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PACKAGING PROCEDURES TO EXTEND THE SHELFLIFE OF FRESH PORK

THE UNIVERSITY OF ARIZONA M.S. 1985

PACKAGING PROCEDURES TO EXTEND THE SHELFLIFE OF FRESH PORK

by

Antonio Bojorquez Romo

A Thesis Submitted to the Faculty of the

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE
WITH A MAJOR IN FOOD SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

John Anton Marchello
Professor of Animal Science

2/22/85
Date

ACKNOWLEDGMENTS

I wish to thank all my professors and friends who helped me to accomplish this thesis project. To Dr. John Anton Marchello, for his advice and support; to Dr. Ron Allen and Dr. James W. Berry for serving on my committee; and to Tim Carr, Mario Ochoa, and Hamdi Ahmad for their technical assistance.

I would also like to express my sincere appreciation to my mother, my family, and the Fisher family, for their encouragement and support that enabled me to reach this goal.

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ABSTRACT

Pork Loin chops were used to determine effects of controlled gas atmosphere packaging on microbial growth, color, shrinkage, and fat oxidation during storage. Packaging treatments were: film overwrapping; vacuum packaging; two controlled atmospheres (15% CO_2 + 85% N_2, and 15% CO_2 + 40% O_2 + 45% N_2): and two modified vacuum packaging treatments in which the samples were sprayed with either 1% LA or 1% AA before packaging.

In Trials I and II, except once, vacuum packaged chops had the lowest psychrotrophic and Lactobacilli counts throughout storage. Chops packaged in atmospheres containing 15% CO_2 + 40% O_2 + 45% N_2 had the greatest ($P<.05$) counts for both kinds of flora at the end of the storage time. In Trials III and IV, chops packaged under vacuum-(AA) had the lowest Lactobacilli counts. Except once, film overwrapped chops had higher ($P<.05$) microbial counts than those chops packaged under vacuum, vacuum-(LA) and vacuum-(AA), throughout storage. Frequently, color readings from Trials II, III, and IV showed an increase in meat surface discoloration with increased storage time. Fat oxidation was not detected in any of the trials.

INTRODUCTION

Conventionally, meat carcasses have been the most common raw material shipped into the backrooms of stores for breaking and cutting into retail cuts, which are then packaged and displayed. Due to the very little influence that this packaging system has on the keepability of fresh meat, and due to the recent increases in costs for transportation, labor, equipment and storage space, the necessity for a more appropriate packaging system has become a reality.

In the last few years, many attempts have been made to approach this ideal packaging procedure. Although they may differ from one another, they all share the same two basic principles: (i) processing of carcasses into primal, subprimal, or even consumer-sized cuts at a central location, and (ii) the use of vacuum or modified atmosphere packaging, to prolong the shelflife of fresh meat by minimizing microbial growth, discoloration, shrink loss, and any other factor that could affect meat quality.

The objective of the present study was to compare the effect of different packaging treatments on the quality of fresh pork stored for 9 days at 4°C.

LITERATURE REVIEW

Microbiology of Meat

Muscle tissue of healthy living animals contains few or no microorganisms. They are introduced during various processes related to handling, slaughtering and dressing of animals (Ayres 1955 and Frazier 1967). Ingram and Dainty (1971) reported that under normal methods of production, meat becomes contaminated with a great diversity of microorganisms in substantial numbers and from this spectrum of possibility, the appropriate organisms are selected by particular conditions of treatment or storage.

Blickstad, Enfors and Molin (1981) observed that the initial microflora of pork taken directly from the process line was dominated in order by Flavobacterium spp., Acinetobacter calcoaceticus, Pseudomonas spp., Micrococcus spp., and Moraxella spp. Together these organisms constituted, on average, 80% of the flora.

During refrigeration at temperatures not greatly higher than 0°C, the microorganisms continue to develop, and they do it on the surface, unless the meat has been treated (e.g., by piercing or mincing) so as to distribute extrinsic bacteria throughout the mass (Ingram and Dainty 1971). Under these storage conditions, the microflora becomes dominated by the

Pseudomonas-Achromobacter group (Clark and Lentz 1969, Gardner, Carson and Patton 1967 and Halleck, Ball and Stier 1958). According to Blickstad et al. (1981) and Enfors, Molin and Ternstrom (1979), after storage on air (4°C) during a week period, more than 90% of the flora of pork meat consisted of non-fluorescent Pseudomonas spp. However, Halleck et al. (1958) determined that the non-fluorescent Pseudomonas-Achromobacter type organisms no longer dominated the bacterial flora in the latter part of a four-week storage period. At that time, about 80% of the total count was constituted by bacteria of the Pseudomonas-fluorescent type.

Halleck et al. (1958) did not find an influence of the kind of meat on bacterial growth in prepackaged meat. Leanness or fatness of the meat apparently did not influence the rate of bacterial growth either, but the initial counts obtained from a fatty meat usually were tenfold higher than those from lean meat. Recently, Blickstad and Molin (1983) emphasized that the composition of the microbial flora was very similar, both on fat and lean surfaces of pork meat. However, a tendency towards increasing incidences of Altermonas putrefaciens (strong spoilage bacteria) was detected.

Factors That Affect Microbial Growth

The nature and rate of microbial growth is governed by different factors.

Temperature. The decrease in microbial growth by cool temperatures may be due partially to the delay of metabolism of microorganisms (e.g., respiration), the increased inhibitory effect of carbon dioxide and the increased susceptibility of microorganisms to other restrictive factors like dryness (Ingram 1962).

