 days in ambient oxygen atmospheres, while at high oxygen atmospheres metmyoglobin reached the unacceptable concentration in about 10 days. They further added that oxygen-enriched atmospheres caused the penatration of oxygen deeper than the normal atmosphere and that carbon dioxide was generated as a result of oxygen uptake.

Daun et al. (1971) explained that oxygen uptake occurs in three stages. The highest uptake started immediately after packaging and ended within 2 to 3 days. The second stage started after 3 days and the increase in oxygen uptake was not significant. The third stage started at the end of the adjustment phase (6 to 9 days), at which time a highly significant oxygen uptake was noted. They also stated that there was more carbon dioxide production than oxygen uptake during storage, despite the concentration of oxygen in the storage atmosphere. Similar findings were obtained by Urbin and Wilson (1961) who believed this was due to the releasing of carbon dioxide from bicarbonate due to changes in pH. Daun et al. (1971) further reported that nitrogen, which remained constant in normal atmosphere, increased slightly in oxygen-enriched atmospheres.

Taylor and MacDougall (1973) further reported that the desired red color of fresh meat cuts stored under 60% oxygen lasted after 8 days while the air packaged samples deteriorated after 5 days of storage. Similar results were noted by Marchello, Dryden, Wooten and Teasdale (1974) who reported that the acceptance of physical appearance of meat cuts depended greatly on their color and increasing the shelf-life of meat color can be achieved by storage in an atmosphere which contains high levels of oxygen.

In contrast, Newton, Harrison and Smith (1977) found that lamb chops stored in atmospheres containing air or 80% oxygen had a shorter shelf-life than those stored in low oxygen or oxygen-free atmospheres. Furthermore, Taylor (1973) reported that high oxygen pressure caused rapid development of oxidative rancidity, but that high oxygen levels with normal pressure did not accelerate oxidative rancidity.

Some researchers have reported that the effects of high oxygen atmospheres are not significant on the microbial growth on meat. For example, Huffman, Davis, Marple and McGuire (1975) reported that 100% oxygen atmospheres had only a slight inhibitory effect on total bacterial counts after 16 days of storage. Seideman, Carpenter, Smith, Dill and Vanderzant (1979a) reported that the highest psychrotrophic counts on longissimus muscle steaks were on those samples stored in 100% oxygen compared with those stored in

100% carbon dioxide, 100% nitrogen, and vacuum. They also found that in 100% oxygen there was an increase in the sarcoplamic proteins and a decrease in the myofibrillar proteins, which they attributed to bacterial action on the muscle proteins. Shaw and Nicol (1969) found that even 100% oxygen did not have any inhibitory effect on pseudomonads. Clark and Lentz (1973), however, reported that 50% oxygen or more inhibited the growth of pseudomonads. Furthermore, Newton et al. (1977) reported that the growth of Microbacterium thermosphactum was retarded by 80% oxygen.

Low oxygen and oxygen-free atmospheres reduce the growth rate of aerobic psychrotrophic organisms but did not inhibit them completely (Newton et al. 1977). They further noted that Microbacterium thermosphactum growth rate decreased gradually in such atmospheres. On the other hand, Shaw and Nicol (1969) found that pure culture of Microbacterium thermosphactum was unaffected by oxygen concentrations in the range of 0.2 to 100%.

Nitrogen

Even though nitrogen is used in gas atmosphere packaging primarily to displace oxygen, some researchers have indicated that it may have an effect on meat color and on the development of spoilage organisms. For example, Weideman (1965) reported that beef muscle stored in a dessicator filled with nitrogen did not develop slime at 0 C for

10 weeks. He also indicated that in most of the trials, all the identified isolates were Microbacterium while in some trials, 10% of the isolates were identified as Lactobacillus. It was also claimed that 100% nitrogen atmospheres increased the shelf-life of lamb loins significantly and M. thermosphactum represented a major percentage of the bacterial flora that developed on these loins (Newton et al. 1977). They also added that Enterobacteriaceae increased in the 100% nitrogen atmosphere from undetectable level intially to form 10 to 30% of the total microflora present at the end of the storage period.

In contrast, Huffman et al. (1975) and Seideman et al. (1979a) reported that 100% nitrogen did not lower the total bacterial counts significantly. Huffman et al. (1975) even added that meat color of samples stored in air was more desirable than those stored in 100% nitrogen and 100% carbon dioxide. However, when the storage time was extended to 23 days, only the meat samples stored in 100% nitrogen maintained acceptable color. Seideman et al. (1979a) disagreed with these findings since they reported that steaks held in 100% nitrogen developed a brown color during storage and took longer to bloom after exposure to oxygen, which they attributed to irreversible binding of nitrogen with myoglobin.

Carbon Monoxide

Carbon monoxide reacts with hemoglobin (El-Badawi, Cain, Samuels and Aglemeier 1964) and myoglobin (Williams 1960) to form the bright red pigments, carboxyhemoglobin and carboxymyoglobin, respectively. Pearson (1960) recommended using carbon monoxide to increase meat color stability during storage. El-Badawi et al. (1964) further reported that flushing fresh packaged beef with a mixture of 2% carbon monoxide and 98% air stabilized its fresh color for 15 days due to the formation of carboxymyoglobin which is more resistant to oxidation than oxymyoglobin.

Clark, Lentz and Roth (1976) studied the effect of different concentrations of carbon monoxide on the growth of psychrotrophic spoilage bacteria, color, and flavor of fresh meat stored at 0, 5, and 10 C. They found that carbon monoxide needed to be present continuously around the meat with a minimum concentration of 1% in order to retard undesirable changes in the color. They also reported that with the increase of carbon monoxide concentration and decrease of storage temperature, the odor shelf-life increased. This led the authors to conclude that carbon monoxide had direct inhibitory effects on the psychrotrophic bacteria by increasing the lag phase and decreasing the growth rate during log phase. It was also suggested by Wolf (1980) that low concentrations of carbon monoxide should be added with high levels of carbon dioxide to increase the

overall shelf-life. He also stated that since carboxymyoglobin is the most stable reduced form of myoglobin, it will remove an effective pathway for the oxidation of unsaturated fat.

Clark et al. (1976) suggested that although consumers ingest carbon monoxide by eating smoked and barbecued meats, work must be done to prove the safety of meat stored in atmospheres containing carbon monoxide before its use on a commercial scale. Wolf, Brown, and Silliker (1976) calculated that 16 ppm carbon monoxide by weight was enough to saturate the myoglobin in ground beef and one has to consume several pounds of meat to equal to amount of carbon monoxide obtained by smoking one cigarette. Furthermore, it has been reported that carbon monoxide is an accumulative poison because the lungs release it by the mass action of oxygen (Tappel 1953).

Carbon Dioxide

The use of carbon dioxide for extending the shelf-life of meat began many years ago. Lawrie (1974) reported that workers in New Zealand started to successfully ship whole chilled carcasses to Great Britain in 1933. By 1938, 60% of the exported beef from New Zealand and 26% from Australia was shipped under carbon dioxide atmospheres. The effect of carbon dioxide on microbial growth was observed as early as 1889 by Frankel (as reviewed by Ogilvy and Ayres

1951a). Clark and Lentz (1969) reported that microorganisms varied in their sensitivity to carbon dioxide. Some were completely inhibited and some were less affected depending on the organism, the concentration of the gas, the temperature of incubation, the age of the cells, the time of atmosphere application, and the water activity of the medium.

According to Coyne (1932), Bacillus, Enterobacter, Flavobacterium, and Micrococcus were killed in 100% carbon dioxide atmosphere after 4 days at room temperature, while Proteus (Haines 1933), Lactobacillus (Ogilvy and Ayres 1953), and Clostridium perfringens (Parekh and Solberg 1970) were not affected significantly. Valley and Rettger (1927) said that high concentration of carbon dioxide inhibited, but did not kill, most yeasts, molds, and bacteria. However, low concentrations stimulated their growth. In addition, Ogilvy and Ayres (1951a) reported that increasing the concentration of carbon dioxide up to 50% increased the growth inhibition depending on microflora and food.

The reduction of storage temperature increased the effectiveness of carbon dioxide against meat spoilage (Ogilvy and Ayres 1951b). More specifically, Clark and Lentz (1972) stated that the maximum effectiveness of carbon dioxide in increasing the shelf-life of meat was at 0 C and decreased gradually to negligible levels at 20 C. It was important to note, however, that this inhibitory effect was

the result of a combination of reduced temperature and carbon dioxide.

Even though the application of carbon dioxide increased the generation time during log phase, its main effect was increasing the lag phase (Clark and Lentz 1969). They found that a 24 hour delay in application of 20% carbon dioxide reduced the shelf-life of meat from 11 days to 2.4 days at 5 C and from 4.4 days to 20 hours at 10 C. In addition, they noted that increasing the concentration of carbon dioxide to 40% with application after 24 hours still had less inhibition than 20% carbon dioxide applied immediately. Therefore, they concluded that at least 20% carbon dioxide was needed to affect inhibition once the cells passed the lag phase. King and Nagel (1967) and Ogilvy and Aryes (1951a) found that the generation time increased linearly with the increase in carbon dioxide concentration. King and Nagel (1967) further reported that generation time did not change with time which led them to conclude that microbial metabolic systems do not adapt during growth under carbon dioxide atmospheres and there is no selection for more carbon dioxide resistance.

Frankel in 1889, as reviewed by Ogilvy and Aryes (1951a), reported that the inhibitory effect of carbon dioxide was reversible, due to the presence of the gas itself and not to the removal of oxygen. Conversely, Silliker, Woodruff, Lugg, Wolfe and Brown (1977) stated that

there was marked residual effects to the carbon dioxide on meat spoilage organisms after further storage in air. The inhibition of microbial growth by carbon dioxide, as postulated by King and Nagel (1967), was due to displacement of oxygen and the lowering of pH of unbuffered medium. They also found that carbon dioxide inhibits succinate dehydrogenase of Pseudomonas aeruginosa. King and Nagel (1975) later found that carbon dioxide reduced the activities of isocitrate dehydrogenase and malate dehydrogenase of Pseudomonas aeruginosa. Furthermore, they believed the mass action of carbon dioxide inhibited the decarboxylating enzymes. Swanson and Ogg (1969) noted that formate hydrogenlyase of Escherichia coli was inhibited by carbon dioxide that was produced metabolically.

Carbon dioxide concentrations above 5% inhibit several meat spoilage organisms, particularly gram negative, aerobic psychrotolerant organisms. Ogilvy and Ayres (1951a) reported that carbon dioxide inhibited the psychrotolerant slime-forming organisms such as Pseudomonas and Alcaligenes. In addition, Clark and Lentz (1969, 1972) stated that the slime layer caused by pseudomonads and Acinetobacter-Moraxella on meat surfaces can be inhibited by storage in an atmosphere containing 20% carbon dioxide.

It has been claimed that facultative anaerobic and anaerobic microorganisms, such as Lactobacillus and Enterobacteriaceae can replace the gram negative aerobes

when high carbon dioxide (about 20%) and low oxygen (about 1%) are used in the storage of packaged meat (Sutherland et al. 1975b, Seideman et al. 1976, and Newton et al. 1977). Similar findings were obtained by Banks, Nickelson and Finne (1980) on fish stored in high carbon dioxide atmospheres at low temperature since they reported an inhibition of gram negative rods (e.g., pseudomonads) and stimulation of gram positive organisms (e.g., lactobacilli). Enfors, Molin and Tenstorm (1979) reported that 100% carbon dioxide decreased the total counts about 7 times compared with storage in air. They further reported that the dominating organisms on pork stored in carbon dioxide were lactobacilli.

Most of the food borne pathogens can tolerate high concentrations of carbon dioxide, while others are easily inhibited by the same atmosphere. For example, Salmonella (Shaw and Nicol 1969) and Clostridium perfringens (Parekh and Solberg 1970) were not affected by carbon dioxide, while inhibition of Staphylococcus aureus (Hays, Burroughs and Warner 1959) was noted. On the other hand, the isolation of various strains of Yersenia enterocolitica (Hanna et al. 1976) from vacuum packaged beef and lamb increased the concern about carbon dioxide application, especially when these organisms were found to be psychrotolerant and resist the effects of carbon dioxide. In addition, Schmidt, Lechowich and Folinazzo (1961) stated that the minimum temperature for growth and toxin production by Clostridium

botulinum type E is 3.3 C. For that reason, Wilhelm (1982) cautioned that the safety from botulism should be studied before the application of carbon dioxide by the retailer.

Even though some investigators (Clark and Lentz 1969 and Gill and Tan 1979) had reported that carbon dioxide had a maximum effect at a specific partial pressure above which any increase in carbon dioxide concentration will not increase its inhibitory effect, others (Blickstad, Enfors and Molin 1981) reported a continuous increase in inhibition with concentration. Therefore, Blickstad et al. (1981) tried to demonstrate the effect of hyperbaric carbon dioxide pressure (760 and 3800 mm Hg) on spoilage microflora of the meat at 4 and 14 C. At 760 mm pressure, lactobacilli were 66% of the total flora after 6 days at 14 C and 100% after 40 days. At pressures of 3800 mm of carbon dioxide, they formed the total flora at both temperatures.

The effect of carbon dioxide on the color of fresh meat is uncertain. Pohja (1967), Ledward (1970), and Finne (1982) found that high carbon dioxide atmospheres discolored meat surfaces, while Taylor (1972) and Seideman, Smith, Carpenter, Dutson and Dill (1979b) reported no detrimental effects due to high carbon dioxide concentrations.

Gas Mixtures

In recent years, the use of carbon dioxide, oxygen, nitrogen, and carbon monoxide in various mixtures has been

thoroughly investigated in order to combine the benefits of each gas. Gee and Brown (1978) reported that a mixture of 50% carbon dioxide and 1% carbon monoxide resulted in a significant inhibition of microbial growth at the meat surface. They concluded, as did Clark et al. (1976), that the carbon dioxide was responsible for inhibiting microbial growth while the carbon monoxide significantly extended the shelf-life of the fresh meat color. According to El-Badawi et al. (1964), carbon monoxide reacts with the myoglobin pigment to form carboxymyoglobin which is more stable against oxidation than oxymyoglobin, while it has similar visible spectral characteristics.

Brody (1970) found that using carbon dioxide enriched with oxygen increased the shelf-life of fresh meat color but he believed that oxygen enriched atmospheres increased the incidence of fat oxidation. Taylor and MacDougall (1973) reported that oxygen/carbon dioxide atmospheres kept meat color red longer due to the formation of a deep layer oxymyoglobin which took longer to dilute by the brown color of metmyoglobin. In studies by Partmann in 1975, as reviewed by Seideman et al. (1979a), the color of meat samples appear relatively fresh after 6 weeks storage in either 80% oxygen or nitrogen and 20% carbon dioxide compared to storage in air.

Newton et al. (1977) noted the growth of psychrotrophic flora decreased successively in oxygen atmospheres

containing 20% carbon dioxide. However, Sutherland, Patterson, Gibbs, and Murray (1977) reported that in an atmosphere containing 80% oxygen and 20% carbon dioxide, both Alcaligenes and lactobacilli grew at the same temperature. Seideman et al. (1979a) noticed that the purge loss was minimal in this same atmospheric mixture. They further added that roasts stored in 80% nitrogen and 20% carbon dioxide had a more desirable color at several time intervals up to 35 days, but over 35 days there was no significant differences in color as compared to samples stored in air. Seideman et al. (1979b) noted that 80% nitrogen and 20% carbon dioxide was better than vacuum packaging in extending the shelf-life of stored pork.

