

1981

# Quality changes in vacuum packaged fresh pork

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**QUALITY CHANGES IN VACUUM PACKAGED FRESH PORK**

*Iowa State University*

**PH.D. 1981**

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Quality changes in vacuum packaged fresh pork

by

William Joseph Lulves

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Graduate Faculty in Partial Fulfillment of the  
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## INTRODUCTION

The use of prepackaged fresh meat prepared in a centralized facility has several advantages over cutting and packaging the meat at the retail level. One of these advantages is reduced transportation costs: when prepackaged, much more product can be placed in a truck or railcar than when shipped as hanging carcasses. Another advantage is more efficient use of labor and by-products. Meat trimmings and fat can be utilized at centralized facilities which are discarded at the retail level. Other advantages of a centralized facility include improved quality assurance and sanitation facilities, greater merchandising flexibility, improved inventory control, and simplification of retail operations (Tatum et al., 1978).

Some progress in this move toward centralized processing and packaging has been made: over two-thirds of all beef shipped in the United States is transported as vacuum-packaged wholesale cuts (Ayres, 1978). The fabrication and packaging of retail cuts of meat represents additional advantages over packaged wholesale cuts. While the prepackaging of retail portions of meat at a centralized location has been almost universally accepted for cured meats, this concept has not yet been accepted as widely in the area of fresh meats.

Vacuum packaging of fresh meats does have some disadvantages not shared with cured meats. The extension of shelf life of fresh meats, while increased by vacuum packaging, does not always compensate for the increased transportation and handling time necessary for centralized processing. Problems have arisen regarding some quality factors, e.g., color of meat in vacuum packages, distortion of retail cuts, and increased purge (drip loss) due to vacuum packaging. The challenge of overcoming these problems is presently an active area of research in food technology.

The purpose of this work was to investigate the effects of various types of processing methods and packaging films on the shelf life of fresh pork packaged as retail cuts. Several of these practices may be applied by the industry. The ultimate objective of this area of research is to provide the food industry and consumer with an improved method of packaging and merchandising of fresh pork.

Specifically, this particular study had several objectives:

1. To determine the effects of packaging films of different permeabilities to oxygen on the shelf life and quality characteristics of fresh pork. This has been the subject of several studies with other fresh meats such as beef, lamb, and poultry,

but there are few investigations concerning pork reported in the literature.

2. To determine the effect of various processing methods and practices, such as deboning of the pork, cutting procedures, and initial shell freezing, on the quality of prepackaged pork chops.
3. To determine and compare the effect of holding times of fresh cuts prior to retail type display and the effect of the form of this meat during holding (loins or retail cuts) on shelf life.

## LITERATURE REVIEW

## Muscle Chemistry

For purposes of brevity, and since the chemistry of meat and meat color are peripheral aspects of this study, only a few of the more recent reviews and research reports will be reviewed here. Meat is a complex composition of water, protein, fat, some carbohydrates, and inorganic constituents. Living muscle and meat generally have the same chemical composition: 53-76% water, 15-22% protein, 1-15% lipid and less than 4% other constituents. It can be seen from these figures that the main constituent of meat on a dry weight basis is protein. Muscle proteins are divided into three general classes: sarcoplasmic proteins, which are the various metabolic enzymes within the muscle cell; stroma proteins, which include collagen and elastin, and impart toughness and elasticity to muscle; and the myofibrillar proteins, which are responsible for the contractile ability of the muscle. The myofibrillar proteins are the largest fraction of proteins in muscle (Bodwell and McClain, 1971).

In the process of the change of muscle into meat, one of the major events is rigor mortis. An important sequence of events takes place during rigor mortis that has consequences relating to the keeping quality and general bacteriology of meat.

When respiration ceases at the time of slaughter, the muscle tissue quickly becomes anaerobic. In this state, oxidative phosphorylation of ATP in the muscle cannot take place. To compensate for this, the muscle utilizes glycogen to provide energy through anaerobic glycolysis. The end product of anaerobic glycolysis is lactic acid, which lowers the pH of the muscle tissue. In beef, the pH of the muscle will fall from approximately pH 7.0 to pH 5.5 (Newbold, 1966). This fall in pH plays a significant role in the prevention of subsequent microbial growth. If the animal has been excited or starved prior to slaughter and depletes the glycogen reserve in the muscle, the ultimate pH reached will be higher than pH 5.5. The closer to neutrality the ultimate pH is, the faster the growth of microorganisms in or on the meat (Dainty, 1971; Rey *et al.*, 1976; Patterson and Gibbs, 1977).

#### Meat Color

Color in red meats comes from the pigments myoglobin and, to a lesser extent, hemoglobin from blood retained in the muscle. Myoglobin is a conjugated protein containing a non-peptide heme group, iron protoporphyrin IX. The factors determining the color of the myoglobin complex are the type of bond formed between the iron in the heme group and the molecule at the sixth coordination position of the iron,

the oxidation state of the iron, and the physical state of the globin portion of the molecule. Myoglobin in the unoxygenated state in the muscle has a purplish-red color. This can be oxygenated to oxymyoglobin, which is bright red. Both myoglobin and oxymyoglobin can be oxidized to metmyoglobin, where the iron in the heme complex is oxidized to the ferric state. Metmyoglobin is a brown color (Bodwell and McClain, 1971).

The color of meat is used by the consumer as a guide to freshness: meat which has the cherry-red oxymyoglobin color is accepted, while meat with the brown metmyoglobin color is rejected as being old. The purplish color of myoglobin is not distinguished from the brown metmyoglobin color by consumers (Watts, 1954). Consumer acceptance is dependent on color. Although the color is not closely related to nutrition or palatability, the relationship between the level of discoloration in terms of metmyoglobin content and proportion of total sales of discolored meat is linear: the greater the metmyoglobin content, the lower the sales (Hood and Riordan, 1973). Thus, when marketing meat in self-service operations, it is important that the meat have the desirable red oxymyoglobin color.

Bacterial Reservoirs in the  
Live Animal

Certain organs in the living, normal healthy animal may harbor viable bacteria. Lepovetsky et al. (1953) found a great number of gram-positive and gram-negative bacteria in the lymph nodes of cattle. These bacteria included the genera Clostridium, Alcaligenes, Escherichia, Flavobacterium, Pseudomonas, and Streptococcus. They also found viable organisms in some bone marrow and muscle samples but with a much lower frequency. In a survey of bovine liver from newly slaughtered animals, Canada and Strong (1964) isolated Clostridium perfringens from 12% of the samples. In this same study, 26% of bovine liver samples from retail markets contained clostridia. Although the liver and lymph nodes may contain living organisms, very few viable bacteria are found inside the muscle tissues (Lechowich, 1971).

Most of the contamination of muscle tissue occurs during slaughter. The stick-knife used in bleeding the animal was shown by Jensen and Hess (1941) to allow the blood system to carry microorganisms to the heart and lungs. Scalding tanks, skinning, evisceration and other processing procedures also contribute to contamination of the carcass (Ayres, 1955).

Microorganisms Associated with Fresh Meat  
Immediately Postmortem

The total microbial flora from meat immediately after slaughter is quite different from that which develops during subsequent storage. In the review by Ayres (1955) on the microbiological implications in the slaughtering of meat animals, the results of eight studies are presented. Of the 19 bacterial genera cited, those genera most often isolated from freshly slaughtered beef included Pseudomonas, Micrococcus, Streptococcus, Achromobacter, Flavobacterium, Escherichia and Bacillus. It is interesting to note that two genera which are often associated with meat spoilage, Lactobacillus and Clostridium, are among those isolated least often, being reported by only one and two authors, respectively, of the eight reviewed. Eighteen different genera of molds and yeasts were also cited as being recovered from meat.

The microbial populations on fresh meat immediately after slaughter and in retail markets were investigated by Stringer et al. (1969). They found that the flora of beef carcasses in the packing plant consisted of Pseudomonas, Micrococcus, Sarcina, Flavobacterium and Bacillus, with Pseudomonas and Micrococcus predominating. At the retail level, however, micrococci were absent and Pseudomonas and Achromobacter predominated. The muscles of various animals



at the time of slaughter and during refrigerated storage were examined by Vanderzant and Nickelson (1969). Of genera isolated from pork, lamb and beef, 87.8%, 40.6% and 55.5, respectively, were found to be Staphylococcus species. A high percentage of these staphylococci were coagulase positive. Other isolates from pork were Micrococcus, coryneform bacteria, Bacillus, Alcaligenes, and Herellea (Acinetobacter).

As indicated by several investigators, of the organisms recovered from the freshly slaughtered animal, many are transient adventitious types. The majority are not identified with defects of subsequent spoilage. Postmortem development of the microflora on fresh meats depends on a number of factors, among which are temperature, oxygen availability, humidity, and availability of substrate for microorganisms to assimilate.

#### Bacteriology of Meat at Warm Temperatures

Reports in the literature have shown that there are important differences between the bacterial flora developing on meat at warm (25°C), intermediate (5°C to 25°C) and chill (5°C) temperatures. Under warm conditions, mesophilic bacteria such as the clostridia will grow at an extremely rapid rate. An important organism that initially grows in meat at warm temperatures is Clostridium perfringens. This

organism breaks down carbohydrates and proteins in the meat, producing gas, putrefactive compounds, and lowering the redox potential of the meat so that other species of clostridia can proliferate. The presence of large numbers of putrefactive anaerobes acts as an indicator for the possible presence of Clostridium botulinum (Ingram and Dainty, 1971). The temperature of meat must be reduced quickly after slaughter to temperatures where clostridia and other mesophilic bacteria will be inhibited. Cooling meat also has an inhibitory action on other potential pathogens such as Salmonella and Staphylococcus, as well as other organisms associated with pathogenicity or spoilage (Angelotti et al., 1961).

#### Bacteriology of Meat at Intermediate and Chill Temperature

The presence or absence of oxygen and other gases plays an important role in the development of the microflora of meat. The effect of various gases and packaging techniques will be discussed in a later section. This section will cover only the development of the microflora under aerobic conditions.

The flora that develops at intermediate temperatures (5°C to 25°C) has been shown by several authors to be somewhat different than that which develops at chill temperatures

(5°C or less). Gardner et al. (1967) found that on pork held at 16°C the Pseudomonas-Achromobacter group constituted 49% of the flora, while other organisms, such as Kurthia (27%) and Enterobacter-Hafnia (13%) were also present in significant numbers. At 2°C, however, Pseudomonas-Achromobacter group was 96% of the flora. At both temperatures Lactobacillus species and Microbacterium thermo-spachtum were minor constituents of the flora. An interesting paper on the microbiology of hung pheasants by Barnes et al. (1973) also noted a difference in flora with differing temperatures. They found that clostridia remained a large part of the flora of the intestine of the pheasants hung at 15°C while they were found at a very low level in pheasants hung at 5°C.

It is generally accepted that spoilage at temperatures of 5°C or less is a surface phenomenon unless the meat has been treated in some way, such as grinding, to distribute the bacteria throughout the mass (Ingram and Dainty, 1971). The first reported studies on the bacterial flora of spoiled meat were made by Glage (1901) as cited by Ayres (1955). Glage stated that slime on meat was caused by bacteria which he called "Aromobakterien". These were oval to rod shaped microorganisms that grew well at 2°C but poorly at 37°C. He noted that the growth of these bacteria was accompanied by a characteristic odor. Haines (1933) found

that most of the bacteria forming slime on meat surfaces at low temperatures were members of the Achromobacter group, with some Pseudomonas and Proteus also being present. He observed that slime formed on meat when microorganisms reached a level of  $10^7$  to  $10^8$  bacteria per  $\text{cm}^2$ . Another result of his experiments was the demonstration that meat that had a low population of bacteria initially had better keeping quality and developed slime later than meat that was heavily contaminated. The bacterial flora of fresh pork sausage was investigated by Sulzbacher and McLean (1951). They found that 75% of the organisms isolated from fresh pork sausage were members of six genera: Pseudomonas, Microbacterium, Achromobacter, Bacillus, Alcaligenes, and Bacterium.

The bacteriology of ground beef was studied by Kirsch et al. (1952). They showed that the total bacterial count of hamburger when spoilage was first noticed in the form of sour odor was approximately  $5.0 \times 10^8$  microorganisms per gram. The microbial flora of the ground beef when purchased consisted of a mixture of gram negative rods, bacteria belonging to the family Micrococcaceae, and lactobacilli. At spoilage, bacteria of the genus Pseudomonas predominated.

There are indications that the microflora of lamb chops stored at chill temperatures seems to be different from that of other meat. Barlow and Kitchell (1966) found that the

microflora of lamb chops that had been stored at 5°C in air consisted largely of Microbacterium thermosphactum, while beef steaks treated identically, developed a flora of 81.3% gram negative rods. Newton et al. (1977) observed that in an oxygen and carbon dioxide atmosphere Microbacterium thermosphactum made up the major constituent of the flora while in other atmospheres Pseudomonas predominated on lamb chops.

The ability of different organisms to produce odor when grown on meat has been studied by McMeekin (1975). Using sterile chicken breast muscle and gas chromatographic head-space analysis he found that nonpigmented Pseudomonas were naturally selected for in spoiling chicken muscle and that they became the major portion of the flora at 2°C. These types produced strong off-odors when inoculated into sterile muscle sections. These results were corroborated that same year by Cox et al. (1975) using sensory analysis. They found that the only significant difference in sensory scores of cultures of microorganisms grown on chicken media was due to pigmented versus nonpigmented strains of Pseudomonas. The nonpigmented strains of Pseudomonas produced more intense off-odors.

It is generally agreed that the bacterial population must be approximately one million organisms per square

centimeter for off-odor to take place on meat (Kraft and Ayres, 1952). The number of bacteria necessary for slime formation is usually higher than that for off-odor, reported to be from three million (Moran, 1935) to 100 million (Kraft and Ayres, 1952) per square centimeter. The time that is required to reach the population necessary for off-odors varies, however, and is affected under aerobic conditions by temperature, initial load of microorganisms, and substrate. Several authors (Jaye et al., 1962; Gardner et al., 1967) have found that there is a pronounced lag in the growth of the total bacterial population at temperatures around the freezing point. The effect of chill temperatures is that the generation time of the bacteria are lengthened, thus increasing the length of time necessary to reach the off-odor and slime-forming populations.

The initial bacterial load on a product has an important effect on the shelf life. The bacterial counts of finished fresh pork sausage were shown by Surkiewicz et al. (1972) to be primarily dependent on the bacterial condition of the pork trimmings used in the manufacture of the sausage. The higher the initial bacterial population, the shorter the time necessary to reach the spoilage point.

Characteristics of the meat itself can affect both the numbers and types of bacteria which grow on it. This selective

action can take place not only between species but also within a species. Rey et al. (1976) investigated the microbiology of pale, dark and normal pork. They found that differences in pH between pale, soft and exudative (PSE) pork and normal pork were significant. Most rapid bacterial growth took place on dark meat and slowest growth on PSE pork. They attributed this to the difference in pH between the two types of meat.

#### Effect of Fluctuating Temperature on Bacterial Growth

Fresh meat, although kept under continuous refrigeration, may not be maintained at a constant temperature. Fluctuations in temperature can take place at many places in the distribution system. Temperature on the packing production floor is different from the storage cooler temperatures. In some large coolers, the temperature may vary 15°C depending on the time of day, day of the week, or season (unpublished data, William Joseph Lulves, Department of Food Technology, Iowa State University, Ames, Iowa). The product may be aged at a different temperature than subsequent storage (Minks and Stringer, 1972). Trucks and railcars vary in temperature according to the season and driver (personal observation, William Joseph Lulves, Department of Food Technology, Iowa State University, Ames, Iowa).

Finally, the display case of the retail store will usually have a defrost cycle which allows the temperature of the refrigerated portion of the display case to vary. For these reasons, some incidental temperature abuse of the product is inevitable.

Peterson and Gunderson (1960) studied the effects of environmental conditions on the elaboration and activity of proteolytic enzymes by Pseudomonas fluorescens. They found that extracellular proteolytic enzyme elaboration was inversely proportional to the temperature at which the culture was grown between 0°C and 30°C. The activity of the proteolytic enzyme, however, showed an increase from 0°C to 15°C. This report would suggest that meat spoilage in a fluctuating temperature environment would be faster than at a constant temperature because of increased enzyme elaboration and activity. Microbial growth, however, would not necessarily be greater. Frank et al. (1972) found that catalase activities for the same organisms were similar at different temperatures. This reversed an earlier finding of theirs (Frank et al., 1963) that catalase activity was reduced at the lower temperature.

Ng et al. (1962), working with Escherichia coli, showed that there was no lag in attainment of growth rate characteristic of a particular temperature with temperature shifts



of 10-15°C, but that there was a lag with temperature shifts of 25°C. They concluded that the cell was damaged at low temperatures in a way that reduced the growth rate.

While investigating the influence of temperature on some biochemical characteristics of Pseudomonas associated with the spoilage of chicken, Rey et al. (1969) found that the proteolytic and lipolytic enzyme production of the cultures were greater at 5°C than at 15°C. They suggested that an increased production of extracellular enzymes may be induced at 5°C to compensate for the reduced rate of enzyme activity at the lower temperature. They also noted that freezing and thawing does not affect the extracellular lipase and protease enzymes.

When temperature fluctuates downward there is an inhibiting effect on spoilage. Rey et al. (1970) found that bacterial counts were higher and spoilage was more rapid for retail cuts from carcasses aged at low temperature for long time periods than for retail cuts from carcasses subjected to accelerated aging at high temperatures. They concluded that the reason for this was that a psychrotrophic population was already established on the carcasses held at low temperature while a lag in adaptation was exhibited by the microflora on the carcasses aged at high temperature.

Howell et al. (1971) noted that temperatures occurring

in nature are constantly fluctuating, while virtually all research involving temperature effects on organisms has been carried out at constant temperatures. Using a thermal gradient bar to cycle cultures of organisms, they found that the response to cycling effects appeared to be greater when temperature was cycled down. In other words, organisms grew better when they started out "warm" rather than starting out "cold." These experiments were carried out using pure cultures, however.

In studying the effect of fluctuating storage temperatures on microorganisms on beef shell-frozen with liquid nitrogen, Rey et al. (1972) found that bacteria on frozen-thawed loins had a longer lag time and reached a lower total population than bacteria on loins that were held at a constant temperature. It should be noted that in this study, the temperature was cycled up.

#### Potential Foodborne Pathogens and Meat

Clostridium perfringens is a ubiquitous organisms occurring as a saprophyte in soil, dust, and human and animal feces (Ayres et al., 1980). It is a natural and unavoidable contaminant of all foods. Clostridium perfringens has been isolated from 47.4% of ground beef in the Denver, Colorado area (Ladiges et al., 1974). Hall and Angelotti (1965) found

Clostridium perfringens in 37% of pork cuts sampled in their survey of meat products in the Cincinnati area. The food intoxication produced by Clostridium perfringens is characterized by profuse diarrhea, abdominal cramps and flatulence. Ordinarily there is no vomiting or nausea. Reducing the temperature of foods to 20°C or less rapidly kills vegetative cells (Traci and Duncan, 1974) but spores survive refrigeration and freezing well (Trakulchang and Kraft, 1977). Most reported incidences of C. perfringens food poisoning are caused by temperature abuse of prepared food products such as stews, gravies or meat pies. The heating of foods which contain Clostridium perfringens vegetative cells and toxin to 63°C destroys both (Ayres et al., 1980). The common rule of keeping food below 40°F or above 140°F results in the control of Clostridium perfringens (National Research Council, 1975).

Staphylococcus aureus causes a food poisoning which is one of the most common reported foodborne illnesses in the United States. The causative organism is easily destroyed by heat but the enterotoxin responsible for the disease is very heat resistant. Staphylococcus aureus produces a variety of toxins, including enterotoxins A through F. The toxin most commonly implicated in food poisoning is enterotoxin A (Thatcher and Clark, 1968). This enterotoxin is a protein which exerts its effect upon the

intestine and central nervous system. The intoxication is characterized by nausea, diarrhea, vomiting and cramps. Symptoms seldom continue for more than 12 to 15 hours (Holvey, 1972). Staphylococcal enterotoxin is most often produced in moist or fluid foods that have been heated, thus destroying competing bacterial populations. The manufacture of fermented foods such as cheese and fermented sausage presents a special problem since Staphylococcus aureus will proliferate at the temperatures used in processing these foods. In these cases (Tatini, 1973) the organism is best controlled by not utilizing contaminated raw material, such as mastitic milk or pork cheeks and other tissues containing lymph nodes, which have high levels of microorganisms (National Research Council, 1975). Another control measure in foods where Staphylococcus aureus is likely to grow is strict sanitation and temperature control. Food handlers must be impressed with sanitary practices since greater than 50% of the healthy adult population are asymptomatic carriers of coagulase-positive staphylococci (Holvey, 1972).

Salmonellosis is actually a food borne infection by one of the more than 1200 serotype species of Salmonella. The reservoir of infection is in the domestic and wild animal populations where carrier rates may be as high as 40%

Wintrobe et al., 1970). Clinical manifestations of the disease are fever, diarrhea, nausea, vomiting, and, in serious cases, septicemia. Weissman and Carpenter (1969) found that 56% of pork carcasses, 38% of fresh pork sausage, and 9% of smoked pork sausage in Georgia were contaminated with Salmonellae. Because 50% of the brands of sausage were positive for salmonellae they expressed the concern that there was general contamination of fresh pork sausage. A later survey of the Toronto, Canada, area (Duitschaever and Buteau, 1979) found lower but still significant contamination in samples from retail markets. In this survey, 14% of pork chops and 20% of ground pork samples were positive for salmonellae or Salmonella. Control of the disease consists of preventing cross-contamination of raw meats with sources of salmonellae and by the practice of strict sanitation measures when dealing with meats at all levels of production and consumption from the feed lot to the table. It is significant that the incidence of Salmonellae in raw pork is higher than that in raw beef, yet outbreaks from beef are more frequent than from pork because pork is usually cooked more thoroughly than beef (National Research Council, 1975).

Interactions of Lactobacillus with other microorganisms

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Lactobacilli are often isolated from meat. Their total effect on the microflora of meat is unknown but there are some reports that they are inhibitory to other spoilage bacteria and some potential pathogens. Price and Lee (1970) isolated Lactobacillus species from seafoods and found that these organisms were capable of inhibiting Pseudomonas, Bacillus and Proteus species. They attributed this inhibition to the production of hydrogen peroxide by the lactobacilli. The effect of lactobacilli on the growth of Microbacterium thermosphactum was investigated by Roth and Clark (1975). They showed that on artificially inoculated fresh beef the lactobacilli markedly restricted the growth of Microbacterium thermosphactum when the beef was vacuum packaged. With unpackaged, aerobic storage this inhibition did not take place. The authors concluded that anaerobic conditions were necessary for the antagonism to take place and that lactobacilli were important in the storage life of vacuum packaged beef. Gilliland and Speck (1977) found that Lactobacillus acidophilus exerted antagonistic actions on the growth of Staphylococcus aureus, Salmonella typhimurium, enteropathogenic Escherichia coli, and Clostridium perfringens. The amount of inhibition varied among strains of Lactobacillus acidophilus and appeared to be due to lactic acid

and hydrogen peroxide production.

### Vacuum Packaging

Vacuum packaging refers to the placing of an article in a container and removing much of the air prior to sealing the container. In regard to fresh meat the primary container is usually a flexible film which can be heat sealed or sealed with a clip. Vacuum packaging developed between 1945 and 1950 in response to a need for better meat packages from the standpoint of appearance, convenience and protection of the product in self-service markets (Ramsbottom, 1971).

Vacuum packaging is presently used for the retail packaging of many kinds of processed and cured meats such as bacon, frankfurters, dried beef and similar items. Fresh meat is vacuum packaged at the packer level as wholesale cuts which are then shipped to supermarkets or institutions where the meat is further reduced to retail or portion cuts. The vacuum packaging of retail cuts at a central processing operation has been hindered because of problems with color retention and shelf life. However, if a means can be developed to assure adequate retention of product quality during its necessary shelf life centralized vacuum packaging of retail cuts offers several advantages. These are reduced labor and transportation costs, more efficient utilization of by-products such as fat, and greater control of quality and

sanitation at the packaging plant level (Ayres, 1978).

### Films used for Vacuum Packaging

Films for vacuum packaging are rarely made of one compound because, aside from the fact that different products which are vacuum-packaged demand different film properties, no one material combines all requirements for thermoforming, abrasion resistance, clarity, sealability and gas barrier characteristics. Most films are combinations of base films into laminates or base films with coatings. The most common films for vacuum packaging will be discussed.

#### Polyethylene

This was one of the first films used as a material for vacuum packaging. Polyethylene is made in three density ranges: low, medium and high. As the density increases, the film's stiffness and resistance to moisture, fat and oxygen permeability increase. Polyethylene is moisture proof but has high porosity to oxygen. Because it has excellent heat sealability, polyethylene is often laminated to other films with better oxygen barrier properties to enable them to seal properly.



Polyvinylidene chloride (Saran)

Saran has high barrier properties in regard to moisture, oxygen and fat. It may be used as a heat processing package as well as a consumer package because it is resistant to heat up to 270°F.

Polyester (Mylar)

The principal property of Mylar is high strength and resistance to wide temperature ranges. It is often coated to allow it to seal properly.

Polyamide (Nylon)

Nylon's main desirable property is its toughness and stretch. Nylon based laminates are often used for forming deep-draw packages having a high ratio of depth to width for processed meats.

Ionomers (Surlyn)

Surlyn is an ethylene acrylic acid copolymer combined with metallic ions, based on low density polyethylene and having the ionic bonds for strength. It has high clarity, gloss, toughness, is moisture proof, and has high porosity to oxygen. It is often combined with other films (Sacharow, 1979; Sacharow and Griffin, 1980).

### Composition of Atmosphere within the Vacuum Package

Even the most effective vacuum packaging machines leave some oxygen within the package (Ingram, 1962). After the package is sealed the composition of the atmosphere that develops over a period of days depends on the permeability of the film and the metabolic gases that accumulate from respiration of the meat and from microorganisms on the meat. Gardner et al. (1967), using metal cans half-filled with pork samples which were covered with a gas impermeable film, but not vacuum packaged, reported that within 3 hours of preparation, the atmosphere in the headspace of the can contained 3-5% carbon dioxide. At 2°C, there was a steady increase of carbon dioxide for four days up to 13% CO<sub>2</sub>, followed by a slower evolution up to 15% CO<sub>2</sub>. In cans held at 16°C, CO<sub>2</sub> evolved to 30% at 4 days while the oxygen level fell to 1%. Similar results were reported by Hall et al. (1980). With pork loins vacuum packed in a high barrier film they found that the relative weight percentage of CO<sub>2</sub> increased from 1.6% initially to 16.03% in seven days, while the O<sub>2</sub> content of the atmosphere within the package dropped from 21.59% initially to 1.34% at 14 days. From these studies, it may be noted that with meat in high barrier films the oxygen level drops to about 1% or less while the carbon dioxide level rises to at least 16% at

refrigeration temperatures. The cause of this change is initially aerobic glycolysis in the muscle after death. After several days, the microbial flora within the package becomes responsible for the change in atmosphere. It should be noted that, while some packaging films may allow enough gas exchange to allow the atmosphere within the package to resemble the atmosphere outside the package, many films regarded as being permeable do restrict exchange enough to alter the microflora within the package (Ingram and Dainty, 1971).

#### Effect of Gas Atmosphere on the Microflora and Keeping Quality of Meat

The gas atmosphere within the package is one important determinant of the type of microflora which develops on the meat. Carbon dioxide has been found to inhibit aerobic bacterial growth while not affecting lactic acid bacteria. Ogilvy and Ayres (1952) found that CO<sub>2</sub> extended the storage life of frankfurters by increasing the lag time of microorganisms associated with spoilage of the meat. Their results showed that CO<sub>2</sub> had a selective action on the types of organisms developing on frankfurters. Most microorganisms were effectively retarded by the CO<sub>2</sub> but lactic acid bacteria were only slightly affected. At CO<sub>2</sub> concentrations above 50%, they had little competition from other

organisms. Baran et al. (1970), working with bacon packaged in an atmosphere of carbon dioxide, reported that total aerobes in CO<sub>2</sub> packaged bacon had a lag time of 14 days. Numbers of lactobacilli on bacon packaged in CO<sub>2</sub> increased during the first three days and then declined. They also reported a residual effect of CO<sub>2</sub> since the packages of bacon treated with CO<sub>2</sub> inhibited growth after 3 days while air and vacuum packaged bacon did not. An inhibitory effect of spoilage bacteria in cottage cheese by CO<sub>2</sub> has also been reported by Scott and Smith (1971).

Huffman (1974) showed that pork chops stored in CO<sub>2</sub> had lower counts for a 5 week storage period than did chops stored in O<sub>2</sub> or N<sub>2</sub>. He concluded that, since the counts in O<sub>2</sub> and N<sub>2</sub> were similar, this proved that CO<sub>2</sub> had a definite inhibitory effect on the total bacterial count. In this experiment aerobic counts on a gas mixture of 70% N<sub>2</sub>, 25% CO<sub>2</sub> and 5% O<sub>2</sub> were consistently higher than on CO<sub>2</sub> only. He speculated that this may have been due to a decline in pH because of absorbed CO<sub>2</sub>. However, King and Nagel (1967) and King and Nagel (1975), reporting on inhibition of Pseudomonas by CO<sub>2</sub>, claimed that CO<sub>2</sub> inhibits by blocking several metabolic enzymes needed for growth. Newton et al. (1977) found that on lamb chops stored in various gas mixtures, Microbacterium thermosphactum became the predominant organism on chops stored in an oxygen plus carbon

dioxide atmosphere. In all other oxygen containing atmospheres, Pseudomonas species made up a large portion of the flora. Lactobacillus species occurred in all oxygen-free atmospheres.

Experiments by Silliker et al. (1977) indicated that on pork and beef CO<sub>2</sub> exhibited a strong inhibitory effect on total count and also had some residual effect. After 14 days storage in air at 1°C, pork loins were spoiled while loins packed in CO<sub>2</sub> were still satisfactory. Roasts cut from the loins stored in CO<sub>2</sub> showed better keeping quality than air stored loins. They noted that the color and odor of CO<sub>2</sub> packed pork remained good but that beef treated with CO<sub>2</sub> showed rapid and severe color degeneration.

The microbiology and sensory characteristics of pork packaged in various combinations of O<sub>2</sub>, N<sub>2</sub> and CO<sub>2</sub> were studied by Christopher et al. (1979). They found little difference in psychrotrophic and Lactobacillus counts between roasts stored in modified gas atmospheres and those stored in vacuum packages. In roasts stored in 100% O<sub>2</sub>, however, Pseudomonas became the dominant flora where as in all other atmospheres Lactobacillus dominated after one week storage. In a companion paper, Seideman et al. (1979) noted that roasts stored in O<sub>2</sub> containing atmospheres had a higher incidence of off odor and chops from these

roasts had lower appearance ratings than did comparable vacuum packed meat. They suggested that a modified gas atmosphere containing 20% CO<sub>2</sub> and 80% N<sub>2</sub> be used as an alternative to vacuum packaging.

In a series of papers reporting results of experiments comparing vacuum packaging with modified gas atmosphere packaging (Seideman et al., 1980; Christopher et al., 1980a, 1980b; Hall et al., 1980) it was stated that psychrotrophic bacterial counts of lean and fat surfaces of pork loins stored in 40% CO<sub>2</sub> and 60% N<sub>2</sub> were frequently significantly lower than counts of comparable sites from vacuum packaged loins. Lactobacillus counts were in most instances higher on the fat surfaces of the loin than on the lean surfaces in loins stored in 40% CO<sub>2</sub> and 60% N<sub>2</sub>. Lactobacillus became a predominant part of the microflora after 28 days in all packages, though Pseudomonas persisted to a greater degree in the vacuum packaged loins than in loins stored in CO<sub>2</sub> plus N<sub>2</sub>. Few differences were observed between the treatments in the physical and sensory characteristics of the loins or in the chops cut from the loins. Retail cuts packaged in CO<sub>2</sub>-N<sub>2</sub> atmospheres and then subjected to retail display usually had lower psychrotrophic counts than vacuum packaged chops. However, in contrast to loins stored in modified gas atmospheres, both vacuum packaged retail cuts

and retail cuts stored in modified gas atmospheres sustained extensive surface discoloration and were of unsatisfactory appearance after only 7 days of storage and 1 day of retail display.

Effect of Vacuum Packaging on the Microflora  
and Keeping Quality of Meat

The comparison of the microbial flora and the keeping quality of meat in air and vacuum packaging has been the subject of many studies since the packaging method was developed. Halleck et al. (1958) studied the effect of package characteristics on the bacterial flora of meat. Their results were that the primary effect of packaging material on bacterial growth was correlated with the oxygen permeability of the packaging films used. Nonpermeable packaging films influenced bacterial growth by the atmosphere produced within the package. Growth in the impermeable packages was marked by a longer lag phase than in permeable films.

Vacuum packaging of beef was investigated by Pierson et al. (1970). Using oxygen permeable and oxygen impermeable films they showed that the total bacterial count increased more rapidly and was always higher with the aerobic packaging method. Under aerobic conditions, fluorescent pseudomonads increased in number while there was no change in pseudomonad count in the anaerobic beef. Lactobacilli were 90 to 95% of

the total count in the anaerobic package. Microbacterium thermosphactum and gram-negative bacteria decreased in number after 15 days storage at 3.3°C in anaerobically packaged samples. Baran et al. (1970) had similar results with fresh hamburger. They found that growth of aerobes on vacuum packaged meat was slower than growth on meat packaged in air. Growth of anaerobes, however, occurred earlier in the vacuum packaged meat. They also noticed that there was a tendency for fresh meat packaged in films having high oxygen permeability to support growth of aerobic organisms as compared with less permeable films.