Holland (1977) mentioned that potential food poisoning bacteria such as Staphylococcus aureus, Clostridium perfringens and Salmonella, usually are not a problem since they are not capable of growing at low refrigeration temperatures. On the other hand, Hanna, Zinc, Carpenter and Vanderzant (1976) postulated that Yersinia enterocolytica-like organisms could be a potential health hazard due to their ability to grow at refrigeration temperatures in vacuum conditions.

Initial Psychrotrophic Flora. Newton and Gill (1980) concluded that the initial psychrotrophic flora has an influence in the spoilage pattern followed by meat. Studies done by Halleck et al. (1958) showed that the initial bacterial count influences the rate and duration of active bacterial growth. These studies also showed that by increasing the initial bacterial counts by inovulation with non-pigmented Achromobacter-pseudomonads cells, an earlier population maximum of similar magnitude to those of non-inoculated material was reached.

Oxygen. When pork meat was stored in air, at cool temperatures, the increase of the bacterial counts was much faster than it was in carbon dioxide, and the flora was dominated by Pseudomonas species (Blickstad and Molin 1983).

To effect aerobic bacterial growth, the oxygen concentration must be drastically reduced (Baran, Kraft and Walker 1970 and Clark and Lentz 1969).

Carbon Dioxide. The ability of carbon dioxide (>10%) to retard the growth of Gram-negative spoilage flora of meat has been reported (Clark and Lentz 1972). These authors claimed that 15% carbon dioxide was the preferred concentration since it was nearly as effective at 20% and almost twice as effective as 10%. Blickstad et al. (1981) proposed that high partial pressures of carbon dioxide have a considerable shelflife prolonging effect by selecting the microflora towards Lactobacillus spp. and by reducing the growth rate of these Lactobacillus spp. According to Silliker and White (1980), lactic acid bacteria, such as Streptococci and Lactobacilli, are less affected by elevated levels of carbon dioxide.

The mechanism of the inhibitory effect produced by carbon dioxide has been studied by King and Nagel (1967 and 1975). They strongly believe that the inhibitory effect of this gas on unbuffered biological systems is in part due to pH change. Furthermore, they mentioned that carbon dioxide may have a

small effect upon all enzymatic reaction rates and that there is a linear relationship between the amount of inhibition and the concentration of carbon dioxide and bicarbonate ion in solution.

pH. Jay (1978) mentioned that it has been well established that most microorganisms grow best at neutral pH. Bacteria tend to be more fastidious in their relationships to pH than molds and yeasts, with the pathogenic bacteria being the most fastidious. With respect to the keeping quality of meats, it has been noted that muscle which is deficient in glycogen is characterized by a high ultimate pH (>6.0). The high pH allows growth of potent spoilage organisms, notably Alteromonas putrefaciens, which have the ability to produce strong spoilage odors. This results in bacterial spoilage becoming evident at an early stage of growth of the meat microflora (Newton and Gill 1980). According to Gill and Newton (1982), Pseudomonas spp. are the microorganisms less affected by pH changes of normal meat, followed by members of the Enterobacteriaceae group.

Humidity. The water activity of most fresh foods is above 0.99, and the approximate minimum water activity for the growth of most spoilage microorganisms is 0.91 (Jay 1978). The water activity of raw meat is near to 0.985, well above the limit for Pseudomonas-Achromobacter organisms (Ingram 1962). This explains why this type of organisms is found on

the meat, forming a superficial slime, under humid conditions (Ingram and Dainty 1971). In this same study, the reported that if conditions are such that the meat surface becomes dry, bacterial growth in hindered and the surface is attacked by molds.

Concentration of Glucose. Newton and Gill (180) concluded that meat spoilage is a result of microbial attack upon amino acids, but this is delayed until preferentially utilized carbohydrate substrates are exhausted at the meat surface. For most bacteria the only significant carbohydrate present in meat is glucose. These same investigators also stated that the absence from "dark, firm, dry" meat of the small quantity of glucose which occurs in normal meat results in immediate attack on amino acids so that spoilage odors and flavors become evident at far lower cell densities than normal.

Spoilage

The spoilage of refrigerated meat is usually caused by common aerobic psychrophilic bacteria such as Pseudomonas spp. (Clark and Lentz 1969 and 1972, Ingram and Dainty 1971, and Seideman, Carpenter, Smith, Dill and Vanderzant 1979). These organisms have proteolytic and lipolytic activities, which are responsible for quality deterioration (Christopher, Carpenter, Dill, Smith and Vanderzant 1980a). Ingram and Dainty (1971) reported that by the time the supervidial numbers exceed 10^7/ sq. cm the meat has a distinct off-odor and it begins to

feel tacky - the first stage in slime formation - when the numbers reach 10^8/sq. cm. On the other hand, Gardner et al. (1967) pointed out that, if the Pseudomonas-Achromobacter element is suppressed (e.g., bu packaging with impermeable film), the flora will become dominated by strains more tolerant to lower oxygen and higher carbon dioxide concentrations, such as Enterobacter-Hafnia spp., Microbacterium thermosphactum and Lactobacilli spp. In these circumstances, the putrid odors of spoilage do not appear, and the bacterial numbers may rise to high levels (e.g., 10^9/gr) before there is any obvious change. Later, Enfors et al. (1979) confirmed that the level where the total aerobic count reaches the critical meat spoilage point is higher when the flora is mainly lactic acid bacteria than it is with the usual aerobic spoilage organisms. Another statement that may help to understand why spoilage seems to be delayed when the flora is dominated by anaerobic microorganisms is that lactic acid bacteria which lower the pH cause the fixing of amino compounds that are the main putrefactive agents (Ingram 1962).