Wolfe et al. (1976) reported that a mixture of 50% carbon dioxide, 30% oxygen, and 1% carbon monoxide inhibited the gram negative bacteria in ground beef, i.e., pseudomonads and Achromobacter, which were not detected for 14 days of storage. They further stated that gram positive organisms might even decrease in such atmospheres. Also in this study, they claimed that immediately after the meat samples were treated with this atmosphere, the metmyoglobin percentage was reduced to almost zero after 1 day, even though carboxymyoglobin concentration was only about 15%. For that reason, they believed that the combination of carbon dioxide/carbon monoxide inhibited the enzymes that catalyze myoglobin oxidation.

Huffman et al. (1975) reported that a mixture of 70% nitrogen, 25% carbon dioxide, and 5% oxygen controlled both aerobic and anaerobic counts of beef samples stored at 1.1 C in the absence of light for 27 days, although the color was adversely affected. Marriott, Smith, Hoke, Carpenter, and West (1977) found that the use of 60% carbon dioxide, 25% oxygen, and 15% nitrogen in shipping meat cuts for long distances improved the appearance and desirability.

MATERIALS AND METHODS

This study has been conducted in two separate experiments. Each experiment was conducted utilizing two repilcated trials. All materials and methods used in both experiments were identical, varying only in sample preparation and packaging procedure.

Sample Preparation and Packaging

Experiment I

Loin strip steaks were purchased from Swift Packing Company in Tucson, Arizona. Samples were transferred to the Meat Laboratory at the University of Arizona at which time they were deboned and fat trimmed. Each steak was cut into two samples with an area of 40 to 50 sq cm and approximately 2.5 cm in thickness. Aseptic techniques were used throughout the preparation of the steaks. The knives were cleaned and sanitized before each cutting by washing thoroughly with hot water and detergent. They were rinsed with distilled water, then dipped in 95% ethanol and flamed before and after each cutting. Table surfaces were sanitized with Roccal II, which contains Alkyl (C_{14}-50%, C_{12}-40%, C_{16}-10%) dimethyl benzyl ammonium chloride (approximately 800 ppm), at frequent intervals. Precautions were

taken to eliminate direct contact of the meat to reduce external microbial contamination.

The steaks were placed on mobile foam trays (5 x 5 x 5/8 inches) for packaging. Each steak was randomly assigned to one of the following treatments: (1) Borden's Resinite film overwrap (oxygen transmission 310 to 387.5 $cc/cm^2/24$ hr and carbon dioxide transmission 2480 to 2790 $cc/cm^2/24$ hr); (2) gas flush (1% CO, 40% O_2, 59% N_2) for two minutes then overwrap with Resinite film; and (3) loose packaging (100 to 150 cc ambient air) in Cryovac B620 Barrier Bags having two layers of ethylene vinyl chloride copolymer (oxygen transmission 0.0035 $cc/cm^2/24$ hr and carbon dioxide transmission 0.0250 $cc/cm^2/24$ hr).

The growth and succession of microorganisms that occur on the top and bottom surfaces of steaks may vary and required these parameters to be compared. Therefore, this design allowed comparison of the growth of psychrotrophs and specific microbial genera that can succeed on steak surfaces during retail display with varying levels of oxygen and carbon dioxide. Two separate steaks were randomly assigned to each packaging treatment for each specific sampling period (0, 3, 6, 9, and 12 days).

Experiment II

The steaks used in this experiment were obtained from the same source and prepared in the same manner as in

Experiment I. Four packaging treatments were used in this experiment, three of which were different gas atmospheres in Cryovac Barrier Bags. The treatments were as follows: (4) 15% CO_2, 40% O_2, 45% N_2 gas atmosphere; (5) 60% CO_2, 40% O_2 gas atmosphere; (6) 10% CO_2, 5% O_2, 85% N_2; and (7) film overwrap (Borden's Resinite film) as a control. In the gas atmosphere treatments, the samples were placed in the barrier bags and evacuated first using a Multivac Packaging Unit (Koch Supplies, Inc., Kansas City, Missouri) and then filled with approximately 500 cc of the appropriate gas mixture.

This design allowed comparison of the growth of psychrotrophs and specific microbial genera that can succeed on steaks during retail display with varying levels of oxygen, carbon dioxide, and nitrogen. Two separate steaks were randomly assigned to each packaging treatment for each specific sampling period (0, 3, 6, 9, and 12 days).

Sample Storage

After packaging, the steaks were placed in Master-Bilt open-top display cases (Model 1230, 17 x 56 x 59 cm) on racks 14 cm above the botton of the case and 10 cm away from the internal sides. The temperature was held at 4 C throughout the storage period and was controlled automatically. The light intensity at the level of the steaks was 60 ft-c in all trials for 12 hr/day, with the remaining 12

hours in darkness. The light source were Sylvania F 96T 12 cool white fluorescent bulbs. At the predetermined sampling periods, steaks were removed and sampled for psychrotrophic growth.

Microbiological Evaluation

The swab technique was used in the sampling of the steak surfaces. A sterile 15.2 cm dry cotton swab was firmly and thoroughly rolled over the surface in all directions. Sterile aluminum templates having an exposed circular area of 3.8 sq cm were used to define the sample zone. The swab was rinsed in 99 ml of sterile Butterfield's buffered phosphate solution (Speck 1976). The swab was pressed on the inner surface of the dilution bottle and rolled a second time across the same area for efficient sampling. The cotton tip was then broken off into the buffer solution. This entire process was repeated with a fresh swab and aluminum template, which was relocated on the steak surface (same side), yielding a total sampling area of 7.6 sq cm. Microbial samples were taken in this same manner from both sides of each steak and recorded as "top" and "bottom".

Serial dilutions were made of the samples using sterile dilution bottles containing 99 ml of the phosphate buffer solution, pH 7.Ø. The appropriate dilutions were transferred to petri dishes and labelled. Standard Plate

Count Agar (Difco) was poured into each dish and incubated at 7 C for 7 to 10 days. (Preliminary experiments showed this nutrient agar to be more conducive to psychrotrophic growth than Trypticase Soy Agar.) Each dilution was replicated and those plates containing between 30 and 300 colonies after incubation were counted.

Five sampling days were used in each experiment, beginning at initial Day 0 followed by sampling at three day intervals (Day 3, 6, 9, and 12) until a definite putrid odor was observed. The psychrotrophic counts were reported as the $\log_{10}$ number of organisms per cm^2 as determined by the following equation:

$$\text{Counts/cm}^2 = \frac{\text{Total Counts x Dilution Factor}}{7.6\ \text{cm}^2}$$

Microbial Identification

The number of microbial colonies were counted for each countable plate and the square root of that number was determined. This provided the number of colonies that were selected from each plate to be streaked on plate count agar for further cultivation to facilitate identification of each microbial type. Colonies were randomly removed with a sterile needle. The plates were incubated for 24 hours at 32 C before identification procedures were employed.

Colonial and Cellular Morphology

The general colonial and cellular morphological characteristics were examined after growth on nutrient agar for 24 hours at 32 C. The same colonies were gram stained (ready gram stain, Difco) to study the cellular morphology. This step enabled the separation of gram positive and gram negative groups and also separation of the rod and cocci organisms.

Selected Tests for Microbial Identification

Production of Acid from Glucose. The distinction between oxidative and fermentative types of both gram positive and gram negative organisms in the production of acid from glucose was made by the Paraffin Layer Technique (Hugh and Leifson 1953). For each organism, two glucose tubes (Gibco) were stabbed several times to distribute the innoculum and one of the tubes covered by a paraffin layer (1" thick) and incubated at 32 C for 24 hours. A semisolid medium structure was used to localize the acid produced. The organisms considered fermentative produced acid in both tubes and throughout the entire medium from top to bottom, while the orgamisms considered oxidative produced acid only in the tube which was not covered with the paraffin layer.

Catalase Test. The catalase test was used for the identification of gram positive bacteria. The center of a young colony (18 to 24 hours) was picked up by a wood stick

and placed over clean microbiological slide and a drop of 30% hydrogen peroxide was placed over the organisms. Vigorous bubbling was the indication for the positive reaction.

Oxidase Test. A commercially prepared Kovac's Reagent (obtained from Analytical Profile Index) was used in this test. Two drops were placed on an isolated colony. A positive reaction was recorded by a color change to dark purple in approximately 10 seconds.

Motility Test. Motility was observed by stabbing tubes containing Semi-gel Bacto Motility Test Medium (Difco) and incubated at 35 C. If the organisms migrated from the stab line and diffused in the medium, causing turbidity, they were considered motile. If the bacterial growth was along the stab line and the medium was clear, the organisms were considered non-motile. The non-motile organisms after 48 hours were further incubated at 21 to 25 C for 5 days to confirm the lack of motility (MacFaddin 1979).

Growth on MacConkey Agar. A petri dish containing 15 to 20 ml of MacConkey Agar was divided into four quadrants. Each quadrant was labelled appropriately and the plates were incubated at 35 C and observed between 24 hours and 5 days.

Flagella Stain. Some of the motile isolates were stained by the method of Gray (1926). Cells from 16 to 20 hour cultures on nutrient agar slopes were obtained by adding 8 ml sterile distilled water down the side of the culture tube. The flooded cultures were allowed to stand for 30 minutes and then a drop was taken from the surface and put on a clean slide and tilted to distribute the drop over a large area. The smear was left to dry in air before staining.

Gram Positive Identification

After recording the colony characteristics of the randomly isolated microorganisms, they were divided mainly into two groups; gram positive and gram negative. Gram positive isolates were identified to the genus level by the Vanderzant and Nickelson (1969) scheme which is presented in Figure 1. Identification was further aided by reference to Bergey's Manual of Determinative Bacteriology (1974).

Gram Negative Identification

The gram negative rods were primarily separated to the genus level by the schemes illustrated in Figures 2 and 3, depending on their reaction to the oxidase test. Identification as to the species level was assisted by using the API-20E system for the identification of Enterobacteriaceae and non-fermentative gram negative rods. The system

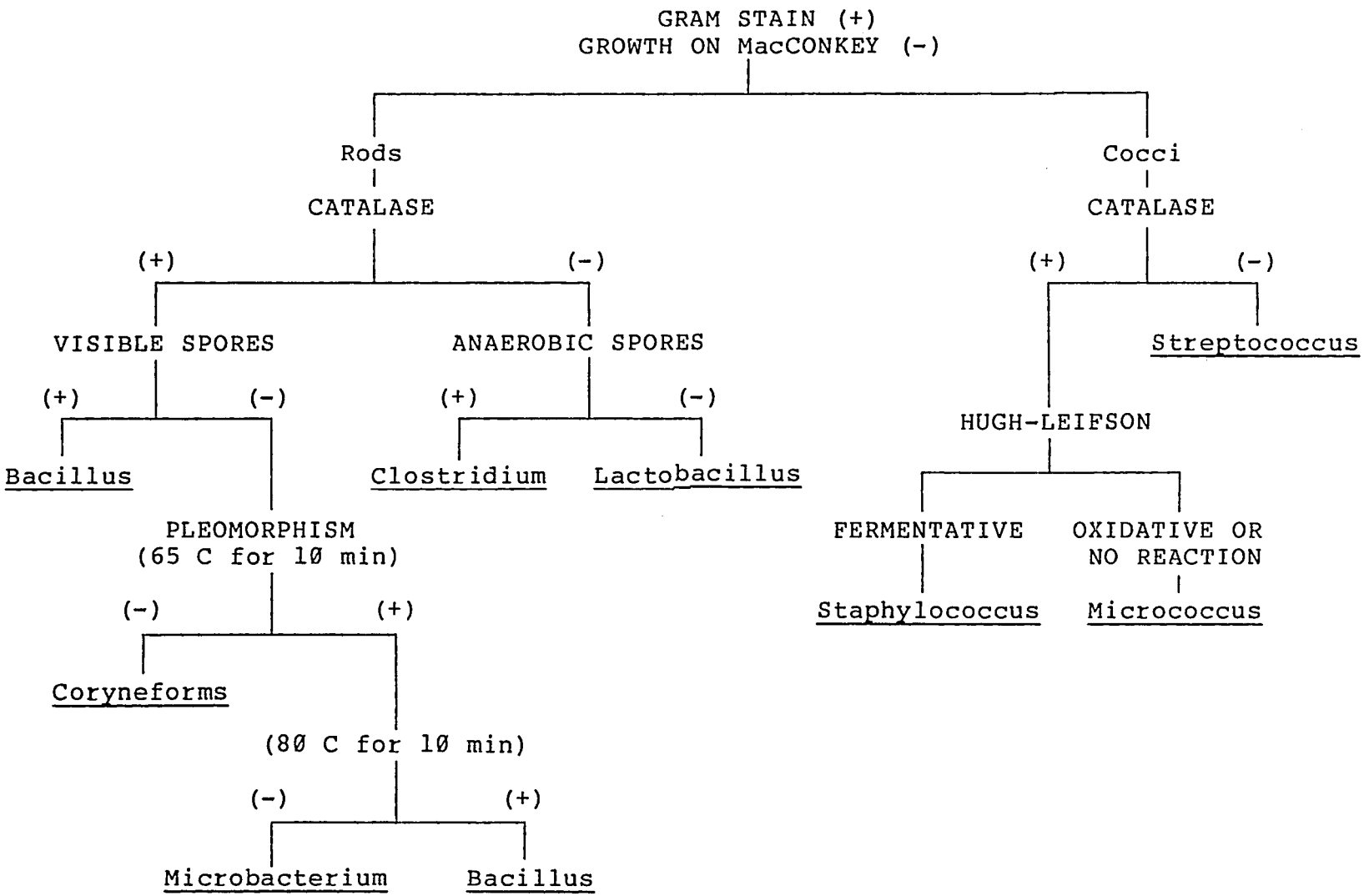

Figure 1. Scheme for Identification of Gram Positive Bacteria.