Microbial growth on vacuum packaged lamb at storage temperatures of 0°C and 7°C was studied by Reagan et al. (1971). They found that spoilage occurred earlier on vacuum packaged lamb stored at 7°C than at 0°C and thus concluded that vacuum packaging was not a substitute for low temperature storage. They also found that the subsequent retail case-life of chops cut from vacuum packaged loins is adversely affected if the loins are stored at either temperature for periods in excess of eight days.

Minks and Stringer (1972) studied the influence of aging wholesale cuts of beef in vacuum. Their results were that there was no significant difference in palatability between beef aged in vacuum packaging and that aged with no packaging,



but the vacuum packaged cuts did have significantly lowered bacterial counts. They also noted that vacuum packaging had a substantial effect on weight losses during the storage period, the vacuum packaged beef having lower losses than beef stored in air. Smith et al. (1974) also worked with wholesale cuts, but of pork rather than beef. In experiments where the cuts were wrapped in parchment paper or polyvinyl chloride (PVC) film, they found that the vacuum packaged pork loins sustained less surface discoloration and were higher in consumer acceptability than either loins wrapped in PVC film with no vacuum packaging or loins wrapped in parchment paper. Another interesting result of their experiments was that loins wrapped in PVC film with no vacuum drawn had higher bacterial counts than those wrapped in parchment paper or vacuum packaged. This may have been due to a drying effect with parchment paper since the parchment paper wrapped loins sustained the highest shrinkage losses. Hodges et al. (1974) demonstrated that wholesale cuts of beef could be aged successfully in vacuum packages, thus reducing weight loss during storage. However, in their experiments, bacterial growth rendered the product in vacuum packages unacceptable between 14 and 28 days of storage at chill temperatures.

The effect of various types of vacuum packages and film on wholesale and retail beef cuts was tested by Seideman

et al. (1976a,b). They found that a combination of a chamber vacuumizing machine and the use of a high barrier packaging film resulted in superior fat appearance ratings, reduced surface discoloration, and higher total desirability scores as opposed to a nozzle type machine and a low barrier film. Differences in psychrotrophic, mesophilic and Lactobacillus counts were not significant for any of the packaging systems tested. They did note, however, that psychrotrophic and mesophilic counts in defective packages (leakers) increased faster than those on meat in intact packages. Also, the flora of the vacuum packages showed a significant difference from that in the leaker packages: on the vacuum packaged meat Lactobacillus dominated the microflora after 28 days of storage while Pseudomonas dominated the flora of the leaker packages. Both types of bacteria were found on all packages, along with Microbacterium and Moraxella-Acinetobacter. The reason for the superior ratings of the chamber vacuumizing machine may have been provided by Bowling et al. (1977) who compared a chamber heat seal system and a nozzle clip seal system using subprimal beef cuts. They noted that the chamber system produced a higher visual vacuum score than the nozzle system. Few other differences were noted between the two systems, however.

Patterson and Gibbs (1977) also observed that the

microflora of vacuum packaged meat was quite different from that of meat stored aerobically. With dark-cutting beef, they found that lactobacilli were the major component of the microflora after 6 weeks of storage at 1°C in vacuum packages. At eight weeks storage, however, psychrotrophic Enterobacteriaceae represented a major portion of the flora.

One problem in reviewing the bacteriology of fresh meats is that the microbial flora of meat shows some variation between investigators. As Ingram (1962) has pointed out, certain bacteria cannot be selected for unless the meat has been contaminated with them beforehand. If this contamination is lacking, the microbial picture will be different. Sometimes it is difficult to eliminate this chance variation, and this may have influenced results reported by various investigators in this review.

#### Effect of Vacuum Packaging on Color of Fresh Meat

The maintenance of bright red color in fresh meat requires a supply of oxygen to form oxymyoglobin at the meat surface. For this reason fresh meats are often packaged in tray-wrap type coverings with an oxygen permeable film for retail display. Since films are normally five times as permeable to carbon dioxide as they are to oxygen (Sutherland, et al., 1975) the inhibiting effect of carbon dioxide is lost when

low barrier film is used and the package is not evacuated. Use of a high barrier film preserves the inhibiting effect of carbon dioxide by retaining metabolic respiratory gases and yet causes problems in the area of color. This paradox does not prevent the use of vacuum packaging in wholesale cuts but so far has limited the use of vacuum packaging in retail cuts of meat.

#### Effect of Bacteria on Fresh Meat Color

Bacterial growth has been reported to have an effect on the color of fresh meats. Butler et al. (1953) showed that bacteria caused an increase in the rate of metmyoglobin formation in prepackaged retail beef cuts. This effect was greatest during the logarithmic growth phase. Robach and Costilow (1961) added cell suspensions of aerobic bacteria to steaks in an effort to determine the role of bacteria in the oxidation of myoglobin. They showed that visible changes in color were associated with the oxygen demand at the surface of the meat, including the respiration demand of any contaminating microorganisms. They concluded that the role of bacteria in meat discoloration was in the reduction of oxygen tension in the surface tissue, thus enhancing the formation of metmyoglobin.

### Effect of Light on Fresh Meat Color

Light is usually not a factor in fresh meat color. Kraft and Ayres (1954) showed that although germicidal ultraviolet light caused darkening of beef, the soft white fluorescent light used in display cases did not cause discoloration over a three day display period. It should be noted, however, that although light does not affect myoglobin, light has a strong effect on accelerating oxidative changes in fat provided that oxygen is available. Illumination of fats not only causes more rapid peroxidation during the period when the fat is exposed to light, but also increases the rate of peroxide formation after the light is removed (Watts, 1954).

### Oxidation of Pork Fat

Lipid represents one of the major components of pork. Furthermore, it has been shown that pork fat is more easily oxidized than fats from other red meat animals (Watts, 1954). The reason for this is that pork fat is much more unsaturated than fats from other animals: the linoleic acid content of the fat from beef varies between 1 and 2% of the total triglycerides, whereas with pork, linoleic acid ranges from 7 to 10% (Chang and Watts, 1952). Ordonez and Ledward

(1977) claimed that lipid oxidation in pork may be the limiting factor in the use of oxygen containing atmospheres for storage, rather than bacterial spoilage or metmyoglobin formation. In their experiments, rancidity became apparent in a few day's storage in air.

#### Gas Chromatography of Oils to Determine Volatiles

Dupuy et al. (1973) developed a method of measuring volatiles in oil by placing the oil on glass wool in the liner of the inlet of a gas chromatograph, thus allowing oil volatiles to be eluted through the liner and onto the chromatographic column. This procedure eliminated time consuming and tedious methods such as solvent extraction, steam distillation and vacuum distillation. In an extension of this work, Jackson and Giacherio (1977) used a similar method to determine the volatiles in soybean oil. In their method, the oil and glass wool are placed in a tube separate from the gas chromatograph and the volatiles are adsorbed onto a Porapak P column which is then connected to a gas chromatograph. They reported that the method shows good correlation with the flavor of soybean oil samples as determined by flavor panel scores. Porapak P is an amorphous polymer composed of ethyl vinyl benzene cross-linked with divinylbenzene to form a uniform cross-structure of a distinct pore

size. It is unique as a chromatographic column packing material in that there is no liquid phase or solid support used: the polymer beads serve the function of both the liquid phase and the solid support (McNair and Bonelli, 1969).

It can be seen from the preceding reports that vacuum packaging has complex effects on the product being packaged. These effects vary with each different product and the packaging method used. As new products and products previously not vacuum packaged are sold using this packaging method, each must be tested individually for quality, safety, and shelf life.

## MATERIALS AND METHODS

### Experimental Design

Five packaging or processing variables were evaluated for their effect on the keeping quality of vacuum packaged pork chops. These variables were: presence of bone in the chop, oxygen barrier characteristics of the packaging film, storage time of loins prior to cutting, method of cutting the loins into chops, and chop storage time prior to display.

Two studies were performed. The first study was a three factorial design in which the variables of presence of bone, type of packaging film, and length of storage time of loins on the keeping quality of pork chops were examined. The second study was a two factorial experiment which tested the effect of cutting method and chop storage time prior to retail display. With this design the interactions of the different variables in each study can be assessed. Each study was replicated three times.

### Preparation of Samples

In the first study, boxed loins were obtained from a commercial meat packing plant (Wilson Foods Corporation, Des Moines, Iowa) and were held in a meat cooler at 5°C until cut into chops. In the first replication of the first



study, the loins were held in the cooler 0, 3, 7, and 10 days prior to cutting. In the second and third replications of the first study, loins were held for 0 and 7 days prior to cutting since the other storage times were not considered to be necessary and made a very complex design. The 3 and 10 day holding periods were therefore eliminated from the last two replications. In the case of the 10 day holding period, the spoilage of chops was too rapid for the chops to be of any practical value. The 3 day holding period did not show enough change when compared to the 0 and 7 day holding periods to justify performing that particular segment of the study.

After the loin holding period, the loins were divided into two groups. In one group, the bone was removed from the loin. In the other group the bone was left attached. The loins were then cut by saw (A. E. W. Engineering Co., Norwich, England, Model MK3), into chops 1 inch thick. The chops were further divided into two groups. One half of the chops were packaged using high vacuum (29 mm Hg) on a Bivac #1 machine (American Can Company, Neenah, Wisconsin) with a high barrier film. The other half were packaged using high vacuum on a Koch Multivac machine (Model Ag-800) with a low barrier film. In addition, for the first replication chops from each processing treatment were tray packaged in air with a stretch wrap film. This packaging treatment was

discontinued for the second and third replications of the first study because of the very quick spoilage (less than 3 days) of these chops. Films in both studies were provided by the American Can Company, Neenah, Wisconsin. Table 1 shows the oxygen permeability of the films used.

Table 1. Oxygen permeability of the films used

Film	Oxygen permeability (cc/M <sup>2</sup> /24 hrs at 73°F, 0% R.H.)
High Barrier (HB)	18
Low Barrier (LB)	2000
Stretch Wrap (SW)	6500

Vacuum-packaged samples were checked closely for package integrity prior to storage and leakers were eliminated from the study. Samples were stored in a refrigerated display case at 5°C ± 1°C under constant fluorescent illumination of about 200 foot candles to simulate supermarket display conditions. Samples were taken at 0, 3, 7, 14, and 21 days of display for microbiological analyses, color, odor, drip loss, pH, and sensory evaluations. Figure 1 illustrates the procedures used in the first study.

In the second study, boxed loins were obtained from the same commercial meat packing plant. These loins were

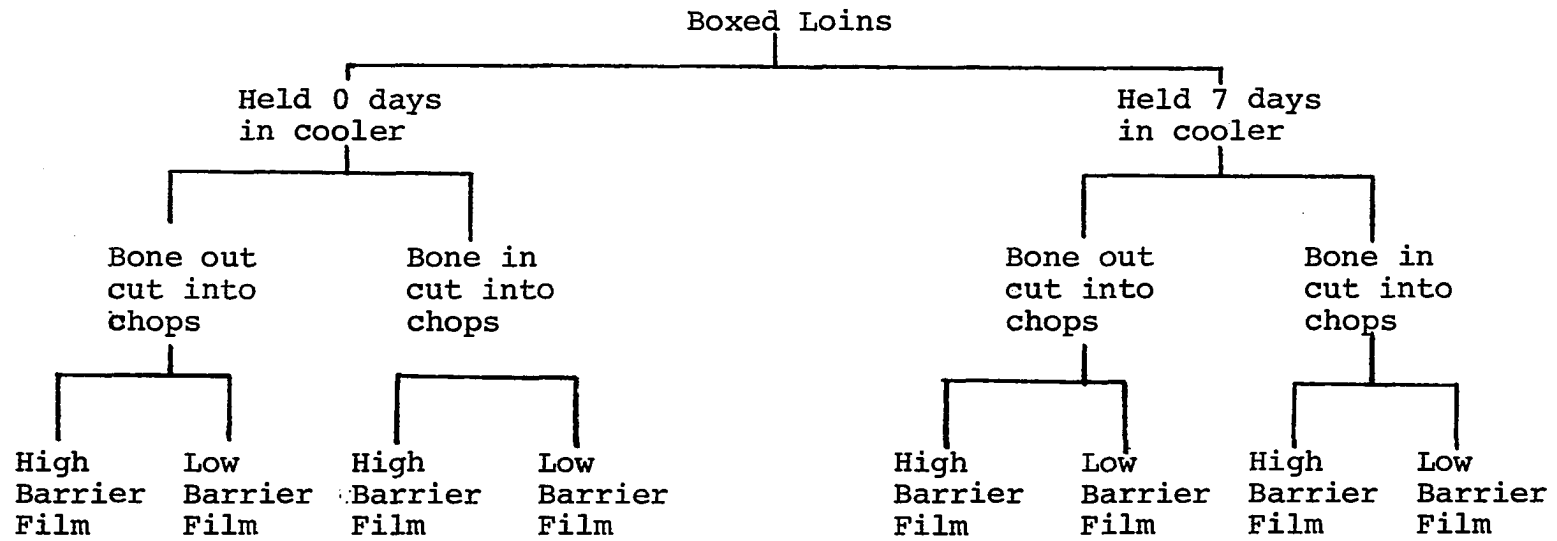


Figure 1. Flow chart describing the first study (3 replications)

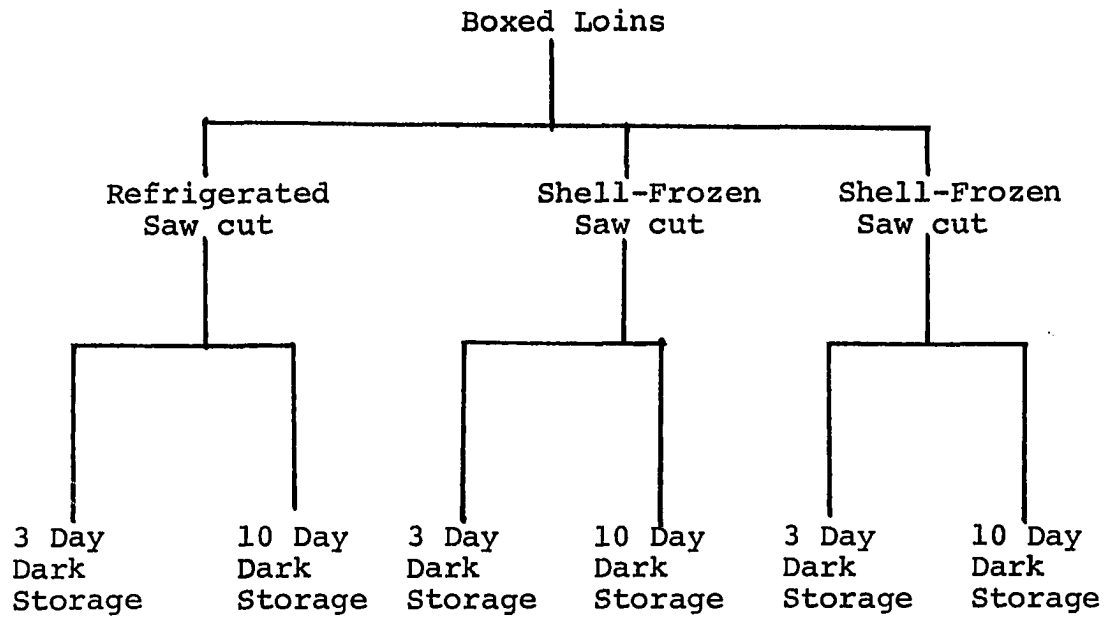


Figure 2. Flow chart describing the second study (3 replications)

divided into three groups. One group of loins was kept in a meat cooler at 5°C for 3 days prior to cutting with a saw into 1 inch chops. The other two groups were placed on wire racks and shell-frozen in a -30°F blast freezer for 1 hour. After this treatment, which froze the outer 1/2 inch of the loin, but left the center unfrozen, they were kept in a meat cooler at 5°C for 3 days prior to cutting with one of two methods. One group of shell-frozen loins was cut into 1 inch chops with a saw. The other group of shell-frozen loins was cut into chops using a power cleaver (Bettcher Industries, Inc., Birmingham, Ohio, Model 39). After cutting, all chops were packaged on a Bivac #1 machine using high vacuum with a high barrier film.

After packaging, the chops were divided into two groups and stored for different time periods in the dark at 5°C. One group was stored for 3 days in the dark and then placed in a refrigerated display case at 5°C under constant fluorescent illumination to simulate supermarket conditions. The other group was stored in the dark for 10 days before being placed in the display case. Samples were taken at the time of packaging and at 0, 3, 7, 14, and 17 days of display storage. Figure 2 illustrates the procedures used in the second study. All procedures were modeled after conditions

that might be used in commercial practice.

#### Measurement of Color

All color determinations were made with the packaging film intact prior to opening the sample for other analyses. Color was measured using objective and subjective methods for both lean and fat. Lean color measurements were taken at the center of the chop. Subjective color measurements of the lean portion were made using a Pork Standards Color Chart (Rust and Topel, 1969). This chart rated the color of the meat on a scale of 1 (light) to 5 (dark). Objective color measurements were made using a Color Reflectance Meter (Photovolt, Inc., New York, New York, Model 670). This instrument was equipped with three glass color filters (amber, green, and blue) to determine reflectance of light of a certain wavelength from a surface. The instrument was calibrated by placing the search unit with the appropriate glass filter on a white enamel plate and adjusting the unit to a predetermined value. The search unit was then placed on the meat sample and the reflectance measured directly. The value represents the reflection from the meat as a percentage of the reflectance from the white enamel plate. Color values can be converted to CIE tristimulus values, chromaticity coordinates or to a yellowness

scale. Yellowness is calculated according to the following formula:  $\text{yellowness} = \frac{\text{amber value} - \text{blue value}}{\text{green value}}$ , negative values for yellowness indicate a bluish white while positive values indicate yellowness (Hunter, 1942).

Fat color was determined at a point on the edge of the chop where the chop was covered with fat. Fat was judged subjectively on a scale of 1 (pearly white) to 5 (yellow) and also by the use of the Color Reflectance Meter.

#### Measurement of Odor

Odor was determined subjectively by sniffing the meat and the packaging material immediately after opening the package. In the second study, an additional odor determination was made 15 minutes after opening the package and separating the meat from the packaging material. Odor was rated on a scale of 1 (no off odor) to 5 (high off odor, almost putrid).

#### Measurement of Microbial Growth

For microbiological samples, the vacuum package was opened aseptically by cutting the packaging film around the edge of the chop with a scalpel until the film could be lifted off the chop without contaminating the top surface. An area

Figure 3. Cutting frozen tempered loin into chops with a power cleaver. Loin is loaded into power cleaver

Figure 4. Cutting frozen tempered loin into chops with a power cleaver. Cut chops are dropping onto a revolving table below the cleaver blade



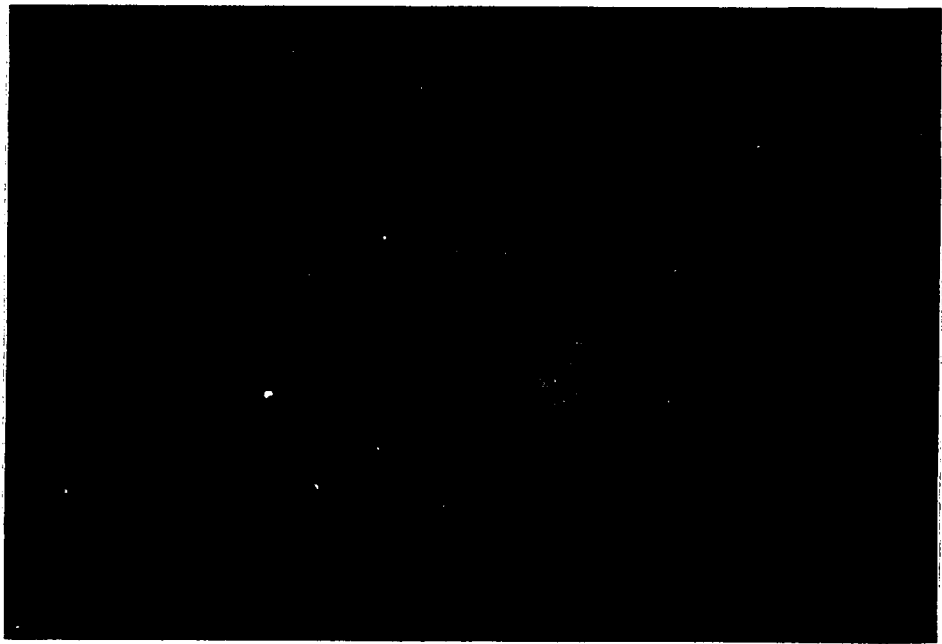
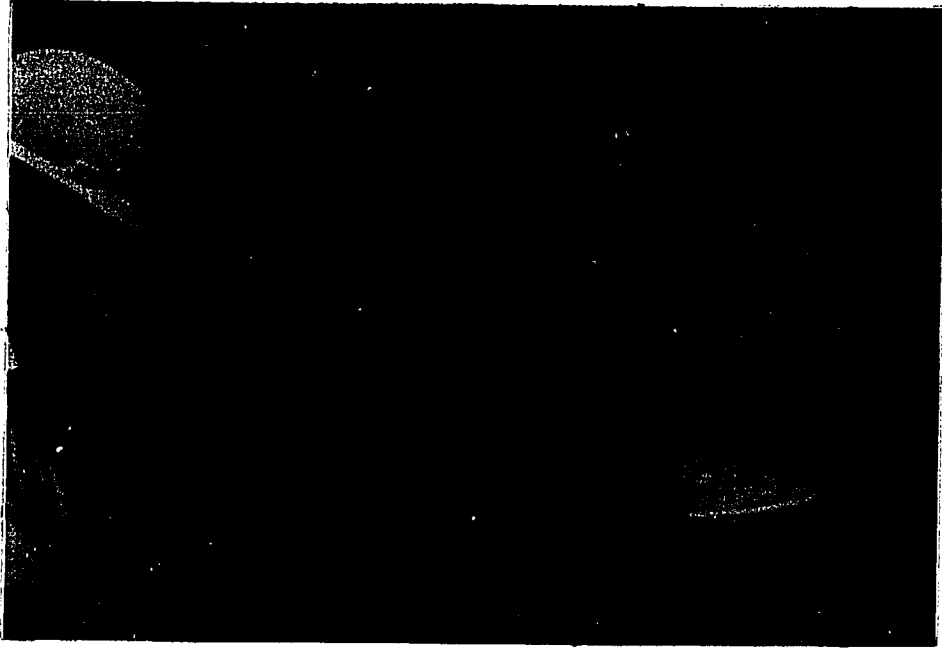
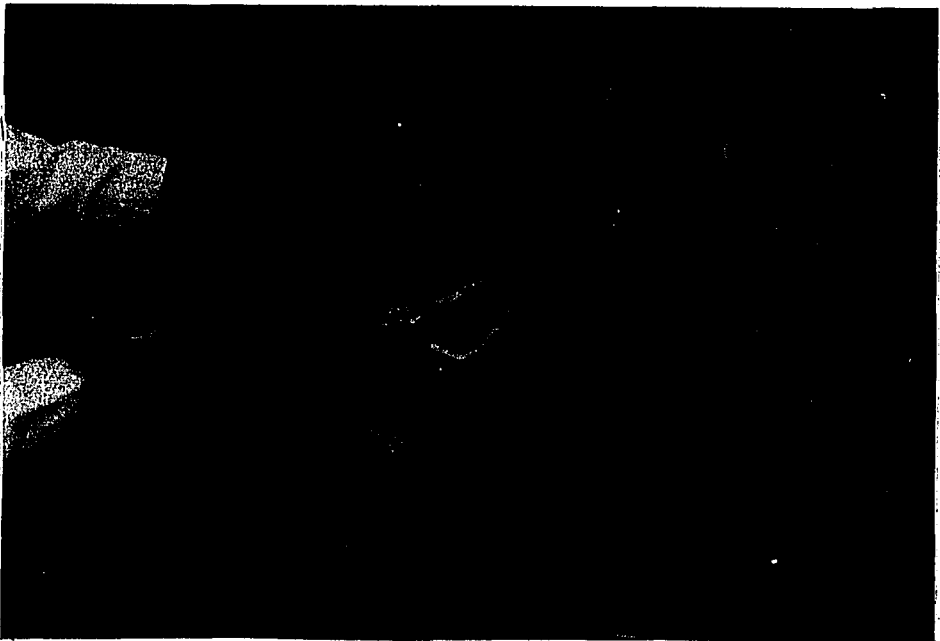


Figure 5. Vacuum packaging pork chops with low barrier film on the Koch Multivac

Figure 6. Vacuum packaging pork chops with high barrier film on the Bivac 1 machine



of 2 cm<sup>2</sup> in the center lean portion of the chop was sampled with a sterile swab using a sterile aluminum template (Ayres et al., 1959). This swab was then broken off into a 99 ml. sterile distilled water dilution blank which was shaken until the swab was disrupted. Ten-fold serial dilutions of the initial dilution were then made using 9 ml. distilled water blanks. Duplicate 1 ml. portions of the dilutions were flooded on the surface of previously dried agar plates and spread evenly over the surface by rocking the plates back and forth (Hentges, 1962), except in the case of dilutions for the culture of Clostridium perfringens where the pouch method was used (Bladel and Greenberg, 1965). Surface (flood) plating was performed to avoid disadvantages of conventional pour plating and for convenience.

Sampling for Salmonella was performed by swabbing the entire top surface of the chop with a sterile swab after the chop was sampled for other bacteria. The swab was then broken into a tube of lactose broth for incubation.

Bacteriological procedures for the enumeration or isolation of microorganisms are summarized in Table 2.

Table 2. Bacteriological procedures employed in enumerating organisms or incidence of organisms

Types of microorganisms	Growth media	Plating technique	Incubation time	Confirmatory tests
Mesophiles	Plate count agar (Difco)	Flood plate (Hentges, 1962)	30°-24/36 hours	
Psychrotrophs	Plate count agar (Difco)	Flood plate (Hentges, 1962)	5°C/10 days	
<u>Lactobacilli</u>	LBS agar (BBL)	Flood plate with overlay	30°C/5 days	
<u>Staphylococcus</u>	Tellurite glycine agar with egg yolk	Flood plate (Hentges, 1962)	37°C/24-48 hrs	Tube coagulase test
<u>Salmonella</u>	Procedures for meats (Galton <u>et al.</u> , 1968) using BGS (BBL), SS (Difco), Tetrathionate broth (Difco), and Selenite Cystine broth (Difco)			TSI agar (Difco) and LIA agar (Difco), Slide agglutination test (Difco) (Galton <u>et al.</u> , 1968)
<u>Clostridium perfringens</u>	SPS agar (BBL)	Anaerobic pouch (Bladel and Greenberg, 1965)		

### Measurement of Drip Loss

Drip loss was calculated according to the following formula:

$$\% \text{ Drip loss} = \frac{\text{Chop weight at time of packaging} - \text{Chop weight at time of sampling}}{\text{Chop weight at time of packaging}} \times 100$$

### Measurement of pH

pH of the meat was determined using a pH meter (Beckman Instruments Company, Fullerton, California, Model SS-3) with a combination electrode (Markson Scientific Company, Del Mar, California, Model Polymark 1888). The method described by Lees (1975), in which the electrode is pushed into the meat to insure contact and the reading taken, was used.

### Sensory Analysis

Pork samples were judged by an eight member trained sensory panel. Almost all members of the panel had served on meat taste panels previously. Three or six samples were evaluated at each session. Judges sat in individual booths designed for organoleptic testing. Each booth had red fluorescent lights to mask any color differences that might be present. Room temperature tap water was provided for each judge.

Chops for sensory analysis were packaged at the same time and in the same manner as chops for other analyses. Each package for sensory analysis contained two chops. At each sampling day, the packages were opened and the chops broiled on an electric broiler (Wells Manufacturing Corporation, San Francisco, California, Model B 506) to an internal temperature of 70°C. The center portion of the chop was removed and cut into pieces approximately 1 inch square which were presented to the judges in pre-warmed aluminum weighing pans. Each chop was evaluated for flavor, tenderness, juiciness, and overall acceptability according to an 8 point hedonic scale with 8 being the most desirable and 1 being the least desirable score. An example of the score sheet used for taste panel evaluation is shown in Figure 7. For some sampling days at the end of a series within a replication, sensory analysis was eliminated because of obvious spoilage of the meat.

#### Statistical Analysis

Data obtained were analyzed by analysis of variance using the Statistical Analysis System (SAS Institute, Inc., Raleigh, North Carolina). Methods were explained by Snedecor and Cochran (1967).

Figure 7. Score sheet for evaluation of flavor, tenderness, juiciness, and overall acceptability of pork chops



SENSORY EVALUATION OF PORK CHOPS

Judge \_\_\_\_\_

Date \_\_\_\_\_

FLAVOR		TENDERNESS		JUICINESS		OVERALL	
Extremely Desirable	8	Extremely Tender	8	Extremely Juicy	8	Extremely Desirable	8
Very Desirable	7	Very Tender	7	Very Juicy	7	Very Desirable	7
Moderately Desirable	6	Moderately Tender	6	Moderately Juicy	6	Moderately Desirable	6
Slightly Desirable	5	Slightly Tender	5	Slightly Juicy	5	Slightly Desirable	5
Slightly Undesirable	4	Slightly Tough	4	Slightly Dry	4	Slightly Undesirable	4
Moderately Undesirable	3	Moderately Tough	3	Moderately Dry	3	Moderately Undesirable	3
Very Undesirable	2	Very Tough	2	Very Dry	2	Very Undesirable	2
Extremely Undesirable	1	Extremely Tough	1	Extremely Dry	1	Extremely Undesirable	1

SAMPLE	FLAVOR SCORE	TENDERNESS SCORE	JUICINESS SCORE	OVERALL SCORE
1				
2				
3				
4				
5				
6				

Comments:

## Gas Chromatography

### Preparation of samples

All samples for gas chromatographic analysis were prepared at the same time. Pork chops from the second study were repackaged under low vacuum after other analyses were performed and stored at  $-29^{\circ}\text{F}$  for four weeks. At the time of sample preparation, the package was opened and 25 grams of fat and lean in approximately equal proportions were cut from the chop. Twenty-five milliliters of distilled water were added to the sample in a blender jar. This mixture was blended at high speed in an Osterizer blender (Oster Corporation, Milwaukee, Wisconsin) for two minutes. The resulting emulsion was placed into a plastic centrifuge tube and tempered to  $40^{\circ}\text{C}$  for 15 minutes. This step was found to be necessary in order to facilitate separating the lipid portion of the emulsion from the water layer. The tube was placed in a centrifuge (Ivan Sorvall, Inc., Norwalk, Connecticut, Model SS-L) and centrifuged at 17,000 RPM for 30 minutes. After centrifugation, the emulsion had broken into three layers: a solid layer at the bottom of the tube, an aqueous layer in the middle, and a clear fat layer at the top. The fat layer was drawn off using a disposable pasteur pipette and placed in a 5 ml. vial under nitrogen for storage in the cooler at  $5^{\circ}\text{C}$  until needed for analysis.

The procedure for gas chromatographic analysis which follows is essentially that of Jackson and Giacherio (1977). Some minor changes were made in the procedure, however.

#### Gas chromatograph

A gas chromatograph (Varian, Inc., Palo Alto, California, Model 2440-10), equipped with a linear temperature programmer and flame ionization detector, was used. A Varian Model A-25 recorder was attached to the chromatograph.

#### Preparation of chromatographic column

Five feet of 1/8 inch stainless steel tubing was rinsed with acetone. One end of the tubing was plugged with glass wool and the tube was packed with Porapak P (Waters Associates, Inc., Milford, Massachusetts), prior to plugging the other end with glass wool. The entire tube was then bent into a spiral approximately 5 inches in diameter. Stainless steel Swagelok ferrules were attached to both ends of the column. The column was then conditioned overnight at 235°C with a constant flow of nitrogen at the rate of 30 ml/minute.

#### Preparation of internal standard

10  $\mu$ l of N-nonane (Fisher Scientific Company, Fairlawn, New Jersey) were added to 200 ml of fresh soybean oil.