Bacterial growth also causes or speeds meat discoloration, due to an increased rate of metmyoglobin formation. This effect is greatest during the logarithmic growth phase, when the oxygen uptake and carbon dioxide production are increased (Butler, Bratzler and Mallman 1953). Because the lactic acid bacteria do not consume oxygen, it is possible to say that they do not affect color as much as aerobic microorganisms do.

Newton and Gill (1980) suggested that spoilage becomes apparent when bacteria attack amino acids. This does not occur under aerobic conditions until bacteria exhaust the glucose at the meat surface.

Meat Color

The color of fresh meats is an important quality attribute which determines the consumer appeal of the product. Westerberg (1971) described that the consumer relates meat freshness and general quality to the presence of the bright cherry red color of normal meat bloom.

Myoglobin is the major, but not the only pigment responsible for fresh meat color. The blood heme pigment, hemoglobin, constitutes as much as 12 to 30% of the total pigment (Govindarajan 1973). According to Fox (1966), as meat color is concerned, myoglobin and hemoglobin are identical in their reactions. However, they may have different reaction rates.

Color Cycle of Fresh Meats

This is a dynamic cycle and in the presence of oxygen the three pigments, oxymyoglobin, myoglobin and metmyoglobin, are constantly being interconverted. The uptake of oxygen by myoglobin converts the purple reduced pigment to the bright red oxygenated pigment, oxymyoglobin. This process produces the familiar "bloom" of fresh meats; at high oxygen pressures

myoglobin is oxygenated, and the concentration of oxymyoglobin increases. This red complex, once formed, is stabilized by the formation of a highly resonant structure; and as long as the oxygen remains complexed to the heme, the pigment will undergo no further color changes. However, the oxygen is continually associating and dissociating from the heme complex, a process which is accelerated by a number of conditions, among them low oxygen pressures. When this occurs, the reduced pigment is subject to oxidation by oxygen or other oxidants. There is a slow and continuous oxidation of the heme pigments to the met form. When the meat is fresh, the production of reducing substances endogenous to the tissue will constantly re-reduce the pigment to the purple form, and the cycle continues if oxygen is present (Fox 1966).

Factors Affecting Meat Color

Effect of Oxygen. Discoloration of fresh meats is a function of several factors, of which oxygen availability is the most important (Landrock and Wallace 1955). Westerberg (1971) mentioned that if high concentrations of oxygen are present, the myoglobin forms oxymyoglobin, which has the attractive, bright, cherry red color. However, if low concentrations of oxygen are present, oxidation of myoglobin is favored to form the unattractive brown or gray color of metmyoglobin. This is probably the most important consideration in designing a package (Govindarajan 1973). Ledward (1970) showed that the

formation of metmyoglobin in beef was maximal at 6 ± 3 mm Hg of O_2 at 0°C and 7.5 ± 3 mm Hg of O_2 at 7°C for semitendinosus muscles.

Immediately after a cut surface is exposed to air, the purple-red ferrous myoblobin is observed. After a short time, the surface becomes increasingly red, because of the oxygenation of the pigment. This reaction is commercially known as "bloom" (Haas and Bratzler 1965). These investigators stated that many factors influence oxygenation, including myoglobin concentration, temperature, relative humidity, biological agents, oxygen pressure and oxygen penetration.

Hermansen (1983) discussed that the oxygenation of myoglobin can be reversed as long as the meat is fresh. If the meat surface is constantly exposed to oxygen, the oxymyoglobin will gradually be transformed into metmyoglobin due to chemical oxidation. Only in rare cases can metmyoglobin be reduced back to myoglobin. This investigator also pointed out that the most pronounced formation of metmyoglobin will occur about 2 mm below the oxymyoglobin zone, where the partial oxygen pressure is about 6 mm Hg. According to Taylor (1982), the depth to which oxygen penetrates meat is proportional to the square root of its partial pressure. With 80% O_2 at the surface, the oxygenated layer is twice as thick as in air, and the metmyoglobin layer forming at the penetration limit is so far from the surface that it remains obscured for several days.

The effect of oxygenation upon Munsell renotations was studied by Haas and Bratzler (1965). These renotations appeared to increase in redness, or hue, and intensity of chroma, while value remained constant. Hue progressed from the yellow-red toward the red region.

Effect of Microorganisms. The effect of bacteria on the color of fresh meat has been studied by several investigators, yet the supporting evidence for detrimental or beneficial effects has been either incomplete or absent.

Benedict, Strange, Palumbo and Swift (1975) concluded that the slow growing Lactobacilli and Microbacterium flora which can grow in low O_2 and high CO_2 appear to have little effect on the formation of metmyoglobin during storage. Christopher et al. (1980a) determined in their study that changes in surface discoloration or overall appearance do not seem to be related to changes in psychrotrophic bacterial counts. Studies done by Cutaia and Ordal (1964) did not show a marked effect of the initial bacterial population of beef on the conversion of oxymyoglobin to metmyoglobin, and of metmyoglobin to reduced myoglobin. Daun, Solberg, Franke and Gilbert (1971) did not find any relationship between microbial growth and initial formation of metmyoglobin either.