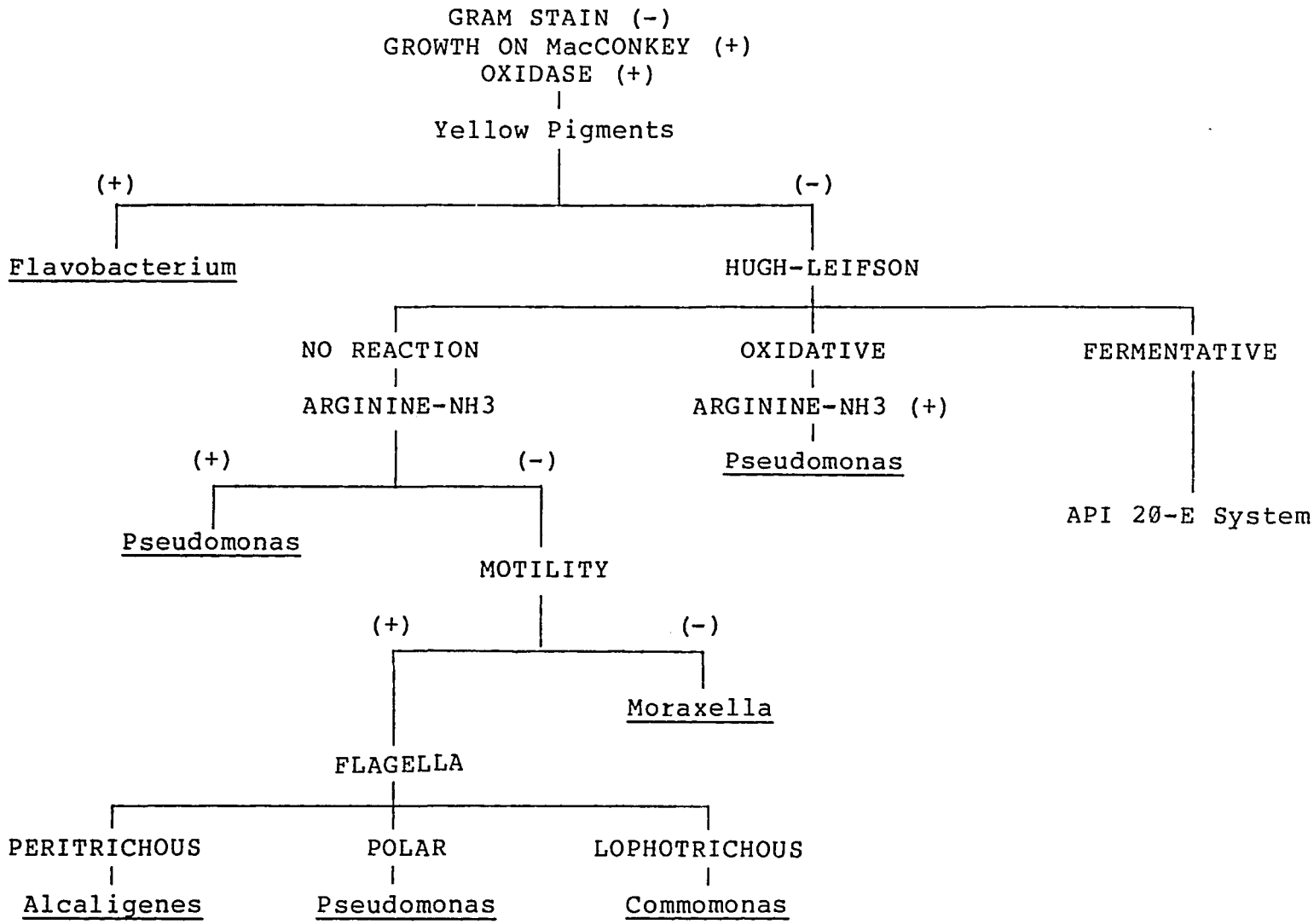

Figure 2. Scheme for Identification of Gram Negative Oxidase-positive Bacteria.

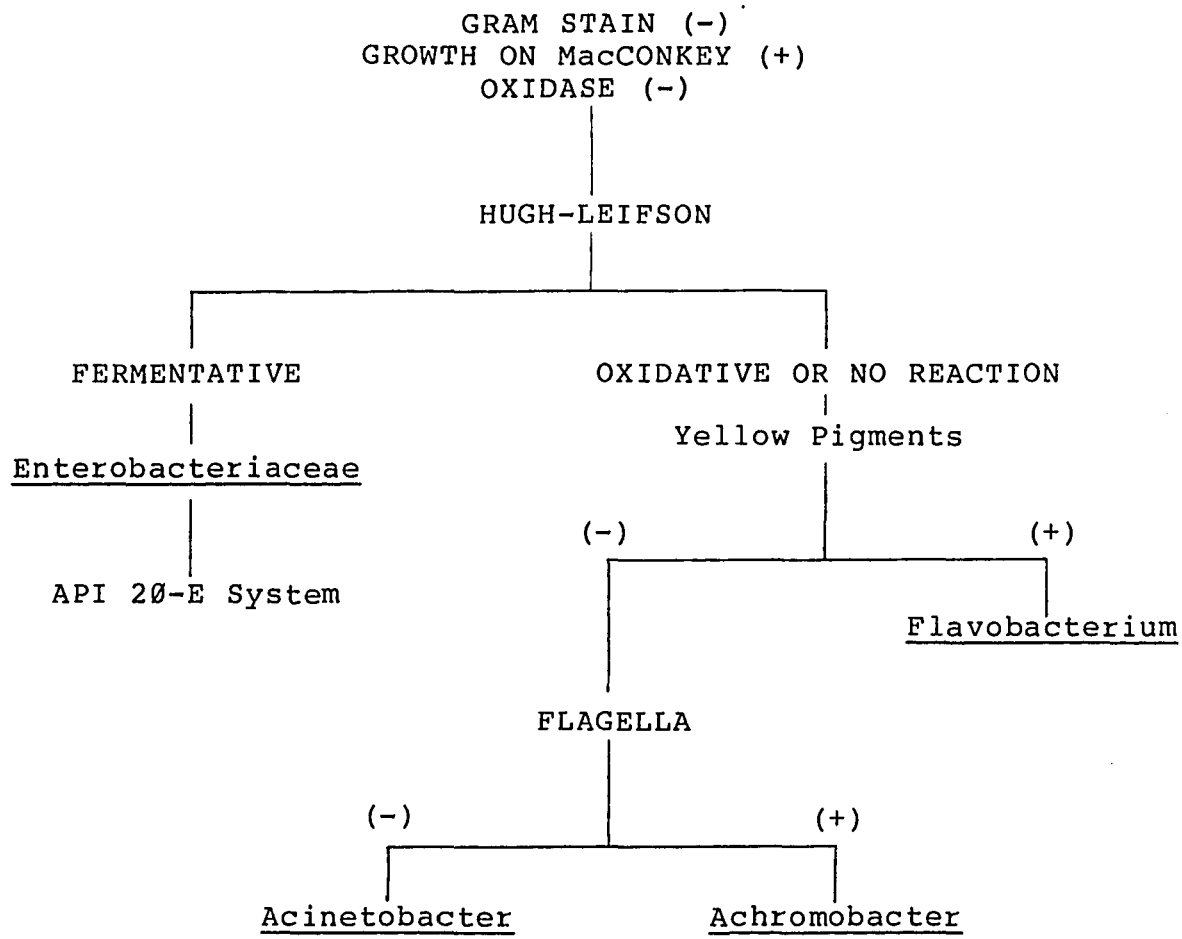

Figure 3. Scheme for Identification of Gram Negative Oxidase-negative Bacteria.

consists of strips with 20 plastic wells containing the following dehydrated biochemical substrates:

O-nitrophenyl B-d-galactopyranoside (ONPG)
arginine dihydrolase (ADH)
lysine decarboxylase (LDC)
ornithine decarboxylase (ODC)
citrate utilization (CIT)
hydrogen sulfide production (H_2S)
urea hydroysis (URE)
trytophane deaminase (TDA)
indol production (IND)
Voges-Proskauer test (VP)
gelatin hydrolysis (GEL)
glucose fermentation (GLU)
manitol fermentation (MAN)
inositol fermentation (INO)
sorbitol fermentation (SOR)
rhamnose fermentation (RHA)
saccharose (sucrose) fermentation (SAC)
melibiose fermentation (MEL)
amygdalin fermentation (AMY)
arabinose fermentation (ARA)

Five more test were conducted to further assist in the identification of non-fermentative gram negative rods. These tests include (1) nitrate reduction to the level of NO_2 or N_2, (2) motility test, (3) growth on MacConkey medium, (4) oxidative utilization of glucose, and (5) fermentative utilization of glucose.

Phenol red was added as an indicator to the ADH, LDC, ODC, and URE wells, while bromothymol blue was added to CIT, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY, and ARA. The indicators in the GEL and H_2S wells were charcoal and iron salts, respectively. No indicators were added to the TDA, IND, and VP wells.

Bacterial cells were grown on nutrient agar for 24 hours and streaked again on nutrient agar plates for more activation. The plates were incubated at 37 C for 24 hours. A well isolated colony was picked up in a loop and suspended in 5 ml of Ø.85% sterile saline solution, then shaken slightly until slight turbidity was observed. Each API-2ØE system well was inoculated with the bacterial suspension using a sterile Pasteur pipet (obtained from API) according to the manufacturer's procedure and incubated overnight at 37 C. If any three reactions were positive after 24 hours of incubation, the reagents were added and the biochemical test results recorded. If three reactions were not positive, the strips were incubated another 24 hours at 37 C according to the manufacturer's recommendation. If no reactions were positive after the subsequent 24 hour period, the viability of the organism was confirmed by streaking a loopful of suspension from the VP well onto a nutrient agar plate and the growth observed after 24 hours of incubation at 37 C. The reactions of the oxidative gram negative rods were recorded as nine-digit numbers and organisms belonging to the Enterobacteriaceae family were recorded as seven-digit numbers. The isolates finally were identified by using the Computerized Analytical Profile Index (API) and the isolates which did not have a profile in the index were identified by the API Computer Teleservice.

Statistical Analysis of Data

Significance testing of the main effects and interactions was done by analysis of variance (Nie, Hull, Jenkins, Steinbrenner, and Bent 1975). Duncan's New Multiple Range Test (Duncan 1955) was used to isolate the effects of packaging treatment, days of storage, and steak side.

RESULTS

Experiment One

Experiment One was conducted using two replicate trials. Due to similarities in the results, Trial I will be thoroughly discussed and only those differences between trials will be mentioned for Trial II.

Trial I

Microbial Growth. Psychrotrophic growth on the steaks which were stored under different environments (varying levels of oxygen available to the steak surface) are presented in Table 1. These numbers represent the means of the $\log_{10}$ numbers of the total plate counts per cm^2 of both top and bottom surfaces of steaks displayed in mobil-foam trays. Generally the microbial numbers were higher on the top surface of the steaks than on the bottom. However, only a few of the differences noted between the two surfaces were statistically significant.

More specifically, the increase in psychrotrophic numbers on the top side of the steaks in all three treatments was found to be one log cycle more than the growth on the bottom side. At Day 3 of sampling, all the treatments showed a slight decrease in psychrotrophic growth on the top

Table 1. Means of Psychrotrophic Organisms[a] Compared by Steak Side within Packaging Treatment and Day of Sampling, Trial I.

Packaging Treatment[b]	Steak Side	Days of Sampling				
		Ø	3	6	9	12
1	Top	2.83^{c}	2.80^{c}	6.66^{d}	9.23^{e}	8.81^{e}
	Bottom	2.91^{c}	3.46^{c}	5.73^{d}	8.36^{e}	8.49^{e}
2	Top	2.99^{c}	2.82^{c}	7.20^{d}	8.44^{e}	9.45^{fx}
	Bottom	3.13^{c}	2.95^{c}	7.06^{d}	7.97^{e}	8.69^{ey}
3	Top	4.36^{c}	4.19^{cx}	6.22^{d}	8.14^{ex}	7.79^{e}
	Bottom	4.38^{c}	5.46^{dy}	6.46^{e}	6.92^{fy}	7.70^{g}

[a]Log numbers of organisms/cm^2.

[b](1) Gas permeable Resinite film overwrap (control steaks), (2) gas flushed with 1% CO, 40% O_2, 59% N_2 for 2 minutes and film overwrap, (3) loose packaging in gas impermeable barrier bag.

^{c,d,e,f,g}Means on the same line which bear unlike superscripts differ significantly ($P<.05$).

^{x,y}Means within the same column for each packaging treatment which bear unlike superscripts differ significantly ($P<.05$).

side while only the gas flushed overwrapped samples showed a slight decrease in growth when the bottom surface was sampled. This decrease in growth can be attributed to the adaptation of the organisms to the new environments and/or to the increase in the lag phase under low storage temperature. Similar findings were reported by Ayres (1960) up to two days of storage at temperatures between 0 and 5 C.

The microbial numbers on the top side of the steaks which were overwrapped with Resinite film (control steaks) increased 6.5 log cycles during the entire storage period. The numbers increased from a log value of 2.83 per cm^2 (initially) to 9.23 at Day 9. However, the increase was only significant between Days 3 and 9. Similar results were obtained by Luiten, Marchello, and Dryden (1982) under the same conditions with mesophilic organisms.

The steaks packaged within Treatment 2 (flushed with 1% CO, 40% O_2, 59% N_2) showed significant increase in the psychrotrophic growth on the top side over the 12 day storage period. This treatment exhibited similar total increases to the control steaks. However, Treatment 2 had highest growth values by Day 12, whereas highest counts were observed by Day 9 on the control steaks.

Those samples which were packaged in gas impermeable barrier bags (Treatment 3) showed a gradual increase in psychrotrophic growth on the top surface of the steaks during the retail storage period. Microbial numbers

increased only 4 log cycles from 4.36 to 7.79 by Day 12, although significant increases only occurred between Days 3 and 9. A slight drop in total counts was observed between 9 and 12 days of storage. The gradual increase in growth may be due to the gradual decrease in oxygen and the accumulation of carbon dioxide, since the barrier bags were impermeable to these gases.

Psychrotrophic numbers within each treatment on the bottom of the steaks are also presented in Table 1. Control steaks (Treatment 1) showed an increase in microbial growth during the entire storage period. This increase was only 5.5 log cycles (2.91 initially to 8.49 at Day 12), which was one log cycle less than the microbial increase observed on the top side of the steak. In addition, significant increases in growth were obtained only between 3 and 9 days of sampling.

Similar growth patterns were noted on the bottom surfaces of steaks packaged for Treatment 2 as those for Treatment 1, except at Day 3, were total counts decreased slightly from that found initially.

Treatment 3 steaks exhibited a gradual increase in psychrotrophic growth on the bottom side of the steaks throughout the entire storage period. Although the microbial numbers increased only 3 log cycles (4.38 initially to 7.70 at Day 12), significant increases were observed at all sampling periods between Day 0 and Day 12. The increase

(P<.Ø5) obtained between initial sampling and Day 3 can be attributed to the accumulation of small concentrations of carbon dioxide due to aerobic and anaerobic metabolic activities in the meat tissue and on the steak surface. A small concentration of carbon dioxide can stimulate the growth and multiplication of microbial cells (Banwart 1981).

The psychrotrophic growth on steaks among each packaging treatment within sampling days is presented in Table 2. Treatment 3 was designed to determine if the natural build-up of carbon dioxide produced by the microorganisms might be sufficient to eventually suppress microbial growth. Fewer numbers (P<.Ø5) of organisms were observed on the top surfaces of steaks within Treatment 3 after 9 and 12 days of storage when compared to Treatments 1 and 2. At sampling Day 3, however, growth was higher (P<.Ø5) on steaks within Treatment 3 than that found in Treatment 1 and 2. In addition, the data in Table 2 indicated that Treatment 3 had lower (P<.Ø5) psychrotrophic growth on the bottom side of the steaks after 9 and 12 days of storage. It was also found that microbial numbers on the bottom surfaces at Day 3 were higher (P<.Ø5) than on steaks packaged in Treatments 1 and 2.

Treatment 3 had more (P<.Ø5) growth at Day 3 on both steak surfaces than the growth found for Treatments 1 and 2. The trend of the growth pattern was reversed since Treatment 3 demonstrated lower growth after Days 9 and 12 on both

Table 2. Means of Psychrotrophic Organisms[a] Compared by Packaging Treatment within Day of Sampling and Steak Side, Trial I.

Packaging Treatment[b]	Days of Sampling				
	Ø	3	6	9	12
			Top Side of Steak		
1	2.83^{c}	2.80^{c}	6.66^{c}	9.23^{c}	8.81^{c}
2	2.99^{c}	2.82^{c}	7.20^{d}	8.44^{c}	9.45^{d}
3	4.36^{d}	4.19^{d}	6.22^{c}	8.12^{c}	7.79^{c}
			Bottom Side of Steak		
1	2.91^{c}	3.46^{c}	5.73^{c}	8.36^{d}	8.49^{d}
2	3.13^{c}	2.95^{c}	7.06^{d}	7.97^{d}	8.69^{d}
3	4.38^{d}	5.46^{d}	6.46^{cd}	6.91^{c}	7.70^{c}

[a]Log numbers of organisms/cm^2.