#### Preparation of silanized glass wool

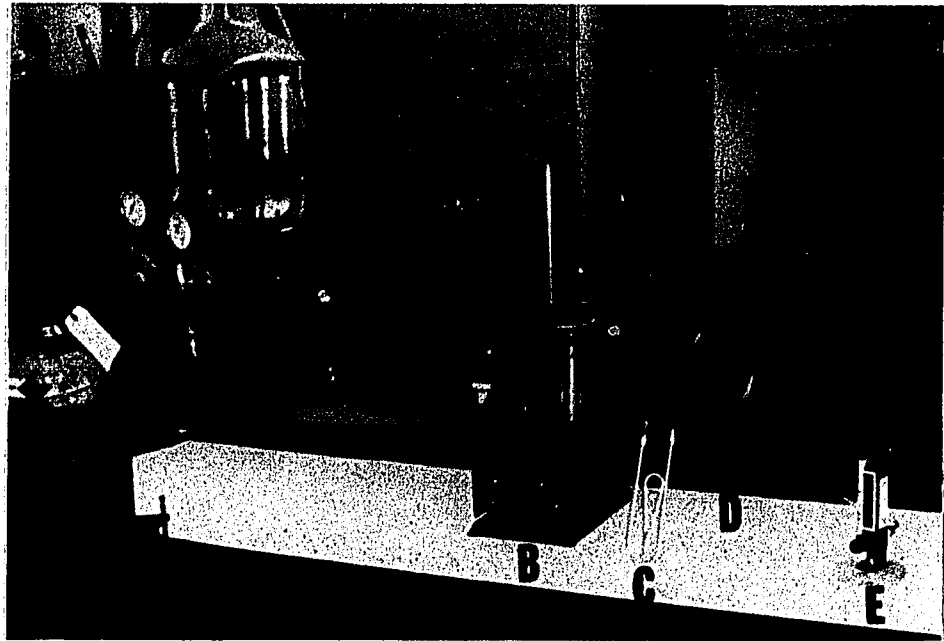
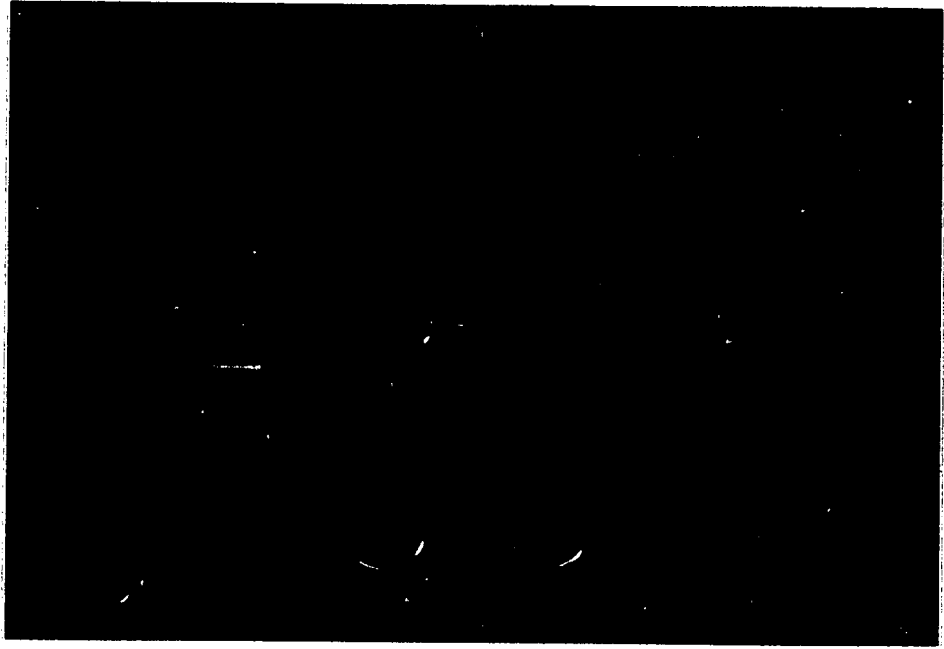
6.0-6.5 grams of glass wool were soaked overnight in a mixture of 40 ml pesticide grade hexane (Fisher Scientific Company, Fairlawn, New Jersey), 20 ml pyridine (J. T. Baker Chemical Company, Phillipsburg, New Jersey), 2 ml chlorotrimethylsilane (Eastman Kodak Company, Rochester, New York), and 4 ml hexamethyldisiloxane (Eastman Kodak Company, Rochester, New York). The next day the glass wool was rinsed in distilled water until the pyridine was removed and soaked in pesticide grade hexane for 4 hours. The glass wool was then squeezed to remove excess hexane and dried overnight at 100°C. After drying, the glass wool was stored at room temperature in a clean, airtight jar until used.

#### Preparation of extracting tubes

One-fourth inch O.D. soft aluminum tubing (Vincent Brass and Aluminum Company, Cedar Rapids, Iowa) was rinsed with acetone and cut into 2 foot lengths. The tubing was bent with a tube bender at the middle so that it was in a "U" configuration. One end of the tube was marked and the tip partially reamed out to facilitate stuffing with .6 to .7 grams of silanized glass wool. Both arms of the tube were then bent so that a "W" shape was formed. One quarter inch reducing Swagelok ferrules were attached to the ends of the tubing. The tube was conditioned by placing it in

Figure 8. Preparation of aluminum extracting tube. (A) Shape of the tube when stuffed with silanized glass wool; (B) Shape of the tube after second bending; (C) Finished tube with Swagelok ferrules attached, ready for sample injection

Figure 9. Apparatus for extracting volatiles. (A) High purity nitrogen gas; (B) Oil bath for heating aluminum extracting tubes; (C) Aluminum extracting tube; (D) Chromatographic column packed with Porapak P.E. Flowmeter



an oven at 170°C for at least 4 hours and allowing it to cool prior to use.

#### Purging the sample of volatiles

Three-tenths ml of the fat sample, previously warmed in a water bath to 40°C to liquify it, was injected into the end of the aluminum tube which contained the silanized glass wool. At the same time, 30  $\mu$ l of the n-nonane internal standard were also injected onto the silanized glass wool. The end of the tube containing the glass wool and sample was attached to a one-eighth inch copper tube from a nitrogen tank. The exit end of the aluminum tube was attached to the inlet end of the chromatographic column. A flowmeter (Airco, Inc., Murray Hill, New Jersey, Model 6-2130-1) was attached to the exit end of the column.

The extraction tube-chromatographic column-flowmeter assembly was allowed to purge with nitrogen (flow rate: 40 ml/min.) at room temperature for five minutes. During this time, all joints were checked for leaks. After purging, the extraction tube was lowered into an oil bath held at 170°C and allowed to purge at that temperature for 20 minutes. The gas chromatographic column was then disconnected from the extraction tube and flowmeter and attached to the gas chromatograph. Table 3 shows the

Table 3. Gas chromatograph operating conditions

Operating condition	Value
Carrier (N) flow rate	30 ml/min
Air flow rate	350 ml/min
Hydrogen flow rate	25 ml/min
Injector temperature control	Off
Detector temperature	265-275°C
Oven temperature	Programmed from 60°C to 235°C
Temperature advance rate	6°C/min.
Attenuation	4-8 X 10 <sup>-10</sup> amps/mv
Recorder speed	$\frac{1}{2}$ inch/min

conditions under which the chromatograph was run. After reaching the programmed end temperature of 235°C, the column was allowed to purge for 30 minutes to remove higher boiling components which could interfere with subsequent analyses.

#### Analysis of chromatographic data

A rising base line beginning at 205°C, prevented peaks eluting past 215°C from being included in the analysis of data. The area under each peak was calculated according to the following formula (McNair and Bonelli, 1969):



Peak area = Height X base at  $\frac{1}{2}$  height

Total volatiles relative to nonane (TVRN) were calculated by adding all the peak areas and dividing the sum into the peak area of N-nonane. This resulted in reducing the effect of differences in sample size so that different sample analyses could be compared.

### Identification of Bacteria

#### Isolation of pure cultures

The qualitative as well as the quantitative aspects of the development of the microflora were followed by the isolation and identification of pure cultures. After counting the number of colonies on a particular mesophilic or psychrotrophic plate, each type of colony that could be visually discriminated from others on the same plate were isolated on tubes of Plate Count Agar (Difco). The tubes were incubated at room temperature for 48 hours and were then stored in the cooler at 5°C until identification could take place. Total length of storage was from 1 to 21 months with transfer of organisms at 6 month intervals. In total for the two studies, 517 colonies were isolated from mesophilic plates and 321 colonies from psychrotrophic plates.

It should be noted here that these colonies were picked from the plates which were counted. In the case of samples

where the microbial flora had increased to a high population, therefore, these colonies represented only those bacterial types that formed a significant percentage of the population. It is possible that bacteria that were present on the chops in low numbers were diluted out and not chosen for isolation and identification, except in the case of fresh chops where a low total number of bacteria were present and so a low dilution was used when enumerating the bacterial population on the chop.

#### Identification of isolates

Cultures from Plate Count Agar plates not more than 24 hours old were used for characterization of isolates. Techniques used for the characterization of isolates are summarized in Table 4. Gram positive cocci were identified according to tests summarized in Table 5, Gram positive rods by tests summarized in Table 6, and Gram negative rods by tests summarized in Table 7. The schemes for identification of organisms were derived from Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). The procedures used in following the identification scheme are, for the most part, described in the Manual of Clinical Microbiology (Lennette et al., 1974). A reference culture of Salmonella st. paul from the Food Technology Laboratory,

Table 4. Tests used in the classification of isolates

Test	Technique
Gram stain, cell morphology	Hucker modification
Metabolism of glucose, lactose, and sucrose	TSI agar slant
Inhibition of Gram negative organisms and metabolism of lactose	MacConkey agar plate
Motility	Needle inoculation of motility test medium
Oxidase test	Kovacs method using N-N tetra-methyl-p-phenylenediamine dihydrochloride with platinum loop
Gelatinase formation	Gelatin stab
Sensitivity to penicillin	Paper disc method
H <sub>2</sub> S production	TSI agar slant
Catalase production	Slide catalase test
Coagulase test	Tube coagulase test
Citrate utilization	Stab and streak of Simmons Citrate agar, incubated for 48 hrs
Lysine decarboxylase	LSI agar
Methyl red	Methyl red added to glucose-peptone broth
Phenylalanine deaminase	Phenylalanine agar
Growth on mannitol salt agar	Streak plate
Oxygen utilization	Thioglycolate medium with resazurin
Colonial morphology	Growth on Plate Count Agar

Table 5. Tests for gram positive cocci

Characteristic	Micrococcus	Staphylococcus	Aerococcus	Streptococcus	Leuconostoc	Pediococcus
Spherical morphology	+	+	+	+	+	+
					or lenticular	
Cell arrangement	Singly, pairs, irregular clusters, tetrads, or cubical packets	Singly, pairs, irregular clusters	Singly on solid medium	Pairs or chains in liquid medium, not on solid media	Pairs of chains	Tetrads on solid media
Colonial morphology	Yellow or red pigmented colonies	Yellow or white colonies	Small colonies, growth sparse and beaded	Small, round	Smooth, around greyish white	Scant beaded growth
Catalase	+	+	-	-	-	-
Growth in mannitol salt agar (7.5% NaCl)	<u>+</u>	+	+	-	<u>+</u>	N.A.
Oxygen utilization	Aerobic	Facultatively anaerobic	Micro-aerophilic	Facultatively anaerobic	Facultatively anaerobic	Micro-aerophilic

Table 6. Tests for identifying gram positive rods

Characteristic	Bacillus	Clostridium	Lactobacillus	Corynebacterium	Arthrobacter	Microbacterium
Cell morphology	Rods	Rods	Rods: long, slender, short coccibacilli	Straight, slightly curved rods; club-shaped swellings	Short rods, curved or club-shaped; older cells colloid	Small diphtheroid rods, rounded rods
Cell arrangement	Singly	Singly	Chains	Angular, palisade arrangement	V formations, coccoid cells in older colonies	Angular, palisade arrangement
Endospores	+	+	-	-	-	-
Oxygen utilization	Aerobic or facultatively anaerobic	Anaerobic	Aerobic or facultatively anaerobic	Aerobic or facultatively anaerobic	Aerobic	Aerobic or facultatively anaerobic
Catalase	+	-	-	+	+	+
Motility	+	+	-	-	<u>+</u>	-

Table 7. Tests for oxidase negative gram negative rods

Characteristic	Pseudo- monas	Escheri- chia	Edward- siella	Citro- bacter	Salmo- nella	Shig- ella
Cell morphology- rods	<u>+</u>	+	+	+	+	+
Strictly aerobic	+	-	-	-	-	-
Characteristic feature						
H <sub>2</sub> S production	<u>+</u>	-	+	<u>+</u>	+	-
Metabolism of lactose	-	<u>+</u>	-	<u>+</u>	<u>+</u>	<u>+</u>
Metabolism of sucrose	-	<u>+</u>	-	<u>+</u>	-	<u>+</u>
Metabolism of glucose	-	+	+	+	+	+
Cytochrome oxidase	<u>+</u>	-	-	-	-	-
Methyl red test	N.A. <sup>a</sup>	+	+	+	+	+
Citrate utilization	N.A.	-	-	+	+	-
Phenylalanine deamination	N.A.	-	-	-	-	-
Lysine decarboxylate	N.A.	+	+	-	+	-
Gelatine hydrolysis	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

<sup>a</sup>N.A. = not applicable.

Klebsiella	Enterobacter	Hafnia	Serratia	Proteus	Yersina	Acinetobacter
+	+	+	+	+	+	Coccobacilli, pairs and chains, occasional filaments
-	-	-	-	-	-	+
Glistening colonies of varying degrees of stickiness			Red or magenta pigment			
-	-	-	-	<u>+</u>	<u>+</u>	-
<u>+</u>	+	-	-	-	-	-
+	+	<u>+</u>	+	<u>+</u>	<u>+</u>	-
+	+	+	+	+	+	-
-	-	-	-	-	-	-
<u>+</u>	-	-	-	+	+	N.A.
<u>+</u>	+	+	+	N.A.	-	N.A.
-	-	-	-	+	-	N.A.
<u>+</u>	<u>+</u>	+	+	<u>+</u>	-	N.A.
N.A.	N.A.	-	+	N.A.	N.A.	N.A.

Table 8. Tests for oxidase positive gram negative rods

Characteristic	Moraxella	Pseudomonas	Aeromonas	Flavobacterium	Alcaligenes
Cell morphology					
Strictly aerobic	+	+	-	-	+
Characteristic feature				Pigmented colonies; red, orange or yellow	
H <sub>2</sub> S production		<u>+</u>		<u>+</u>	Trace on slant
Metabolism of lactose	-	-	+	<u>+</u>	-
Metabolism of sucrose	-	-	<u>+</u>	<u>+</u>	-
Metabolism of glucose	-	-	+	<u>+</u>	-
Cytochrome oxidase	+	<u>+</u>	+	+	+
Penicillin sensitivity (2.5-5.0 I.U./ml)	S	R	R	R	R
Motility	-	+	<u>+</u>	<u>+</u>	+



Iowa State University, was used as a control in the identification of Salmonella isolates.

RESULTS AND DISCUSSION  
Microbiological Quality

Effect of bone on bacterial growth

Figure 10 shows the relationship between presence of bone and growth of mesophiles, psychrotrophs, and Lactobacillus. Deboning reduced the growth of mesophiles slightly when compared to chops with the bone intact. With regard to psychrotrophic growth, the lack of bone in the chop produced a slight increase in lag time as evidenced by lower counts for the deboned chops at the start of the experiment. However, by the third day of storage the psychrotrophic population in the deboned chop was equal to or greater than the psychrotrophic count in the chops with bone left in. Lactobacillus counts show a similar situation in that growth of lactobacilli by the third day of storage in the deboned chops is equal to that of the chops with the bone in. None of the differences in population in either the mesophilic, psychrotrophic, or Lactobacillus counts were statistically significant between the bone in or boneless chops. This is a curious result in light of odor scores which will be discussed in detail later. Odor scores for the chops with the bone in were significantly higher, especially after 14 and 21 days of storage, than the chops with the bone removed. One would expect that the higher odor scores would

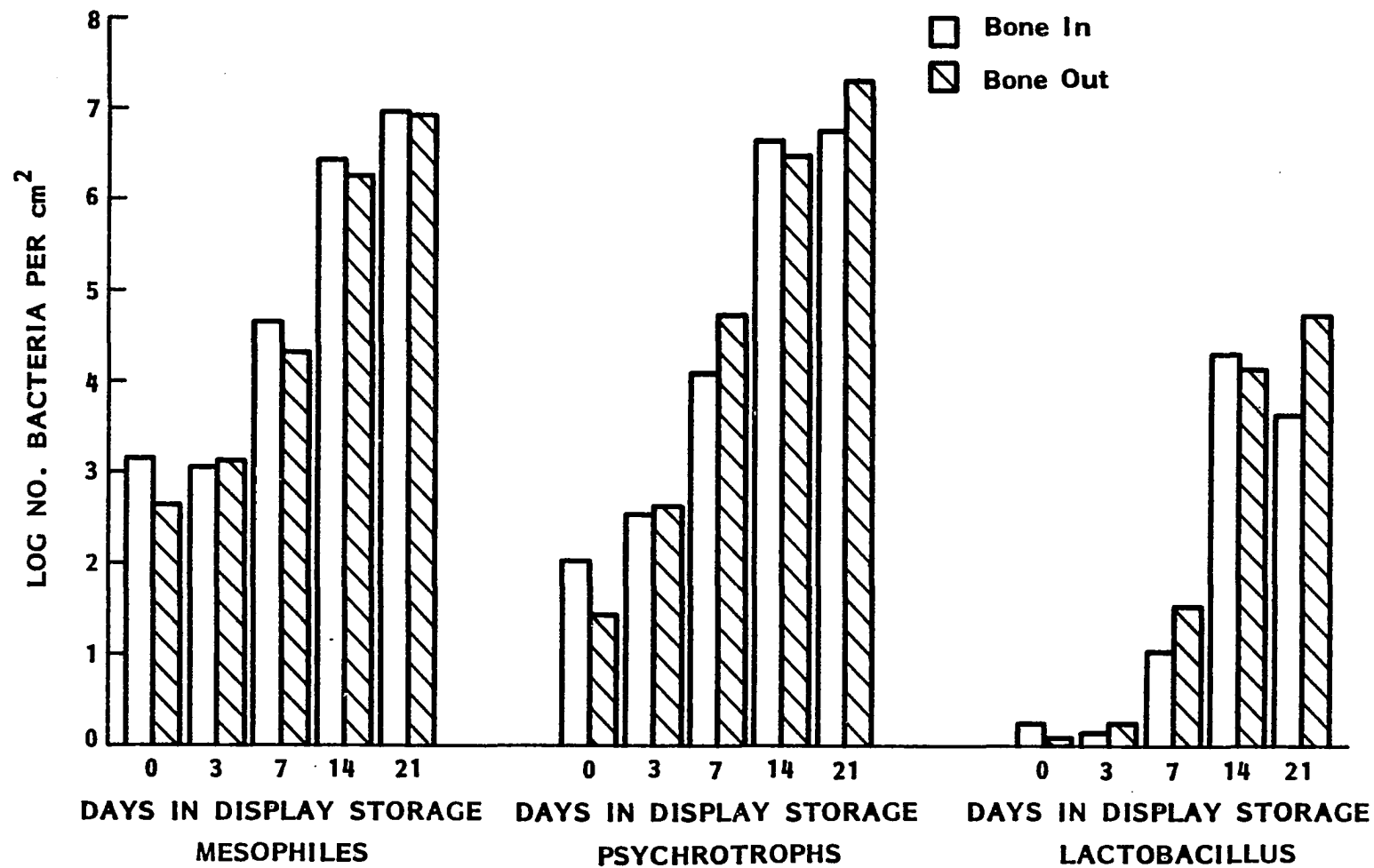


Figure 10. Effect of bone on growth of mesophiles, psychrotrophs, and Lactobacillus

be reflected by the bacterial counts on the meat.

It is possible that the reason for higher odor in the bone in chops may be found in Table 9, which represents isolations from plate count agar plates from bone in and deboned chops. In this table, the differences in frequency between certain bacterial types may be less important than the total number of isolates from each type of chop. The total number of isolates from chops with the bone in is almost four times that isolated from deboned chops, even though the number of chops is equal. This indicates that the diversity of the bacterial flora on the bone intact chops was greater than that on the deboned chops. This may be due to the fact that the presence of the bone in the vacuum package allowed pockets of air or moisture to be retained more often than in the deboned chops, which provide a more snug fit of the packaging film on the meat. The bone itself may contribute to the inoculum diversity as well as allowing a more diverse ecologic system to develop within the package by adding nutrients not found in the muscle or fat tissue. At any rate, no firm connection can be drawn between odor, bacterial population, and presence of bone, but a possible explanation is that a combination of bacterial types is necessary for increased odor production.

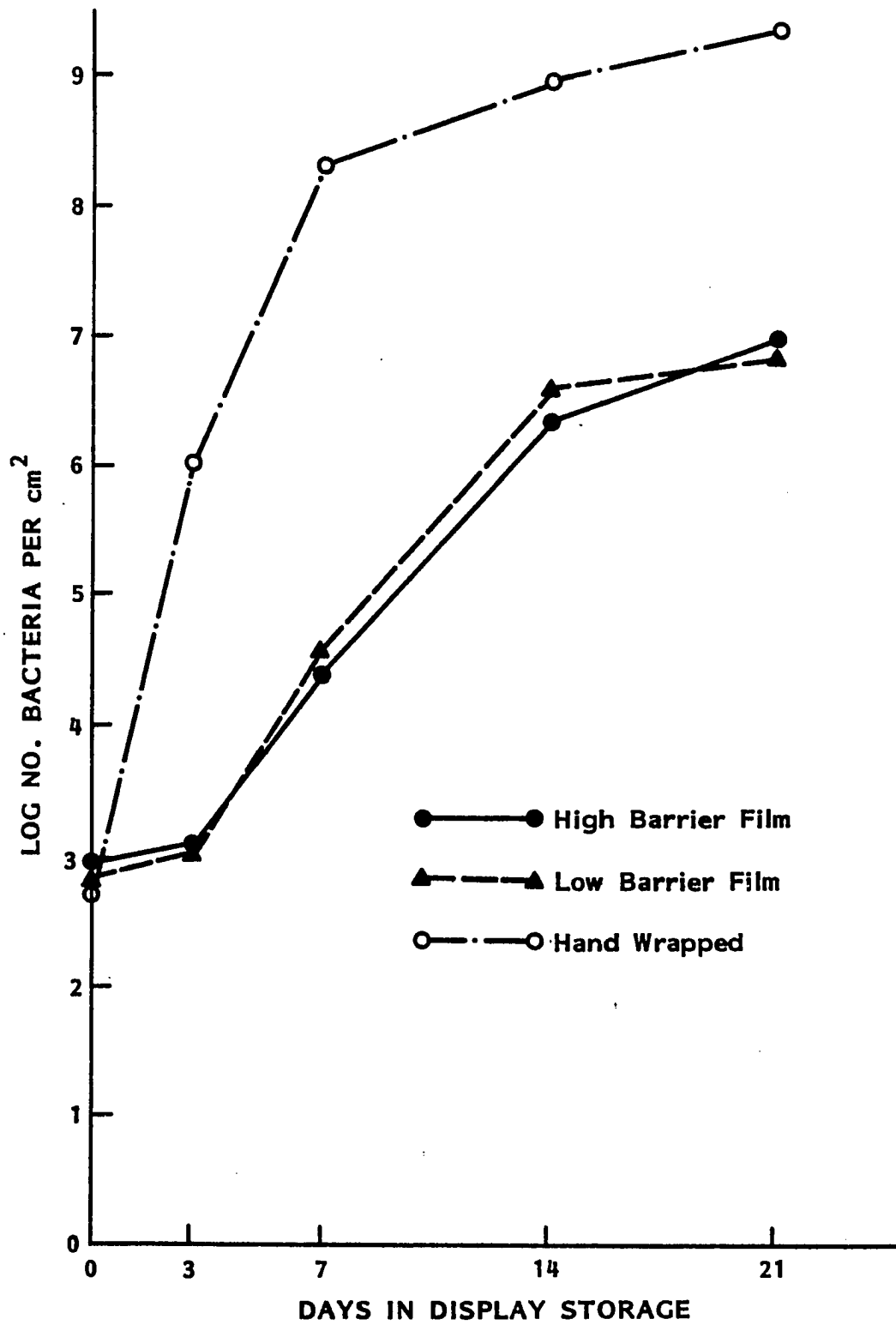


Figure 11. Effect of film on the growth of mesophiles

Table 9. Numbers and percent frequency of organisms isolated from bone in and deboned vacuum packaged chops after 14 and 21 days of display case storage at 5°C

Organism	Bone In		Bone Out	
	Frequency	Percent	Frequency	Percent
<u>Aerococcus</u>	1	0.81	-	-
<u>Aeromonas</u>	11	8.94	1	3.03
<u>Enterobacteriaceae</u> <sup>a</sup>	53	43.09	18	54.54
<u>Flavobacterium</u>	1	0.81	-	-
<u>Lactobacillus</u>	41	33.30	8	24.24
<u>Pseudomonas</u>	11	8.94	4	12.12
<u>Staphylococcus</u>	-	-	1	3.03
Unidentified	4	3.25	1	3.03
Yeast	1	0.81	-	-
TOTAL	123		33	

<sup>1</sup>Includes bacteria classified as Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, and Yersinia.

#### Effect of Packaging Film on Bacterial Growth

Figure 11 represents the effect of the packaging film used to wrap the pork on the growth of mesophilic bacteria on the pork chops. The hand-wrapped pork chops reached a population of bacteria indicating spoilage (Log 6.03 viable

bacteria per  $\text{cm}^2$ ) in 3 days, while chops in the two vacuum packaged films required two weeks to reach the same population. The differences in mesophilic bacterial count between the chops packaged with the two different films used for vacuum packaging are not statistically significant. Apparently, the oxygen permeability of both films was sufficiently low and the integrity of the vacuum pack was maintained well enough to inhibit the growth of aerobic organisms.

Differences in bacterial types isolated from chops packaged in different films are apparent in Table 10. Of the bacteria isolated from the hand-wrapped chops, after two weeks or more of storage at  $5^\circ\text{C}$ , 64.7 percent were Pseudomonas. These aerobic, psychrotrophic organisms far out grew other competing types of bacteria. There is also some indication that the low barrier film did admit more oxygen to the surface of the chops than did the high barrier film. Pseudomonas made up 12.50 percent of the isolates after two weeks or more storage from the chops packaged in the low barrier film but only 5.00 percent of the isolates from the high barrier film. In the high barrier film, facultative anaerobic members of the family Enterobacteriaceae and Lactobacillus formed the majority of the isolates with 56.25 percent and 28.75 percent, respectively. In the low barrier film the percentage of lactobacilli (34.04%) was higher than in the high barrier film (28.75%), but facultative anaerobic

bacteria were lower in proportion of the total (34.57%), being supplanted somewhat by more aerobic organisms such as yeasts, Staphylococcus, Aerococcus, Aeromonas, and as previously mentioned, Pseudomonas.

Table 10. Bacteria isolated from pork chops after 14 and 21 days of storage at 5°C (includes both mesophiles and psychrotrophs)<sup>a</sup>

Bacterial type	Film					
	High Barrier		Low Barrier		Hand-wrapped	
	Number	Percent	Number	Percent	Number	Percent
<u>Aerococcus</u>	-	-	1	1.23	-	-
<u>Aeromonas</u>	5	6.25	7	8.64	-	-
<u>Alcaligenes</u>	-	-	-	-	2	11.76
<u>Enterobacteriaceae</u> <sup>b</sup>	45	56.25	28	34.57	2	11.76
<u>Flavobacterium</u>	1	1.25	-	-	-	-
<u>Lactobacillus</u>	23	28.75	30	37.04	-	-
<u>Pseudomonas</u>	4	5.00	10	12.50	11	64.71
<u>Staphylococcus</u>	-	-	1	1.23	-	-
Yeast	-	-	1	1.23	2	11.76
Unidentified	2	2.50	3	3.70	-	-
TOTAL	80		81		17	

<sup>a</sup>Totals of organisms isolated include three replications for high barrier and low barrier films and one replication for hand-wrapped chops.

<sup>b</sup>Includes bacteria identified as Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, and Yersinia.



Viable counts comparing films for psychrotrophic bacteria are shown in Figure 12. The results were similar to those for mesophilic bacteria in that the hand-wrapped chops showed a quick rise in bacterial population to the spoilage level, while the vacuum-packed chops had population levels similar to each other and did not reach a count of one million per  $\text{cm}^2$  until the 14th day of storage. A lag period may be seen in the psychrotrophic population when comparing it to the mesophilic counts for all three films. The lag is approximately 3 days before the psychrotrophic population on the chops attained the same level as the mesophilic population in the hand-wrapped chops. The lag period was even longer in the vacuum packages, the psychrotrophic count did not reach the mesophilic count until after 7 days of storage.

The effect of film type on Lactobacillus growth is seen in Figure 13. There was a pronounced lag in Lactobacillus growth in the vacuum packages for 7 days of storage, after which there was a steady increase in population. In the hand-wrapped chops the growth of Lactobacillus was quite different. Rapid growth of Lactobacillus occurred in the first seven days of storage but these organisms were not recovered later. As may be seen in Table 10, this was accompanied by the quick growth of Pseudomonas in the

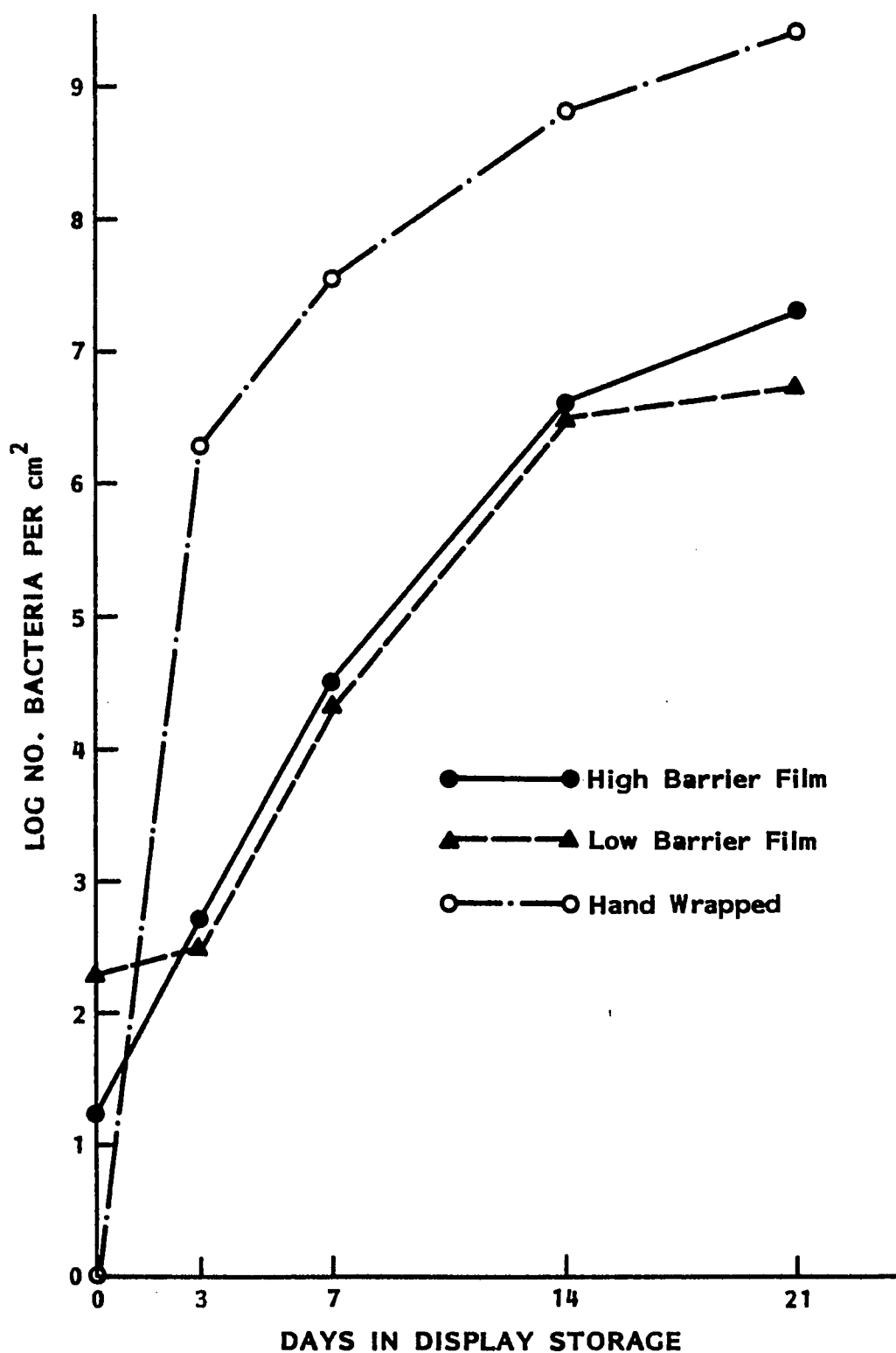


Figure 12. Effect of film on the growth of psychrotrophs

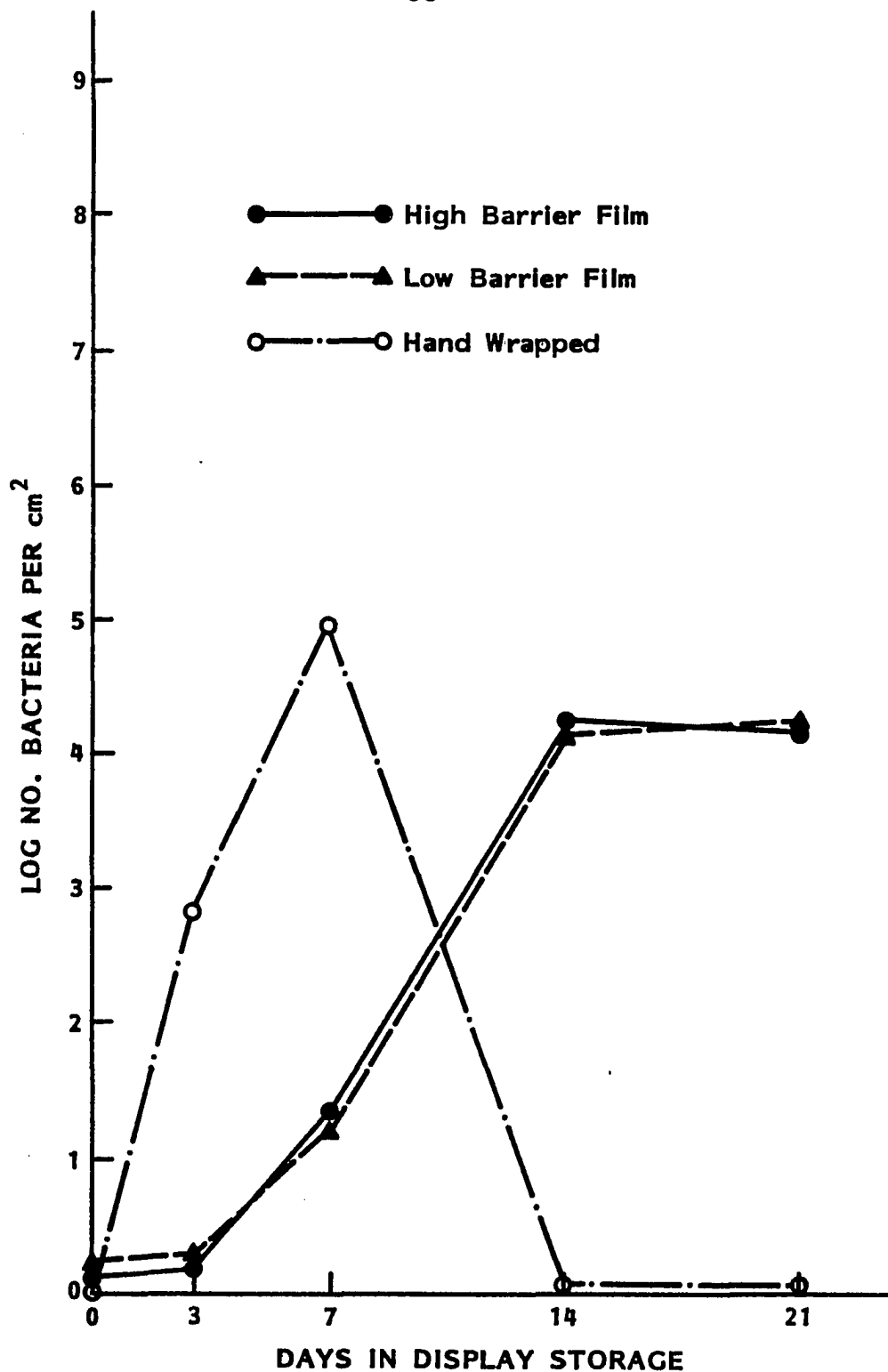


Figure 13. Effect of film on the growth of Lactobacillus

aerobic package. It appeared that, in an aerobic environment, Lactobacillus cannot compete with Pseudomonas. This effect was also illustrated in Table 11, which compared films with frequency of isolation of Lactobacillus and Pseudomonas with regard to days in storage. In the vacuum packages, the numbers of Lactobacillus isolates increased from the start of storage until 14 days of storage and remained high, while Pseudomonas isolates dropped to a low level by 21 days of storage. The opposite effect was seen in the hand-wrapped chops: Lactobacillus was not isolated from mesophilic plates while Pseudomonas steadily increased.