On the other hand, some other investigators have discussed some kind of evidence for the effect of bacteria on meat color. For example, Robach and Costilow (1961) observed that

the addition to steaks of cell suspensions of a number of aerobic bacteria and of Saccharomyces cerevisiae greatly increased the rate of discoloration. Low inocula resulted in the more rapid appearance of the brown color of metmyoglobin, whereas high cell poulations quickly produced the purple color of myoglobin. These visible changes in color were associated with the oxygen demand of the surface tissue including, of course, the demand of any contaminating micro-organisms. They also stated that the primary role of the bacteria in meat discoloration is in the reduction of the oxygen tension in the surface tissue. Butler et al. (1953) proved that bacteria discoloration of meat was greatest during the logarithmic growth phase. This is believed to be due to the high oxygen demand of aerobic bacteria in that generation period, which coincides with a rise in the metmyoglobin formation. According to Robach and Costilow (1961) some species of aerobic bacteria such as Pseudomonas fluorescens, P. aeruginosa, P. geniculata, Achromobacter liquefaciens and Flavobacterium rhenanus have proven to discolor meat by reducing the O_2 tension on the meat surface, while the facultative anaerobe Lactobacillus plantarum did not cause metmyoglobin formation because it does not consume O_2 to any extent.

Effect of Dehydration. Brody (1970) explained that removal of water from the surface can lead to surface dehydration and consequent concentration of salts that can

cause color changes. Sacharow (1974) postulated that loss of moisture produces surface discoloration. He explained that fresh meat turns to a dark reddish-brown color. Dehydration occurs and the concentration of pigment increases on the surface of the meat. Moisture, containing dissolved pigments, migrates to the surface, evaporates and causes pigment concentration.

Effect of Preslaughter Conditions. Fresh meat color is also found to be a function of preslaughter conditions. These would include animal breed, maturity, feed, and stress susceptibility of the animal. The incidence of "dark cutting beef" is the best example illustrating the effect of abnormal pre slaughter conditions giving rise to poor quality beef. This happens when the animal is subjected to stress conditions prior to slaughter (Govindarajan 1973).

Effect of Temperature. Cutaia and Ordal (1964) determined in their study that at higher temperatures, the metmyoglobin formed is more rapidly converted to reduced myoglobin and the maximum amount of metmyoglobin present is lowered. The differences in the maximum amounts of metmyoglobin detected in the samples were explained by visualizing an interaction between two separate systems. In one system, an autoxidation of oxymyoglobin to metmyoglobin is taking place. The second system is generated by the meat itself. Because of the macerated condition of the beef, metabolic activity proceeds at a rate greater than that which would be observed

in a solid cut of meat. A reducing potential generated by this enzymatic activity is capable of converting the metmyoglobin to reduced myoglobin. Since the activity of the enzyme systems can be considered to accelerate as the temperature increases, these investigators have concluded that the metmyoglobin conversion rate is influenced by temperature. On the other hand, Taylor (1982) mentioned that an increase in temperature from 0°C to 5°C doubles the rate of surface discoloration.

Other Factors. Some researchers have reported the effect of other factors on the color of fresh meat. For example, Cutaia et al. (1964) detected that, as the amount of fat in the ground beef was increased, the rate of metmyoglobin formation was increased, as well as its rate conversion to reduced myoglobin. Fellers, Whaba, Caldano and Ball (1963) observed that of three major cuts of beef they studied (round, loin, and chuck), the loin had the highest degree of bloom and the chuck the lowest.

Color Measurement

Although the methodology of color measurement has been studied extensively, there is not yet a single method completely free from criticism. The measurement of meat color has proved to be a difficult task due to two major reasons: the complexity of myoglobin distribution in the muscle and the dynamic nature of the pigment. Further

complicating the situation is the presence of intramuscular fat which tends to interfere with the color measurement (Govindarajan 1973). This investigator pointed out that meat color is a surface property and that it can be measured by subjective evaluation or objectively by instruments. Although subject evaluation has been used extensively, he realizes that there is no uniformity in the color scales used.

Govindarajan (1973) also explained that meat color can be expressed in international CIE units (X,Y,Z) or Hunter L,a,b units. Butler et al. (1953) used disk colorimetry to measure the color. The hue, value and chroma were combined into one number for statistical treatment by use of the Nickerson (1936) formula for index of fading. The index of fading was reported to increase with increases in metmyoglobin content of the sample. Munsell spinning disks were also used by Robach and Costilow (1961). They calculated an index of fading on the basis of the standards proposed by Butler et al. (1953). Govindarajan (1973) explained that fresh meat color can also be expressed in terms of relative concentrations of oxidized and reduced forms of the pigment. Broumand, Ball and Stier (1960) extracted these two forms of myoglobin using aqueous solvents, and determined them spectrophotometrically. Snyder (1965) discussed that practical problems arise when using this absorption method. Some of these problems are: (1) in extracting the pigment from meat to give a clear solution, (2) in selecting the surface volume of

meat to be analyzed, (3) changes in the form of the pigment (mainly from myoglobin to oxymyoglobin) during extraction and analysis.