[b](1) Gas permeable Resinite film overwrap, (2) gas flushed with 1% CO, 40% O_2, 59% N_2 for 2 minutes and film overwrap, (3) loose packaging in gas impermeable barrier bag.

[c,d]Means within the same column and for each steak side which bear unlike superscripts differ significantly ($P<.05$).

steak surfaces. However, only the bottom side had significantly lower growth values.

Microbial Succession. The data in Table 3 show the different microorganisms that were found initially on the top surface of the steaks and their subsequent changes during storage in various atmospheres. Three different genera were the main initial contaminants of the meat, Pseudomonas, Coryneforms, and Microbacterium. Yersenia and Acinetobacter were also present but in smaller percentages. Pseudomonas spp. clearly dominated the growth of the other microorganisms present. Simard, Zee, and L'heureux (1984) reported that Pseudomonas was predominant on the whole beef carcass. They found 82% of the microflora isolated from the front of the carcass to be Pseudomonas and the other 18% was Brochothrix. All of the microflora isolated from the rear portion of the carcass was Pseudomonas. Different findings were made by Vanderzant et al. (1982) as they reported that the major initial contaminants isolated from steaks were Micrococcus and Flavobacterium spp.

In both Treatments 1 and 2, the percentages of Pseudomonas spp. decreased at Day 3 of sampling from that found initially (Day 0). This decrease may be due to: (1) the total growth, as was determined by Plate Count Agar Method, which decreased at Day 3 of storage; (2) the percentages of some of the initial contaminants other than

Table 3. Microbial Succession[a] on the Top Side of Steaks by Day of Storage within Packaging Treatment, Trial I.

Microorganism	Day 0	Treatment 1[b] 3	6	9	Treatment 2[b] 3	6	9	Treatment 3[b] 3	6	9
Pseudomonas	45.3	37.5	53.9	80.0	33.3	75.0	82.0	62.5	50.0	30.0
Ps. putida	4.8	--	15.4	20.0	--	37.5	18.3	25.0	25.0	20.0
Ps. stutzeri	8.1	12.5	15.4	40.0	--	12.5	27.3	--	25.0	10.0
Ps. fluorescens	32.4	25.0	15.4	20.0	33.3	25.0	36.4	37.5	--	--
Ps. aeruginosa	--	--	7.7	--	--	--	--	--	--	--
Coryneforms	11.4	--	--	--	16.6	--	--	12.5	--	--
Microbacterium	17.6	12.5	15.4	--	--	--	--	--	--	--
Micrococcus	--	25.0	15.4	--	33.3	25.0	--	--	--	--
Alcaligenes	--	12.5	7.7	--	--	--	--	--	--	--
Yersenia enterocolitica	9.5	--	--	--	--	--	--	--	--	--
Acinetobacter	4.8	--	--	--	--	--	--	--	--	--
Serratia liquifaciens	--	--	--	--	--	--	--	12.5	25.0	60.0
Unidentified	11.4	12.5	7.6	20.0	16.8	--	18.0	12.5	25.0	10.0

[a]Values represent the percentage of each genus for each day of storage, except where species are indicated.

[b](1) Gas permeable Resinite film overwrap, (2) gas flush with 1% CO, 40% O_2, 59% N_2 for 2 minutes then film overwrap, (3) loose packaging in gas impermeable barrier bags.

Pseudomonas spp. which increased on this sampling day; and (3) the decrease in Pseudomonas spp. which may have been due to the appearance of other gram negative rods and/or Micrococcus spp. which were not detected at Day 0.

The percentages of Pseudomonas spp. increased by Day 6 and reached a maximum by Day 9 which were 80.0% and 81.2% for Treatments 1 and 2, respectively. The meat samples were completely spoiled by Day 9 resulting in intense off-odors. In addition, slime layers developed on the top surfaces in all of the treatments and the microbial number exceeded 10^8 per cm^2.

Treatment 3 exhibited a major difference in the types and percentages of the predominant microorganisms at all sampling days throughout the storage period. For example, the percentage of Pseudomonas spp. did not decrease at Day 3 from that found initially. At Day 3, the pseudomonads accounted for 62.5% of the total counts which was the highest Pseudomonas percentage during the entire storage period. The limitation of oxygen in the sealed barrier bags (Treatment 3) allowed the strict aerobic organisms to increase in growth up to Day 3. After that the percentages of Pseudomonas spp. dropped to 50.0% by Day 6 and 30.0% by Day 9. These decreases were accompanied by concomitant increases of Serratia liquifaciens (Table 3). This bacterium is facultative and competed with Pseudomonas spp. on the meat surface with limited amounts of oxygen. Serratia

liquifaciens was identified first at Day 3 of sampling and its percentage increased from 12.5% (Day 3) to 25.0% by Day 6 and 60.0% by Day 9. The atmosphere for Treatment 3 started with ambient oxygen levels and probably was consumed by the aerobic microbes present (Gardner et al. 1967). At the same time, carbon dioxide accumulation (and oxygen depletion) may have been responsible for the decrease in Pseudomonas and the increase in Serratia liquifaciens.

The changes in the microorganisms on the bottom surfaces of the steaks are shown in Table 4. In addition to Pseudomonas, Coryneforms, and Microbacterium, which were found on the top surfaces, specific organisms were found on the bottom surface. These microorganisms were Micrococcus, Acinetobacter, Achromobacter, Alcaligenes, and Yersinia. Pseudomonas growth showed the same pattern in both Treatment 1 and 2. Furthermore, Pseudomonas demonstrated similar growth patterns upon comparing the top and bottom surfaces with two exceptions. First, pseudomonad growth in Treatment 2 did not decrease at Day 3 from that found initially. Second, the highest Pseudomonas percentage (66.7%) on the bottom side was obtained between Days 6 and 9 of sampling. Pseudomonad growth on the top side had the highest percentage at Day 9, exceeding 80%.

Pseudomonas spp. dominated the microflora at the bottom side of the steaks. Pseudomonas species found regularly were Ps. putida, Ps. stutzeri, and Ps. fluorescens.

Table 4. Microbial Succession[a] on the Bottom Side of Steaks by Day of Storage within Packaging Treatment, Trial I.

Microorganism	Day 0	Treatment 1[b] 3	6	9	Treatment 2[b] 3	6	9	Treatment 3[b] 3	6	9
Pseudomonas	31.1	45.5	60.0	66.7	37.5	64.3	60.0	30.8	50.0	50.0
Ps. putida	12.8	18.2	10.0	16.7	12.5	7.1	10.0	7.7	16.7	10.0
Ps. stutzeri	8.6	9.1	10.0	33.0	25.0	14.3	10.0	7.7	16.7	--
Ps. fluorescens	9.7	9.1	40.0	16.7	--	42.9	40.0	15.4	16.7	40.0
Ps. aeruginosa	--	9.1	--	--	--	--	--	--	9.1	--
Coryneforms	9.7	--	20.0	33.3	12.5	7.1	10.0	23.1	33.3	10.0
Microbacterium	17.4	36.4	--	--	25.0	14.3	--	7.7	--	--
Micrococcus	20.2	--	20.0	--	--	--	10.0	--	--	--
Alcaligenes	6.1	--	--	--	--	--	--	--	--	--
Yersenia enterocolitica	8.3	--	--	--	--	--	--	--	--	--
Acinetobacter	3.0	9.1	--	--	25.0	14.3	--	--	--	--
Serratia liquifaciens	--	--	--	--	--	--	--	23.1	16.7	20.0
Achromobacter	--	9.1	--	--	--	--	--	--	--	--
Unidentified	4.2	--	--	--	--	--	20.0	7.7	--	20.0

[a]Values represent the percentage of each genus for each day of storage, except where species are indicated.

[b](1) Gas permeable Resinite film overwrap, (2) gas flush with 1% CO, 40% O_2, 59% N_2 for 2 minutes then film overwrap, (3) loose packaging in gas impermeable barrier bags.

Their growth and distribution was similar to that found on the top side since they did not demonstrate a regular increase or decrease with storage time.

The relatively low percentage of Pseudomonas on the bottom surface was followed primarily by the consistent appearance of Coryneforms in Treatment 1. Coryneforms, Microbacterium, and Acinetobacter appeared regularly on Treatment 2 steaks, while the latter two genera were found irregularly in Treatment 1. Coryneforms are described in Bergey's Manual of Determinative Bacteriology (8th edition) as gram positive, non-spore forming rods. These organisms are either aerobic or facultative; therefore, they appear to be able to compete to a certain extent with Pseudomonas on the bottom surface of the steaks. The Microbacterium species normally found on meat is M. thermosphactum, which has more recently been renamed to Brochothrix thermosphacta. It has been reported that this bacterium can compete with the pseudomonads at lower oxygen concentrations (Sutherland et al. 1975, Seideman et al. 1976, and Newton et al. 1977) even though its maximum growth is obtained in air and its lowest growth obtained in 100% carbon dioxide atmospheres (Blickstad and Molin 1984).

The microflora which developed on the bottom side of the steaks in Treatment 3 consisted mainly of Pseudomonas, Coryneforms, and Serratia liquifaciens. Even though the pseudomonads were the predominant organisms throughout the

entire storage period, they never accounted for more than 50% of the total microflora. In addition, the pseudomonad percentage dropped to 44.4% by the last day of sampling (Day 9). The Coryneforms increased gradually by the first 6 days of storage to represent approximately one third of the total microflora, then decreased to about 10% by Day 9 of storage.

The percentage of Serratia liquifaciens did not demonstrate a large change during the storage period. This organism was first identified at Day 3, forming 23.1% of the total microflora, with 16.7% at Day 6 and 20.0% at Day 9. Microbacterium, which can generally grow and compete with Pseudomonas at low oxygen levels, was not detected at either Day 6 or Day 9.

Trial II

Microbial Growth. The microbial numbers on both top and bottom surfaces of the steaks are presented in Tables 5 and 6. Similar results were obtained in Trial II as compared to Trial I (Table 5). Generally, the growth on the top side was greater by about one log cycle than the growth on the bottom side. However, only a few of the differences noted between the two surfaces were significantly different ($P<.05$).

Two major differences in growth pattern were noted from that of Trial I. First, the microbial numbers on the top surface of the steaks in Trial II increased ($P>.05$) at

Table 5. Means of Psychrotrophic Organisms[a] Compared by Steak Side within Packaging Treatment and Day of Sampling, Trial II.

Packaging Treatment[b]	Steak Side	Days of Sampling				
		Ø	3	6	9	12
1	Top	3.06^{c}	4.05^{c}	6.75^{dx}	8.43^{e}	9.21^{ex}
	Bottom	3.70^{c}	4.67^{d}	5.80^{ey}	7.13^{f}	8.55^{gy}
2	Top	2.38^{cx}	2.55^{c}	6.33^{dx}	8.39^{e}	8.81^{e}
	Bottom	2.91^{cy}	3.92^{d}	5.35^{ey}	7.99^{f}	8.40^{f}
3	Top	2.76^{c}	3.62^{c}	5.45^{d}	7.71^{dx}	8.52^{e}
	Bottom	3.33^{c}	4.13^{c}	5.33^{d}	6.73^{ey}	8.11^{f}

[a]Log numbers of organisms/cm^2.

[b](1) Gas permeable Resinite film overwrap, (2) gas flushed with 1% CO, 40% O_2, 59% N_2 for 2 minutes and film overwrap, (3) loose packaging in gas impermeable barrier bags.

[c,d,e,f,g]Means on the same line which bear unlike superscripts differ significantly ($P<.05$).

[x,y]Means within the same column for each packaging treatment which bear unlike superscripts differ significantly ($P<.05$).

Table 6. Means of Psychrotrophic Organisms[a] Compared by Packaging Treatment within Day of Sampling and Steak Side, Trial II.

Packaging Treatment[b]	Days of Sampling				
	Ø	3	6	9	12
			Top Side of Steak		
1	3.Ø6^{c}	4.Ø5^{c}	6.76^{d}	8.43^{c}	9.21^{d}
2	2.38^{c}	2.55^{c}	6.33^{cd}	8.39^{c}	8.81^{cd}
3	2.76^{c}	2.63^{c}	5.45^{c}	7.71^{c}	8.52^{c}
			Bottom Side of Steak		
1	3.7Ø^{c}	4.67^{c}	5.8Ø^{c}	7.13^{cd}	8.55^{c}
2	2.91^{c}	3.92^{c}	5.35^{c}	7.99^{d}	8.4Ø^{cd}
3	3.33^{c}	4.13^{c}	5.33^{c}	6.73^{c}	8.11^{d}

[a]Log numbers of organisms/cm^2.

[b](1) Gas permeable Resinite film overwrap, (2) gas flushed with 1% CO, 4Ø% O_2, 59% N_2 for 2 minutes and film overwrap, (3) loose packaging in gas impermeable barrier bags.

[c,d]Means within the same column and for each steak side which bear unlike superscripts differ significantly ($P<.Ø5$).

Day 3 from that found initially. Second, extending the storage time from 9 days to 12 days also caused an increase in the total microbial numbers in Trial II, although not significantly greater.

On the bottom surface of the steaks, a significant increase was obtained after 3 days of storage in Treatments 1 and 2 while in steaks from Treatment 3 did show a significant increase at this sampling time. In addition, extending the storage time from 9 to 12 days caused a significant increase in psychrotrophic numbers in Treatments 1 and 3 but not in Treatment 2.

The psychrotrophic growth among packaging treatments within sampling days for Trial II are compared in Table 6. Generally, Treatment 3 steaks possessed lower microbial numbers on both surfaces of the steaks than those packaged in Treatments 1 and 2, although these differences were not significant. The exception to this occurred in Treatment 1 at Days 6 and 12 (Table 6) where growth was higher ($P<.05$) on the top side of the steaks. Treatments 1 and 2 showed higher ($P<.05$) microbial numbers than Treatment 3 when sampled on the bottom side at Days 9 and 12, respectively.

Microbial Succession. The various types of microflora that are succeeded on the top surface of the steaks are presented in Table 7. As in Trial I, the initial contaminants represented a wide spectrum of different

Table 7. Microbial Succession[a] on the Top Side of Steaks by Day of Storage within Packaging Treatment, Trial II.

Microorganism	Day Ø	Treatment 1[b]			Treatment 2[b]			Treatment 3[b]		
		3	6	9	3	6	9	3	6	9
Pseudomonas	42.9	25.Ø	6Ø.Ø	66.7	44.4	84.6	87.5	66.7	66.7	44.4
Ps. putida	11.6	12.5	3Ø.Ø	16.7	22.2	3Ø.7	12.5	16.7	--	22.2
Ps. stutzeri	14.1	--	--	33.3	11.1	7.7	5Ø.Ø	--	25.Ø	22.2
Ps. fluorescens	11.1	12.5	2Ø.Ø	16.7	--	46.2	25.Ø	5Ø.Ø	66.7	--
Ps. aeruginosa	6.1	--	1Ø.Ø	--	11.1	--	--	--	--	--
Coryneforms	8.6	--	--	16.6	--	--	12.5	--	16.7	--
Microbacterium	5.6	--	2Ø.Ø	--	22.2	--	--	--	--	11.1
Micrococcus	19.7	5Ø.Ø	--	16.6	11.1	15.4	--	16.7	--	11.1
Acinetobacter	--	12.5	--	--	11.1	--	--	--	--	--
Serratia liquifaciens	5.6	--	--	--	--	--	--	16.7	16.7	22.2
Achromobacter	6.1	--	--	--	--	--	--	--	--	11.1
Unidentified	11.5	12.5	2Ø.Ø	--	12.2	--	--	--	--	--

[a]Values represent the percentage of each genus for each day of storage, except where species are indicated.