The results of this study regarding film are similar to many other reports concerning the effect of various packaging films on microbial growth. Kraft and Ayres (1952) found that materials of low gas permeability were most effective in retarding spoilage of fresh beef. They compared Pliofilm and aluminum foil-Pliofilm laminates to aluminum foil and cellophane, showing that the aluminum foil-Pliofilm laminate provided the best shelf life for fresh beef. In their study, shelf life was generally correlated with the gas permeability of the packaging material. Jaye et al. (1962) found that in hamburger packed in cellophane, fluorescent pseudomonads increased rapidly in number, but when the hamburger was packed in Saran the Pseudomonas count remained

Table 11. Comparison of percentage of isolates of Lactobacillus and Pseudomonas by films and time in storage

Film	Organism	Days in storage					Total number of isolates
		0	3	7	14	21	
High Barrier	<u>Lactobacillus</u>	10.51	2.63	26.32	34.21	26.32	38
	<u>Pseudomonas</u>	20.00	35.00	35.00	5.00	5.00	40
Low Barrier	<u>Lactobacillus</u>	4.26	17.02	25.53	31.91	21.28	47
	<u>Pseudomonas</u>	21.43	35.71	25.00	17.86	0	56
Hand-wrapped	<u>Lactobacillus</u>	0	0	0	0	0	0
	<u>Pseudomonas</u>	0	7.69	7.69	53.85	30.77	13

constant rather than increasing. In their study temperature played a relatively minor role in the growth of Pseudomonas: growth at 38°F (3°C) and 30°F (-1°C) was approximately the same in Saran and cellophane. In contrast, Lactobacillus growth at 30°F (-1°C) in cellophane and Saran was less than growth at 38°F (3°C) in cellophane and Saran.

Baran et al. (1970) reported that packaging method affected the microflora of hamburger which was vacuum packaged or packaged in air. Growth of anaerobes occurred earlier in fresh meat packaged under vacuum, while growth of aerobes occurred earlier in meat packaged in air. In the present study, growth of anaerobes showed a constant increase in vacuum packaging, while strictly aerobic bacteria virtually disappeared. In the aerobic (hand-wrapped) package, aerobic bacteria became the dominant flora. Roth and Clark (1972) compared vacuum packaging of fresh beef in a gas impermeable film with air packaging in a gas permeable film. They found that vacuum packaging with gas impermeable film reduced the growth rates of most bacteria, favored the development of lactobacilli, and increased the shelf life of the meat with regard to color and odor.

High barrier and low barrier packaging films, although not the same as those used in the present work, were tested for their effect on the microbial growth and flora of wholesale

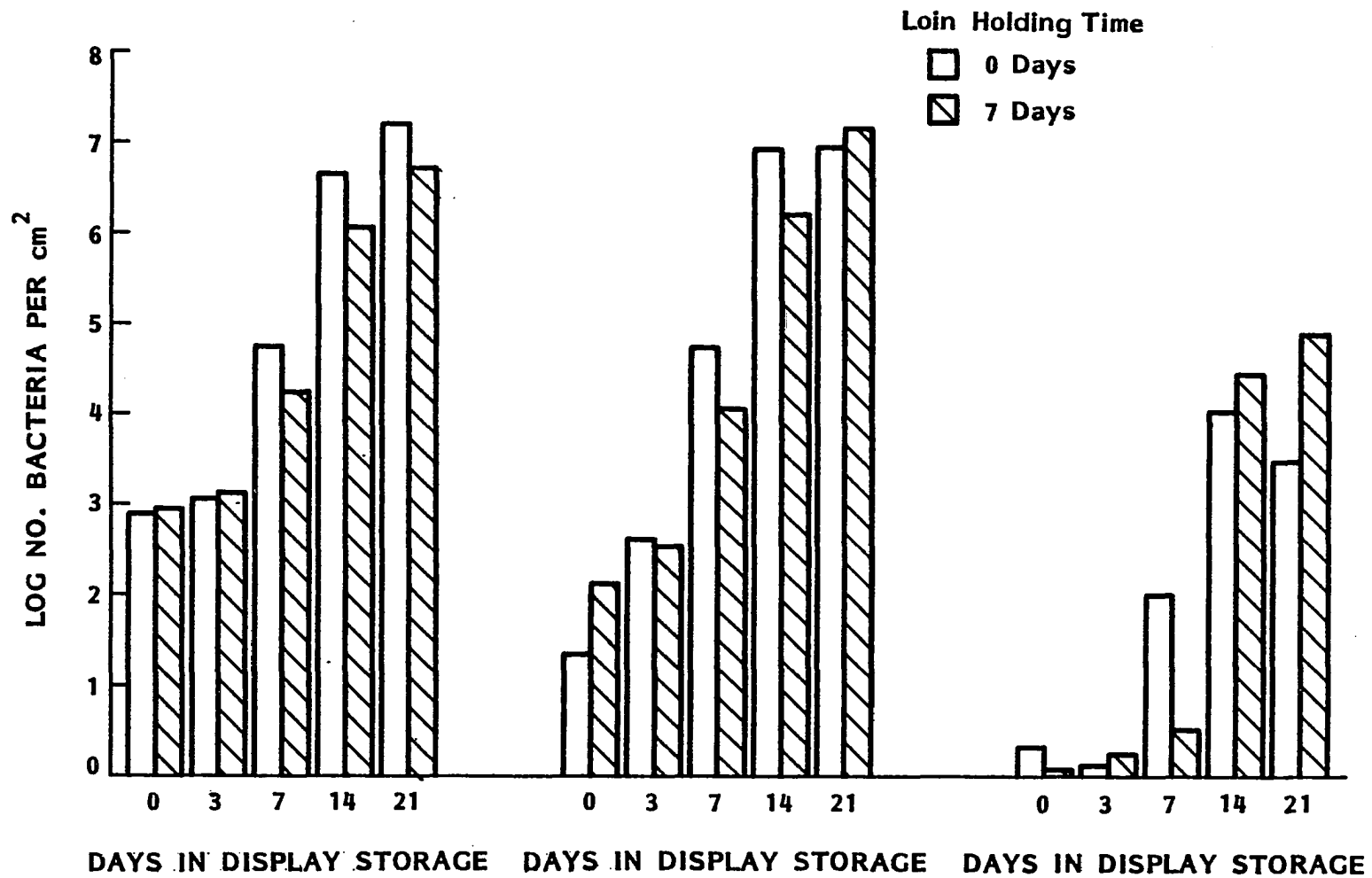


Figure 14. Effect of loin storage time on the growth of mesophiles, psychrotrophs, and Lactobacillus

and retail cuts of beef by Seideman et al. (1976). Their results were similar to this study in that there were few significant differences between high barrier and low barrier films in the growth of mesophiles, psychrotrophs, or Lactobacillus.

#### Effect of loin storage time on bacterial growth

The effect of loin storage time on mesophilic count, psychrotrophic count, and growth of Lactobacillus is shown in Figure 14. Holding the loins for seven days prior to cutting into chops allowed a psychrotrophic flora to become established as evidenced by higher psychrotrophic counts for chops on the first day of sampling each loin. However, the total aerobic flora did not increase at a rapid rate on the loins held seven days at cooler temperatures as on those loins cut immediately after slaughter. This shows that loins may be held for seven days prior to cutting with no increased spoilage potential due to bacterial growth. The aerobic flora established on loins held for seven days evidently did not adapt as well to vacuum packaging as did the flora on chops vacuum packaged immediately after slaughter. Lactobacilli counts also showed a longer lag time for the bacterial population to begin increasing: chops cut from loins with no holding period began a significant rise in Lactobacillus counts 7 days after packaging while the Lactobacillus



population on chops cut from loins held seven days did not show a large increase in numbers until after 14 days of storage. Ultimate numbers of lactobacilli, however, were not significantly different from each other on chops cut from either loin.

These results differ somewhat from those of Rey et al. (1970) who found that beef carcasses aged for 11 days at 2°C had higher bacterial counts and spoilage was more rapid for retail cuts from those carcasses than when carcasses were aged for only 4 days at 2°C. After 3 days storage at 5°C, steaks from carcasses aged for 11 days at 2°C had counts of  $1.5 \times 10^6$  fluorescent bacteria per  $\text{cm}^2$ , while steaks cut from carcasses aged 4 days at 2°C had counts of  $8.0 \times 10^3$  fluorescent bacteria per  $\text{cm}^2$ . We should note, however, that their work was done on beef carcasses rather than loins, and the total storage time before beef steaks were cut and packaged was 4 days longer than in the present study. Christopher et al. (1980) found that there was little difference in psychrotrophic counts on chops cut from loins held 0 and 7 days after 5 days retail display storage. Their work was on vacuum packaged pork loins and pork loins stored in various gas atmospheres. Unfortunately they did not run an aerobic control. The results presented here, however, agree with theirs in that there is little difference in

mesophilic or psychrotrophic counts after 7 days retail storage on chops cut from loins held 7 days at 5°C or chops cut from loins immediately after slaughter.

#### Effect of cutting method on bacterial growth

Cutting method had no significant effect on the growth of mesophiles (Figure 15) or on Lactobacillus growth (Figure 16) although, in the case of Lactobacillus, there was a slight lowering of the population for the refrigerated loins cut with a saw. In the case of psychrotrophic growth, there was a significant difference between the population on the refrigerated, saw-cut chops and the population on the frozen tempered, cleaver-cut chops. The refrigerated, saw-cut chops had a lower population at every sampling period. As noted in Figure 17, the refrigerated, saw-cut chops had a lag period of approximately six days behind the frozen tempered, cleaver-cut chops in psychrotrophic population. From these results, it appeared that refrigerated, saw-cut chops had slightly improved microbial keeping quality when compared to chops processed by the other methods tested. At least some of the increase in rate of growth for the psychrotrophs and Lactobacillus on the cleaver-cut chops may have been due to the increased availability of nutrients because of the freeze-thawing process the meat had undergone. This is suggested by the fact that the frozen

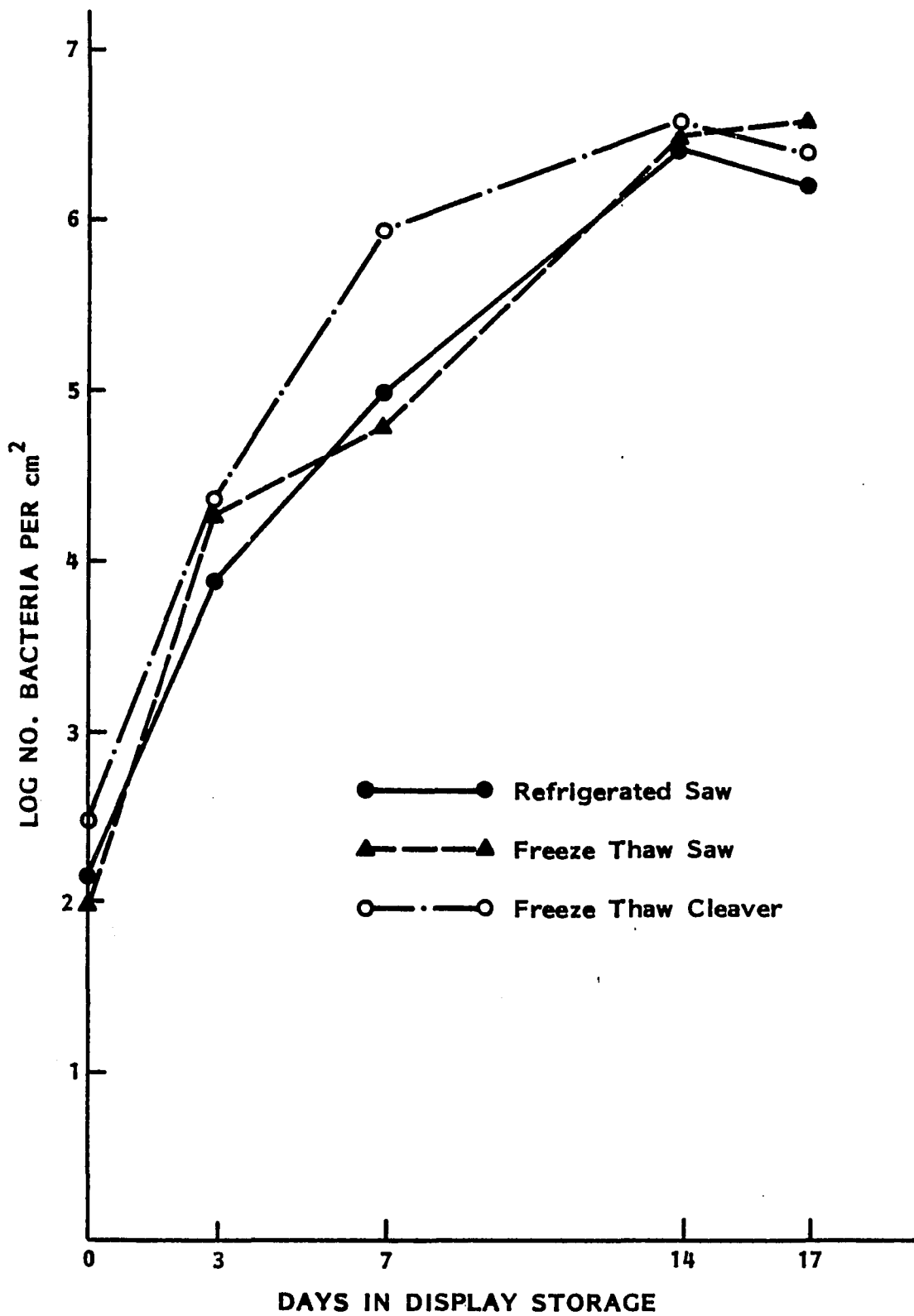


Figure 15. Effect of cutting method on growth of mesophiles

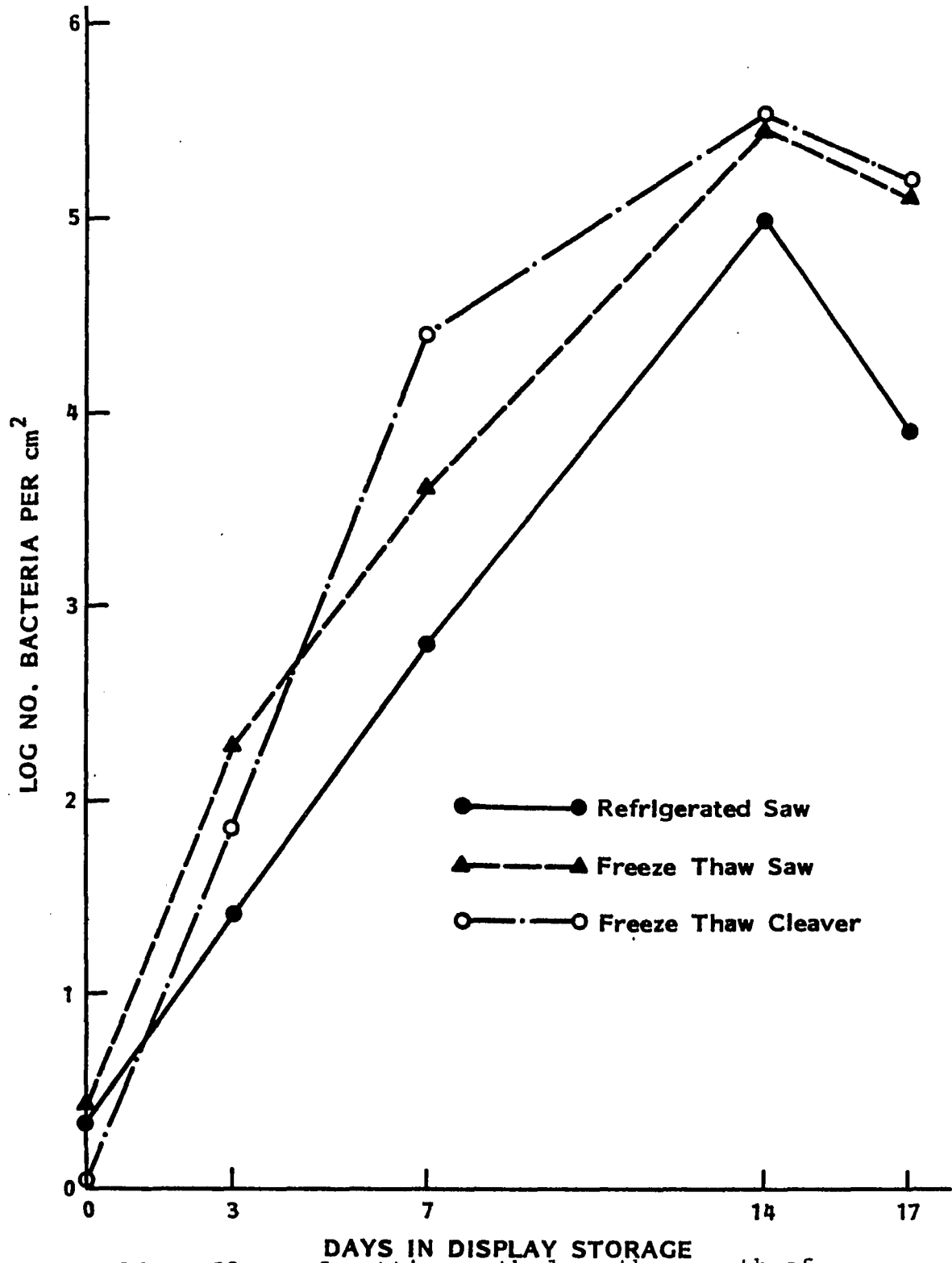


Figure 16. Effect of cutting method on the growth of Lactobacillus

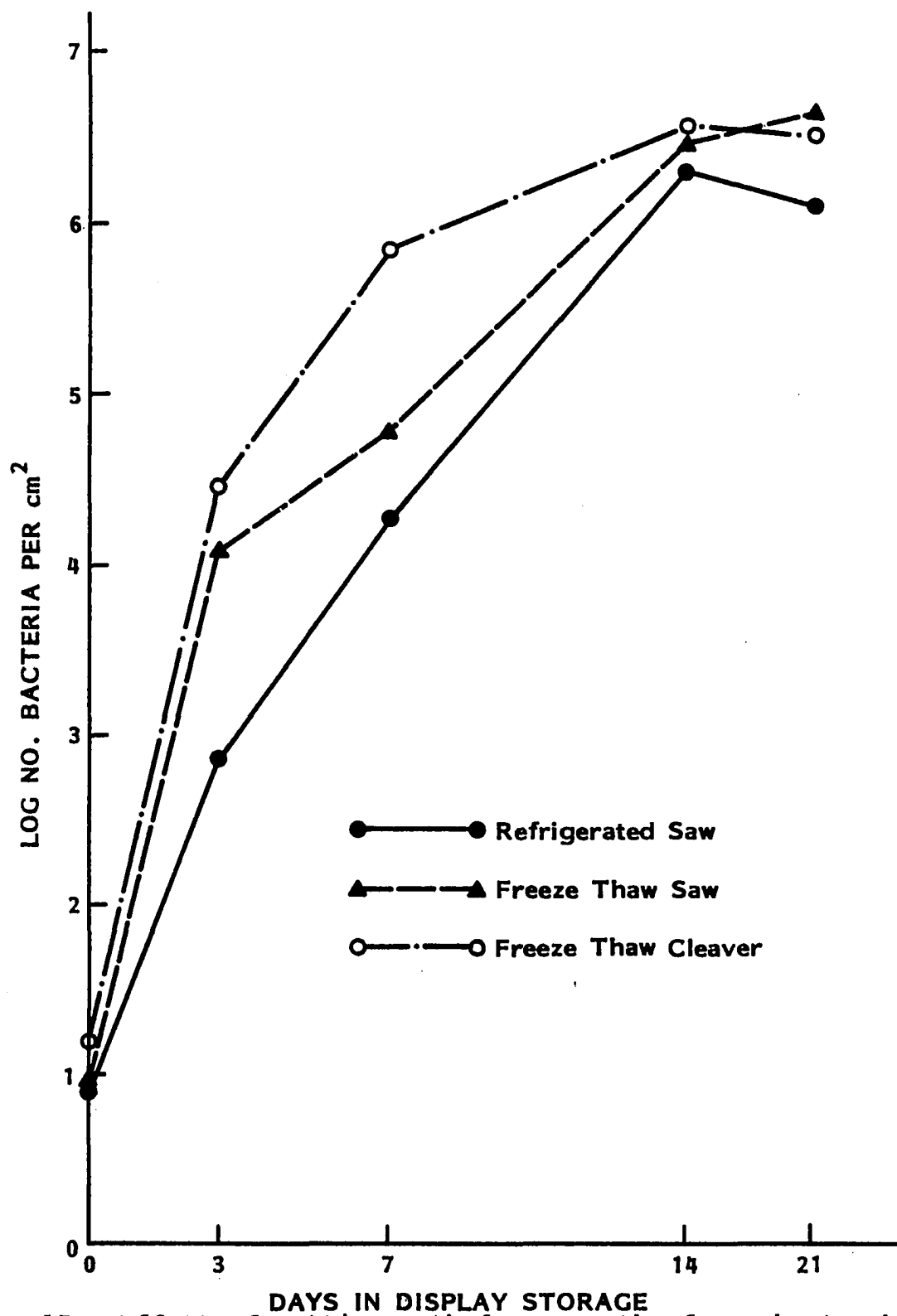


Figure 17. Effect of cutting method on growth of psychrotrophs

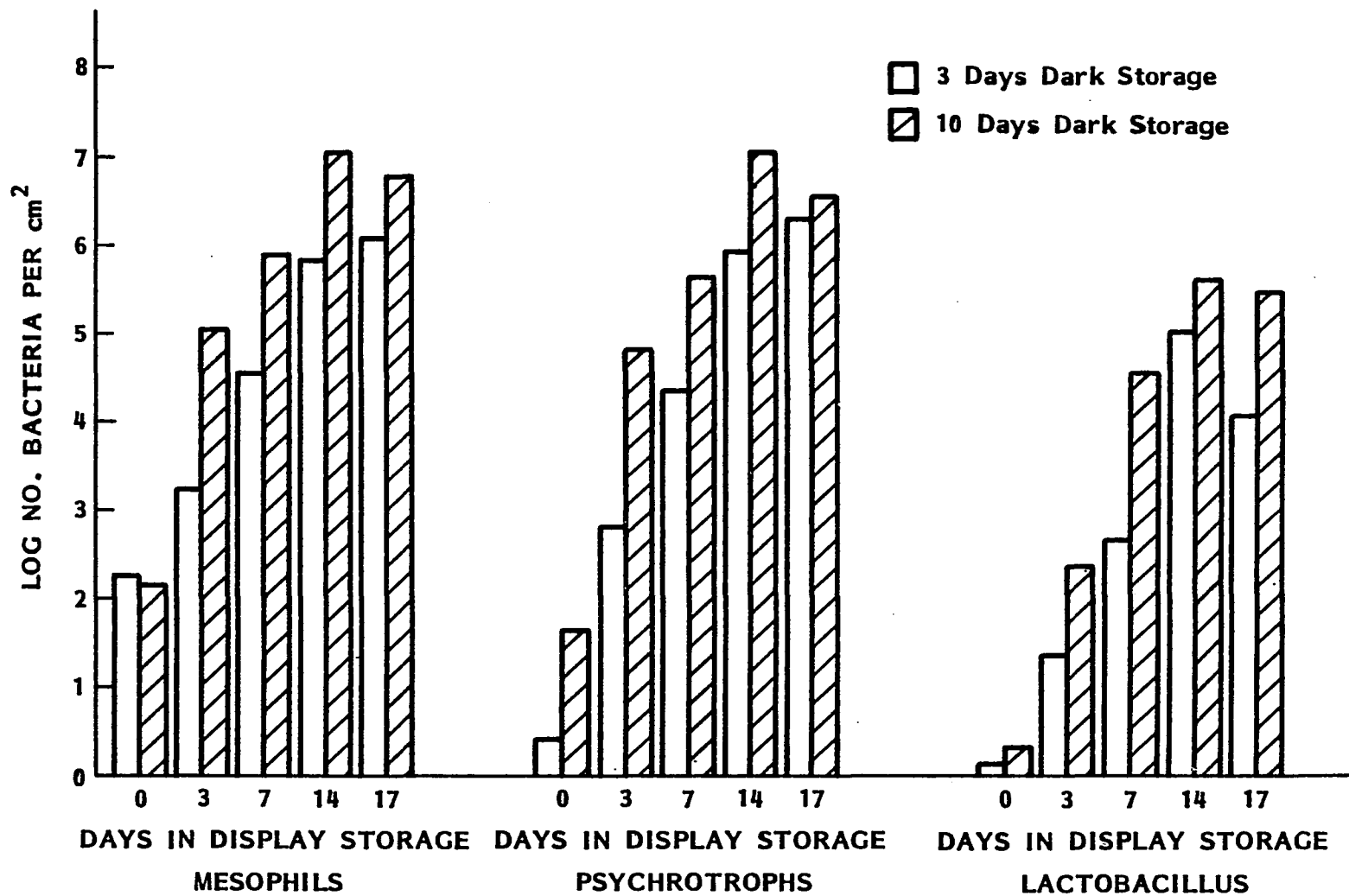


Figure 18. Effect of dark storage period on growth of mesophiles, psychrotrophs, and Lactobacillus

tempered saw-cut chops had bacterial growth curves that, while not equaling those of the cleaver cut chops, more closely approximated them than the growth curves for the refrigerated, saw-cut chops, indicating increased microbial growth resulting from freezing and thawing, regardless of cutting method.

These results are at variance with those of Sulzbacher (1952) for ground meat which was frozen and thawed or refrigerated. He found no indication that frozen meat became more perishable after thawing than fresh meat. Rey and Kraft (1971) found that, on chicken, the microflora was changed somewhat after freezing, the result being an increase in the proportion of biochemically active psychrotrophs which could cause rapid spoilage on defrosted chicken.

#### Effect of dark storage period on growth of bacteria

Dark storage at low temperatures for 10 days resulted in some inhibition of mesophilic growth as indicated by the bacterial population on chops held 10 days being slightly below the population on chops held for 3 days at the initial sampling. However, as indicated by Figure 18, the mesophilic population on chops held 10 days soon surpassed the population on chops held in the dark for only three days. This was to be expected since, at equivalent sampling times, the chops held in dark storage for 10 days are aged 7 days

longer than chops held for 3 days. The reason for the inhibition of total count by dark storage is not completely clear but it probably was due to temperature: the meat cooler used to hold the chops had less temperature fluctuation than the display case, which had a daily defrost cycle in which the temperature increased about 10°C and then decreased to normal operating temperature over a 2 hour period.

Dark storage also provided some inhibition of psychrotrophs, although not of the same magnitude as that of the mesophiles. Psychrotrophs for the first sampling period for the ten day storage chops were above the population for comparable three day chops, although the psychrotroph population was not as high as it would have been if the chops had been held under display conditions. Once the chops were placed under display conditions, the psychrotrophic population increased at a rapid rate, approaching spoilage populations within three days of display storage.

Lactobacillus growth was similar to that of mesophiles and psychrotrophs, being inhibited somewhat by the dark storage but recovery after the chops were placed in the display case.

These results are similar to those of Marriott et al. (1967) with pre-packed, nonvacuum packaged beef. They found



that over a ten day period, steaks in a display case had higher bacterial counts than those in continuous dark storage. This did not appear to be a temperature effect since they claimed that the display case was held at a continuous 30°F (-1°C). They made no attempt to explain the difference in bacterial counts between the dark storage steaks and display case steaks.

The effect of the dark storage period on composition of the microbial flora is given in Table 12. The composition of the microflora changed during the storage period. The

Table 12. Numbers and percent frequency of organisms isolated from chops after low temperature storage for three days and ten days in darkness

Organism	3 Days		10 Days	
	Frequency	Percent	Frequency	Percent
<u>Acinetobacter</u>	1	3.00	-	-
<u>Arthrobacter</u>	5	15.15	-	-
<u>Bacillus</u>	-	-	1	3.22
<u>Corynebacterium</u>	1	3.00	3	9.68
<u>Enterobacteriaceae</u> <sup>a</sup>	-	-	7	22.58
<u>Lactobacillus</u>	2	6.06	5	15.15
<u>Leuconostoc</u>	1	3.00	-	-
<u>Microbacterium</u>	-	-	2	6.45
<u>Micrococcus</u>	7	21.21	1	3.22
<u>Pseudomonas</u>	1	3.00	7	22.58
<u>Staphylococcus</u>	15	45.45	5	16.13
TOTAL	33		31	

<sup>a</sup>Includes bacteria identified as Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, and Yersinia.

isolates from the chops stored three days were composed primarily of aerobic bacteria, with types such as Staphylococcus, Micrococcus, and Arthrobacter making up the majority of the population. By the tenth day of storage, however, the population was composed of a greater proportion of lactobacilli and members of the family Enterobacteriaceae, along with psychrotrophic pseudomonads. Thus, the change in population brought about by the ten day storage period seemed to have prepared the bacterial population on the chops for quick growth in the environment of the display case.

#### Succession of the microflora within the vacuum package

As pointed out by Ingram (1962), the bacterial flora within a vacuum package is constantly changing because conditions within the package are also constantly changing. The particular sequence of events that takes place is called "succession", meaning an alteration of conditions brought about by the preceding stage. Table 13 shows frequency of isolates and the relative percentages of isolates from vacuum packaged pork chops packaged with a high barrier film. All isolates came from the second study. Figure 19 through Figure 22 are condensed from Table 13 for purposes of illustration. The figures clearly show the influence of the vacuum packaging and indicate that, when a high barrier film is used,

Table 13. Frequency of isolates relative to total number of isolates from pork chops vacuum packaged in high barrier film stored at 5°C

Organism	Days in storage at 5°C							
	0-3		6-10		13-17		20-27	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
<u>Acinetobacter</u>	2	3.13	-	-	1	0.68	-	-
<u>Aeromonas</u>	1	1.56	5	4.00	8	5.41	6	4.80
<u>Alcaligenes</u>	-	-	1	0.80	1	0.68	-	-
<u>Arthrobacter</u>	7	10.94	1	0.80	2	1.35	-	-
<u>Bacillus</u>	2	3.13	1	0.80	2	1.35	4	3.20
<u>Corynebacterium</u>	1	1.56	3	2.40	-	-	-	-
<u>Enterobacteriaceae</u> <sup>a</sup>	5	7.81	45	36.00	66	44.59	66	52.80
<u>Flavobacterium</u>	-	-	-	-	2	1.35	-	-
<u>Lactobacillus</u>	6	9.38	24	19.20	47	31.76	45	36.00
<u>Leuconostoc</u>	2	3.13	-	-	1	0.68	-	-
<u>Microbacterium</u>	-	-	3	2.40	1	0.68	1	0.80
<u>Micrococcus</u>	10	15.63	5	4.00	1	0.68	1	0.80
<u>Pediococcus</u>	1	1.56	1	0.80	-	-	-	-
<u>Pseudomonas</u>	2	3.13	25	20.00	15	10.14	1	0.80
<u>Staphylococcus</u>	25	39.06	11	8.80	1	0.68	1	0.80
TOTAL isolates	64		125		149		126	

<sup>a</sup>Includes bacteria identified as Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, and Yersinia.

the dominant factor affecting the microbial flora is the oxygen tension within the package.