Moisture Loss

According to Brody (1970), meat may undergo a loss of water that leads to economic losses as well as color changes. Since meat is sold on a weight basis, loss of water by evaporation means a dollar loss. Removal of water from the surface can lead to surface dehydration and consequent concentration of salts that can cause color changes. He also explains that loss of water is a physical phenomenon in which vapor pressure of water in the surrounding atmosphere has a direct influence. As water-molecule activity increases with increasing temperature, the rate of water escape from within the meat increases; so this parameter is temperature dependent. When the relative humidity is high, and equilibrium is established, loss from the meat is negligible. Taylor (1982) pointed out that the water activity of chilled meat is very high and, if unprotected, meat is almost certain to lose weight by evaporation and its appearance will deteriorate. Further weight loss occurs when meat is cut, since the exposed surfaces exude liquid, detracting also from the appearance of meat packages. Although efficient chilling can reduce the quantity of exudate, a certain amount will

always be present when meat cuts are held for retailing. Pirko and Ayres (1957) related loss of moisture from the meat to the size and thickness of the sample.

Most meat coolers maintain the product at 35-40 F°with 80-85% relative humidity. Accurate records show that if 30°F and 85-90% relative humidity are steadily maintained, meat storage life can be extended greatly and shrinkage minimized (Sacharow 1974). Seideman, Carpenter, Smith, Dill and Vanderzant (1979, 1980) stated that vacuum packaging tends to minimize shrink losses.

Fat Oxidation

Oxidation is the chief factor in quality degradation of fats and fatty portions of foods. This oxidative deterioration results in off-flavors and off-odors which are organoleptically potent and adversely affect the marketability of the food (Stuckey 1972).

DeMan (1982) explains that the unsaturated bonds present in all fats and oils represent active centers which, maong other things, may react with oxygen. This reaction leads to the formation of primary, secondary and tertiary oxidation products which can make the fat or fat-containing food unsuitable for consumption. He makes the comment that the process of autoxidation and the resulting deterioration in flavor of fats and fatty foods are often described by the term "rancidity". Lundberg (1961) distinguishes several types of rancidity. One of them is oxidative rancidity, and

it usually results in fats such as lard on exposure to oxygen, and is characterized by a sweet but undesirable odor and flavor which becomes progressively more intense and unpleasant as oxidation continues.

Among the many factors which affect the rate of oxidation, DeMan (1982) considered the amount of oxygen present, the degree of unsaturation of the fat, presence of antioxidants, presence of prooxidants, nature of packaging materials, light exposure and temperature of storage.

Seideman et al. (1979) noted that flavor desirability decreased significantly with increased storage for pork meat stored in atmospheres initially containing 80% or more oxygen. Ordonez and Ledward (1977) claimed that lipid oxidation - rather than bacterial spoilage or metmyoglobin formation - may be the limiting factor in the use of O_2-containing atmospheres for storage of pork.

Meat Distribution

Present Distribution System

According to Brody (1970) animals are killed, dressed and broken into carcases at meat-packaging plants near the livestock feeding area. Carcasses chilled to below 10°C may be shipped, hanging, to the retailer or to his warehouse for subsequent reshipment. Sides or quarters unpackaged are still the most frequent raw materials shipped into the backrooms of stores for breaking and cutting into retail cuts. These

consumer-sized pieces are then placed in pulp, foamed-polystyrene or transparent oriented-polystyrene trays. Filled trays are subsequently overwrapped with a transparent film. This researcher also points out that all of this packaging is performed today at slightly below ambient tempeartures under varying sanitation conditions, and that workers have varying degrees of training and generally a lack of appreciation of the sanitary implications of their activities.

Centralized Packaging

Economic pressure from increasing costs of labor, time and space has stimulated interest in centralized cutting and packing operations (Taylor 1982). Brody (1970) considered that centralized packaging would elevate meat from a commodity to a branded product. Consumers could then purchase uniform quality fresh meat on the basis of brand, and a skillful processor and marketer could operate on a profitable basis, just as do other sectors of the food manufacturing business. This investigator described a system to approach this ideal packaging system. It consists in breaking the carcass into primal or subprimal cuts, so that they can be enclosed in saran-type bags and then vacuum packaged. In this form they are distributed to retail stores for breaking and cutting into retail cuts. Seideman et al. (1980) explained other possible systems that could be used: a) to further process subprimal

cuts at a central location by cutting and trimming to form actual retail cuts and then "reforming" the subprimal cuts followed by vacuum packaging for transport-distribution to retail stores; b) to perform complete retail cut preparation, including wrapping, at a central location for subsequent distribution to retail outlets.

Hermansen (1983) compared modified atmosphere versus vacuum packaging to extend the shelflife of retail fresh meat cuts, and stated that both systems have potential for centralized packaging of meat. Furthermore, Brody (1970) suggested that if only basic principles of sanitation, temperature and relative humidity control were applied, centralized packaging could be feasible.

Meat Packaging

The purpose of packaging is to preserve the initial quality factors of meat for as long a period as necessary to attain marketing objectives. According to Hermansen (1983), packaging cannot improve the quality of the product. It can only delay the onset of spoilage by regulating the factors that contribute to it. The product, therefore, is only protected for a limited amount of time, determined by the system that is used. The parameters that he mentions that affect the shelflife of meat are the bacteriological standard of the raw materials, the hygiene and temperature during cutting and packaging, the gas atmosphere, pH value,

relative humidity, composition of the microflora, and the storage temperature, which he refers to as the most important.

Packaging without a preservation mechanism can have little beneficial effect beyond exclusion of surface contamination. The most commonly used meat preservation process is refrigeration. At these cool temperatures, just above meat's freezing point, microbiological, enzymatic, and biochemical changes can be slowed sufficiently to enable a longer retention of initial quality (Brody 1970).