[b](1) Gas permeable Resinite film overwrap, (2) gas flush with 1% CO, 4Ø% O_2, 59% N_2 for 2 minutes then film overwrap, (3) loose packaging in gas impermeable barrier bags.

genera. The genus Micrococcus was second to Pseudomonas in the initial percentages, although it was not detected in Trial I initially. The pseudomonad percentage decreased after 3 days of storage and was accompanied by the domination of Micrococcus (50.0% of the total microflora present). In addition, Acinetobacter was also detected at Day 3 and formed 12.5%, even though it was not detected initially. The pseudomonad percentage was not as high in Trial II (66.7%) as in Trial I (80.0%). However, the percentage of Pseudomonas was similar throughout the storage periods in both trials. Steaks overwrapped in Resinite film after they were flushed in 1% CO, 40% O_2, and 59% N_2 exhibited the same growth pattern in both Trial I and Trial II. In Treatment 3, Pseudomonas continued to dominate the total microflora in Trial II, whereas Serratia liquifaciens dominated at the end of the storage period in Trial I. S. liquifaciens did not demonstrate a large increase in Treatment 3, forming only about one fifth of the total microflora at Day 9.

The microbial succession on the bottom surface of the steaks for Trial II is presented in Table 8. The same succession patterns were noted in Trial II as in Trial I, with little difference in the major microorganisms. Pseudomonas putrifaciens, which was not detected in Trial I, was present in Trial II.

Table 8. Microbial Succession[a] on the Bottom Side of Steaks by Day of Storage within Packaging Treatment, Trial II.

Microorganism	Day 0	Treatment 1[b] 3	Treatment 1[b] 6	Treatment 1[b] 9	Treatment 2[b] 3	Treatment 2[b] 6	Treatment 2[b] 9	Treatment 3[b] 3	Treatment 3[b] 6	Treatment 3[b] 9
Pseudomonas	37.9	42.8	70.0	58.3	30.0	69.2	70.0	27.3	50.0	44.4
Ps. putida	18.7	14.3	20.0	16.7	--	15.4	10.0	--	20.0	22.2
Ps. stutzeri	11.7	14.3	20.0	16.7	--	15.4	--	--	--	22.2
Ps. fluorescens	7.5	--	30.0	16.7	30.0	30.8	60.0	18.2	30.0	--
Ps. aeruginosa	--	14.3	--	--	--	--	--	--	--	--
Ps. putrifaciens	--	--	--	7.2	--	--	--	--	--	--
Coryneforms	14.5	14.3	30.0	25.0	30.0	15.4	20.0	18.2	10.0	11.2
Microbacterium	5.6	--	--	--	10.0	--	--	--	--	--
Micrococcus	24.8	--	--	--	20.0	15.4	--	--	--	--
Alcaligenes	12.5	--	--	--	10.0	--	--	--	--	--
Yersenia enterocolitica	--	--	--	--	--	--	--	9.1	--	--
Acinetobacter	--	28.6	--	--	--	--	--	--	--	--
Serratia liquifaciens	--	--	--	--	--	--	--	27.3	40.0	44.4
Achromobacter	4.8	14.3	--	--	--	--	--	--	--	--
Unidentified	--	--	--	16.7	--	--	--	18.2	--	--

[a]Values represent the percentage of each genus for each day of storage, except where species are indicated.

[b](1) Gas permeable Resinite film overwrap, (2) gas flush with 1% CO, 40% O_2, 59% N_2 for 2 minutes then film overwrap, (3) loose packaging in gas impermeable barrier bags.

Experiment Two

Experiment Two was conducted using two replicate trials. Due to similarities in the results, Trial III will be thoroughly discussed and only those differences between trials will be mentioned for Trial IV.

Trial III

Microbial Growth. Psychrotrophic growth on the steak surfaces which were packaged in different atmospheres are presented in Table 9. These numbers represent the means of the $\log_{10}$ numbers of total psychrotrophic counts per sq cm of steak surface for the top and bottom surfaces as displayed in the mobilfoam trays.

When the microbial growth on the top and bottom surfaces is compared, a higher growth was obtained on the bottom side in the gas packaged atmospheres (Treatments 4, 5, and 6). However, steaks packaged in Treatment 7 (control steaks) exhibited different results, growth on the bottom side was lower than the growth on the top side. Only steaks from Treatment 4 (15% CO_2, 40% O_2, 45% N_2) and Treatment 7 had significant differences between the growth on the two surfaces. Treatment 4 steaks had higher ($P<.05$) growth on the bottom surface at Days 0, 3, and 6. However, Treatment 7 steaks had higher ($P<.05$) psychrotrophic growth on the top side at Days 6, 9, and 12.

Table 9. Means of Psychrotrophic Organisms[a] Compared by Steak Side within Packaging Treatment and Day of Sampling, Trial III.

Packaging Treatment[b]	Steak Side	Days of Sampling				
		Ø	3	6	9	12
4	Top	3.41^{cx}	3.10^{cx}	4.85^{dx}	7.63^{e}	8.43^{f}
	Bottom	4.58^{cy}	5.04^{dy}	6.86^{ey}	7.42^{f}	8.55^{g}
5	Top	4.04^{cx}	5.68^{d}	6.94^{e}	7.51^{e}	9.50^{f}
	Bottom	5.36^{cy}	5.99^{d}	7.88^{e}	7.97^{e}	8.01^{e}
6	Top	4.41^{c}	4.83^{cx}	5.32^{d}	6.73^{e}	8.10^{f}
	Bottom	4.89^{c}	4.96^{cy}	5.72^{d}	6.49^{e}	8.19^{f}
7	Top	3.62^{c}	5.06^{d}	7.22^{ex}	9.16^{fx}	9.40^{fx}
	Bottom	3.42^{c}	4.58^{d}	6.02^{ey}	7.77^{fy}	8.54^{fy}

[a]Log numbers of organisms/cm^2.

[b](4) Gas packaged in 15% CO_2, 40% O_2, 45% N_2; (5) gas packaged in 60% CO_2, 40% O_2; (6) gas packaged in 10% CO_2, 5% O_2, 85% N_2; (7) gas permeable Resinite film overwrap (control steaks).

[c,d,e,f,g]Means on the same line which bear unlike superscripts differ significantly ($P<.05$).

[x,y]Means within the same column for each packaging treatment which bear unlike superscripts differ significantly ($P<.05$).

The log of the microbial numbers on the top surface of the control steaks (Treatment 7) increase approximately 6 log cycles during the storage period (Table 9). Numbers increased from 3.62 at Day Ø to 9.4Ø at Day 12. However, a significant increase were not obtained between Day 9 and Day 12. Extending the storage time to 12 days did not increase the microbial number significantly from the preceeding sampling time. The reason for this may be due to slime layer development on the top surface which might prevent the diffusion of oxygen to the microbial cells.

In the gas atmosphere treatments, the microbial growth at the top surface of the steaks increased between 3.7 and 5.5 log cycles during the storage period depending on the treatment. For example, microbial numbers in Treatment 4 increased from log value 3.41 initially to 8.43 at Day 12. However, the only significant increase occurred between Day 3 and Day 12. This may indicate that this atmosphere caused an increase in the psychrotrophic lag phase for 3 days. Steaks packaged in Treatment 5 had a significant increase in growth on the top side between Day Ø (log value of 4.Ø4) and Day 12 (log value of 9.5Ø). Treatment 6, on the other hand, appeared to have the highest detrimental effects on growth since the microbial numbers increased only 3.7 log cycles during the entire storage period. Numbers increased from 4.41 initially to 8.1Ø at Day 12. The significant increase occurred between Day 3 and

Day 12, as in Treatment 4, which also increased the lag phase for at least 3 days of storage since there was no significant increase in microbial numbers during that time.

The psychrotrophic growth within each packaging treatment on the bottom surface of the steaks is presented in Table 9. The steaks overwrapped in Resinite film (control steaks) had a similar growth pattern on both sides of the steaks. Microbial numbers increased ($P<.05$) over the first 9 days of storage. However, extending the storage time from 9 to 12 days did not significantly increase the psychrotrophic numbers. The total increase in the microbial numbers was about 5 log cycles; from an initial value of 3.42 to 8.54 at Day 12.

The steaks packaged in the gas atmospheres increased between 3 and 5 log cycles in total microbial numbers on the bottom surface, depending on the atmosphere. Organism numbers increased significantly on the steaks packaged in Treatment 4. The increase was 4 log cycles, from an initial count of 4.58 to 8.55 at Day 12 of sampling. Psychrotrophic organisms also increased on steaks packaged in Treatment 5 (60% CO_2 and 40% O_2). Significant increases, however, were observed only between Day 0 and Day 6. Statistically, this may indicate that the microbial growth reached the stationary phase of growth by Day 6 of storage. The reason for that may be due to a higher initial contamination level on the steaks that were packaged in this atmosphere.

The steaks packaged in Treatment 6 (10% CO_2, 5% O_2, 85% N_2) also exhibited a continuous increase in psychrotrophic numbers during the 12 day storage period. Log values increased from an initial 4.89 to 8.19 at Day 12, with significant increases occurring between Days 3 and 12. The gas mixture used in this treatment is the only mixture that caused an increased lag phase of growth on the steaks.

Growth comparisons between different packaging treatments on both steak surfaces within each sampling day are presented in Table 10. Some significant differences between the treatments were present at Day 0. These differences are unquestionably due to varying levels of initial contamination on each steak.

Samples packaged in Treatment 4 (15% CO_2, 40% O_2, 45% N_2) had lower ($P<.05$) microbial numbers on the top surface than steaks from the other three treatments, except at Day 6 and Day 12.

The samples packaged in Treatment 5 (60% CO_2, 40% O_2) had higher ($P<.05$) psychrotrophic growth on the top side at Day 3 than steaks from the other three treatments. Between Days 6 and 12, steaks packaged in Treatment 5 and Treatment 7 (control) had significantly higher growth than samples packaged in Treatment 6 (10% CO_2, 5% O_2, 85% N_2). On the other hand, the growth observed in Treatments 5 and 7 did not differ significantly except at Day 9, when Treatment 7 steaks showed higher growth.

Table 10. Means of Psychrotrophic Organisms[a] Compared by Packaging Treatment within Day of Sampling and Steak Side, Trial III.

Packaging Treatment[b]	Days of Sampling				
	Ø	3	6	9	12
			Top Side of Steak		
4	3.41^{c}	$3.1Ø^{c}$	4.85^{c}	7.63^{d}	8.43^{c}
5	$4.Ø4^{d}$	5.68^{e}	6.94^{d}	7.51^{d}	$9.5Ø^{d}$
6	4.41^{de}	4.83^{d}	5.32^{c}	6.73^{c}	$8.1Ø^{c}$
7	3.62^{cd}	$5.Ø6^{d}$	7.22^{d}	9.16^{e}	$9.4Ø^{d}$
			Bottom Side of Steak		
4	4.58^{d}	$5.Ø4^{c}$	6.86^{d}	7.42^{d}	8.55^{d}
5	5.36^{c}	5.99^{d}	7.88^{e}	7.97^{d}	$8.Ø1^{c}$
6	4.89^{d}	4.96^{c}	5.72^{c}	6.49^{c}	8.19^{cd}
7	3.42^{c}	4.58^{c}	$6.Ø2^{c}$	7.77^{d}	8.54^{d}

[a]Log numbers of organisms/cm^2.

[b](4) gas packaged in 15% CO_2, 4Ø% O_2, 45% N_2; (5) gas packaged in 6Ø% CO_2, 4Ø% O_2; (6) gas packaged in 1Ø% CO_2, 5% O_2, 85% N_2; (7) gas permeable Resinite film overwrap.

[c,d,e]Means within the same column and for each steak side which bear unlike superscripts differ significantly ($P<.Ø5$).

Psychrotrophs on the bottom surface of the steaks were not reduced by the gas atmosphere treatments when compared with the control packages during the first 6 days of storage. It is important to note that some gas atmosphere treated steaks had significantly higher microbial growth than the overwrapped (control) steaks. For example, steaks packaged in Treatment 4 at Day 6 and Treatment 5 at Days 3 and 6 had higher ($P<.Ø5$) counts than the control steaks. The reasons for this may be due to the high level of contamination and/or the possibility that the microorganisms have passed their lag phase, by which the effect of the gas atmospheres on microbial growth will be reduced.

Those samples packaged in Treatment 6 (1Ø% CO_2, 5% O_2, 85% N_2) had lower ($P<.Ø5$) growth than steaks packaged in Treatments 4 and 5 when sampled at Days 6 and 9. This may imply that gas atmosphere 6 reduces the growth rate of psychrotrophic organisms more than gas atmospheres 4 and 5. In addition, Treatment 6 had higher growth than Treatment 7 in the first 3 days of storage. The growth pattern was reversed by Days 9 and 12, since Treatment 6 had lower growth (although not significantly at Day 9).

Microbial Succession. Results of microflora development which occurred on the top surface of steaks packaged in the various treatments are presented in Table 11. The initial psychrotrophic flora consisted of a wide spectrum of

Table 11. Microbial Succession[a] on the Top Side of Steaks by Day of Storage within Packaging Treatment, Trial III.

Microorganism	Day 0	Treatment 4[b] 3	6	9	12	Treatment 5[b] 3	6	9	12	Treatment 6[b] 3	6	9	12	Treatment 7[b] 3	6	9	12
Pseudomonas	34.7	22.2	50.0	16.7	12.5	28.6	50.0	57.1	50.0	55.5	33.3	28.5	22.2	44.4	85.7	83.4	100.0
Ps. putida	—	11.1	16.6	16.7	—	—	—	—	25.0	22.2	33.3	—	—	—	28.6	16.7	—
Ps. stutzeri	12.5	—	16.6	—	—	—	16.7	28.6	25.0	—	—	—	—	11.1	57.1	25.0	16.7
Ps. fluorescens	18.6	11.1	16.6	—	12.5	14.3	33.3	14.3	—	33.3	—	28.5	11.1	22.2	—	41.7	83.3
Ps. paucimobilis	—	—	—	—	—	—	—	—	—	—	—	—	—	11.1	—	—	—
Ps. aeruginosa	3.6	—	—	—	—	14.3	—	14.3	—	—	—	—	—	—	—	—	—
Ps. cepacia	—	—	—	—	—	—	—	—	—	—	—	—	11.1	—	—	—	—
Coryneforms	5.0	22.2	16.7	16.7	—	14.3	—	—	—	22.2	16.7	28.5	22.2	11.1	—	—	—
Microbacterium	8.1	11.1	—	—	—	—	—	—	—	—	—	—	—	22.2	14.3	—	—
Micrococcus	20.7	11.1	16.7	—	—	14.3	—	—	—	11.1	16.7	14.3	—	—	—	—	—
Alcaligenes	3.1	—	—	—	—	—	—	—	—	—	—	—	—	11.1	—	—	—
Yersenia enterocolitica	—	—	—	—	12.5	—	—	—	—	—	—	—	—	—	—	—	—
Acinetobacter	5.0	—	—	—	—	7.1	—	—	—	—	—	—	—	—	—	—	—
Serratia liquifaciens	—	—	16.7	50.0	12.5	14.3	16.7	14.3	25.0	—	25.0	28.5	55.5	11.1	—	—	—
Achromobacter	—	—	—	—	25.0	—	33.3	14.3	—	11.1	—	—	—	—	—	—	—
Pasteurella	5.0	11.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Aeromonas	3.6	22.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Enterobacter aerogenes	—	—	—	—	25.0	—	—	14.3	12.5	—	—	—	—	—	—	—	—
Moraxella	6.3	—	—	—	—	21.4	—	—	—	—	—	—	—	—	—	—	—
Flavobacterium	5.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chromobacterium	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8.3	—
Unidentified	3.6	—	—	16.7	12.5	—	—	—	12.5	—	8.3	—	—	—	—	8.3	—

[a]Values represent the percentage of each microorganism genus for each day of storage, except where species are indicated.