The microbial flora initially consisted of a mixed population of aerobic organisms such as Staphylococcus, Micrococcus, Pseudomonas, and a high proportion of miscellaneous organisms ("Other" in Figures 19 through 22). After six days of storage the proportion of facultative anaerobic Enterobacteriaceae has risen to 36 percent, lactobacilli to 19 percent, and the number of staphylococci and micrococci has fallen to 13 percent. That some oxygen remained within the package after sealing is indicated by the fact that the percentage of Pseudomonas had risen after 6 days to 20 percent of isolates from 3 percent at the initial period. Pseudomonas is evidently favored in the period immediately after sealing by the low storage temperature and is able to scavenge enough oxygen from the little remaining within the package to grow. From 13 to 27 days of storage, the microbial flora became less complex, the progression favored only the facultative anaerobic organisms. At 20 to 27 days of storage, the proportion of Enterobacteriaceae had risen to 53 percent of the total, and lactobacilli to 36 percent. At the same time, the aerobic pseudomonads became reduced to 1 percent of the isolates, and staphylococci and micrococci to 2 percent collectively. Staphylococcus, although a facultative

Figure 19. Frequency of isolates relative to total number of isolates from pork chops vacuum packaged in high barrier film after 0 to 3 days display case storage at 5°C (note predominance of aerobic types) (adapted from Table 13)

Figure 20. Frequency of isolates relative to total number of isolates from pork chops vacuum packaged in high barrier film after 6 to 10 days display case storage at 5°C (adapted from Table 13)

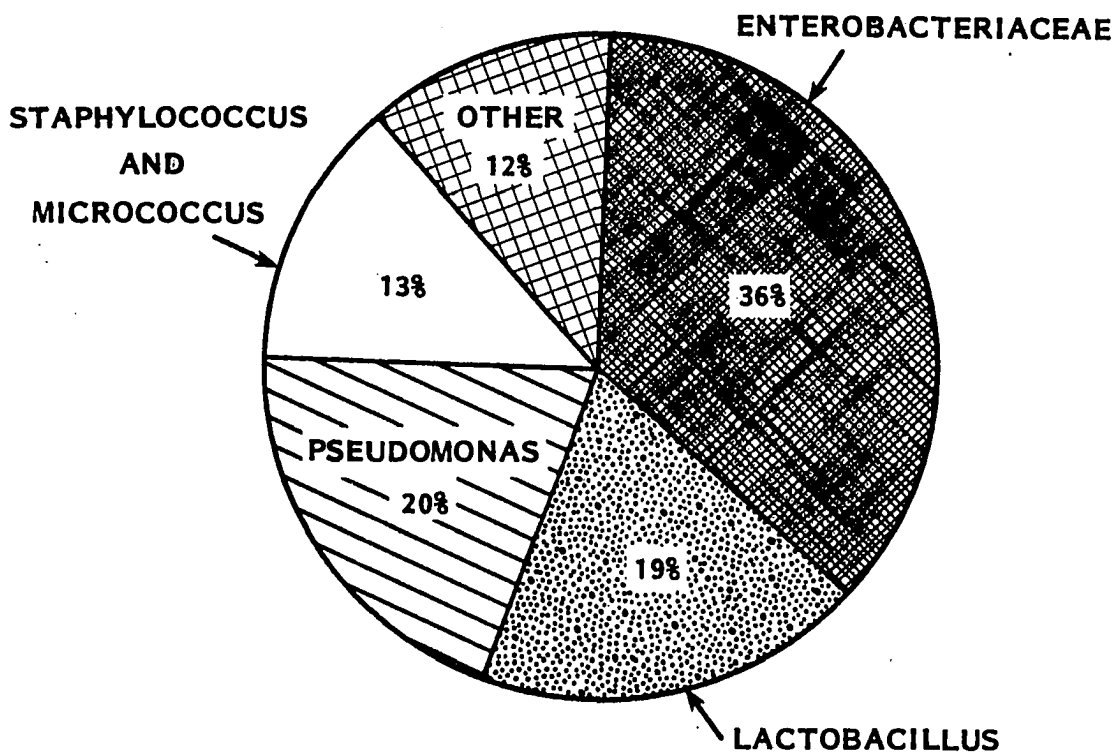
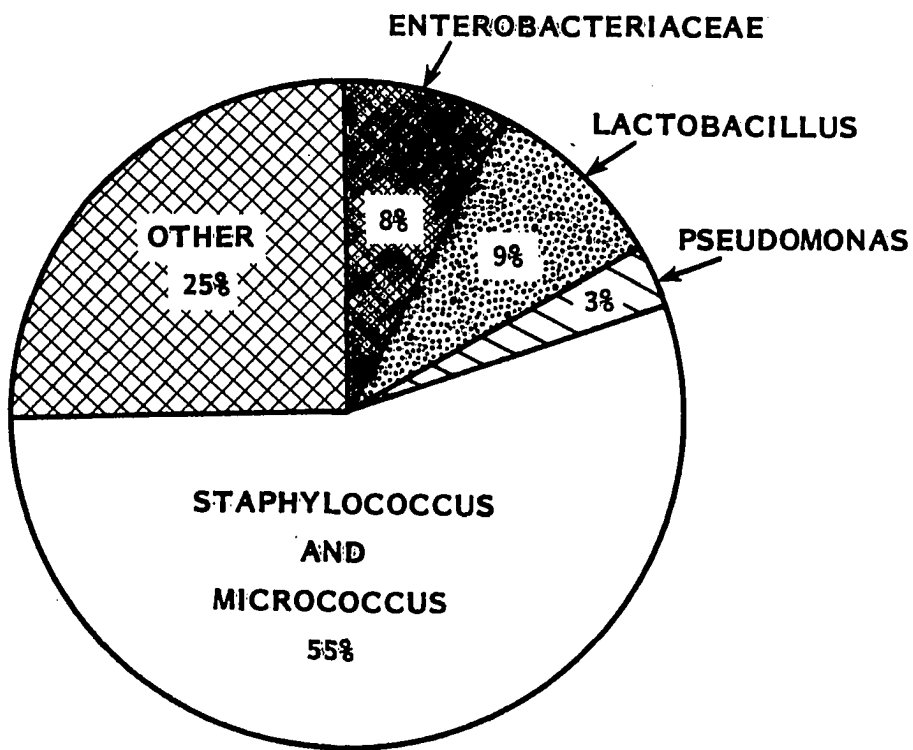
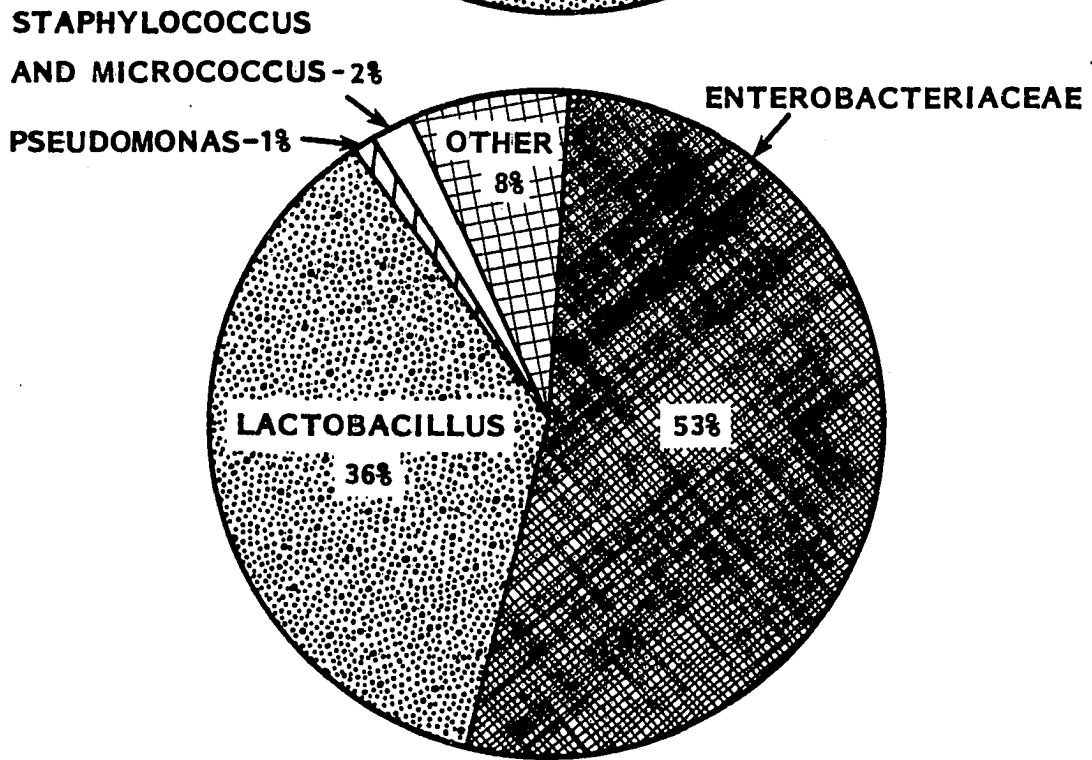
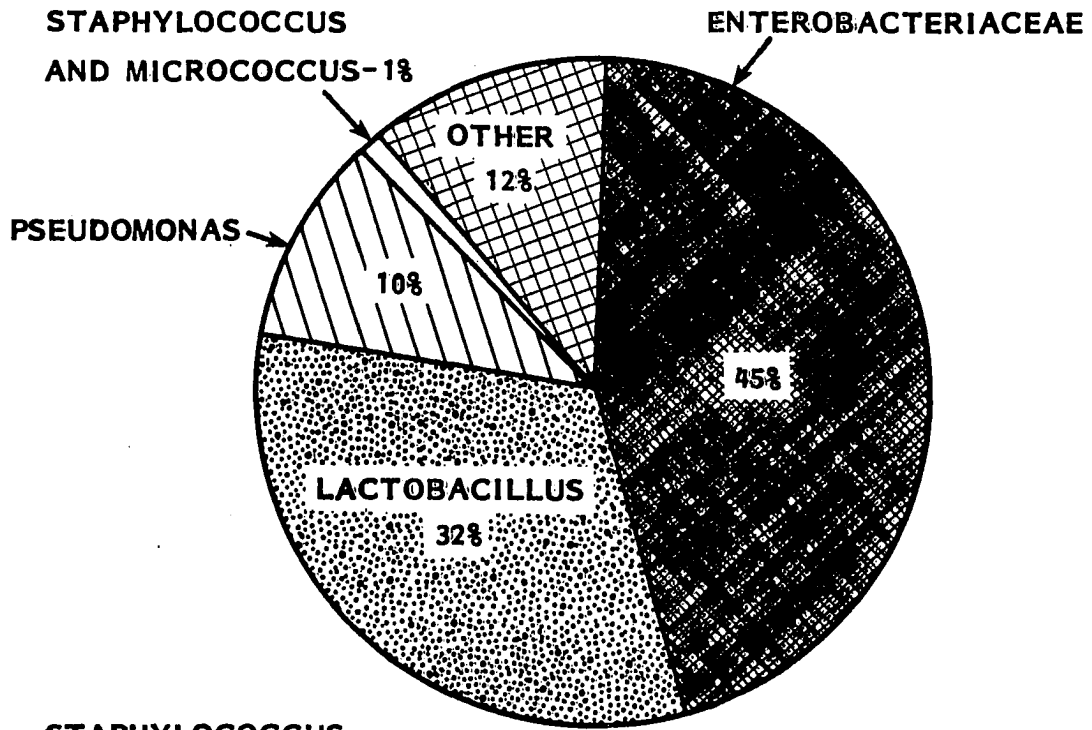


Figure 21. Frequency of isolates relative to total number of isolates from pork chops vacuum packaged in high barrier film after 13 to 17 days display case storage at 5°C (adapted from Table 13)

Figure 22. Frequency of isolates relative to total number of isolates from pork chops vacuum packaged in high barrier film after 13 to 17 days display case storage at 5°C (note predominance of facultative anaerobic bacteria) (adapted from Table 13)





anaerobe, evidently cannot withstand the combined stress of low temperatures and lack of oxygen as well as the Enterobacteriaceae and lactobacilli can. That the general flora undergoes a simplification is shown in the reduction of miscellaneous organisms to 9 percent of the total isolates from 25 percent at 0 to 3 days of storage.

In meat which is stored at refrigeration temperatures or frozen under aerobic conditions a high proportion of Pseudomonas invariably develops. Jaye et al. (1962) found that psuedomonads represented 56 percent of the isolates from beef spoiled at refrigerator temperatures. Other authors have found similar results (Roth and Clark, 1972; Seideman et al., 1976; Kraft et al., 1979). Usually there is a mixed flora during aerobic spoilage of meat which includes other genera such as Lactobacillus, Microbacterium, and Moraxella-Acinetobacter as well as Pseudomonas.

On vacuum packaged meat a different microflora develops, most reports showing similarities to these results in that Lactobacillus forms a high percentage of the population after several weeks storage. Seideman et al. (1976) reported that at 28 days the bacterial population on vacuum packaged beef cuts was primarily Lactobacillus. In contrast to the present study, however, they stated that Enterobacteriaceae represented only a small percentage of the microflora at that time.

In the results presented here, Pseudomonas reached a peak of 20 percent of isolates from 6 to 10 days after packaging and then declined. This may have been due to exhaustion of the oxygen supply within the package, but could also be caused by carbon dioxide produced by lactobacilli, which show a steady increase in numbers, or by carbon dioxide produced by other bacteria. Several reports (King and Nagel, 1967; Roth and Clark, 1972) state that Pseudomonas is inhibited by carbon dioxide. Lactic acid bacteria are not as affected by carbon dioxide, according to Kraft and Ayres (1952). Pseudomonas has also been reported by Price and Lee (1970) to be inhibited by hydrogen peroxide produced by Lactobacillus species. Roth and Clark (1975) also show that lactobacilli can inhibit Microbacterium thermosphactum on fresh beef. Their results indicated that this inhibition did not occur in air, thus establishing that anaerobic conditions are necessary for it to take place. Antagonistic actions by lactobacilli on the growth of several other microorganisms such as Staphylococcus, Salmonella, Escherichia, and Clostridium were reported by Gilliland and Speck (1977).

Potential foodborne pathogens on fresh pork

Staphylococci are a common contaminants of fresh meat, being almost ubiquitous in nature. Surkiewicz et al. (1972) found staphylococci at low levels in 75 percent of fresh pork sausage, and Vanderzant and Nickelson (1969), in a survey of pork carcasses immediately after slaughter, found Staphylococcus species to be the predominant isolates from pork.

Staphylococci were isolated quite often during the course of these studies. For example, during the second day, Staphylococcus species comprised 6.19 percent of all bacteria isolated from mesophilic plate count agar plates. At times, especially in the early sampling periods of each replication, staphylococci could reach major proportions of all the isolates (Table 13). There are several reasons, however, for believing that no significant health hazard exists from staphylococci in vacuum packaged fresh pork. One reason is that the actual frequency of coagulase positive staphylococci was small: 4 of 67 samples (6.97%) were found to be coagulase positive, indicating that the probability for incidence of enterotoxin production was low. This is in contrast to the findings of Vanderzant and Nickelson (1969) who stated that a high percentage of isolated bacteria were found to be coagulase positive staphylococci. The reason for this

discrepancy may be due to random inoculation of the packing plant surveyed by Vanderzant and Nickelson or may be due to the fact that they were testing carcasses immediately or soon after slaughter.

Another factor of public health benefit regarding staphylococci in fresh pork is that the staphylococci are not able to compete well at low temperatures with other spoilage bacteria on meats such as Lactobacillus and members of the family Enterobacteriaceae. As can be seen in Table 13, after two weeks of storage at 5°C, staphylococci had virtually disappeared from the scene with regard to the ability to be recovered from the meat. These results agree with population results from the first study wherein the staphylococcal population was followed by plating on Tellurite Glycine agar (Figure 23). There were no significant interactions between loin storage time, bone presence, or films and Staphylococcus populations. The results showed that Staphylococcus started out at a low population and the population decreased with increasing storage time at low temperatures. More important, at no time did the population of staphylococci come close to the number generally regarded as being necessary for toxin production that would represent a health hazard ( $10^5$  to  $10^6$  organisms per gram). As Tatini (1973) observed, while Staphylococcus has the ability to grow and produce toxin at low temperatures and under

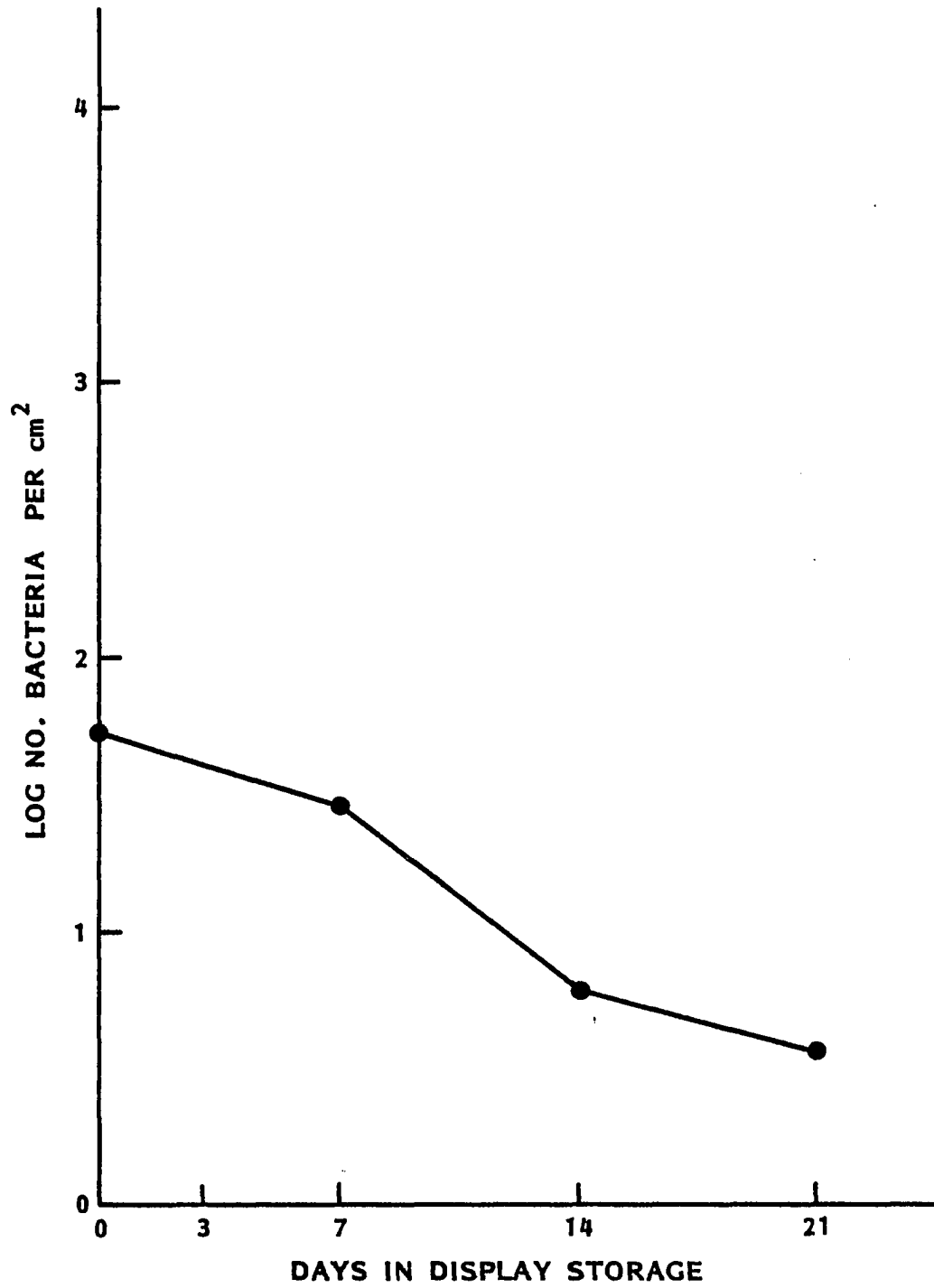


Figure 23. Growth of Staphylococcus on vacuum packaged fresh pork

anaerobic conditions, the optimum situations for both of these activities are at higher temperatures (37-45°C) and under aerobic conditions. Thus, it would appear that as long as the product is not abused there is little cause for concern over staphylococcal growth on vacuum packaged pork.

Clostridium perfringens was isolated from 11 chops of the total of 290 chops tested during the course of the two studies, an isolation rate of 3.74 percent. However, all of these isolations were from the first replication of the first study. Weather conditions may have had something to do with these isolations; this particular replication was performed during a very hot August in Iowa and the meat may have suffered some inadvertent temperature abuse which allowed the clostridia to survive, although not multiply, during the first few days of the replication. This explanation is supported by the observation that all the clostridia were isolated from chops stored in the display case for ten days or less. No clostridia were isolated from chops refrigerated for more than ten days and no clostridia were recovered at any other time during any of the other replications or other study. From the few observations in this case, there does not seem to be an effect on Clostridium perfringens other than that of time in cold storage. These results support findings of Traci and Duncan (1974). They reported that

at 4°C, 90 percent of an initial population of exponential phase cells of Clostridium perfringens was killed upon cold shock and 93 percent of the remaining population was killed within 90 minutes of continued exposure at 4°C. Other than cold shock lethality, another reason for the low incidence of Clostridium perfringens may be the high sanitation standards at the Meat Laboratory of Iowa State University. As Rey et al. (1970) and Bryan and Kilpatrick (1971) have pointed out, handling and contact with workers appears to be a major factor in contamination with Clostridium perfringens. When the chops were cut for these studies handling was at a minimum, food contact surfaces were exceptionally clean, and the potential for cross-contamination from other meats was practically nonexistent. From these results, it would appear that as long as meat is not subject to temperature abuse there should be no hazard from Clostridium perfringens in vacuum packaged fresh pork.

No Salmonella were found during the entire course of this study. This is curious in light of several studies (Weissman and Carpenter, 1969; Childers et al., 1973; Duitshaeffer and Buteau, 1979) that have found Salmonellae on pork carcasses or chops. However, Childers et al. (1973) observed that while the incidence of Salmonellae on the carcass may be high, the incidence in chops may be quite low.

They also point out that sanitation of product contact surfaces has much to do with the level of contamination. As mentioned above, the facilities where the pork was cut into chops was exceptionally clean. This may be the reason for the failure to isolate Salmonellae during these studies. Statistical analyses for microbiological data are presented in Appendix Tables 17 and 18.

#### Other Quality Characteristics

##### Effect of packaging and processing treatments on organoleptic quality

The data illustrated and discussed with regard to organoleptic quality extend only to the 14th day of display case storage because, in almost all cases, chops held longer than this were unacceptable for presentation to a taste panel because of high off odor. Figure 24 illustrates the effect that packaging films had on organoleptic quality. There was no significant difference between the two films (high barrier and low barrier) on flavor, juiciness, or overall acceptability (see Appendix Table 17). Tenderness showed a slight trend toward greater desirability with the low barrier film but this was not statistically significant. The difference between storage up to and including 7 days, on the one hand, and storage up to 14 days on the other, is significant. All organoleptic qualities remain high until



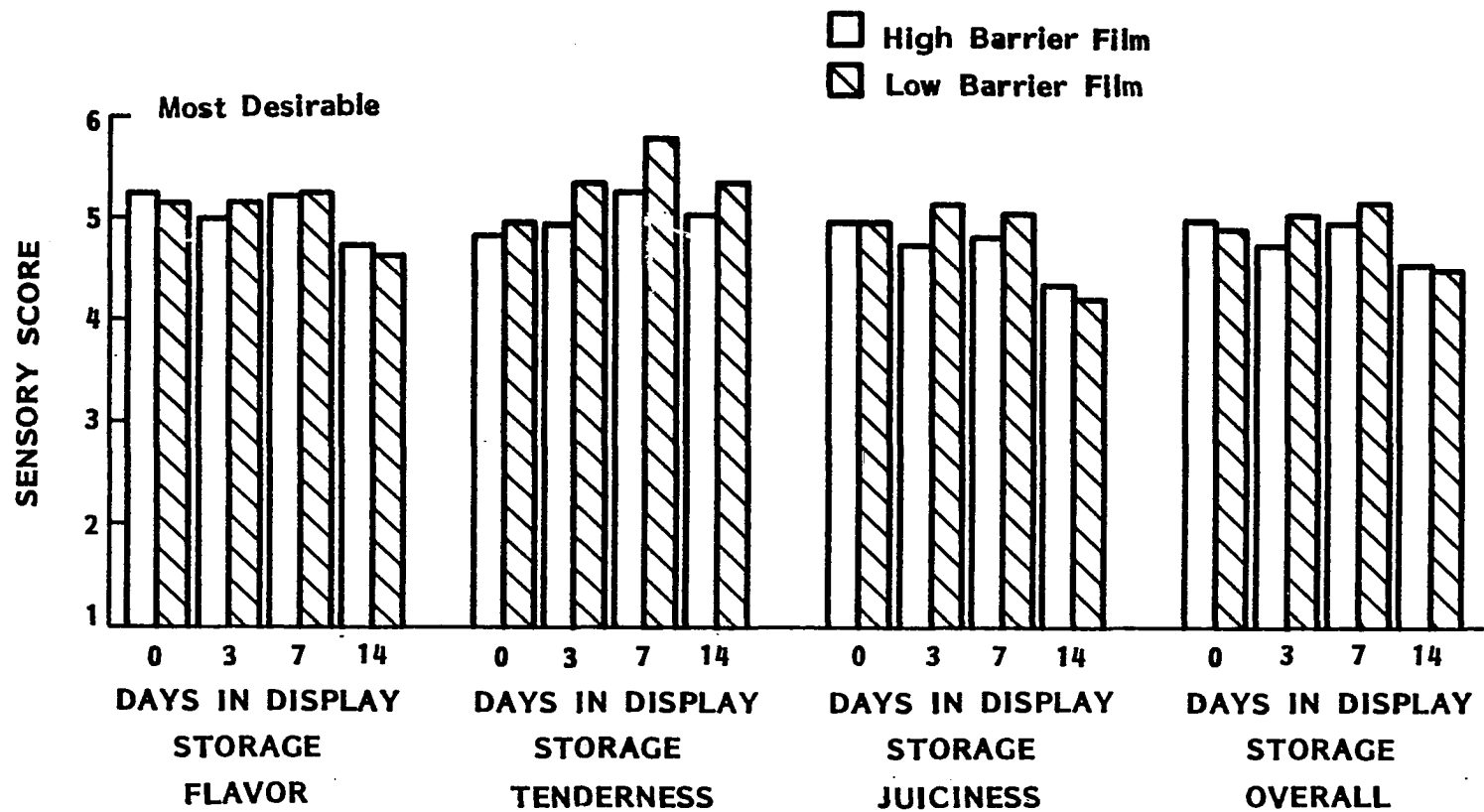


Figure 24. Effect of film on organoleptic quality

the 14th day of display case storage, when there is a drop in ratings for flavor, juiciness and overall acceptability. Tenderness, however, actually increases from the initial sample and remained high after two weeks of display case storage.

Loin storage time had a significant effect on the flavor, juiciness, and overall acceptability of the chops, as shown in Figure 25. From the initial sampling period until the 7th day of display case storage the chops that had been cut from loins immediately after slaughter were more desirable in flavor, juiciness, and overall acceptability. By the 14th day of display case storage, these values were reversed, with the chops from loins stored 7 days prior to cutting being rated higher than the chops cut from loins that were not stored. However, by this time, the chops from both loins were rated significantly lower in acceptability. Tenderness for both loin storage periods was approximately the same and remained high for two weeks of storage.

Chops with the bone left in were rated higher in all categories than boneless chops, but this effect was significant only for tenderness (Figure 26). Flavor and overall acceptability show lower, but consistent increases in the desirability of chops with the bone left in. Juiciness was rated negligibly higher for the chops with the bone left in.

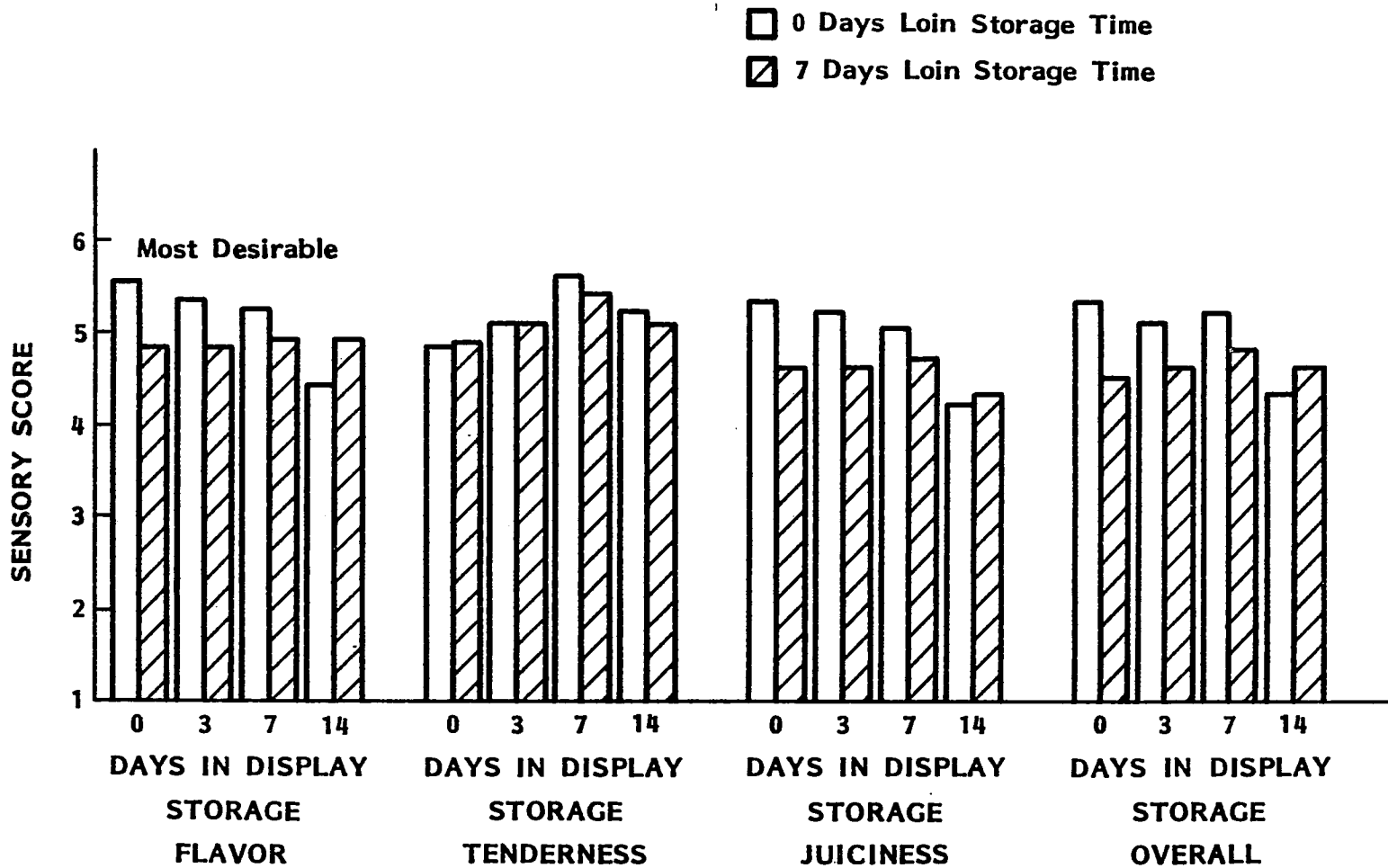


Figure 25. Effect of loin storage time on organoleptic quality

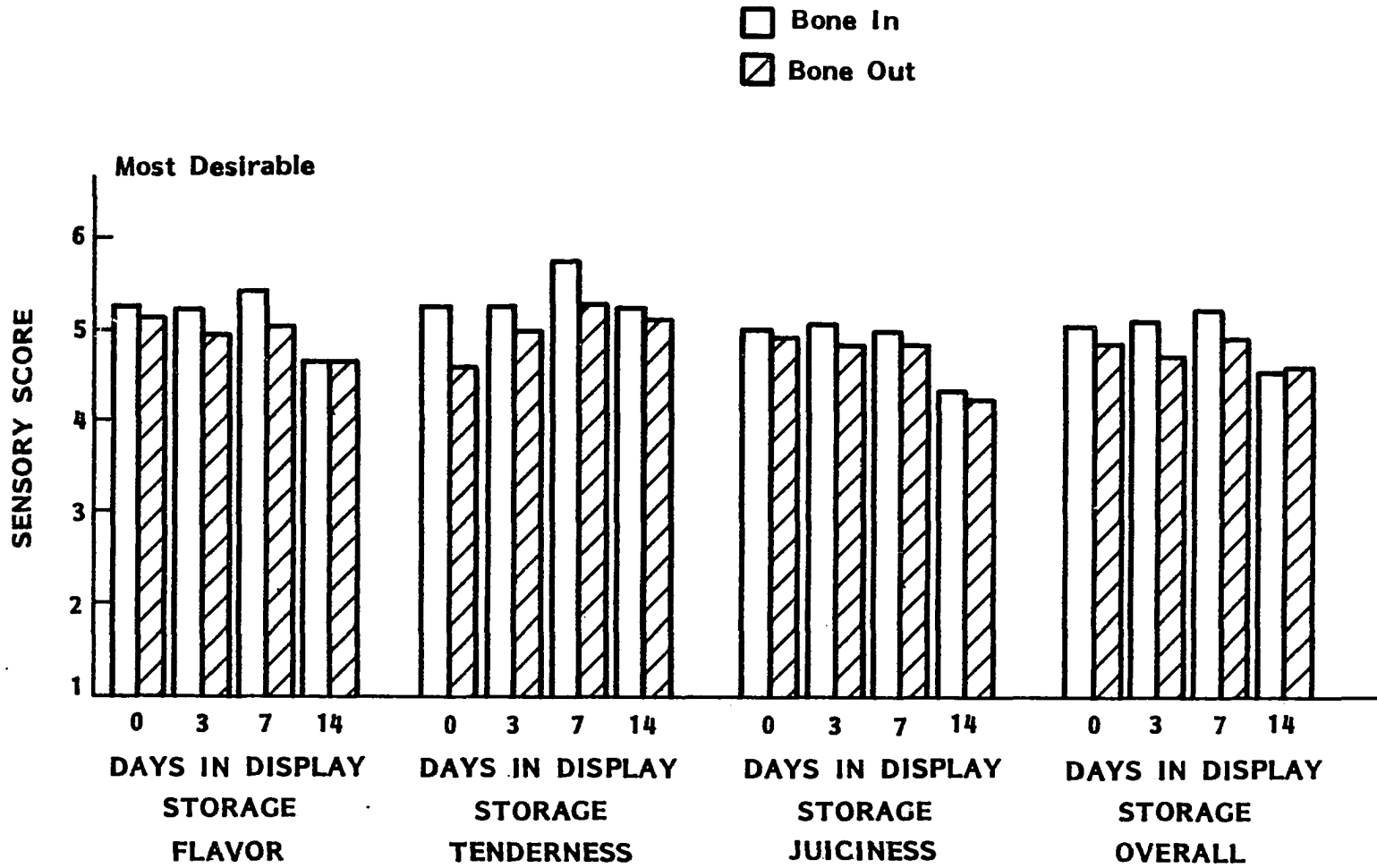


Figure 26. Effect of bone on organoleptic quality

The method of cutting the loins into chops did not have a significant effect on the flavor, tenderness, juiciness or overall acceptability. As Figure 27 shows, there was no consistent trend in sensory qualities that favored any cutting method.