The most important causes of meat quality deterioration that must be considered in designing a package are: color changes, moisture loss and handling contamination. The color is very important to the consumer (housewife) since she relates meat freshness and general quality to the presence of the bright, cherry red color of normal meat bloom. Secondly, moisture loss from the package has to be prevented since dehydration reduces meat quality and, if large losses occur, can also lead to problems with governmental inspectors. Prevention of contamination is necessary since the retail cut is handled frequently during normal marketing procedures (Westerberg 1971).

In his studies about the microbiology of prepackaged meats, Ingram (1962) concluded that the effect of packaging may depend not only on permeability of the wrapper to O_2, CO_2, and perhaps water, but equally on the exact kind of

meat and on the nature and number of the bacteria it happens to carry.

Taylor (1982) has proposed that packaging influences the meat's behavior by changing its environment, and that the main change is the modification of the gaseous atmosphere. This atmosphere composition influences the color of the meat and determines the extent and type of microbiological spoilage during storage. This investigator claims that the gases primarily involved are O_2 and CO_2, and that the main difference between packaging systems is the extent to which O_2 is available to the meat, initially and during storage and display. Furthermore, Seideman et al. (1979) determined that continuous changes in gas composition occurred during storage of packaged pork. They suggested that these changes are likely caused by diffusion of gases through the package film (which will depend upon the film permeability), microbial and enzymatic activity, and dissolution of gases into tissue fluids.

Package Forming and Filling

Most supermarket packages are wrapped manually in a film tray combination. The entire wrapping operation is fairly simple and 10 to 15 packs may be produced per minute. In a large volume operation, semiautomatic and automatic machinery become a realistic necessity. Several systems are based on semiautomatic equipment involving sleeving the film

around the tray manually. The package is then fed into the machine for weighing, labeling, and pricing with speeds up to 20 packs per minute possible. Fully automatic machinery involves a roll-fed mechanism. The fill tray is inserted on an in-feed conveyor system. Subsequent operations are fully automatic with speeds up to 35 packs per minute (Sacharow 1980).

Packaging Materials

Films and foils that may be considered for use as packaging materials for self-service fresh and cured meats vary widely in those properties affecting keeping quality of the product (Kraft and Ayres 1952). According to Sacharow (1974), transparent films form the largest single group of packaging materials used in fresh meat packaging. These films must have some important characteristics: since the consumer believes it is necessary to see the meat product she is purchasing to satisfy her belief of quality, the packaging film must possess excellent optical properties to show the meat cut in an attractive manner; it must be a moisture barrier to minimize weight loss and it must retain integrity on handling because the consumer usually punches, pokes and feels the product prior to purchasing and normally does not purchase the first package she examines (Westerberg 1971). To achieve this, Sacharow (1974) stated that a film with an oxygen permeability of 5000 ml of O_2/sq. cm/24 hr/atm at 75°F with 100% relative humidity inside a

52% relative humidity outside the package is necessary. Fox (1966) observed that as the O_2 permeability of the film decreases, a partial pressure is reached where O_2 utilization by the tissue balances O_2 penetration at a pressure level which favors the oxidation of myoglobin.

From the microbiological point of view, films with a high O_2 permeability support aerobic growth. Halleck et al. (1958) noted that permeable packaging materials permitted bacterial growth at approximately the same rate as in unpackaged meat; therefore, their influence on bacterial growth was not appreciable. Later, this was supported by studies done by Ingram and Dainty (1971). These workers pointed out that if the package is adequately permeable to atmospheric gases, the spoilage flora follows the normal pattern. However, Gardner et al. (1967) observed that many packaging films regarded as being permeable do restrict gaseous exchange sufficiently to alter the microflora.

Some examples of permeable films are: coated cellophane, pliofilm, low density polyethylene and stretch polyvinyl chloride which is one of the most widely used (Sacharow 1974).

Gas Impermeable Films. In order to be used in vacuum packaging, or in gas-flushing procedures, a film needs to have some specific characteristics: it must be an excellent barrier to oxygen so that when this gas is removed from

around the product on packaging it cannot reenter the package to degrade product quality; the film must be a barrier to moisture so continued moisture loss does not occur from the meat; it must also form hermetic seals; and should be tough and puncture-proof so that vacuum or gas-flush are not easily lost during normal distribution channels (Westerberg 1971).

Many investigators have observed that films with very low permeability extend the product shelflife by controlling microbial deterioration. For instance, Halleck et al. (1958) noted that bacterial growth was influenced by the atmosphere produced within the package, and that growth was marked by a longer lag phase than in the permeable films. Similarly, Gardner et al. (1967) reported that, when the pork meat packed under a gas impermeable film was analyzed, an increase in CO_2 concentration and a decrease in O_2 concentration showed up. As a result, the Pseudomonas-Achromobacter organisms were inhibited.

An example of gas impermeable films is polyvinylidene chloride (PVDC). It is very strong and durable, and keeps the meat in the purple color (Sacharow 1974).

Rigid Trays. Several years ago, most fresh meat trays were made from molded pulp and paperboard. These trays were absorbent, economical and fairly rigid. Disadvantages included possible decomposition by excessive moisture absorption, adherence to the frozen meat, and poor

visibility. To overcome these problems, polystyrene foam trays appeared on the market. Although moisture absorbency is poor, foam trays offer an aesthetically appealing white background for red meat. Recently, a growing concern by the consumer to full visibility in meat packages has led to the development of clear formed polystyrene trays. They offer improved visibility. Meat juices may create a poor sales effect, although blotters eliminate the juice accumulation. An inherent problem with polystyrene is its poor resistance to cracking and handling (Sacharow 1974).