[b](4) 15% CO_2, 40% O_2, 45% N_2; (5) 60% CO_2, 40% O_2; (6) 10% CO_2, 5% O_2, 85% N_2; (7) gas permeable Resinite film overwrap

genera, including Pseudomonas, Micrococcus, Coryneforms, Microbacterium, Alcaligenes, Acinetobacter, Pasteurella, Aeromonas, Flavobacterium, and Moraxella. Pseudomonas and Micrococcus were considered the predominant genera since they formed the highest percentages of the total growth (34.6% and 20.7%, respectively). Pseudomonad species that were identified were Ps. stutzeri, Ps. fluorescens, and Ps. aerogenosa. Ps. fluorescens was the predominant pseudomonad species, forming over half of the total genus present. In the post-treatment storage, the individual bacteria have grown at a rate dependent on the environment. For example, the bacteria that were more resistant to certain environments dominated within the particular environment at the end of the storage period.

The pseudomonads on the top surface of the steaks that were packaged in Treatment 4 (15% CO_2, 40% O_2, 45% N_2) were reduced from an initial 34.6% to 22.2% at Day 3. Micrococcus percentage also decreased at Day 3 to approximately one-half. The decrease in the number of these two genera was accompanied by an increase of other genera, specifically Coryneforms, Microbacterium, Pasteurella, and Aeromonas. However, the pseudomonads increased sharply by Day 6 to 50.0% of the total microflora present, but decreased sharply thereafter.

Some other genera were identified in at least one sampling day. Coryneforms were able to compete with the

dominant flora up to Day 9 of storage. Micrococcus had survived the treatment up to Day 6 while Microbacterium, Pasteurella, and Aeromonas were only identified up to Day 3.

Even though the pseudomonads dominated the growth on the top side of the steaks in Treatment 4 up to 6 days, member of the Enterobacteriaceae family, such as Serratia liquifaciens and Yersenia enterocolitica, dominated growth after Days 9 and 12. For example, S. liquifaciens was first recovered at Day 6 and increased gradually to form about one-third of the total flora present at Day 9. At Day 12, S. liquifaciens decreased in number to form only 12.5%. Y. enterocolitica and Enterobacter aerogenes were detected on Day 12 to form 12.5% and 25.0% of the total population, respectively.

The pseudomonads were able to maintain a complete domination of the psychrotrophic growth on the top surface of the steaks packaged in Treatment 5 (60% CO_2 and 40% O_2). For example, the pseudomonad percentages increased gradually from 28.6% at Day 3 to form 57.1% at Day 9; however, the percentage dropped to 50.0% by Day 12. The species that were found regularly were Ps. stutzeri and Ps. fluorescens since that were detected at most of the sampling periods. Two other Pseudomonas species that were found within Treatment 5 were Ps. aeruginosa (Days 3 and 9) and Ps. putida (Day 12).

The facultative organisms like Serratia liquifaciens and Enterobacter aerogenes were found to be more competitive in Treatment 5 as storage time increased. S. liquifaciens was first detected at Day 3 to represent 14.3% and increased thereafter to form 25.0% of the microflora present by Day 12. E. aerogenes was not identified until Day 9.

Only four genera were found to survive within Treatment 6 (10% CO_2, 5% O_2, 85% N_2) beyond three days of storage. These genera were Pseudomonas, Coryneforms, Micrococcus, and Serratia. The pseudomonads reached the highest percentage at Day 3 (55.5%) and was followed by subsequent decreases thereafter. The pseudomonads, however, were still the dominant flora at Days 3, 6, and 9. At Day 12 of storage they were overgrown by Serratia spp. The Pseudomonas species that were identified within this packaging treatment were Ps. putida, Ps. fluorescens, and Ps. cepacia.

Coryneforms appeared to compete well with the pseudomonads on the top surfaces when packaged in this low oxygen and high carbon dioxide (Treatment 6) atmosphere. This bacterium increased from 5.0% initially to 28.5% by Day 9 of sampling, with a slight decrease at Day 12 (22.2%), probably influenced by the domination of Serratia liquifaciens. Micrococcus was also able to survive up to 9 days of storage under this environment, although its percentages during the

post-treatment sampling were lower than its initial percentage.

Serratia liquifaciens was the only facultative enteric organism that was detected on the top surface of the steaks in Treatment 6. It was first identified at Day 6 in a relatively high percentage (25.0%), increasing to 28.5% by Day 9. The percentage increased sharply to 55.5% by Day 12 to dominate all the organisms present.

The microflora that developed on the top side of the control steaks (Treatment 7) were completely aerobic except for Serratia liquifaciens, which was detected at Day 3 only. There were several genera identified early in the storage period, including Pseudomonas, Coryneforms, Microbacterium, Alcaligenes, and Serratia. The pseudomonads were the predominant organisms, forming 44.4% of the total microflora. By Day 12 of storage, Pseudomonas was the only genus present. The pseudomonad species that were identified were Ps. fluorescens, Ps. stutzeri, and Ps. putida, although Ps. putida was not detected at either Day 3 or Day 12.

The succession of microorganisms which occurred on the bottom side of the steaks packaged within the various atmospheres is presented in Table 12. These data indicate that the initial contaminants were Pseudomonas, Coryneforms, Microbacterium, Micrococcus, Alcaligenes, and Flavobacterium. Of these genera, Pseudomonas was the predominant

Table 12. Microbial Succession[a] on the Bottom Side of Steaks by Day of Storage within Packaging Treatment, Trial III.

Microorganism	Day 0	Treatment 4[b]				Treatment 5[b]				Treatment 6[b]				Treatment 7[b]			
		3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12
Pseudomonas	38.3	40.0	30.0	16.7	16.7	42.8	55.5	50.0	28.5	50.0	30.0	28.6	22.2	44.4	66.7	80.0	66.7
Ps. putida	6.5	—	10.0	—	—	—	—	25.0	—	20.0	10.0	—	—	11.1	—	20.0	16.7
Ps. stutzeri	7.3	—	—	16.7	—	28.5	—	—	—	—	—	—	—	11.1	16.7	10.0	—
Ps. fluorescens	18.7	20.0	20.0	—	16.7	14.3	55.5	12.5	28.5	30.0	20.0	28.6	22.2	22.2	50.0	50.0	33.3
Ps. aeruginosa	—	20.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ps. cepacia	—	—	—	—	—	—	—	12.5	—	—	—	—	—	—	—	—	—
Ps. putrifaciens	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16.7
other Ps. spp.	5.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Coryneforms	7.8	10.0	—	16.7	8.3	28.6	11.2	—	—	12.5	10.0	28.6	11.1	—	16.7	20.0	25.0
Microbacterium	15.9	40.0	20.0	—	—	—	—	—	—	—	10.0	—	11.1	33.3	—	—	—
Micrococcus	12.9	—	10.0	16.7	—	—	—	—	28.5	25.0	—	—	—	—	16.7	—	—
Alcaligenes	5.0	—	—	—	—	—	—	—	—	12.5	—	—	—	—	—	—	—
Yersenia enterocolitica	—	—	—	—	16.7	—	—	—	—	—	—	—	—	—	—	—	—
Acinetobacter	—	—	—	—	—	14.3	—	—	—	—	—	—	—	—	—	—	—
Serratia liquifaciens	—	—	20.0	50.0	33.3	—	33.3	37.5	42.9	—	30.0	28.6	22.2	—	—	—	—
Achromobacter	7.2	10.0	—	—	—	14.3	—	—	—	—	—	—	—	22.3	—	—	—
Pasteurella	—	—	10.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Enterobacter aerogenes	—	—	—	—	8.3	—	—	—	—	—	10.0	14.3	11.1	—	—	—	—
Flavobacterium	2.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Unidentified	10.8	—	10.0	—	16.7	—	—	12.5	—	—	10.0	—	22.2	—	—	—	8.3

[a]Values represent the percentage of each genus for each day of storage, except where species are indicated.

[b](4) 15% CO_2, 40% O_2, 45% N_2; (5) 60% CO_2, 40% O_2; (6) 10% CO_2, 5% O_2, 85% N_2; (7) gas permeable Resinite film overwrap.

organism as it formed the major part of the microflora present (38.3%).

The growth of pseudomonads on the bottom side of the steaks packaged in Treatment 4 (15% CO_2, 40% O_2, 45% N_2) demonstrated a slight increase in numbers at Day 3, followed by a continuous reduction in percentage to 16.7% at both Day 9 and Day 12. Other aerobic genera were able to compete with the pseudomonads within this atmospheres. These genera were Coryneforms, Microbacterium, and Micrococcus.

The aerobic floras were overgrown by the facultative organisms, such as Serratia liquifaciens, Yersenia enterocolitica, Pasteurella, and Enterobacter aerogenes. For example, S. liquifaciens was first identified at Day 6 to form 20.0% and increased sharply to 50.0% at Day 9. This number decreased to 33.3% at Day 12 of storage. The reason for this decrease may be due to the presence and competition of Yersenia enterocolitica and Enterobacter aerogenes at the last day of sampling.

Steaks packaged in Treatment 5 (40% O_2 and 60% CO_2) showed Pseudomonas growth on the bottom side to be the only strict aerobe to survive and compete with the other microorganisms during the entire storage period. However, Coryneforms, Acinetobacter, and Achromobacter competed during the first 6 days of storage.

Serratia liquifaciens was the only facultative microorganism to be identified in Treatment 5. It was first

detected by Day 6 to form one-third of the total growth, increasing to 37.5% at Day 9 and dominating at Day 12 with 42.9%.

Four main genera appeared to survive and develop on the bottom surface of the steaks packaged in Treatment 6 (10% CO_2, 5% O_2, 85% N_2). These genera were Pseudomonas, Micrococcus, Serratia, and Enterobacter. Genera which appeared in smaller percentages were Coryneforms, Microbacterium, and Alcaligenes. The pseudomonads dominated the growth by Day 3, decreasing constantly to 22.2% by Day 12. Ps. fluorescens was the only species that survived this atmosphere for the 12 day storage period, although Ps. putida was detected during the first 6 days. Coryneforms was able to compete with Pseudomonas under this low oxygen environment, even though its growth pattern was irregular during the storage period.

The common facultative organism in this study, Serratia liquifaciens, was also found on the bottom surface of the steaks packaged in Treatment 6. Its highest percentage (30.0%) was detected at Day 6 and decreased thereafter. In addition, the reduced oxygen concentration appeared to stimulate the growth of Enterobacter aerogenes at the latter sampling days.

The microbial growth on the bottom side of the control steaks (Treatment 7) was completely aerobic. At Day 3, only three genera were identified: Pseudomonas (44.4%),

Microbacterium (33.3%), and Achromobacter (22.3%). At Day 6 of storage, the pseudomonads had increased sharply to 66.7%, while the other two genera were not detected. Coryneforms and Micrococcus were identified at Day 6 and each formed 16.7% of the total microflora. The pseudomonads increased to 80.0% by Day 9 and then decreased by Day 12 to 66.7%. Coryneforms continued to compete with the pseudomonads at Days 9 and 12, forming 20.0% and 25.0%, respectively. Micrococcus was not detected after 6 days of storage.

Trial IV

Microbial Growth. The steaks in this trial were packaged in the same gas atmospheres as in Trial III. Since Trial III and Trial IV have identical designs, only those differences which exist between the trials will be discussed in this section.

When psychrotrophic growth on the top side of steaks is compared with growth on the bottom side for Trial IV, only one major difference between trials was noted. Those steaks packaged in Treatment 4 had higher ($P<.05$) numbers on the top surface in Trial III up to Day 6, while significantly counts were only obtained at Day 12 in Trial IV.

The results for psychrotrophic growth in Trial IV as compared by days of storage within each packaging treatment and steak side are presented in Table 13. The initial contaminants that were found varied from a log value of 1.60

Table 13. Means of Psychrotrophic Organisms[a] Compared by Steak Side within Packaging Treatment and Day of Sampling, Trial IV.

Packaging Treatment[b]	Steak Side	Days of Sampling				
		Ø	3	6	9	12
4	Top	2.26^{cx}	2.74^{c}	$4.\text{Ø}5^{d}$	4.89^{e}	6.85^{fx}
	Bottom	2.88^{cy}	2.84^{c}	3.46^{c}	4.32^{d}	6.35^{ey}
5	Top	2.82^{c}	2.86^{c}	4.84^{dx}	6.43^{e}	6.98^{f}
	Bottom	2.55^{c}	2.87^{c}	5.17^{dy}	6.45^{e}	$7.\text{Ø}5^{e}$
6	Top	1.6Ø^{c}	$2.\text{Ø}8^{cx}$	3.76^{d}	4.64^{e}	5.53^{f}
	Bottom	2.13^{c}	2.49^{cy}	3.74^{d}	$5.\text{ØØ}^{e}$	6.24^{f}
7	Top	2.64^{c}	5.52^{d}	6.83^{ex}	8.49^{fx}	$9.\text{Ø}9^{gx}$
	Bottom	1.99^{c}	5.29^{d}	6.31^{ey}	7.19^{fy}	$8.\text{ØØ}^{gy}$

[a]Log numbers of organisms/cm^2.

[b](4) Gas packaged in 15% CO_2, 40% O_2, 45% N_2; (5) gas packaged in 60% CO_2, 40% O_2; (6) gas packaged in 10% CO_2, 5% O_2, 85% N_2; (7) gas permeable Resinite film overwrap.

[c,d,e,f,g]Means on the same line which bear unlike superscripts differ significantly ($P<.\text{Ø}5$).

[x,y]Means within the same column for each packaging treatment which bear unlike superscripts differ significantly ($P<.\text{Ø}5$).

to 2.65, which is much lower than in Trial III. All of the gas atmospheres in Trial IV increased the lag phase at least up to Day 3 while Treatment 6 was the only atmosphere to increase the lag phase 3 days in Trial III. In addition, for all gas atmospheres microbial numbers did not exceed the log value of 7.Ø on both steak surfaces.

Psychrotrophic growth comparisons between packaging treatments on both steak sides within each sampling days are represented in Table 14. The lower initial contamination in Trial IV allowed better comparisons between the treatments, although the growth pattern was the same in both trials. On the top surface, steaks packaged in 6Ø% CO_2 and 4Ø% O_2 (Treatment 5) had higher counts than those packaged in the 15% CO_2, 4Ø% O_2, 45% N_2 atmosphere (Treatment 4). Numbers were significantly higher only at Days 6 and 9. In addition, Treatment 5 also demonstrated higher ($P<.Ø5$) growth than Treatment 6 (1Ø% CO_2, 5% O_2, 85% N_2) during the entire storage period. However, the control steaks had the highest ($P<.Ø5$) microbial count compared to the steaks packaged in the gas atmospheres.