The dark storage period had a significant effect on flavor (Figure 28). The flavor scores for the chops held 3 days were consistently higher than scores for chops held 10 days at comparable sampling tests. As may be seen in Figure 28, the flavor level for the chops stored 3 days was almost as acceptable after 14 days of display case storage as the chops stored 10 days were after 7 days of display case storage. There is no direct comparison at sampling day 14. Chops stored for 10 days were unacceptable for sensory analysis after 14 and 17 days of display case storage because of high off odor. Dark storage also had a similar effect, although not statistically significant, on the overall acceptability scores. The chops stored for 3 days were superior to those stored 10 days prior to being placed in the display case. The tenderness and juiciness scores for chops stored for the two periods were more nearly equal. In fact, for the initial sampling time (0 days in the display case) the chops stored for 10 days scored slightly higher than those held for 3 days.

It is interesting to compare pork stored as loins with

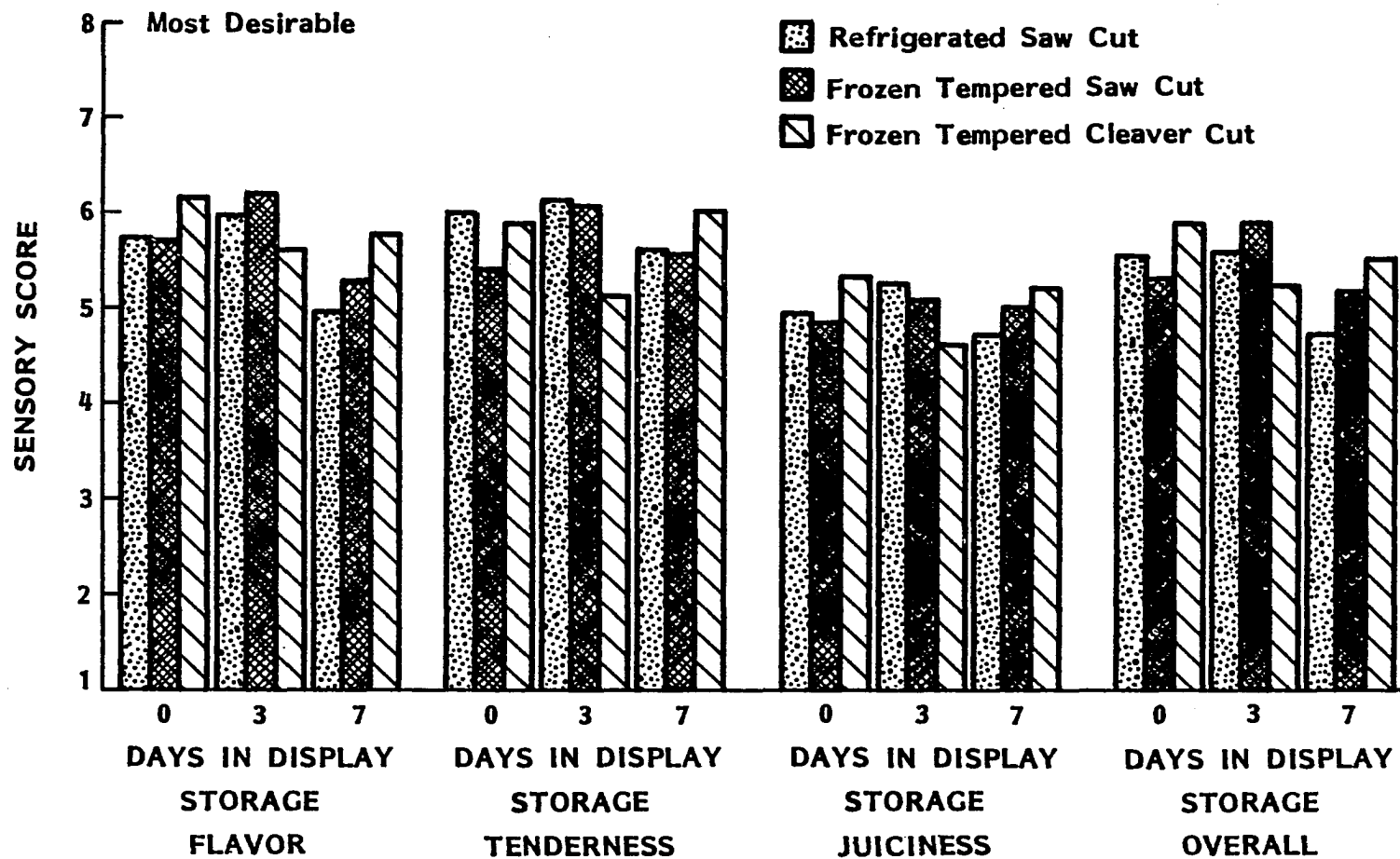


Figure 27. Effect of cutting method on organoleptic quality

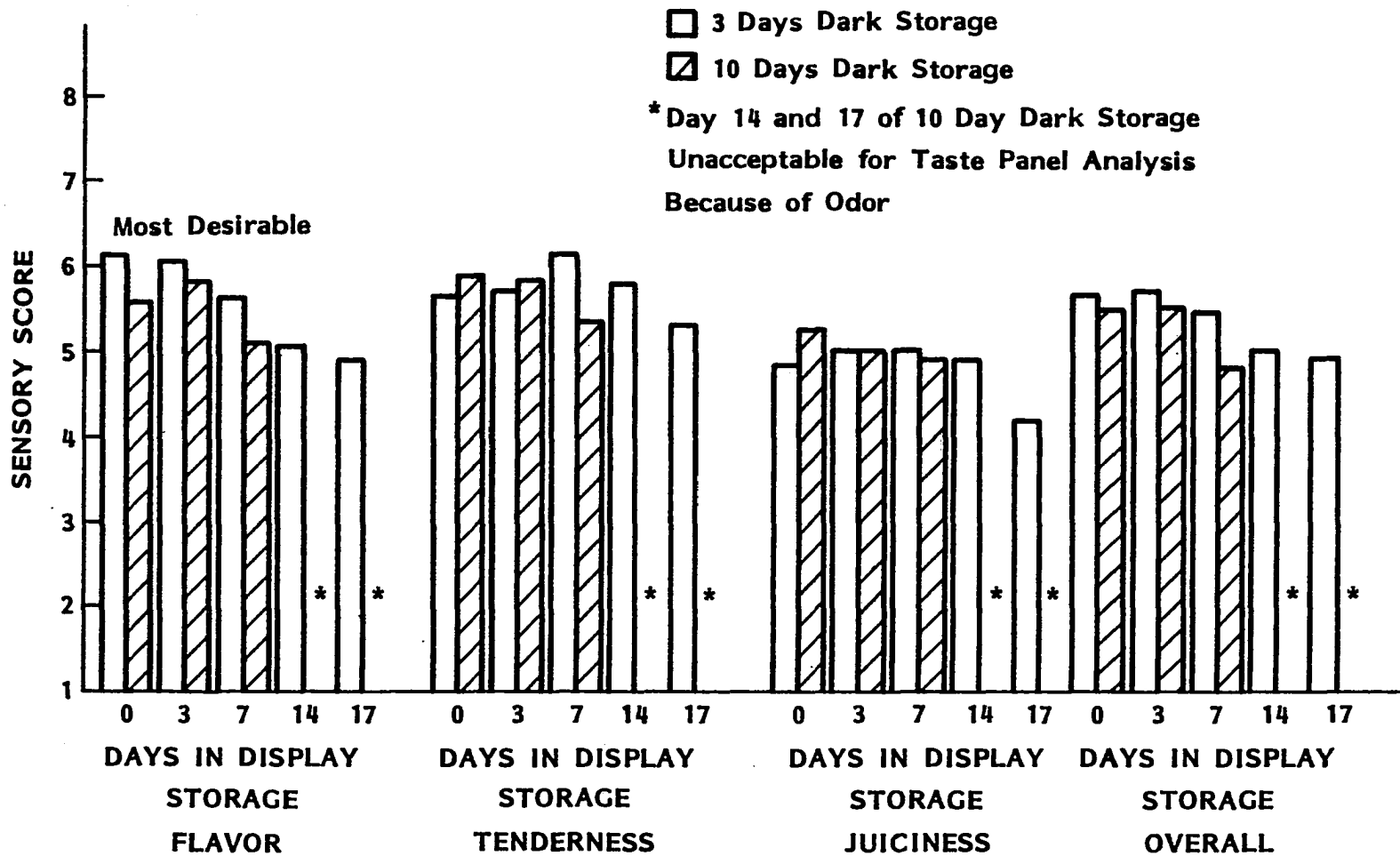


Figure 28. Effect of dark storage period on organoleptic quality

pork stored as vacuum packaged chops to evaluate the two methods of holding regarding quality of chops. In the first study, pork loins were stored for 0 and 7 days prior to cutting into chops. In the second study, pork loins were stored for 3 days, and then cut into chops which were then stored for 3 and 10 day periods. Thus, we may make a "rough" comparison of the two methods for storing meat. Comparison of Figure 25 with Figure 28 shows that there seems to be little difference in the effect of storing pork as loins or chops with regard to organoleptic qualities. In both cases, the loins which were more "fresh" received higher flavor and overall acceptability scores. With both types of storage, the tenderness of the product did not appear to be adversely affected. One difference that emerged is that when stored as loins, the juiciness of the pork stored for 10 days was less acceptable than the more fresh product, while when stored as vacuum packaged chops, there did not seem to be any lowering of the juiciness score of the product stored 10 days when compared to the product stored 3 days.

#### Effect of packaging and processing methods on drip loss

Loin storage time had no significant effect on drip loss, either on chops from loins stored 7 days prior to cutting or on chops cut from loins immediately after



slaughter. Chops from both loins had about 3 percent drip loss. The storage of pork as vacuum packaged chops, however, did have an affect on the drip loss, as shown in Figure 29. The chops stored 10 days had, on average, 0.34 percent greater drip loss than chops stored for 3 days before retail display. This difference, although statistically significant, is small in absolute terms and may be explained by the slight time effect seen on drip loss, since the chops stored 10 days in the dark were, in terms of actual age, 7 days older than the chops stored 3 days in the dark.

Figure 30 shows the effect of bone on drip loss. Chops with the bone in and chops with the bone out begin retail storage with approximately 2.15 percent drip loss. In the boneless chops this increased to 3.35 percent drip loss within 3 days, with slight increases to 21 days of retail display. The chops with the bone left in, however, show a slight, steady increase in drip loss to 2.86 percent after 21 days of storage in the retail display case. That the difference in drip loss is due to large part to the deboning operation is indicated by the majority (77.3%) of the increase taking place within the first 3 days of storage. The increase in drip loss past 3 days storage in the deboned chops was approximately equal to the drip loss in the chops with the bone in (0.36% vs. 0.52%). In other words, the deboning process itself caused the major

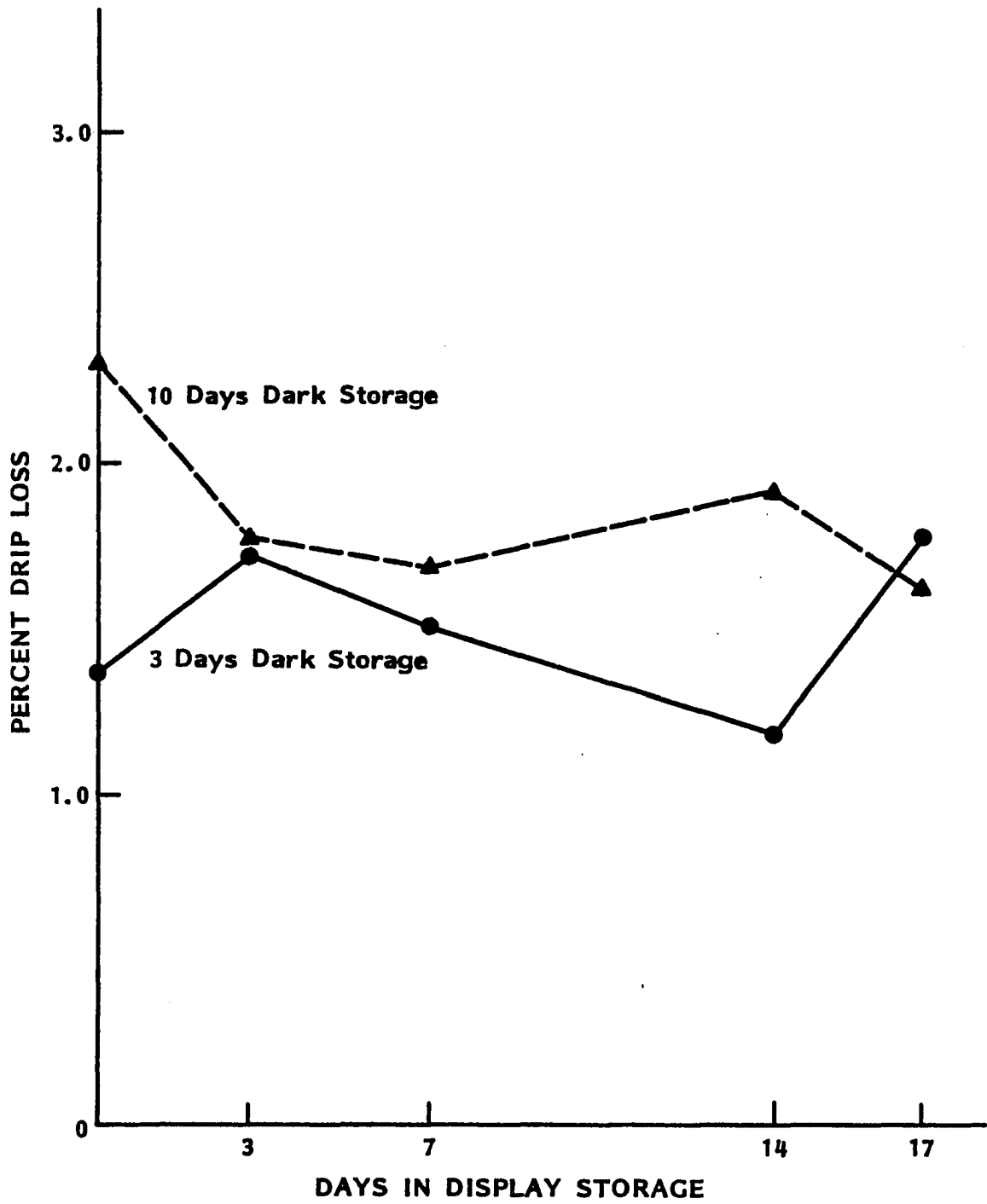


Figure 29. Effect of dark storage period on drip loss

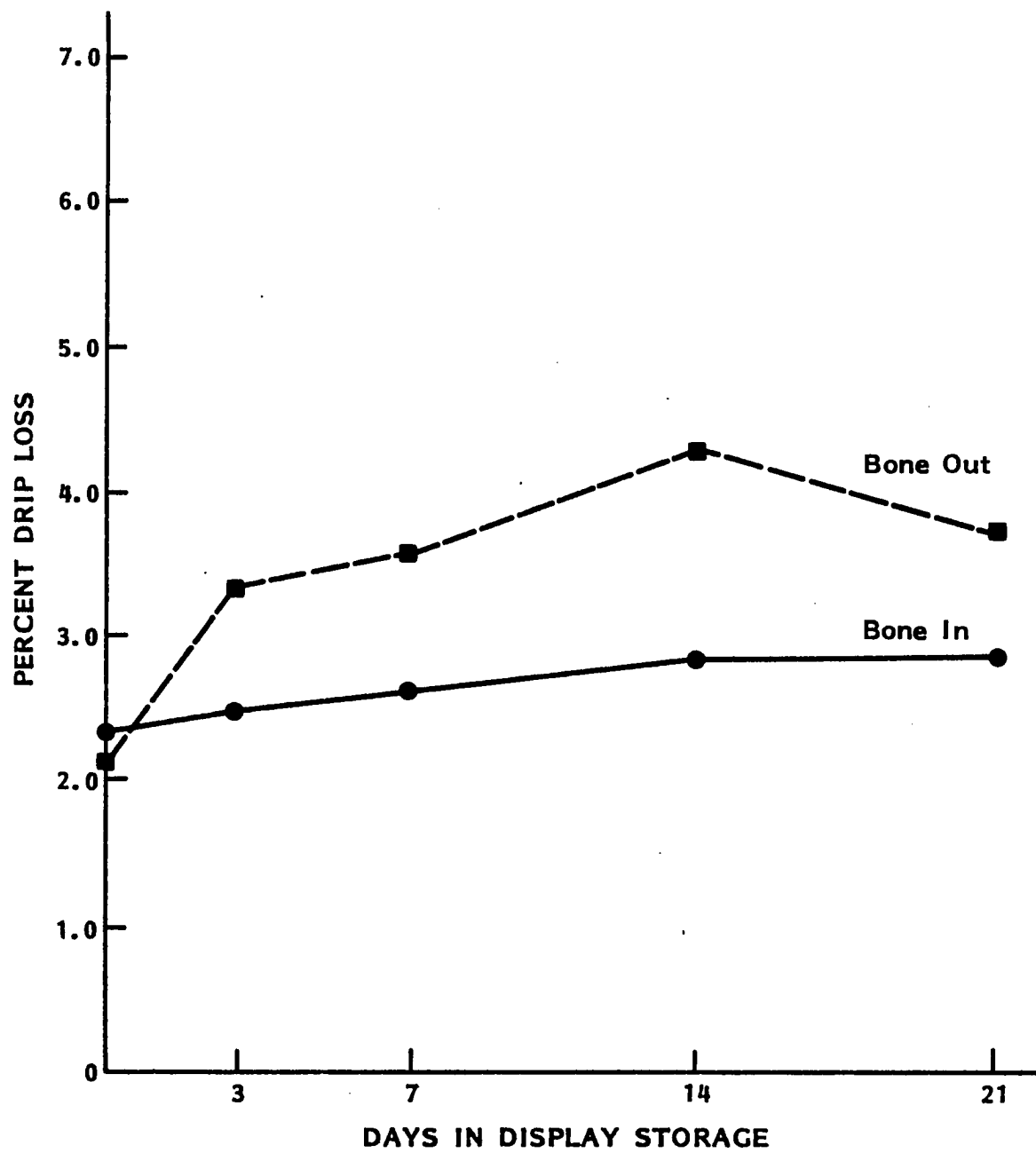


Figure 30. Effect of bone on drip loss

difference in drip loss between the two types of chops, noted by the first three days of storage or immediately prior to it. The deboning operation caused an approximate 1 percent increase in drip loss in the chops.

Film type also influences drip loss, with chops packaged in a high barrier film having much lower drip loss than chops packaged in a low barrier film (Figure 31). Once again, there was a slight time effect. Chops packaged in both films had an increase in drip loss of approximately 1 percent over the course of the 21 day period in the retail display case. However, the rates of increase were the same for drip loss in both films. Because most of the difference in drip loss between the films occurred immediately upon packaging, this difference may be due in part to processing methods. Specifically, different machines were used to package the chops with the respective films. There is no way of determining if this is a true film effect or a packaging method effect from the data obtained here. We can say that there was a difference in drip loss between the two methods of packaging, however. Ball et al. (1957) investigated the loss of weight and change in texture in meat attributed to various packaging films. They could find no differences in drip loss between a low barrier polyethylene-cellophane laminate film and a high barrier pliofilm-cellophane laminate, both types of film showing

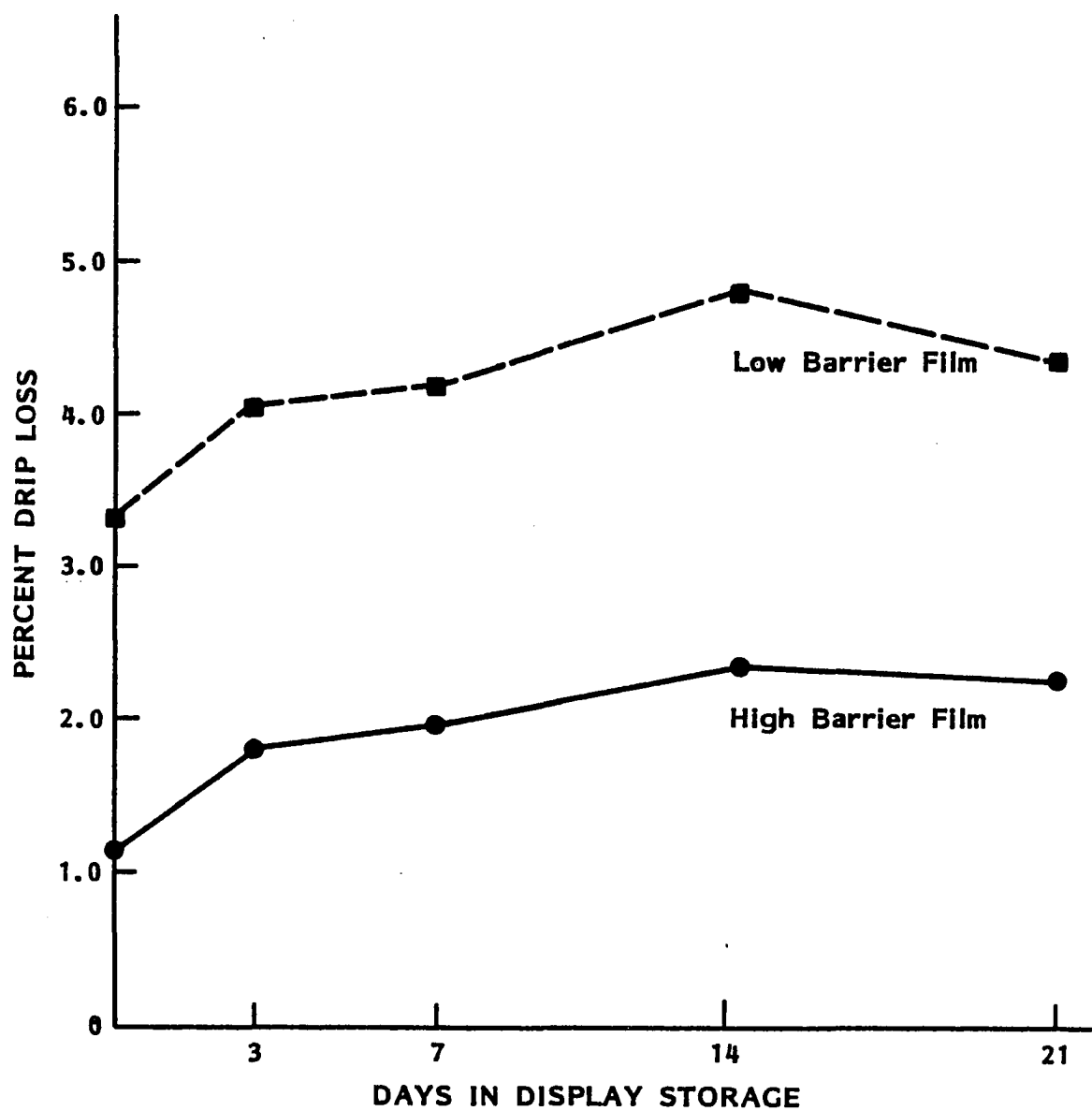


Figure 31. Effect of film type on drip loss

drip losses of about 1.6 percent. They did note that MSAT cellophane when used alone as a packaging film allowed drip losses of 3-4 percent, twice as much as the barrier type laminates.

These effects of bone, film and time can combine to produce differences in drip loss in some chops greater than twice that in other chops. Figure 32 illustrates the combined effects of bone, film and time on the drip loss from chops stored up to 21 days in the retail display case. After 21 days display the drip loss from chops with the bone intact packaged with a high barrier film was only 42 percent of the drip loss of boneless chops packaged with a low barrier film. The actual difference is 2.91 percent. Translated into dollars, 3 percent loss for a processor because of processing or packaging methods can be a considerable economic loss, depending on the price of pork at the time.

The slight time effect on drip loss has been noted by Hodges et al. (1974). They found that the drip loss in vacuum packaged beef increased with time from 0.51 percent to 0.93 percent for a 28 day storage period. They also found a difference in drip loss between cuts of beef, with the higher grade cuts of beef shrinking less than the low grades. They attributed this difference to the greater amount of fat in the high grade cuts. This explanation cannot

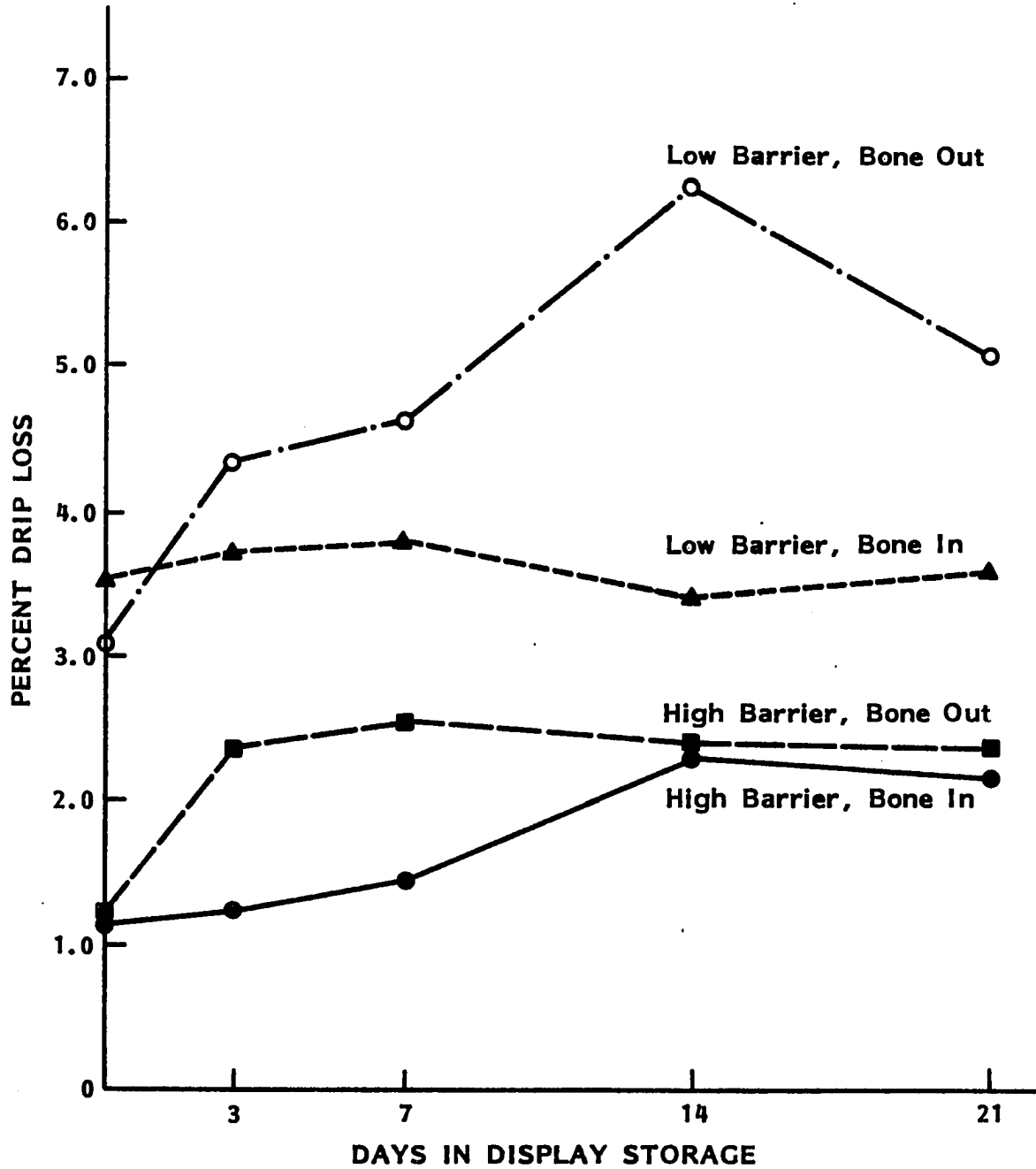


Figure 32. Interaction of film type, bone, and days in display case storage with drip loss

fit the results of this study since, on a weight basis, the boneless chops had a higher amount of fat than the bone in chops, yet also show increased drip loss. A possible explanation for the lower drip loss in the chops with bone intact may be that the intact chops, on a percentage weight basis, have less available drip since the bone adds weight to the chop without adding a proportional moisture loss capability.