Overwrapping

When this system is being used for packaging, the meat is usually retailed in trays of rigid or expanded plastic, which are then overwrapped with a clear plastic film held in place by heat sealing or cling folding. The overwrapping film is generally very thin (15 to 25μ), it has a low moisture transmission rate, and an O_2 permeability of around 10000 cc/sq. m-atm-day. Even with a gas permeability as high as this, overwrapping films can hinder passage of gases sufficiently to cause slight depletion of O_2 in meat packages and a measurable accumulation of CO_2. However, the concentration of O_2 is still high enough to support growth of the normal aerobic spoilage bacteria, and the level of CO_2 is too low to have any positive effect on storage life. In practice, the stability of meat in this type of package is slightly

less than that of hung meat because there is more surface drying (Taylor 1982). This investigator also suggests that, with good refrigeration, prepackaged meat may be acceptable microbiologically for up to a week, but in retailing its life is limited by deterioration of the desirable bright red color, which results from the oxidation of myoglobin.

Igbinedion, Cahill, Ockerman, Parret and Vanstavern (1983) stated that conventional overwrapping of fresh meat has not proven successful for extending shelflife beyond three days at refrigeration temperatures. The determined that the total plate counts accelerated linearly throughout the storage time.

Vacuum Packaging

Vacuum packaging at its simplest is defined as the evacuation of air from a package which then is sealed to maintain an anaerobic environment. Under such circumstances, the essential conditions for bacterial life are changing dramatically, due to a different composition of the residual air. If there is just a small O_2 concentration in the packs, the Pseudomonas spp. will show a certain growth. It is, therefore, very important to use packaging material with a very high grade of impermeability to O_2. At the same time, it is also very important to obtain as high a degree of vacuum as possible (Hermansen 1983). Taylor (1982) mentioned that the small volume of residual O_2 is quickly consumed by tissue respiration and its concentration falls to less than

0.5% within 2 or 3 days. During this time, CO_2 accumulates to well above 20% and remains at this level during storage. The combination of CO_2 and low O_2 concentration inhibits the Pseudomonas spp. normally responsible for meat spoilage, and the developing microbial flora is mainly composed by Lactobacilli which do not produce such strong off-odors. Meat packed in this way, therefore, has an extended storage life. According to Hermansen (1983), a complete dominance of lactic acid bacteria is desirable, as most of these bacteria are harmless in respect to spoilage of the meat.

Ingram (1962) and Gardner et al. (1967) explained that the content of CO_2 in vacuum packaging is known to increase during storage due to respiration of the meat and/or microbial activity. They also proposed that the effect of vacuum packaging in extending shelflife is due to the accumulated CO_2 and not to the reduced O_2 tension. In a study done by Brody (1970), it was suggested that the CO_2 present in vacuum packages reduces microbial growth, enzymatic activity, oxidation, and water loss. Baran et al. (1970) observed that growth of aerobes on vacuum packaged meat was slower than growth on meat packaged on air. On the other hand, growth of anaerobes occurred earlier in fresh meat packaged under vacuum than in air.

The effects of vacuum packaging on meat color have been extensively studied. Taylor (1982) pointed out that the very low partial pressure of O_2 restricts the penetration of

this gas into the meat so that the metmyoglobin layer, which develops at its limit, is virtually on the surface. This layer is very thin, however, and does not mask the purple color of underlying myoglobin pigment. According to him, vacuum packaging retail cuts differs from vacuum packaging larger joints of meat in one respect which is important to color. With large joints, the ratio of residual air to meat is low enough to ensure quick depletion of O_2, but with retail portions, the ratio can be higher and consequent metmyoglobin formation can discolor the meat surface.

It has been proven that upon re-exposure to air, the reduced pigments would oxygenate and the meat would "bloom". However, Hermansen (1983) observed that, during storage, the color may change slowly to the greyish-brown metmyoblobin due to oxidation. Rikert, Bressler, Ball and Stier (1957) suggested high vacuum storage for long-term color preservation of meat.

Advantages and Drawbacks of Vacuum Packaging

The advantages of vacuum packaging are, first of all, the longer shelflife and a significant reduction in drip loss. The packages are very suitable for keeping in the consumer's freezer. Vacuum packed meat requires only little space during distribution and storage. Another advantage is that leakers are very easy to identify. The greatest drawbacks are: the purple color which may be a problem with respect

to consumer acceptance, packaging of bone-in cuts, and the difficulties in recognizing the exact shape and quality of the cuts in vacuum bags (Hermansen 1983).

Modified Atmospheres

The use of modified atmospheres for retail packaging of fresh meat seems to be becoming of increasing interest throughout the world. The reasons for this might very well be its potential for prolonging the shelflife of centralized packaged meat. The shelflife of modified atmosphere-packaged meat is normally limited by the high content of O_2 and by the activity of spoilage bacteria (Hermansen 1983). This investigator has concluded that the gas-mixture, usually CO_2 and O_2, helps to extend the product's shelflife, mainly by maintaining the meat color. Carbon dioxide is primarily added to the gas-mixture in the gas-packed meat because it has a restraining effect on the bacterial growth when the initial counts are low. Oxygen is a reactive gas which influences the flavor as well as the color of the meat. Another commonly used gas is nitrogen which is considered a neutral filler as it influences neither the color of the meat nor its keeping quality.