Samples packaged in Treatment 5 had higher microbial counts than steaks packaged in Treatments 4 and 6 on the bottom surface. Differences were only significant, however, at Days 6, 9, and 12. Treatment 4 and Treatment 6 steaks demonstrated similar growth except at Day 9 where steaks in Treatment 6 had significantly higher growth. The control

Table 14. Means of Psychrotrophic Organisms[a] Compared by Packaging Treatment within Day of Sampling and Steak Side, Trial IV.

Packaging Treatment[b]	Days of Sampling				
	Ø	3	6	9	12
			Top Side of Steak		
4	2.26^{cd}	2.47^{d}	4.Ø5^{c}	4.89^{c}	6.85^{d}
5	2.82^{d}	2.86^{d}	4.84^{d}	6.43^{d}	6.98^{d}
6	1.6Ø^{c}	2.Ø1^{c}	3.76^{c}	4.64^{c}	5.53^{c}
7	2.65^{d}	5.52^{e}	6.83^{e}	8.49^{e}	9.Ø9^{e}
			Bottom Side of Steak		
4	2.88^{c}	2.84^{c}	3.46^{c}	4.32^{c}	6.35^{c}
5	2.55^{c}	2.87^{c}	5.17^{d}	6.45^{e}	7.Ø5^{d}
6	2.13^{c}	2.49^{c}	3.74^{c}	5.ØØ^{d}	6.24^{c}
7	1.99^{c}	5.29^{d}	6.31^{e}	7.19^{f}	8.ØØ^{e}

[a]Log numbers of organisms/cm^2.

[b](4) gas packaged in 15% CO_2, 4Ø% O_2, 45% N_2; (5) gas packaged in 6Ø% CO_2, 4Ø% O_2; (6) gas packaged in 1Ø% CO_2, 5% O_2, 85% N_2; (7) gas permeable Resinite film overwrap.

[c,d,e,f]Means within the same column and for each steak side which bear unlike superscripts differ significantly ($P<.Ø5$).

steaks, however, had higher ($P<.05$) counts on the bottom side than all the atmosphere packaged steaks in the post-treatment storage.

Microbial Succession. The specific organism development and succession on the top surface of the steaks are presented in Table 15. The initial microflora represented a wide spectrum of genera, although more were detected in Trial III. For example, Microbacterium, Alcaligenes, and Aeromonas were not found in Trial IV initially, while they were identified in Trial III. In addition, Micrococcus dominated the initial contaminants in Trial IV while Pseudomonas was the predominant organism in Trial III.

During post-treatment storage, the pseudomonads dominated the floras present on the steaks (top surface) in Treatment 4 while this genus was overgrown by Serratia liquifaciens in the same treatment in Trial III. In the present trial, S. liquifaciens and Coryneforms were found to be less competitive in terms of percentages and frequency of identification in Treatments 4 and 5. Pasteurella was recovered in all three gas atmospheres in Trial IV late in the storage period while this genus was only identified once in Treatment 4 in Trial III (Day 3).

Generally, the pseudomonad growth on the top side of steaks packaged in Treatment 6 were lower in Trial IV and was completely absent at Day 12. In addition to Pseudomonas

Table 15. Microbial Succession[a] on the Top Side of Steaks by Day of Storage within Packaging Treatment, Trial IV.

Microorganism	Day 0	Treatment 4[b]				Treatment 5[b]				Treatment 6[b]				Treatment 7[b]			
		3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12
Pseudomonas	25.6	33.4	66.7	54.5	28.6	20.0	63.6	50.0	21.4	25.0	44.4	12.5	—	25.0	44.4	90.9	100.0
Ps. putida	4.7	8.4	—	9.1	—	6.7	18.2	—	—	12.5	22.2	—	—	25.0	11.1	—	20.0
Ps. stutzeri	—	—	16.7	—	—	13.3	18.2	—	—	12.5	11.1	12.5	—	—	—	18.2	10.0
Ps. fluorescens	16.3	25.0	50.0	36.3	14.3	—	27.2	25.0	21.4	—	—	—	—	—	33.3	36.4	30.0
Ps. aeruginosa	2.3	—	—	—	—	—	—	—	—	—	11.1	—	—	—	—	9.1	10.0
Ps. cepacia	—	—	—	9.1	—	—	—	—	—	—	—	—	—	—	—	—	—
Ps. putrifaciens	—	—	—	—	—	—	—	25.0	—	—	—	—	—	—	—	18.2	30.0
Ps. fragi	2.3	—	—	—	14.3	—	—	—	—	—	—	—	—	—	—	—	—
Coryneforms	9.3	16.6	—	27.3	14.3	—	18.2	—	14.3	—	33.3	37.5	9.1	—	22.2	9.1	—
Microbacterium	—	8.3	—	—	7.1	—	—	12.5	28.6	—	—	12.5	18.2	—	11.1	—	—
Micrococcus	32.5	25.0	33.3	—	—	73.3	—	—	—	37.5	—	—	—	37.5	22.2	—	—
Yersenia enterocolitica	—	—	—	—	—	—	—	—	7.1	—	—	—	—	—	—	—	—
Acinetobacter	4.6	—	—	—	—	—	—	—	—	12.5	—	—	—	—	—	—	—
Serratia liquifaciens	2.3	—	—	—	28.6	—	18.2	—	14.3	—	11.1	25.0	36.4	—	—	—	—
Achromobacter	—	—	—	—	—	—	—	—	—	12.5	11.1	—	—	—	—	—	—
Pasteurella	—	—	—	—	7.1	—	—	12.5	—	—	—	12.5	9.1	—	—	—	—
Aeromonas	—	—	—	—	—	—	—	—	—	12.5	—	—	—	—	—	—	—
Enterobacter aerogenes	—	—	—	18.2	—	—	—	25.0	—	—	—	—	27.3	—	—	—	—
Moraxella	7.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Flavobacterium	18.6	8.3	—	—	—	—	—	—	—	—	—	—	—	25.0	—	—	—
Unidentified	—	8.3	—	—	14.3	6.7	—	—	14.3	—	—	—	—	12.5	—	—	—

[a]Values represent the percentage of each genus for each day of storage, except where species are indicated.

[b](4) 15% CO_2, 40% O_2, 45% N_2; (5) 60% CO_2, 40% O_2; (6) 10% CO_2, 5% O_2, 85% N_2; (7) gas permeable Resinite film overwrap.

and Coryneforms, Micrococcus was the other aerobic genus found in Trial III, whereas Microbacterium was detected in Trial IV. The total facultative microflora were higher in Trial IV, except for Serratia liquifaciens. The reason may due to the appearance of other facultative organisms, such as Pasteurella, Enterobacter, and Aeromonas.

The microflora on the top side of the control steaks (Treatment 7) in both trials were strict aerobic microorganisms. In the first 6 days of storage, the pseudomonads were higher in Trial III. However, in both trials this genus formed 100% of the isolated microorganisms in the latter part of the storage period. Pseudomonas putrifaciens was only found in Trial IV; at Days 9 and 12 of the control steaks and at Day 9 of Treatment 5.

The succession of the different flora developed on the bottom surface of the steaks in Trial IV are presented in Table 16. Micrococcus was the predominant bacterium initially on the bottom side (as on the top) in Trial IV, whereas Pseudomonas was predominant in Trial III.

In post-treatment storage, the floras that developed on the bottom surface differed greatly between trials than growth found on the top surface. The pseudomonads increased continuously in Treatment 4 up to Day 9 (75.0%) then decreased to 44.4% at Day 12. In Trial III, the pseudomonads had reached their highest percentage by Day 3 (40.0%) and dropped continuously to 16.7% by Days 9 and 12. The

Table 16. Microbial Succession[a] on the Bottom Side of Steaks by Day of Storage within Packaging Treatment, Trial IV.

Microorganism	Day 0	Treatment 4[b]				Treatment 5[b]				Treatment 6[b]				Treatment 7[b]			
		3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12
Pseudomonas	15.4	42.9	62.5	75.0	44.4	30.0	80.0	46.1	33.3	33.3	50.0	30.0	23.1	33.3	38.5	60.0	64.3
Ps. putida	7.7	28.6	—	12.5	—	20.0	—	15.3	8.3	—	—	—	—	—	7.7	10.0	7.1
Ps. stutzeri	5.1	—	—	25.0	11.1	—	20.0	7.7	—	22.2	—	—	—	16.7	15.4	20.0	—
Ps. fluorescens	2.6	14.3	50.0	25.0	22.2	10.0	40.0	23.1	16.7	11.1	37.5	30.0	15.4	16.7	15.4	30.0	42.9
Ps. aeruginosa	—	—	12.5	—	—	—	10.0	—	8.3	—	—	—	—	—	—	—	—
Ps. putrifaciens	—	—	—	—	—	—	—	—	—	—	—	—	7.7	—	—	—	—
Ps. fragi	—	—	—	12.5	11.1	—	—	—	—	—	12.5	—	—	—	—	—	14.3
Coryneforms	17.9	14.3	25.0	12.5	22.2	40.0	—	15.4	16.7	22.2	12.5	20.0	15.3	—	30.7	20.0	21.4
Microbacterium	—	14.3	12.5	—	—	—	—	—	8.3	—	—	—	7.7	33.3	15.4	—	7.1
Micrococcus	25.6	7.1	—	—	—	30.0	—	—	—	11.1	—	—	—	—	15.4	—	—
Alcaligenes	—	—	—	—	—	—	—	—	—	22.2	—	—	—	16.7	—	—	—
Acinetobacter	5.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Serratia liquifaciens	—	—	—	12.5	11.1	—	10.0	23.1	33.3	—	12.5	30.0	30.7	—	—	10.0	—
Achromobacter	12.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pasteurella	—	—	—	—	—	—	—	—	8.3	—	—	—	7.7	—	—	—	—
Enterobacter aerogenes	—	—	—	—	11.1	—	—	7.7	—	—	25.0	10.0	7.7	—	—	—	7.1
Moraxella	2.6	14.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Flavobacterium	12.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chromobacterium	7.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Unidentified	—	7.1	—	—	11.2	—	10.0	7.7	—	11.2	—	10.0	7.7	16.7	—	10.0	—

[a]Values represent the percentage of each genus for each day of storage, except where species are indicated.

[b](4) 15% CO_2, 40% O_2, 45% N_2; (5) 60% CO_2, 40% O_2; (6) 10% CO_2, 5% O_2, 85% N_2; (7) gas permeable Resinite film overwrap.

same genera increased to a maximum percentage at Day 6 then decreased thereafter. In Trial IV the pseudomonads reached 80.0% in Treatment 5, while it formed only 55.0% maximum in Trial III. The species Ps. fragi was identified at Day 9 and Day 12 in Treatment 4 only in Trial IV. It was also noted (as on the top surface) that Serratia liquifaciens was less competitive in Trial IV, as was evident on the bottom surface of the steaks packaged within Treatments 4 and 5.

The pseudomonad development on the bottom side of steaks packaged in Treatment 6 was about the same in both trials. However, they reached their highest percentage at Day 6 in this trial and at Day 3 in Trial III. Coryneforms was found to be another competitive organisms in this atmosphere in Trial IV, whereas Micrococcus was the secondary competitive aerobic genus in Trial III.

The control steaks had lower Pseudomonas growth on the bottom side in Trial IV, possibly due to the presence of Microbacterium. In addition, some facultative organisms were recovered twice in this trial. For example, Serratia liquifaciens formed 10.0% of the total microflora at Day 9 and Enterobacter aerogenes formed 7.1% at Day 12.

DISCUSSION

Experiment One

Microbial Growth

The lack of significant differences between microbial growth on the top and bottom surfaces of the steaks in both trials may have been a result of the top surface organisms having ample amounts of oxygen and enough nutrients, which may be a limiting factor, to keep them growing at a maximum rate throughout the storage period. The organisms on the bottom surfaces had the advantage of having excess amounts of primary and secondary nutrients in the exudate. However, when the exudate around the edge of the steaks dried, it created a seal on the bottom surface within the package, which may have created a microaerophilic or anaerobic environment, especially within the Resinite film packages.

The primary nutrient source is glucose, while the secondary source of carbon are amino acids (Eskin, Henderson, and Townsend 1971). Most of the organisms found on the meat surfaces, except *Acinetobacter*, can grow at maximum rate on either the primary or secondary sources of carbon (Gill and Newton 1977). Gill and Newton (1977) also claimed that *Brochothrix thermosphacta*, which is a major

bacterium on meat, can utilize glutamate in addition to glucose as a primary carbon source.

It was determined by subjective observations that the microbial numbers at Day 6, which were between log 6.0 and 7.0, appeared to have detrimental effects on the meat color in all treatments. However, the steaks did not develop off-odors at Day 6. When sampled at Day 9, the steaks had pronounced off-odors and had developed a clear slime layer on their surfaces. Similar findings were reported by Ayres (1960b) and Ingram and Dainty (1971) who claimed the microbial growth must exceed a log value of 7.0 per cm^2 in order to become organoleptically unacceptable due to off-odor formation. Furthermore, Gill (1983) stated that off-odors will develop soon after the depletion of glucose which may occur when the microbial number reaches a log value of 8.0. He added that off-odors could occur at lower levels of growth when Acinetobacter is forming a major portion of the total microflora since this genus cannot utilize glucose and immediately attack the amino acids.

The growth of the microorganisms on meat surfaces is governed mostly by the amount of oxygen available on the surfaces of the cut (Eskin et al. 1971). Since the Resinite film used in wrapping the steaks in Treatments 1 and 2 is very permeable to oxygen, the large increase in growth that was found was expected, particularly when all the required nutrients were available.

Extending the storage time up to 12 days did not increase the total microbial numbers significantly. The reasons for that may be related to the depletion of readily available and low molecular weight compounds suchs as sugars, lactic acid, and free amino acids and/or due to the limitation of the physical space. In addition, Gill and Newton (1977) reported that when the microbial number exceeded $10^9/cm^2$, the growth rate decreased due to the formation of a slime layer which prevents the diffusion of oxygen from the environment to the organisms at the surface of the meat.

The decrease in the psychrotrophic growth rate on the loosely packaged steaks (Treatment 3) can be attributed to the combined effect of accumulated carbon dioxide, depleted oxygen, and low storage temperature. For example, Gardner et al. (1967) reported that the gradual accumulation of carbon dioxide and concomitant decrease in oxygen caused some facultative organisms, such as Enterobacter-Hafnia, to compete with the strict aerobic microflora. That competition may be considered a reason for the reduction the growth rate. In addition, Clark and Lentz (1969) concluded that carbon dioxide inhibits the psychrotolerant bacteria despite the type of meat tissue they grow on. It has also been claimed that even though the combination of carbon dioxide and low temperature increases the shelf-life of fresh meat, the later is the most important environmental factor that

affects the growth and viability of microorganisms (Silliker et al. 1980).