The method of processing and cutting the loin into chops also has a significant effect on the drip loss of the chops. Figure 33 shows that the drip loss from the chops saw-cut from refrigerated loins was less than that from frozen tempered loins either saw-cut or cleaver-cut. There is also a difference, although not statistically significant, between the saw-cut and cleaver-cut frozen tempered loins. By taking the total average difference between the refrigerated saw-cut and frozen tempered cleaver cut loins and then dividing the difference between the various treatments, we can estimate that 70 percent of the total increase in drip loss is due to freezing and thawing while 30 percent is due to the difference between the cutting methods. This large difference due to freezing may be caused by the rupture of cells within the muscle during the freezing process and leakage of the cells during subsequent thawing. The difference



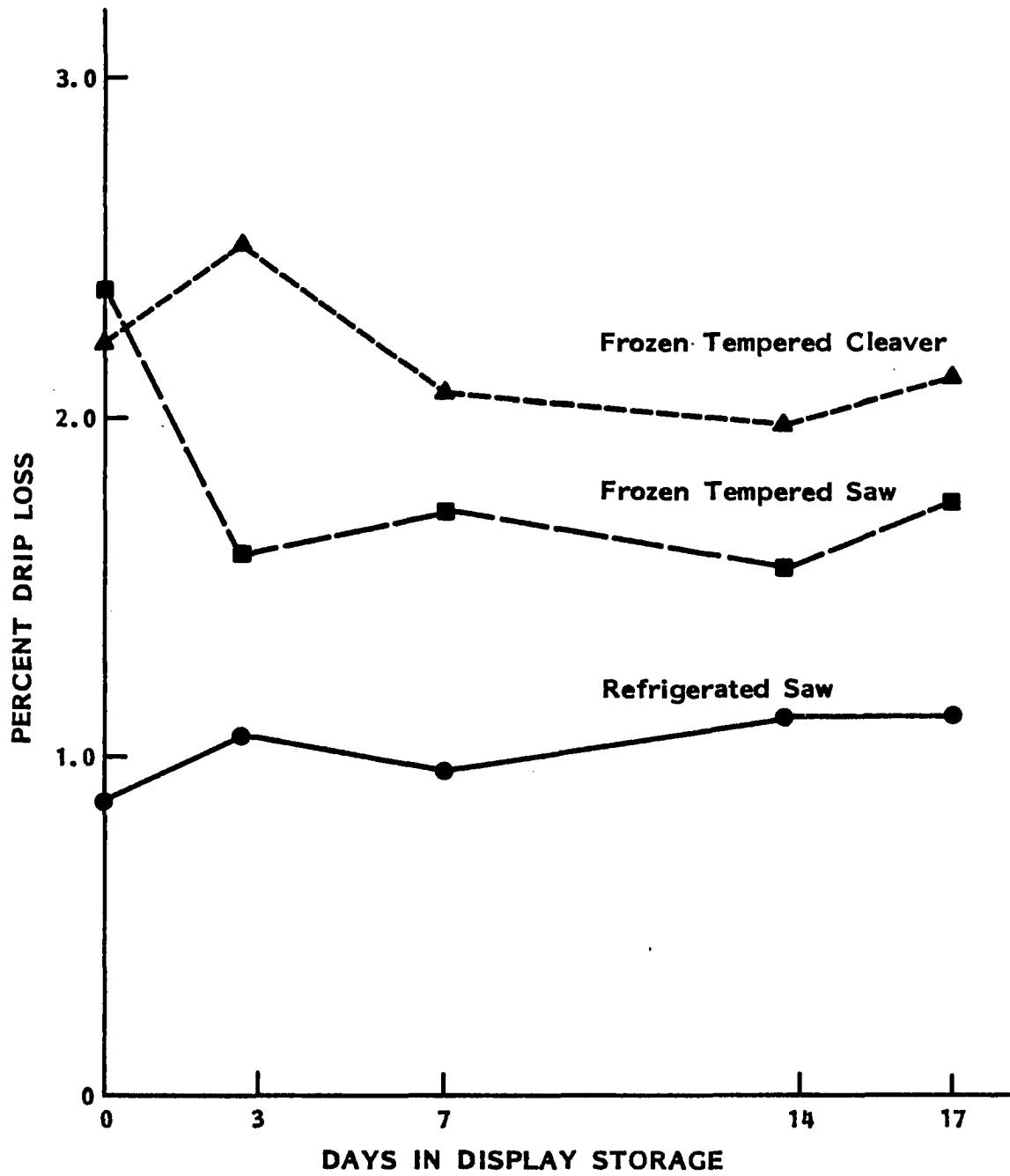


Figure 33. Effect of cutting method on drip loss

between cutting methods is less easily explained and is open to speculation. One would think that the jagged edges of a saw blade would tear and disrupt the muscle tissue much more than the blade of the cleaver. However, it should be noted that these particular results are from chops with the bone in. In order for the cleaver to operate properly, the loin must be crust frozen. An observation made during the production of the chops was that the cleaver tended to shatter the frozen bone somewhat, whereas the saw, refrigerated or frozen, did not do this. This shattered bone may be the source of the increased drip loss from the cleaver method. This question could be resolved by performing the cleaver cutting operation with bone in and boneless loins.

#### Effect of packaging and processing methods on odor

Odor is a difficult attribute to quantify because the subjective observation is one of quality as well as quantity, which tends to affect the magnitude estimation of the judge. The precision of subjective odor judgements is not as close as other more objective tests such as microbial enumeration methods. For this reason, several of the odor scores are not statistically significant. The treatment of loin storage time had no effect on odor development. This is not surprising since off odor in meat is recognized to be caused

in large part by bacterial action and there was little effect of loin storage time on bacterial population. The correlation coefficient between odor and mesophilic growth is 0.6424 and between odor and psychrotrophic growth is 0.5722. Both are significant at the 1 percent level.

Figure 34 represents the effect of film on odor score. Although the differences between the two films are slight, there is a consistently higher off odor score for the chops in the high barrier film. More importantly, there was a qualitative difference in the odor from chops stored in the different films. The odor from the low barrier film can be described as being "putrid" while the odor from the high barrier film was "fruity". Thus, even though the odor from the high barrier film was stronger, it was not as offensive as that from the meat packaged in the low barrier film. Examination of bacterial types isolated from the chops shows that the high barrier film had a higher proportion of Enterobacteriaceae (56.75% vs. 38.35%) and a lower proportion of Pseudomonas (5.26% vs. 13.69%) than did the low barrier film. In future studies, it might be interesting to attempt to correlate type of odor more closely with characteristic types of predominant bacteria and their volatile products.

Figure 35 depicts the effect of cutting method on odor. The off odor from the refrigerated saw-cut chops was somewhat

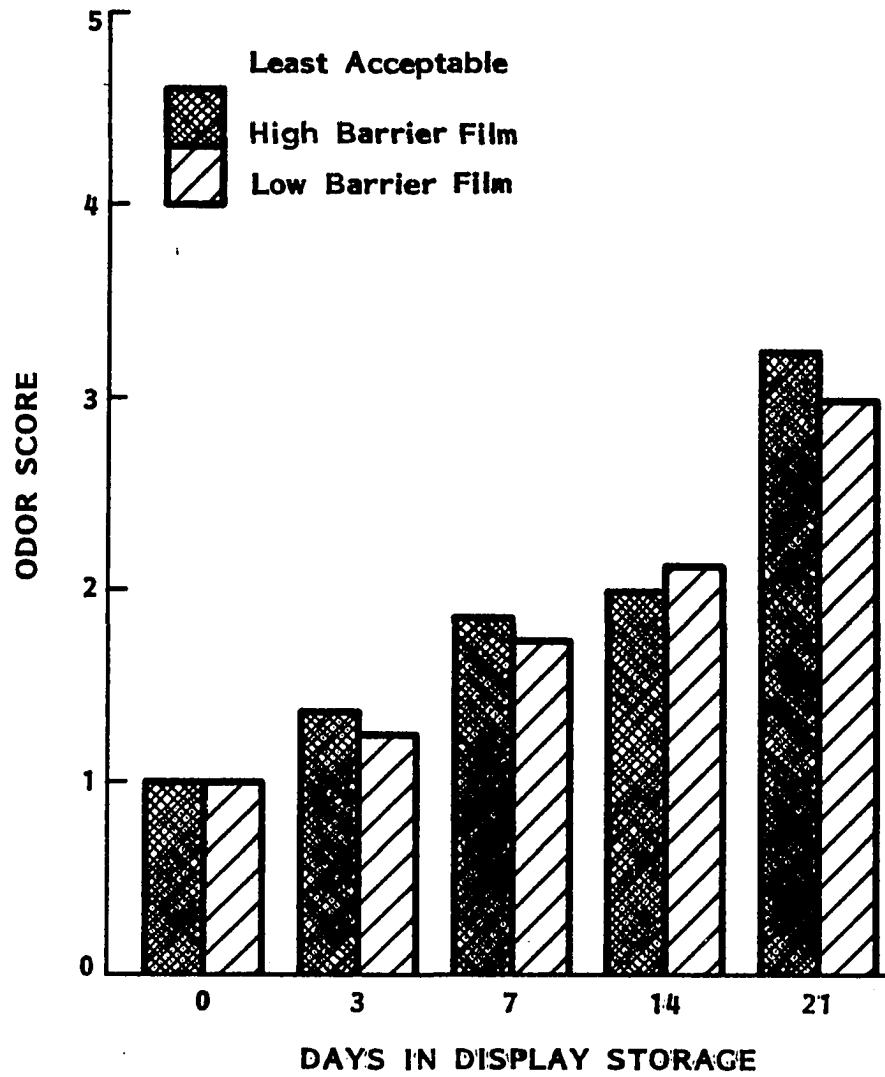


Figure 34. Effect of film type on odor score

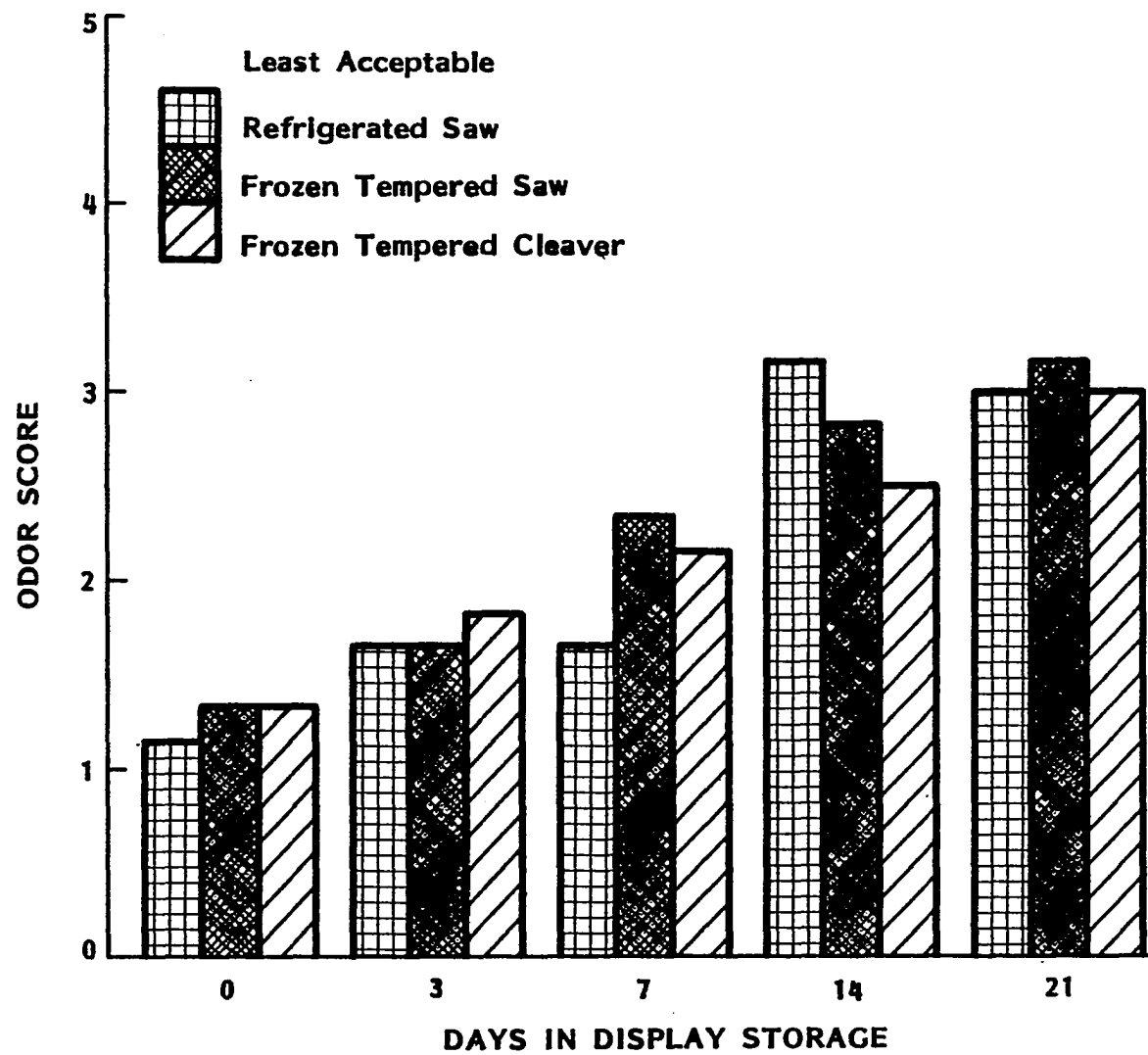


Figure 35. Effect of cutting method on odor score

lower than that from the other two methods for four of the five sample days. This is consistent with the microbiological results which show the refrigerated saw method slightly superior to the other cutting methods with regard to psychrotrophic and Lactobacillus counts.

The effect of bone on the odor score of the chops is shown in Figure 36. For the first three days of storage, the presence or absence of bone in the chop had little influence on odor development. After seven days of storage, the chop with bone in began to develop a higher off odor than the boneless chops and this trend continued and became greater with increasing storage time. Both a bone and time effect was evident since the boneless chops also generally increased in off odor.

Figure 37 illustrates the effect of dark storage period on the development of off odor. The off odor for the chops stored ten days is consistently higher than the off odor for chops that have been stored for only three days. The difference in odor was slight at first, indicating that the dark storage period had inhibited to some extent the development of odor, but increased in magnitude as time in the retail display case increased. This is an expected development in view of the effect of dark storage on bacterial growth. Both psychrotrophic bacteria and lactobacilli showed

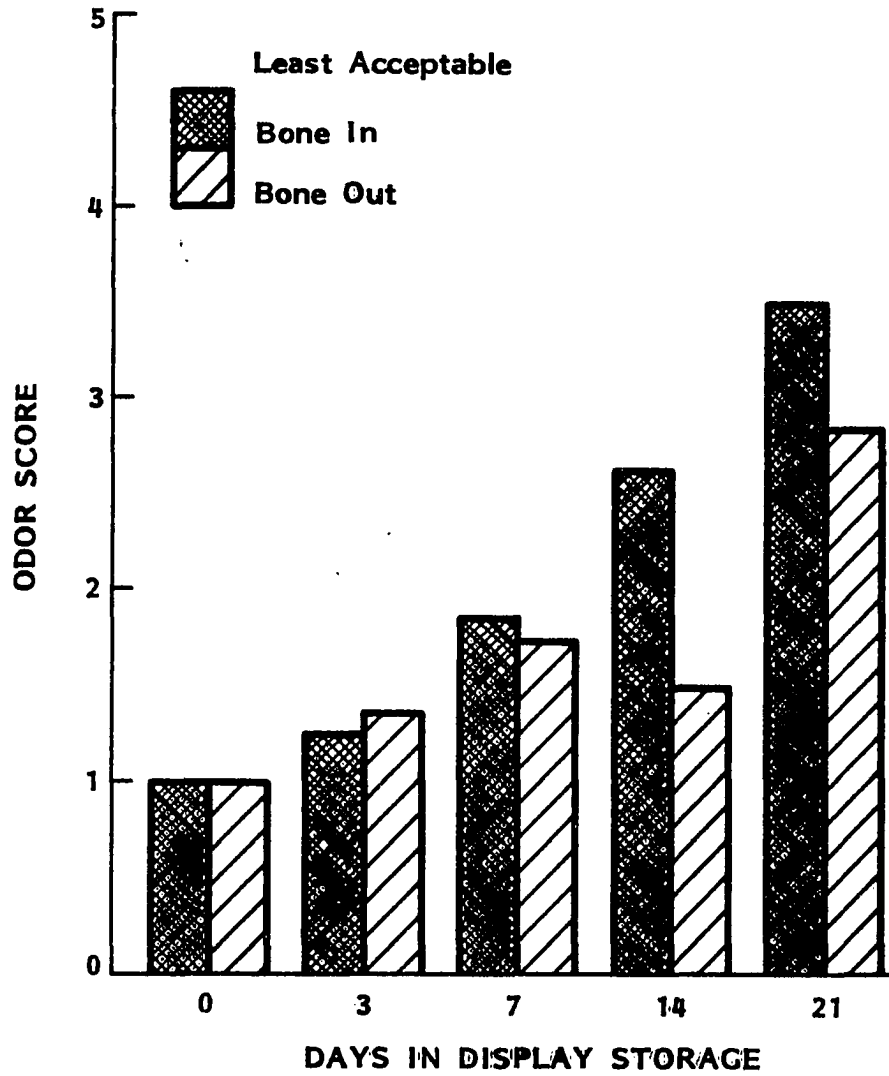


Figure 36. Effect of bone on odor score

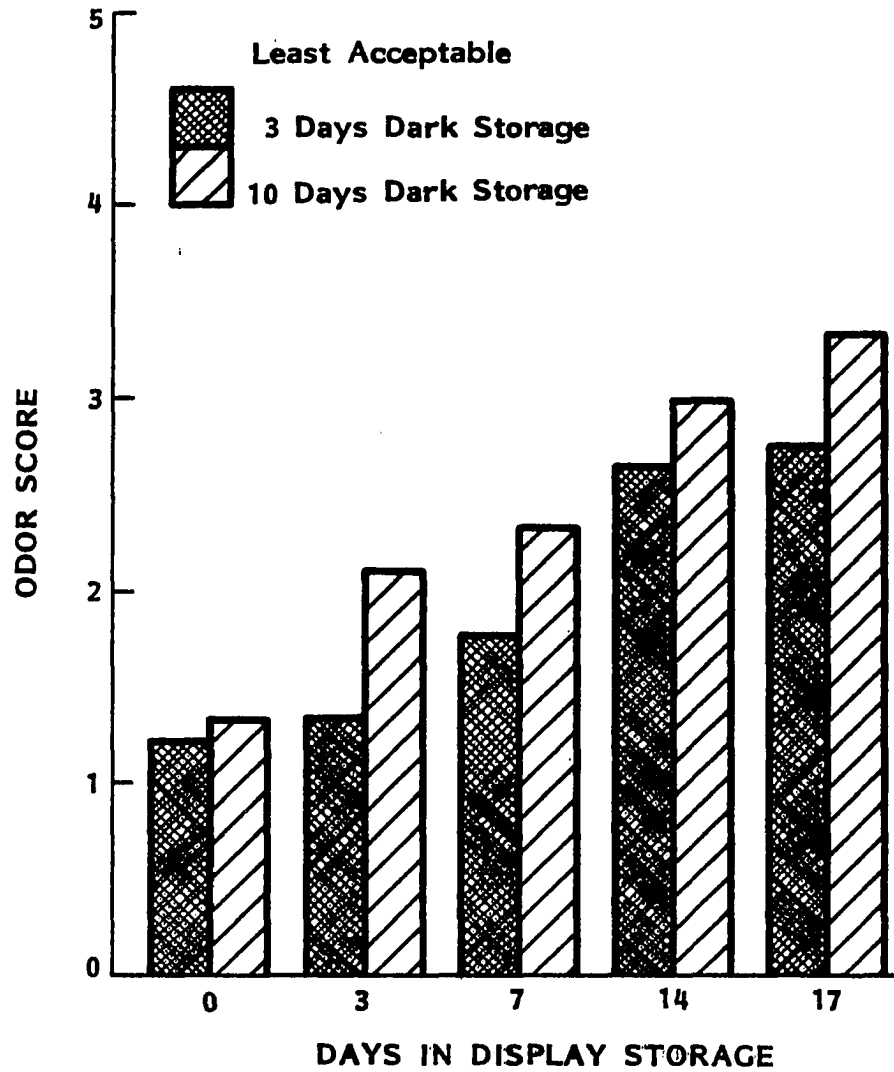


Figure 37. Effect of dark storage on odor score



increased growth for the chops stored for ten days. In addition, as described earlier, the composition of the microflora changed considerably during the ten day storage period and contained more bacterial types which were likely to cause more pronounced odor.

Since odor is caused by volatile compounds, the hypothesis that odor would change if highly volatile compounds were allowed to escape to the atmosphere was tested. Figure 38 shows the results of these experiments, wherein the meat and package were allowed to stand open to the air for a period of 15 minutes prior to being retested for odor. The results are interesting in that they show that many of the off odors seemed to be associated with the packaging film rather than the meat, or with the uptake of odor components from the meat to the film. The meat at opening and at 15 minutes after opening showed no consistent trend: at the first sampling day the odors were equal, whereas for other days the odor 15 minutes later was slightly greater or less than that for the meat at opening. Package odor at opening was similarly usually close to the meat odor at opening, although in some instances it was more marked. The packaging material 15 minutes after opening, however, was invariably higher in off odor than with the other test conditions. One explanation for this may be that at opening some volatile components were capable of masking heavier, more undesirable

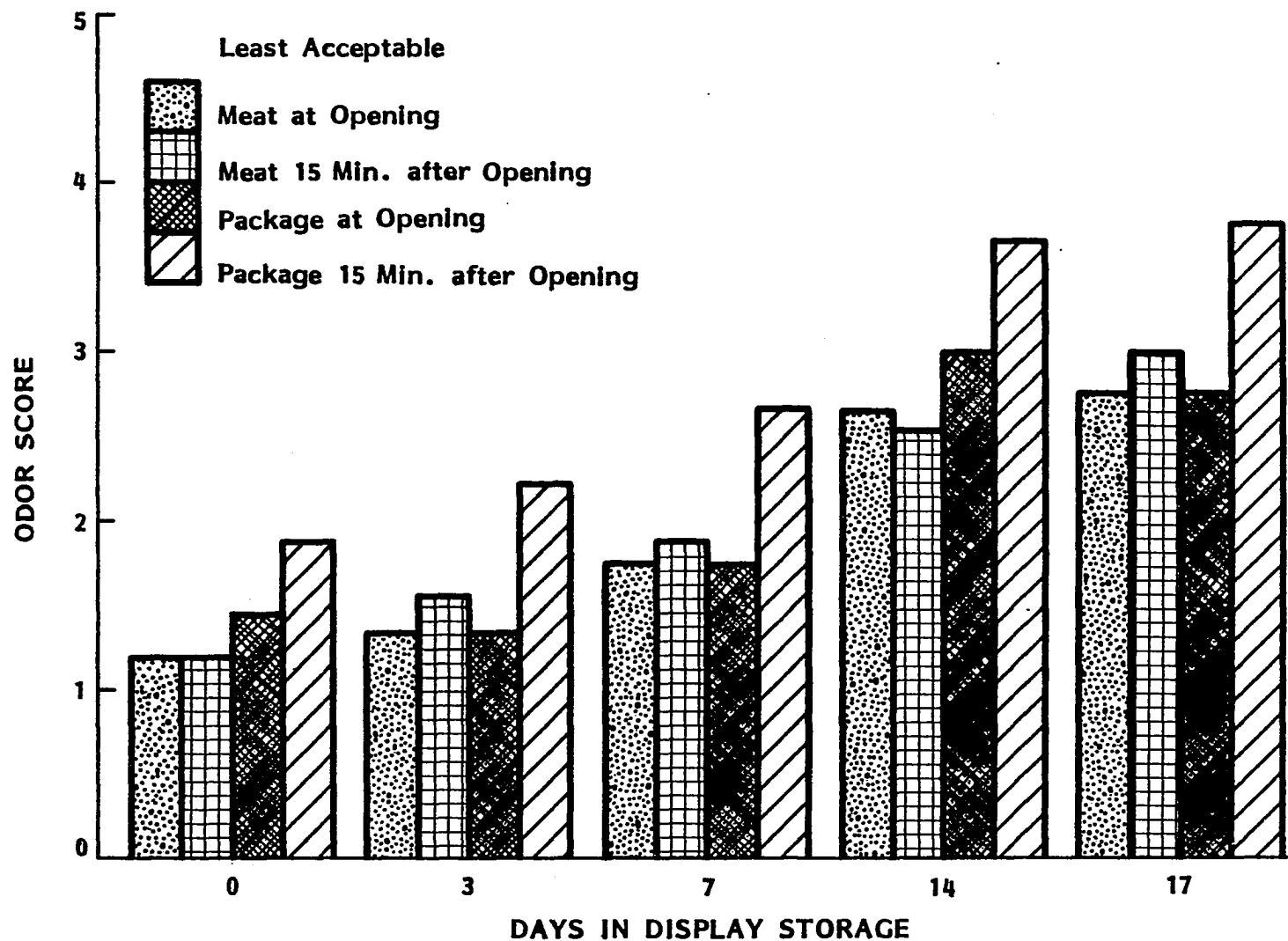


Figure 38. Odor of meat and package, at opening and 15 minutes after opening

odors. After being open to the atmosphere for 15 minutes, these volatiles were removed and higher molecular weight compounds exerted more of an effect on odor. This, of course, is speculation.

#### Effect of packaging and processing methods on color

Color in pork is much more variable than in other meats such as beef. Even though color measurements were taken at the same place on the chop each time samples were taken, there was considerable variation between chops, so much so that the data show few significant effects upon color. There was an interaction between loin storage time and film in the first study. Figure 39 illustrates this effect. Chops cut from loins immediately after slaughter, and chops cut from loins held seven days and packaged in a high barrier film showed a slight time effect with the meat gradually becoming lighter over the course of the study. Chops cut from loins held seven days and packaged in a low barrier film showed a more rapid and consistent lightening of meat color and from three days of storage onward were the lightest of all combinations of loins and films. No other packaging or processing treatment had any effect on color.

In general, it was observed that as time progressed, the meat in the vacuum packages appeared to lose its brightness and become dull by seven days of display case storage. This

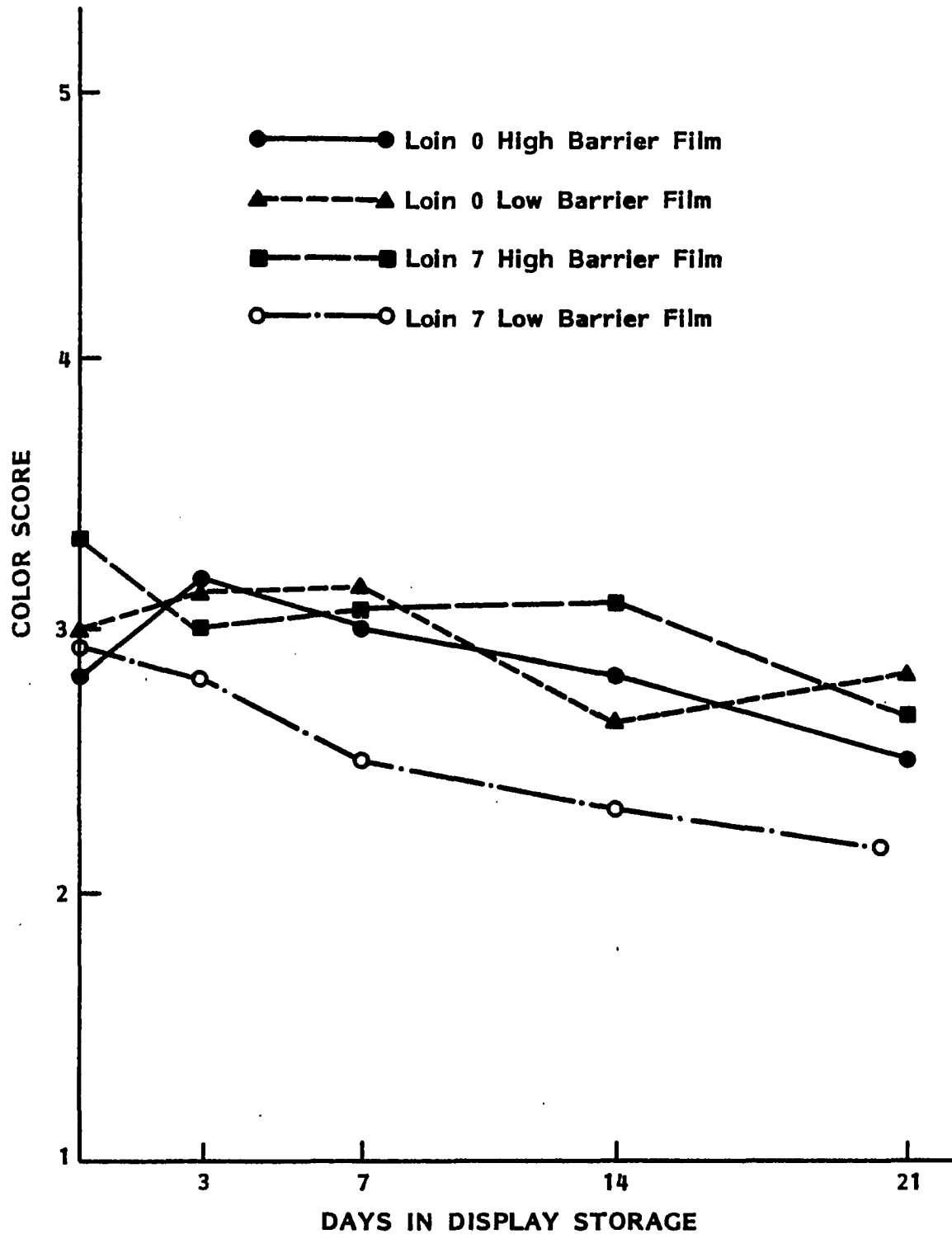


Figure 39. Interaction of loin storage time and film type with meat color score

observation is reflected in the time effect in Figure 39. At no time, however, did the vacuum packaged chops become severely discolored, even after microbial spoilage and high off odor occurred. In the aerobic, hand-wrapped packages, the chops did become severely discolored through yeast, mold and bacterial action. These results are similar to those of Adams and Huffman (1972) who found that pork in air and various gas atmospheres became lighter over a 15 day period. They attributed the change to metmyoglobin formation. However, Seideman et al. (1980) stated that extensive discoloration took place in pork chops stored as retail cuts after 7 days storage and 1 day of retail display. These results do not agree with theirs.

Fat color immediately after packaging was a pearly white. As time progressed the fat lost its white luster and began taking on a more dull white or yellowish cast, although at no time did the fat become severely yellowed. Subjective fat color showed a statistically significant increase in yellowing with days in storage, although the objective reflectance meter readings were not significant. No effects of packaging or processing methods were seen with fat color.

Comparison of subjective color judgements with the Photovolt color reflectance meter

Because subjective color measurements are dependent on uncontrollable or unreproducible conditions such as intensity and quality of light, test surroundings, individual variation in observers' judgement, and other variable factors, an objective method of color measurement for meat and fat color was tested. Table 14 shows the correlation coefficients between the subjective color judgements and the readings from the Photovolt color reflectance meter. For meat color, all of the Photovolt color filter measurements showed a high correlation with subjective judgements, the green filter having the highest correlation coefficient. When meat yellowness was calculated, however, the correlation coefficient was not significant. The fat color correlation coefficients show just the opposite results: none of the correlation coefficients from the filter measurements were significant but the yellowness calculation showed significance at the 5% level. The obvious conclusion to be drawn is that when working with different parts of the chop, different values must be used to judge color.

Table 14. Correlation coefficients of subjective color judgments with Photovolt color reflectance measurements

Photovolt color filter used	Subjective judgement	
	Meat color	Fat color
Green	-0.7226**	-0.2148
Amber	-0.6995**	0.0793
Blue	-0.6902**	-0.0224
Yellowness	-0.1502	0.2909*

\* Significant at the 5% level.

\*\* Significant at the 1% level.

#### Effect of packaging and processing methods on pH

The effect of loin storage time, film type, presence or absence of bone, and dark storage time of chops on pH was negligible. The mean pH of all samples was 5.63 with a standard deviation of 0.2 pH unit. One effect on pH that was statistically significant was that of cutting method (Table 15). In this table, it can be seen that the frozen tempered chops (saw and cleaver cut) had lowered pH values than the refrigerated saw-cut chops. At first glance, it may appear that freezing and thawing was responsible for this effect. However, Van den Berg (1961) stated that the freezing of meat causes an increase in pH, just the opposite effect of these results.

A possible explanation for these results, as well as the variable results with regard to pH in the first study, lies in the fact that there is considerable variation in pH between animals and between muscles, depending on the physiological state of the animal at slaughter, genetic tendency of the animal toward porcine stress syndrome, and other individual variations in physiology among animals. We should note here that the chops used in study two were not randomized. The chops for the various treatments all came from the same loin or group of loins. For this reason, they can be expected to have similar pH values and to differ from other treatments. Also, the analysis of variance indicating a significant difference for cutting method has an  $r^2$  value of .4337, meaning that the model explains only 43.37 percent of the variation in the data. This is a low  $r^2$  value for this data. For the above reasons, more work should be done in this area before it is concluded that cutting method affects pH.

In these studies, there was not a general rise in pH with increasing spoilage as has been observed by some authors (Turner, 1960; Pearson, 1968). However, Sutherland et al. (1975) observed that this trend did not take place in vacuum packaged beef. These results support her findings. Jaye et al. (1962) also noted that, in ground beef packed in an



oxygen impermeable film, pH tended to fall or remain constant rather than rise. They attributed this to the metabolic activity of lactic acid bacteria.

Table 15. Effect of cutting method on pH

Days in display storage	Refrigerated saw	Treatment frozen tempered saw	Frozen tempered cleaver
0	5.67	5.50	5.50
3	5.85	5.53	5.55
7	5.70	5.57	5.62
14	5.86	5.48	5.58
17	5.85	5.60	5.50

Gas chromatographic analyses performed on fat extracted from pork chops

The gas chromatographic method employed measured only fat volatiles. The method was time consuming; each sample took approximately 3 hours to run and samples could not be run simultaneously. For this reason, and because this section of the study was meant to be an initial step in further work, duplicate analyses were not performed on each sample. Examples of typical chromatographic tracings are shown on Figure 40.