Microbial Succession

The initial contamination of the steaks by microorganisms shows a species selectivity. The genera that predominated in the initial contamination were Pseudomonas, Micrococcus, and Microbacterium. Haines (1933), Scott (1936), Krisch et al. (1952), and Ayres (1960ab) also found these three genera to predominate in the initial contamination of fresh meat. As reported by Ayres (1960b), other researchers have also found Coryneforms, Alcaligenes, Acinetobacter, and/or Achromobacter to be involved as initial contaminants of fresh beef. Futhermore, Vanderzant et al. (1982) reported that the only initial contaminants isolated from steaks were Micrococcus and Flavobacterium.

The microflora of steaks packaged aerobically in Resinite film was dominated by the genus Pseudomonas after 3 days of storage and formed at least 80% of the predominating microorganisms by Day 9. Therefore, it is assumed that the pseudomonads were responsible for the spoilage of the steaks stored aerobically. This assumption is strongly supported by the research of Ayres (1951, 1960ab), Kirsch et al. (1952), Wolin, Evans, and Niven (1957), and Gill and Newton (1977). Pseudomonas can dominate the spoilage microbes due to their faster growth rate than other microbes found

initially on fresh meat surfaces when stored between 2 and 15 C (Gill and Newton 1977). These investigators further stated that pseudomonads appear to inhibit the competing organisms by reducing their growth rate and decreasing their maximum cell densities as well as being more competitive for the available oxygen on meat surfaces. In another study, Gill and Newton (1982) found the pseudomonads were not affected by the ultimate pH range of normal meat cuts, whereas Moraxella and Acinetobacter are inhibited by low pH of normal meat (Gill 1983). However, the latter two genera were reported as part of spoilage flora (Ingram and Dainty 1971).

Pseudomonas spp. in Treatment 3 reached a maximum percentage by Day 3 of storage and decreased therafter. The drop in growth was followed by an increase in the percentage of Serratia liquifaciens. From work conducted by Gardner et al. (1967), the percentage of Pseudomonas-Achromobacter dropped from 49% to 15% after 4 days of storage at 16 C and from 96% to 49% when stored 14 days at 2 C. When meat samples were stored in sealed gas impermeable bags, the decrease in the Pseudomonas-Achromobacter percentage was followed by an increase in the Brochothrix thermosphacta and Enterobacter-Hafnia percentages.

Carbon dioxide percentage can increased in a sealed package up to 30% in 4 days at 16 C and up to 15% in 14 days at 2 C (Gardner et al. 1967). Erichsen and Molin (1981)

reported that carbon dioxide can increase in packages containing normal meat up to 19.7% and up to 18.8% in dry, firm and dark (DFD) meat after 14 days at 4 C. Pseudomonads can be inhibited at carbon dioxide levels as low as 10% (King and Nagel 1975 and Enfors and Molin 1980). In addition, facultative organisms can start to develop on meat surfaces when the strict aerobes are inhibited (Vanderzant et al. 1982).

In Treatment 3, it was assumed that the amount of oxygen in the packages when they were sealed was enough to allow the pseudomonads to dominate the growth during the first few days of storage. When carbon dioxide evolved within the packages as a result of meat tissue respiration and aerobic metabolism of the organisms, the Pseudomonas growth rate decreased before the onset of meat spoilage. As a result, the facultative organisms, such as Serratia liquifaciens, began to compete with the strict aerobic flora. The transition of the dominating organisms can be considered an increase in the shelf-life of the fresh meat. It is apparent that having one condition which favors the growth of certain spoilage microorganisms may not be the method of choice for extending fresh meat shelf-life.

Coryneforms was found to be able to compete with Pseudomonas at the bottom surface of the steaks in all three packaging treatments. The reasons for that may be due to lower oxygen availability and/or greater nutrient

availability. Microbacterium also formed a considerable percentage on the bottom side of steaks in Treatment 2. This bacterium was found to be able to compete with the pseudomonads on meat surfaces at lower oxygen concentrations (Sutherland et al. 1975b, Seideman et al. 1976, and Newton et al. 1977) even though maximum growth of Microbacterium is obtained in air and lowest growth obtained in 100% carbon dioxide (Blickstad and Molin 1984).

The individual Pseudomonas species did not show regular growth patterns, probably due to differences in initial contamination of the carcass cuts. Similar results were reported by Ayres (1960a) since he could not establish specific growth patterns for the gelatinase-producing pseudomonads. For example, Ps. geniculata was not identified in the present study, although it was the most isolated species in earlier research (Kirsch et al. 1952, Wolin et al. 1957, and Ayres 1960a). In addition, the absence of Ps. putrifaciens in this study may be a result of inhibition by normal meat pH since pH 5.5 was found inhibit this bacterium (Barnes and Impey 1968, McMeekin 1975, and Gill and Newton 1977).

Even though individual pseudomonad species did not demonstrate regular growth patterns, Ps. putida and Ps. fluorescens were found regularly at all sampling days. Jaye et al. (1962) and Rey, Kraft, Walker, and Parish (1970) also

found these flora on steaks and ground meat initially and after meat was spoiled.

The absence of Flavobacterium spp., which are capable of grow at temperatures close to 0 C (Kirsch et al. 1952), was unexpected. Halleck et al. (1958b) also reported the absence of this genus from the common bacteria which can spoil fresh meat. Empey and Scott (1939) did, however, isolate these organisms from refrigerated food.

Experiment Two

Microbial Growth

The steaks packaged in Treatment 4 (15% CO_2, 40% O_2, 45% N_2) and in Treatment 5 (60% CO_2, 40% O_2) had lower psychrotrophic growth and longer shelf-life than the control steaks. This agreed with the findings of Bartkowski, Dryden, and Marchello (1982) since they concluded that 15% CO_2 concentration is very critical in reducing microbial growth especially when combined with high levels of oxygen (40% or above). Luiten and Marchello (1982) further reported that a mixture of 60% CO_2/40% O_2 was effective in extending the shelf-life of beef steaks.

Although different CO_2 concentrations were used in the present study, there was no significant differences in psychrotrophic growth on steak surfaces packaged in these atmospheres. This implies that elevated O_2 concentrations possess antimicrobial effects when used along with elevated

CO_2 concentrations. Christopher, Seideman, Carpenter, Smith, and Vanderzant (1979) support this conclusion since they stated that using high O_2 concentrations along with CO_2 reduce the total psychrotrophic growth significantly when compared with vacuum packaged treatment during two weeks of storage. The actual effect of elevated O_2 appeared to increase the lag phase only and did not reduce the growth rate at the log phase or reduce the final cell number (Clark and Burki 1972).

The bacteriostatic effect of CO_2, on the other hand, can be increased with the increase in CO_2 concentrations up to 25% (Ogilvy and Ayres 1953). Sutherland et al. (1977) concluded that 20% CO_2 is required to limit the multiplication of gram negative organisms. Furthermore, Seideman et al. (1979b) claimed that 100% CO_2 reduces the psychrotrophic growth more significantly than 100% O_2 due to the significantly higher pH of the meat stored in 100% O_2.

A gas atmosphere mixture has to contain at least 5% O_2 in order to maintain the oxygenated form of myoglobin (Holland 1978). Since typical spoilage flora are aerobic and can be inhibited by reduced O_2 concentrations, steaks were packaged in 10% CO_2, 5% O_2, and 85% N_2 (Treatment 6) in order to keep the O_2 level to a minimum. Steaks packaged in this treatment were found to have lower psychrotrophic counts than steaks in all other packaging treatments. This microbial inhibition may be due to the combined effect of

elevated CO_2 and reduced O_2 levels. Enfors and Molin (1980) supported these findings by stating that the effect of high CO_2 and low O_2 are "additive" or even "synergistic". Molin (1983) later found that O_2 limitation and CO_2 inhibition together cause greater reductions in microbial growth than each factor alone when used in continuous culture. In contrast, Wells (1974) stated that the combination of these two gases is "antagonistic", showing no additional microbial inhibition.

Treatment 6 maintained the shelf-life of the steaks 9 days in Trial III, while Treatments 4 and 5 maintained the shelf-life only to Day 6. However, all the gas atmosphere treatments in Trial IV extended the shelf-life to Day 12. The reason for the differences between the two identical trials can be attributed to the higher initial contamination levels in Trial III. The high initial contamination may indicate that the organisms started to grow and multiply before the steaks were packaged in the gas atmospheres. The inhibitory effect of the gas mixtures appear to be reduced if the microorganisms already started to grow and multiply before the meat is packaged (CLark and Lentz 1969). In addition, Ayres (1960b) concluded that increasing the initial contamination of poultry meat from about a log value of 3.3 to 4.7 caused a reduction in the off-odor shelf-life from 8 to 5 days.

Microbial Succession

The initial contaminants of the steaks represent a wide spectrum of genera and species. In the post-treatment storage, certain bacteria have grown at a rate dependent on the gas mixture. For example, the bacteria that are more suited or more resistant to a certain environment have dominated later during storage.

The elevated CO_2 and O_2 concentrations used in Treatment 4 and Treatment 5 increased the lag phase and reduced the growth rate of the pseudomonads. It was also noticed that the pseudomonad percentages decreased sharply towards the end of the storage period. The reason may be due to the high sensitivity of Pseudomonas to CO_2 (Coyne 1933 and Haines 1933). The high sensitivity of the pseudomonads may also add to the competing ability of Coryneforms on both steak surfaces in these two atmospheres.

The effect of Treatment 6 (10% CO_2, 5% O_2, 85% N_2) on Pseudomonas appeared to be more severe than either Treatment 4 or 5. This indicates that the low O_2 concentration in this atmosphere is depleted quickly and the pseudomonads could not compete well with the Coryneforms or Serratia liquifaciens.

Gram negative facultatively anaerobic rods were found to be competitive with the strict aerobic gram negative rods throughout this study, except on the control steaks. For example, Serratia liquifaciens were found to be highly

competitive in terms of percentage and frequency, particularly during late storage. Other organisms, such as Yersenia enterocolitica, Enterobacter aerogenes, Aeromonas, and Pasteurella, were also identified occasionally.

The reason for the recovery of some facultative organisms can be attributed to the indirect effect of CO_2. Since Pseudomonas is inhibited by CO_2, this inhibition allows the grow of these facultative organisms. Furthermore, facultatively anaerobic organisms were not recovered from the control steaks. It was also claimed that 100% CO_2 can retard the growth of Enterobacteriaceae (Molin and Stenstorm 1984). Therefore, it appeared that the growth of Pseudomonas had a greater inhibitory effect on the facultative organisms than the CO_2 concentrations used in this study.

The lower O_2 concentrations used in Treatment 6 (10% CO_2, 5% O_2, 85% N_2) can also be considered an encouraging factor for the growth of the facultative organisms. This agrees with findings of Newton et al. (1977) who isolated Enterobacter liquifaciens and E. aerogenes from lamb chops stored in low O_2 and O_2-free atmospheres.

The organism of major concern that was identified in this study was Yersenia enterocolitica, since both virulent and avirulent species were isolated from porcine tonque (Doyle, Hugdahl, and Taylor 1981 and Doyle and Hugdahl 1983). Harmon, Swaminathan, and Forrest (1984) reported

that Y. enterocolitica was identified on retail meat purchased in several parts of the world. In addition, Hanna, Zink, Carpenter, and Vanderzant (1976) isolated Y. enterocolitica from vacuum packaged meat. However, Y. enterocolitica can develop faster in oxygen-permeable film than in vacuum packages stored at 1 to 5 C (Hanna, Stewart, Carpenter, and Vanderzant 1977b). Even though Swaminathan, Harmon, and Mehlman (1982) stated that most of the Y. enterocolitica isolated from food and water are non-pathogenic to human, more conclusive studies are necessary with regard to gas atmosphere packaging.

SUMMARY

This study was conducted utilizing two experiments to determine psychrotrophic growth and succession on beef steaks as influenced by different packaging procedures. Each experiment was conducted using two identical trials: Experiment One (Trials I and II) compared psychrotrophic growth and succession on steak surfaces (top and bottom) that were packaged in (1) gas permeable Resinite film overwrap as a control, (2) film overwrap after 2 minute gas flush with 1% CO, 40% O_2, 59% N_2, and (3) gas impermeable barrier bags with 100 to 150 cc ambient air. Experiment Two (Trials III and IV) compared psychrotrophic growth and succession on steaks packaged in (4) 15% CO_2, 40% O_2, 45% N_2, (5) 60% CO_2, 40% O_2, (6) 10% CO_2, 5% O_2, 85% N_2, and (7) Resinite film overwrap as controls.

Loin strip steaks were cut to a thickness of 2.5 cm having a surface area of 40 to 50 cm^2. After packaging, steaks were stored at 4 C in open top display cases with light intensity of 60 ft-c for 12 hours/day, with the remaining 12 hours in darkness.

Microbiological evaluations were at intervals of 3, 6, 9, and 12 days. After determining the total psychrotrophic count, the square root number of the total colonies

were picked for further identification of the specific organisms present. Samples were taken from the top and bottom surfaces of the steaks.

The results from Experiment One showed that psychrotrophic growth on the top side of the packaged steaks compared to the bottom side did not differ significantly, although it was one log cycle higher on the top surface. Trial I demonstrated a decrease in the total counts at Day 3 of storage and an increase ($P<.05$) was observed between Days 3 and 9 on most of the steaks. Generally, similar growth patterns were observed on both the top and bottom surfaces of the steaks.

Even though several bacterial genera were found initially on the steak surfaces in Experiment One, only the bacteria more suited or more resistant to the environment developed and dominated at the conclusion of the storage period. In Treatments 1 and 2, the pseudomonads formed at least 80% of the total microflora present on the spoiled steaks. Serratia liquifaciens appeared to be more competitive in Treatment 3, since it formed up to 60% of the dominating organisms. The bottom side of the steaks had lower Pseudomonas growth than the top side in all treatments, while Coryneforms was more competitive on the bottom side.

In comparing psychrotrophic growth on the top and bottom surfaces of the steaks in Experiment Two, a reversed trend was obtained since the bottom surface has higher

growth. The gas atmospheres used for packaging in this experiment allowed less total increases in microbial numbers compared to the control steaks (Treatment 7). The concentration of CO_2 used in Treatment 4 (15%) and Treatment 5 (60%), in conjunction with 40% O_2 in both treatments, appeared to impose the same amount of bacterial inhibition. However, when lower CO_2 and O_2 concentrations were used in Treatment 6 (10% CO_2, 5% O_2, 85% N_2), a greater Pseudomonas inhibition was observed.

The initial contaminants detected in Experiment Two represented a wide spectrum of genera. The flora which developed during post-treatment storage, however, were dominated by the organisms which were able to grow more rapidly under the prevailing conditions. On the control steaks (Treatment 7), Pseudomonas species has a marked advantage in growth rate over the other genera that were present initially. The growth rate and final number of Pseudomonas appeared to be higher on the top side of the control steaks. This indicates that the inhibition of the other genera is due to their inability to compete with the pseudomonads for the available nutrients at high oxygen levels.

On the steaks packaged within the different gas atmospheres, the pseudomonads dominated early in the storage period. However, members of the Enterobacteriaceae family and other facultative organisms were able to compete with the pseudomonads during later storage. Some aerobic

organisms, such as Micrococcus, Coryneforms, and Microbacterium, were more competitive with the pseudomonads on steaks packaged in the different gas atmospheres.

Higher numbers of initial contaminants reduced the shelf-life of the meat despite the gas atmosphere treatments. However, when the steaks had relatively low psychrotrophic counts initially, the gas atmospheres could extend the shelf-life at least 6 days longer than the control steaks. Furthermore, the effect of the different packaging treatments on microbial growth was more clear when the steaks had lower initial microbial numbers.

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