Total volatiles, nonane, and total volatiles relative

Chops Held 3 Days Dark, 0 Days In Light

TVRN  $\approx$  5.96

Attenuation =  $8 \times 10^{-10}$

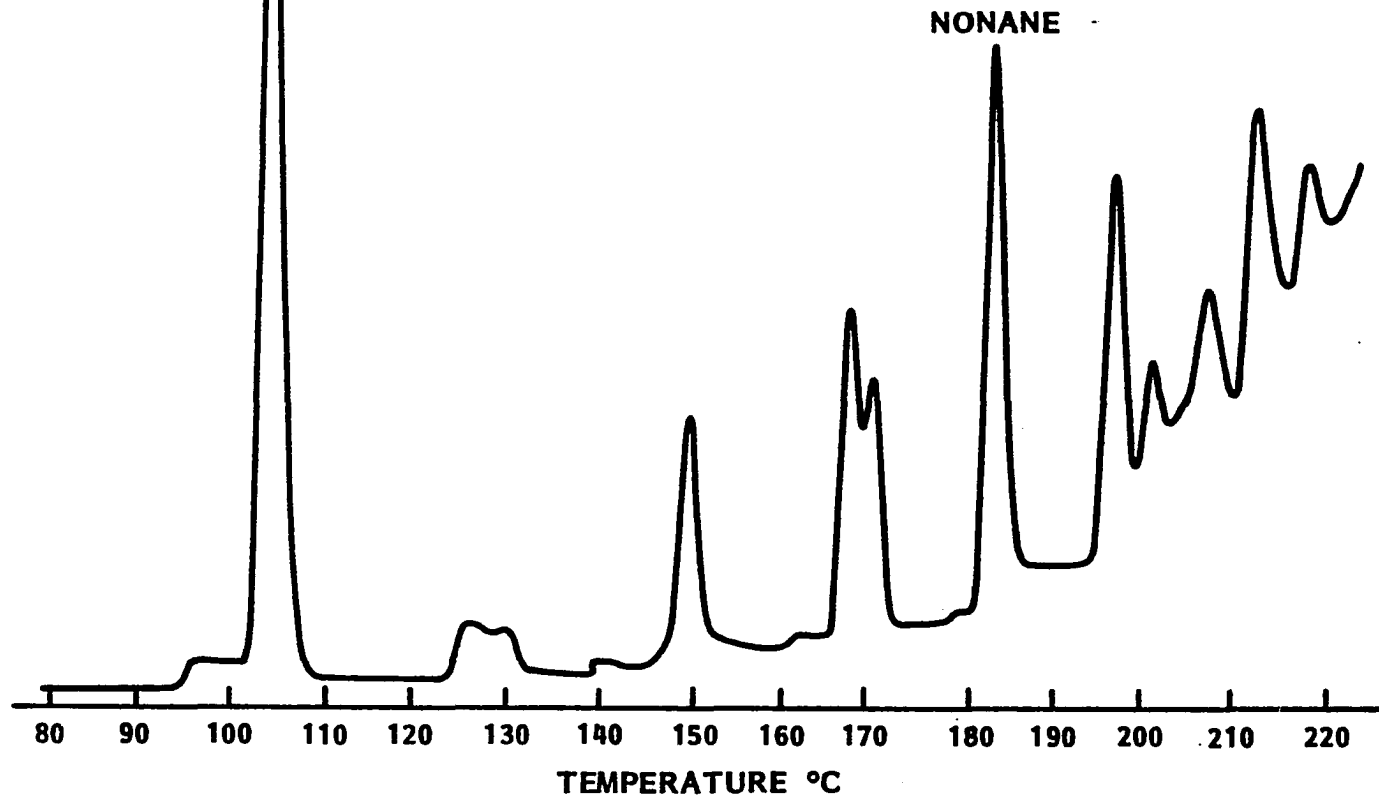


Figure 40. Chromatographic tracing of fat sample from pork chop held 3 days in dark, no days in light storage

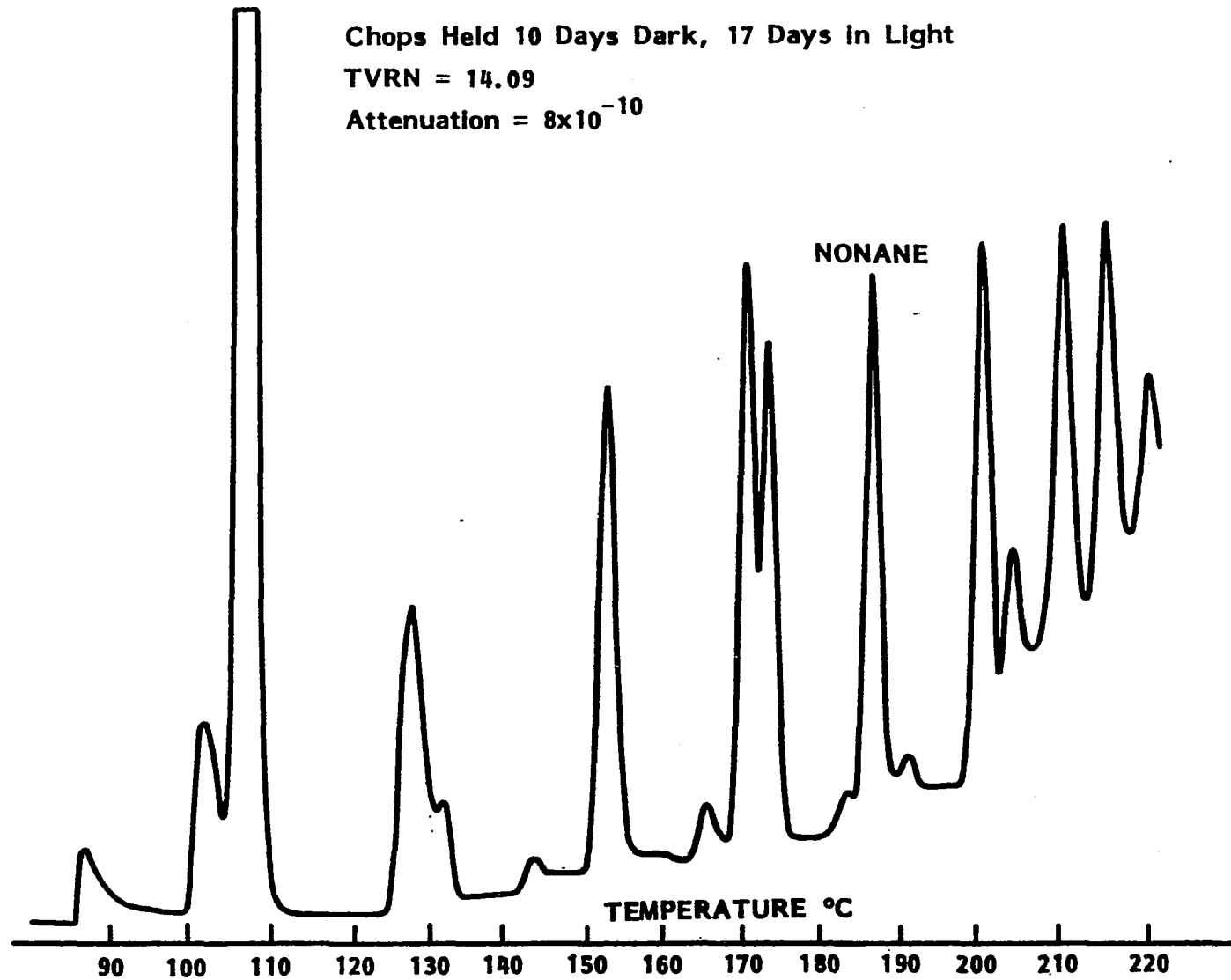


Figure 41. Chromatographic tracing of fat sample from pork chop held 10 days in dark, 17 days in light storage

to nonane (TVRN) are shown in Table 16. Keeping in mind that these values are individual analyses, not means of replications, we can see that time has relatively little effect on TVRN. Chops stored in the dark and light for different periods of time had approximately the same TVRN. The main effect on TVRN appeared to be the effect of light on the oxidation of the fat. Chops that were stored in the dark for 3 and 10 days had TVRN values of 7.71 and 7.61, respectively, while chops stored in the dark for 3 and 10 days and then exposed to display case lighting conditions had TVRN values of 12.08 and 11.62, respectively. Cutting method did not affect the TVRN value of the fat.

These results are supported by Watts (1954) who gave the example of beef kidney fat which was not rancid after 1200 hours in the dark but showed signs of rancidity after 10 minutes in sunlight. Ordonez and Ledward (1977) also discuss the effect of oxidation on pork fat. They stated that, provided oxygen is available, rancidity can take place within 6 days at 1°C.

While oxidative rancidity undoubtedly takes place in the fat in vacuum packaged pork chops, thus affecting the flavor and odor of the chops, further work in this area should also examine the role of water soluble compounds for evidence of off flavors and off odors. Dainty (1971) reported that the breakdown of fats by bacteria is relatively

Table 16. Total volatiles, nonane, and total volatiles relative to nonane for samples run

Cutting method	Days in dark storage	Days in display case	Total volatiles	Nonane	Total volatiles relative to nonane
Refrigerated saw	3	0	123.86	9.90	12.51
Frozen tempered saw	3	0	33.28	7.15	4.65
Frozen tempered cleaver	3	0	41.09	6.90	5.96
Mean					7.71
Refrigerated saw	10	0	84.75	9.18	9.23
Frozen tempered saw	10	0	94.81	12.40	7.65
Frozen tempered cleaver	10	0	41.09	6.90	5.96
Mean					7.61
Refrigerated saw	3	17	39.64	6.03	6.57
Frozen tempered saw	3	17	61.53	6.40	9.61
Frozen tempered cleaver	3	17	37.38	4.68	7.99
Mean					12.08
Refrigerated saw	10	17	140.36	13.68	10.26
Frozen tempered saw	10	17	97.97	6.89	14.09
Frozen tempered cleaver	10	17	133.41	12.69	10.51
Mean					11.62

slow so that in raw meats the lean is usually spoiled before fat breakdown becomes noticeable. Protein and amino acids possibly should be considered for contribution to odor.

#### Summary

The effects of five different packaging and processing methods on various quality changes were observed for vacuum packaged pork chops. These variables included type of packaging film, presence or absence of bone in the packaged chop, length of loin storage time prior to cutting into chops, method of cutting into chops, and length of storage time as packaged chops prior to retail display. The microflora of pork chops was characterized with regard to numbers of microorganisms, types present, and changes in numbers and types over time. Vacuum packaged pork chops were also tested with regard to organoleptic quality, drip loss, odor, color, and pH. Each of the packaging and processing treatments and their effects on quality will be summarized in order.

The presence of bone in the chops had no effect on the microbial population other than a possible qualitative change in bacterial types represented in the flora. Chops with the bone in were rated higher in all sensory qualities, especially tenderness. Leaving the bone in the meat reduced the drip loss upon opening the packaged. The only

negative effect that bone produced was for odor; chops with the bone intact had higher off odor scores than boneless chops after seven days storage. It is suggested that this is caused by a more diverse microbial community whose development is enhanced by the retention of moisture and air in pockets within the vacuum package permitted by the presence of the bone.

The packaging films used to wrap the meat had several interesting effects on keeping quality. Aerobic, hand-wrapped chops spoiled rapidly from bacterial action within three days of storage. Vacuum packaging the chops tripled the microbiological shelf life to about nine days before spoilage was apparent. Growth of mesophiles, psychrotrophs, and lactobacilli were similar in the high barrier and low barrier films, although there was a slight increase in aerobic bacterial types isolated from the low barrier film. Film type had little effect on sensory qualities, with the exception of tenderness which showed a slight improvement in the low barrier film. The high barrier film produced significantly less drip loss than the low barrier film. With regard to off odor, the high barrier film had higher off odor scores but the odor was qualitatively different, somewhat less offensive than the odor from the low barrier chops. Odor throughout the study was difficult to characterize both quantitatively and qualitatively. When loins were

stored for seven days and packaged in low barrier film, the chops became more discolored than chops cut from loins that were not first held, or than chops that were packaged in high barrier film. Film type had no effect on the pH of the chops.

Holding loins for seven days prior to cutting showed no effect on the microbiological quality of the chops other than a slight increase in the initial population of psychrotrophic bacteria. Chops cut from loins immediately after slaughter, however, scored higher sensory scores for flavor, juiciness, and overall acceptability. Tenderness scores were not different for the two loin storage periods. Loin storage period had no effect on odor, drip loss, color or pH.

Cutting method had an effect on the growth of psychrotrophic bacteria and Lactobacillus. The loins which were subjected to a freeze tempering process prior to being cut showed faster growth of psychrotrophs and lactobacilli than the refrigerated loins. The cutting method had no effect on sensory scores but had a large effect on drip loss. Chops from frozen tempered loins, saw or cleaver-cut, had higher drip loss than the refrigerated, saw-cut loins. From analysis of the data, it was estimated that 70% of this effect was due to the freezing and thawing of the meat and 30% due to the cutting method. Consistent with the microbiological results, odor for the refrigerated, saw-cut chops was slightly



lower than chops cut with other methods. Cutting method had no effect on color. The pH of the refrigerated saw-cut chops appeared to be higher than the pH of the chops subjected to freeze tempering but this may not be due to the cutting method as such, but rather individual variation.

A dark storage period at low temperature caused inhibition of mesophilic and Lactobacillus growth but growth rate increased for the stored chops after the dark storage period and holding in a lighted display case. Psychrotrophic bacteria were less inhibited by the dark storage period than the other types specified. During the dark storage period, the microbial flora was changed somewhat, favoring facultative anaerobes and psychrotrophs. Chops stored for 10 days had lower sensory quality scores for flavor and overall acceptability than chops stored for 3 days. Drip loss was slightly higher for chops stored for the longer period, as was odor after comparable time in the display case except for the initial sampling period. Dark storage period had no effect on color or pH.

The succession of the microbial flora within the vacuum package was followed. Initially the flora consisted of mostly aerobic bacteria. After one week storage pseudomonads reached their highest proportion of the flora, approximately 20% of isolates. From one week onward, the

facultatively anerobic Enterobacteriaceae and Lactobacillus dominated the flora, being 89% of total isolates at the end of four weeks.

The fate of potential food borne pathogens, Staphylococcus, Salmonella, and Clostridium perfringens was followed. Salmonella was not isolated during the course of the study. Clostridium perfringens had a very low recovery rate, and the population of Staphylococcus became steadily lower as time progressed in cold storage. None of the potential pathogens appears to be a problem in vacuum packaged pork chops.

Gas chromatographic data showed that light had an important effect in causing oxidation of the fat in the chops.

### Conclusions

1. The initial microbial flora of vacuum packaged pork chops consists of a mixed culture of mostly aerobic bacteria. Under vacuum packaged cold storage conditions, facultative anaerobes soon become the dominant flora.
2. Vacuum packaging pork chops in either a high barrier or low barrier film greater enhances shelf life when compared to stretch wrapping the chops on trays.
3. Chops with the bone in are superior in sensory qualities and percent drip loss to boneless chops. Chops with bone intact, however, have higher odor upon long term

storage.

4. High barrier film is superior to low barrier film with regard to drip loss.

5. Loins may be held for up to one week prior to cutting into chops without adverse impact on quality in the first few days after cutting.

6. Loins which are frozen and thawed prior to cutting have higher drip loss and slightly higher bacterial growth than loins which do not undergo the freeze thawing process.

7. Storage in the dark inhibits growth of most bacteria for the duration of the dark storage period. Subsequently, however, spoilage is more rapid.

8. During dark storage at low temperatures, the microflora within the vacuum package is not static, but changes to favor facultative anaerobic and psychrotrophic bacteria.

9. The potential food borne pathogens, Staphylococcus, Salmonella, and Clostridium perfringens, do not pose a significant hazard in vacuum packaged pork chops.

10. The Photovolt color reflectance meter may be used as an objective method of meat and color measurement. When used on meat the reading for the green filter should be used (correlation significant at 1% level). When used to determine

fat color the yellowness calculation gives the most accurate value of the fat color (correlation significant at 5% level).

11. Light enhances the oxidation of pork fat under display case conditions.

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APPENDIX A: STATISTICAL ANALYSIS OF DATA

Table 17. Analysis of variance for packaging and processing variables, first study

Variable	Source	DF	Sum of squares	Mean square	F value
Mesophiles	Model	43	374.00	8.69	11.77
	Error	76	56.15	0.73	-
	Loin	1	2.71	-	3.67
	Bone	1	1.29	-	1.75
	Film	1	0.04	-	0.06
	Day	4	325.05	0	109.99**
Psychrotrophs	Model	43	670.59	15.59	8.83
	Error	76	134.26	1.76	-
	Loin	1	0.25	-	0.15
	Bone	-	0.23	-	0.14
	Film	1	0.02	-	0.01
	Day	4	524.08	-	74.17**
Lactobacillus	Model	43	473.16	11.00	6.57
	Error	76	127.27	1.67	-
	Loin	1	0.02	-	0.01
	Bone	1	1.97	-	1.18
	Film	1	0.02	-	-0.00
	Day	4	406.84	-	60.73**
Staphylococcus	Model	43	62.18	1.44	1.18
	Error	76	92.92	1.22	-
	Loin	1	0.47	-	0.39
	Bone	1	0.01	-	0.00
	Film	1	0.02	-	0.01
	Day	4	25.69	-	5.25**
Color	Model	43	30.03	0.69	1.11
	Error	76	47.83	0.63	-
	Loin	1	0.30	-	0.48
	Bone	1	1.63	-	2.60
	Film	1	1.20	-	1.91
	Day	4	4.53	-	1.80
	Loin*Film	1	2.70	-	4.28*

\* Significant at the 5% level.

\*\* Significant at the 1% level.

Table 17 (Continued)

Variable	Source	DF	Sum of squares	Mean square	F value
Odor	Model	39	66.96	1.71	5.48
	Error	40	12.52	0.31	-
	Loin	1	0.31	-	1.00
	Bone	1	2.81	-	8.98**
	Film	1	0.11	-	0.36
	Day	4	42.92	-	34.27**
	Day*Bone	4	4.62	-	3.69*
pH	Model	39	2.59	0.06	1.09
	Error	40	2.42	0.06	-
	Loin	1	0.01	-	0.02
	Bone	1	0.06	-	0.10
	Film	1	0.01	-	0.17
	Day	4	0.11	-	0.48
	Drip loss	Model	39	202.28	5.18
Error		40	61.90	1.54	-
Loin		1	0.83	-	0.54
Bone		1	12.17	-	7.87**
Film		1	98.72	-	63.79**
Day		4	16.31	-	2.64*
Flavor		Model	36	22.50	0.62
	Error	59	20.73	0.35	-
	Loin	1	2.94	-	8.37**
	Bone	1	0.92	-	4.47*
	Film	1	0.00	-	0.00
	Day	3	4.67	-	4.44**
	Tenderness	Model	36	28.16	0.78
Error		59	41.60	0.70	-
Loin		1	0.14	-	0.20
Bone		1	3.04	-	4.32*
Film		1	2.70	-	3.82
Day		3	5.11	-	2.42
Juiciness		Model	36	28.16	0.78
	Error	59	41.60	0.70	-
	Loin	1	0.14	-	0.20
	Bone	1	3.04	0	4.32*
	Film	1	2.70	-	3.83
	Day	3	5.11	-	2.42

Table 17 (Continued)

Variable	Source	DF	Sum of squares	Mean square	F value
Overall	Model	36	41.95	1.16	2.12
	Error	59	32.38	0.54	-
	Loin	1	3.08	-	3.55
	Bone	1	0.26	-	0.47
	Film	1	0.30	-	0.55
	Day	3	7.84	-	4.76**

Table 18. Analysis of variance for packaging and processing variables, second study

Variable	Source	DF	Sum of squares	Mean square	F value
Mesophiles	Model	23	280.59	12.19	15.84
	Error	66	50.82	0.77	-
	Cuttin method	2	14.60	-	1.69
	Dark storage	1	21.89	-	28.43**
	Day	4	227.87	-	73.98**
Psychrotrophs	Model	23	442.45	19.23	13.49
	Error	66	94.08	1.42	-
	Cutting method	2	10.41	-	3.65*
	Dark storage	1	31.32	0	21.98**
	Day	4	366.91	-	64.35**
Lactobacillus	Model	23	377.85	16.42	8.85
	Error	66	122.53	1.85	-
	Cutting method	2	9.24	-	2.49
	Dark storage	1	24.17	0	13.02**
	Day	4	318.49	-	42.89**
Drip loss	Model	23	51.72	2.24	3.56
	Error	66	41.64	0.63	-
	Cutting method	2	21.31	-	16.89**
	Dark storage	1	2.58	-	4.09*
	Day	4	0.90	-	0.36
Meat color	Model	23	22.24	0.96	1.00
	Error	66	63.71	0.96	-
	Cutting method	2	2.28	-	1.19
	Dark storage	1	0.17	-	0.18
	Day	4	1.73	-	0.45
Fat color	Model	23	12.15	0.52	1.86
	Error	66	18.17	0.28	-
	Cutting method	2	2.22	-	3.91
	Dark storage	1	0.04	-	0.16
	Day	4	5.55	-	4.89**

\* Significant at the 5% level.

\*\* Significant at the 1% level.

Table 18 (Continued)

Variable	Source	DF	Sum of squares	Mean square	F value
Meat odor at opening	Model	23	49.83	2.16	5.51
	Error	66	25.95	0.39	-
	Cutting method	2	0.28	-	0.37
	Dark storage	1	4.90	0	12.46**
	Day	4	40.17	-	25.34**
Package odor at opening	Model	23	50.92	2.21	4.69
	Error	66	31.17	0.47	-
	Cutting method	2	0.48	-	0.49
	Dark storage	1	2.50	-	5.29**
	Day	4	41.48	-	21.96**
pH	Model	23	2.05	0.08	2.20
	Error	66	2.68	0.04	-
	Cutting method	2	1.14	-	14.07**
	Dark storage	1	0.05	-	1.45
	Day	4	0.07	-	0.46
Flavor	Model	15	11.27	0.75	2.29
	Error	38	12.44	0.32	-
	Cutting method	2	0.73	-	1.11
	Dark storage	1	2.53	-	7.74**
	Day	2	3.55	-	5.43**
Tenderness	Model	15	10.82	0.72	1.43
	Error	38	19.18	0.50	-
	Cutting method	2	0.59	-	0.59
	Dark storage	1	0.16	-	0.33
	Day	2	0.01	-	0.01
Juiciness	Model	15	5.46	0.36	0.75
	Error	38	18.44	0.48	-
	Cutting method	2	0.04	-	0.04
	Dark storage	1	0.04	-	0.34
	Day	2	0.04	-	0.04
Overall	Model	15	8.83	0.58	1.56
	Error	38	14.31	0.37	-
	Cutting method	2	0.56	-	0.75
	Dark storage	1	1.40	-	3.72
	Day	2	2.22	-	2.95

APPENDIX B: NUMERICAL DATA FOR FIGURES

Table 19. Effect of bone on the growth of mesophiles, psychrotrophs, and lactobacillus

Days in display storage	Log no. bacteria per cm <sup>2</sup>					
	Mesophiles		Psychrotrophs		Lactobacillus	
	Bone in	Bone out	Bone in	Bone out	Bone in	Bone out
0	3.19	2.67	2.06	1.45	0.27	0.10
3	3.07	3.12	2.56	2.64	0.19	0.24
7	4.68	4.33	4.11	4.71	1.05	1.53
14	6.45	6.28	6.65	6.49	4.34	4.18
21	6.98	6.92	6.77	7.30	3.67	4.73



Table 20.. Effect of film on the growth of mesophiles<sup>a</sup>

Days in display storage	Log no. bacteria per cm <sup>2</sup>		
	High Barrier	Low Barrier	Hand-Wrapped
0	2.98	2.88	2.88
3	3.14	3.06	6.03
7	4.43	4.58	8.03
14	6.38	6.35	8.98
21	7.01	6.89	9.40

<sup>a</sup>For high barrier and low barrier, N=12. For hand-wrapped, N=4.

Table 21. Effect of film on the growth of psychrotrophs<sup>a</sup>

Days in display storage	Log no. bacteria per cm <sup>2</sup>		
	High Barrier	Low Barrier	Hand-Wrapped
0	1.25	2.26	0.00
3	2.71	2.49	6.26
7	4.51	4.31	7.55
14	6.63	6.50	8.83
21	7.33	6.74	9.44

<sup>a</sup>For high barrier and low barrier, N=12. For hand-wrapped, N=4.

Table 22. Effect of film on the growth of lactobacillus<sup>a</sup>

Days in display storage	Log no. bacteria per cm <sup>2</sup>		
	High Barrier	Low Barrier	Hand-Wrapped
0	0.17	0.20	0.00
3	0.18	0.25	2.86
7	1.38	1.20	4.95
14	4.27	4.25	0.00
21	4.17	4.22	0.00

<sup>a</sup>For high barrier and low barrier, N=12. For hand-wrapped, N=4.

Table 23. Effect of loin holding time on the growth of mesophiles, psychrotrophs, and lactobacillus

Days in display storage	Log no. bacteria per cm <sup>2</sup>					
	Mesophiles		Psychrotrophs		Lactobacillus	
	Loin 0	Loin 7	Loin 0	Loin 7	Loin 0	Loin 7
0	2.90	2.96	1.38	2.14	0.38	0.00
3	3.08	3.12	2.63	2.57	0.14	0.29
7	4.77	4.24	4.73	4.08	2.02	0.56
12	6.66	6.07	6.93	6.21	4.05	4.48
21	7.20	6.70	6.93	7.14	3.50	4.90

Table 24. Effect of cutting method on the growth of mesophiles

Days in display storage	Log no. bacteria per cm <sup>2</sup>		
	Refrigerated saw	Freeze thaw saw	Freeze thaw cleaver
0	2.15	1.99	2.49
3	3.89	4.25	4.25
7	4.98	4.77	5.94
14	6.41	6.46	6.52
17	6.23	6.58	6.40

Table 25. Effect of cutting method on the growth of Lactobacillus

Days in display storage	Log no. bacteria per cm <sup>2</sup>		
	Refrigerated saw	Freeze thaw saw	Freeze thaw cleaver
0	0.32	0.41	0.00
3	1.44	2.30	1.87
7	2.83	3.62	4.40
14	5.05	5.47	5.52
17	3.92	5.13	5.20

Table 26. Effect of cutting method on the growth of psychrotrophs

Days in display storage	Log no. bacteria per cm <sup>2</sup>		
	Refrigerated saw	Freeze thaw saw	Freeze thaw cleaver
0	0.90	0.94	1.22
3	2.86	4.09	4.45
7	4.29	4.78	5.84
14	6.31	6.47	6.58
17	6.12	6.66	6.52

Table 27. Effect of dark storage period on the growth of mesophiles, psychrotrophs, and Lactobacillus

Days in display storage	Log no. bacteria per cm <sup>2</sup>					
	Mesophiles		Psychrotrophs		Lactobacillus	
	3 days	10 days	3 days	10 days	3 days	10 days
0	2.26	2.16	0.40	1.64	0.14	0.34
3	3.23	5.01	2.79	4.80	1.37	2.37
7	4.56	5.90	4.33	5.61	2.67	4.57
14	5.86	7.06	5.90	7.01	5.00	5.69
17	6.04	6.77	6.30	6.56	4.05	5.45

Table 28. Composition of the microbial flora on vacuum packaged pork chops from 0 to 27 days storage

Organism	Days storage in high barrier film							
	0-3		6-10		13-17		20-27	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
<u>Enterobacteriaceae</u>	5	7.81	45	36.00	66	44.59	66	52.80
<u>Lactobacillus</u>	6	9.38	24	19.20	47	31.76	45	36.00
<u>Pseudomonas</u>	2	3.13	25	20.00	15	10.14	1	1.00
<u>Staphylococcus</u> and <u>Micrococcus</u>	35	54.69	16	12.80	2	1.36	2	1.60
Other	16	24.99	15	12.00	18	12.15	11	8.60
TOTAL	64		125		148		125	

Table 29. Growth of Staphylococcus on vacuum packaged fresh pork

Days in display storage	Log no. bacteria per cm <sup>2</sup>
0	1.74
3	1.61
7	1.48
14	0.81
21	0.58

Table 30. Effect of film on organoleptic quality

Days in display storage	Sensory score							
	Flavor		Tenderness		Juiciness		Overall	
	High Barrier	Low Barrier	High Barrier	Low Barrier	High Barrier	Low Barrier	High Barrier	Low Barrier
0	5.25	5.17	4.82	4.96	4.99	4.99	4.98	4.93
3	4.99	5.15	4.94	5.32	4.78	5.13	4.77	5.04
7	5.22	5.25	5.28	5.78	4.81	5.06	4.99	5.17
14	4.73	4.64	5.02	5.35	4.38	4.22	4.57	4.52



Table 31. Effect of loin storage time on organoleptic quality

Days in display storage	Sensory score							
	Flavor		Tenderness		Juiciness		Overall	
	0 Days	7 Days	0 Days	7 Days	0 Days	7 Days	0 Days	7 Days
0	5.59	4.84	4.87	4.90	5.33	4.65	5.35	4.57
3	5.36	4.79	5.13	5.12	5.24	4.67	5.14	4.67
7	5.25	4.95	5.62	5.44	5.08	4.79	5.29	4.87
14	4.44	4.93	5.25	5.12	4.25	4.36	5.38	4.70

Table 32. Effect of bone on organoleptic quality

Days in display storage	Sensory score							
	Flavor		Tenderness		Juiciness		Overall	
	In	Out	In	Out	In	Out	In	Out
0	5.27	5.16	5.21	4.57	5.00	4.98	5.05	4.87
3	5.23	4.92	5.25	5.01	5.07	4.85	5.09	4.72
7	5.42	5.06	5.76	5.31	5.00	4.87	5.22	4.94
14	4.68	4.69	5.23	5.14	4.33	4.27	4.52	4.56

Table 33. Effect of cutting method on organoleptic quality

Cutting method	Days in display storage	Flavor	Tenderness	Juiciness	Overall
Refrigerated saw	0	5.73	5.97	4.97	5.58
	3	5.98	6.12	5.28	5.62
	7	4.98	5.62	4.72	4.75
Freeze thaw saw	0	5.70	5.42	4.87	5.33
	3	6.20	6.07	5.10	5.92
	7	5.30	5.58	5.02	5.20
Freeze thaw cleaver	0	6.12	5.88	5.32	5.88
	3	5.60	5.12	4.62	5.25
	7	5.78	6.00	5.22	5.55

Table 34. Effect of dark storage period on organoleptic quality

Days in display storage	Sensory score							
	Flavor		Tenderness		Juiciness		Overall	
	3 Days	10 Days	3 Days	10 Days	3 Days	10 Days	3 Days	10 Days
0	6.15	5.58	5.62	5.89	4.82	5.82	5.69	5.51
3	6.03	5.82	5.69	5.84	5.00	5.00	5.67	5.52
7	5.61	5.10	6.11	5.35	5.04	4.92	5.49	4.84
14	5.07	-	5.83	-	4.90	-	5.03	-
17	4.87	-	5.27	-	4.20	-	4.93	-

Table 35. Effect of dark storage period on drip loss

Days in display storage	Percent drip loss	
	3 days storage	10 days storage
0	1.37	2.31
3	1.72	1.73
7	1.51	1.69
14	1.19	1.92
17	1.79	1.62

Table 36. Effect of bone on drip loss

Days in display storage	Percent drip loss	
	Bone in	Bone out
0	2.33	2.12
3	2.49	3.35
7	2.62	3.57
14	2.84	4.30
17	2.86	3.71

Table 37. Effect of film type on drip loss

Days in display storage	Percent drip loss	
	High barrier film	Low barrier film
0	1.15	3.30
3	1.81	4.03
7	1.99	4.20
14	2.34	4.80
21	2.25	4.31

Table 38. Interaction of film type, bone, and days in display storage with drip loss

Days in display storage	Percent drip loss			
	High barrier		Low barrier	
	Bone in	Bone out	Bone in	Bone out
0	1.13	1.16	3.53	3.07
3	1.25	2.37	3.73	4.33
7	1.45	2.53	3.79	4.61
14	2.30	2.38	3.39	6.21
17	2.14	2.36	3.57	5.05

Table 39. Effect of cutting method on drip loss

Days in display storage	Percent drip loss		
	Refrigerated saw	Freeze thaw saw	Freeze thaw cleaver
0	0.89	2.39	2.23
3	1.06	1.60	2.51
7	0.97	1.72	2.09
14	1.13	1.56	1.98
17	1.13	1.76	2.23

Table 40. Effect of film on odor score

Days in display storage	Odor score	
	High barrier	Low barrier
0	1.00	1.00
3	1.37	1.25
7	1.87	1.75
14	2.00	2.12
21	3.25	3.00

Table 41. Effect of cutting method on odor

Days in display storage	Odor score		
	Refrigerated saw	Freeze thaw saw	Freeze thaw cleaver
0	1.16	1.33	1.33
3	1.67	1.67	1.83
7	1.67	2.33	2.17
14	3.16	2.83	2.50
17	3.00	3.17	3.00

Table 42. Effect of bone on odor score

Days in display storage	Odor score	
	Bone in	Bone out
0	1.00	1.00
3	1.25	1.37
7	1.87	1.75
14	2.62	1.50
21	3.50	2.75

Table 43. Effect of dark storage on odor

Days in display storage	Odor score at opening	
	3 Days storage	10 Days storage
0	1.22	1.33
3	1.33	2.11
7	1.78	2.33
14	2.67	3.00
17	2.78	3.33

Table 44. Odor of meat and package, at opening and 15 minutes after opening after 3 days dark storage

Days in display storage	Odor score			
	Meat		Package	
	Opening	After	Opening	After
0	1.22	1.22	1.44	1.89
3	1.33	1.56	1.33	2.22
7	1.78	1.89	1.78	2.67
14	2.67	2.56	3.00	3.67
17	2.78	3.00	2.78	3.78

Table 45. Interaction of loin storage time and film with meat color score

Days in display storage	Color score			
	Loin 0		Loin 7	
	High barrier	Low barrier	High barrier	Low barrier
0	2.83	3.00	3.33	3.00
3	3.17	3.17	3.00	2.83
7	3.00	3.17	3.17	2.50
14	2.83	2.66	3.17	2.33
21	2.50	2.83	2.67	2.17