Hermansen (1983) also described the procedure for packaging under modified atmospheres. First, a gas-mixture is back-flushed into the package after vacuumizing. For most products, the head space is approximately three times

the volume of the meat. The packaging material used must be impermeable to gases, to keep the gases within the package.

In the last few years, many attempts have been made to find the right gas-mixture to be used in modified atmosphere packaging. Clark and Lentz (1973) reported that a mixture of 70-85% O_2 and 15% CO_2 gave the best results, increasing the color and odor shelflife, compared to storage in air. The results indicated that the separate beneficial effects of CO_2 and O_2 on odor and color shelflife are approximately additive when the gases are used together. It appears, therefore, that storage in such a mixture could be used in a central prepackaging operation, including transportation, to increase the shelflife of consumer cuts of fresh meat. Christopher et al. (1980a) noted that psychrotrophic bacterial counts and Lactobacilli counts of steaks and chops stored in CO_2-N atmospheres usually were lower, though not often statistically significant, than those of comparable vacuum packaged steaks, chops or loins. Furthermore, Christopher et al. (1980b) claimed that psychrotrophic bacterial counts of lean and fat surfaces of loins stored in 40% CO_2 + 60% N_2 were frequently significantly lower than counts of comparable sites of vacuum packaged loins probably because of the presence of inhibiting concentrations of CO_2 and a more limiting concentration of O_2. There were marked decreases in the percentage of Pseudomonas spp. and increases

in the percentage of Lactobacilli in the microflora of the pork loins. Seideman et al. (1979) suggested that a modified gas atmosphere of 20% CO_2 + 80% N_2 is a suitable alternative to vacuum packaging. The use of this gas-mixture was superior to vacuum packaging in retaining the natural appearance of pork. Similarly, Spahl, Reineccius and Tatini (1981) concluded that the best gas-mixture for extending the shelflife of pork chops at both 2 and 5°C were those containing only CO_2 and N_2.

Advantages and Disadvantages of Modified Atmospheres

This packaging system for fresh meat cuts could eliminate distortion of cuts and theoretically reduce leaker rates and purge losses (Seideman et al. 1980). Hermansen (1983) made a review of the advantages and drawbacks of packaging under modified atmospheres. He mentioned that the appearance of modified atmosphere-packed meat is very attractive to the consumer, because a bright red color seems to be synonymous with freshness. Furthermore, packaging of bone-in cuts may be done without problems. On the other hand, this investigator explained that packaging in modified atmosphere packs gives the meat a shorter life than vacuum packaging, requires more space during distribution and storage, and it is not suitable for freezing.

Oxygen

Recent studies showed that by using a head space of about 90% O_2 the acceptable meat color was prolonged when compared with samples stored in an air atmosphere (Daun et al. 1971).

Tests with O_2-enriched air and pure O_2 showed that both the odor and color shelflife of meat increased with increased O_2 concentrations above 50% (Clark and Lentz 1973). Christopher et al. (1980b) mentioned that O_2 maintains the presence of oxymyoglobin. However, Ricket et al. (1957) did not obtain any added benefit in increasing the partial pressure of O_2 to a value higher than that in air in reference to the red color of meat.

It is also appropriate to consider the negative effects produced by using O_2 atmospheres for packaging of fresh meat.

Seideman et al. (1979) detected a decrease in the quality factors of pork initially stored in O_2-rich atmospheres (because of continuous growth and activity of Pseudomonas spp.). They observed that meat in O_2-containing modified atmospheres usually had light green areas of discoloration at early storage periods and tan to light brown discoloration at later periods of storage; and also noted an increased incidence of off-odor after some period of storage.

Hermansen (1983) explains that O_2-rich atmospheres help to preserve the meat color due to the formation of the

stable oxymyoglobin pigment. However, he also explains that the meat will have a short shelflife, and a gradual decrease of flavor and aroma due to the presence of O_2, which provides good growth conditions for spoilage bacteria and simultaneously reacts with a great number of the chemical components of the meat and the fat.

Christopher, Vanderzant, Carpenter and Smith (1979) and Daun et al. (1971) concluded that air and O_2-enriched packaged meats supported equivalent microbial growth. In the same study, Christopher et al. (1979) also reported that pork chops stored in 100% O_2 or in air had significantly higher aerobic plate counts during the entire storage period than chops stored in 100% CO_2 or in 70% N_2 + 25% CO_2 + 5% O_2. Ordonez and Ledward (1977) claimed that lipid oxidation -- rather than bacterial spoilage or metmyoglobin formation -- may be the limiting factor in the use of O_2-containing atmospheres for storage of pork. Based on the studies done by Seideman et al. (1979) it has been concluded that gas mixtures containing O_2 do not appear to be a satisfactory method of storage of fresh pork meat.

Oxygen concentration must be drastically reduced to affect bacterial growth (King and Nagel 1967; Baran et al. 1970; Clark and Lentz 1969; Gardner et al. 1967). On the other hand, Westerberg (1971) stated that if low concentrations of O_2 are present, oxidation of myoglobin is favored to form the unattractive brown or gray color of metmyoglobin. This is

probably one of the most important factors on designing a package (Govindarajan 1973).

Carbon Dioxide

The ability of CO_2(10%) to retard the growth of spoilage flora of meat has been reported (Clark and Lentz 1972). Gardner et al. (1967) suggested that the selective action is probably not due to a lack of O_2, as there was at least 1% in all packs that they analyzed. This level suffices most organisms to maintain respiration and growth rates similar to those in air, though it has still to be shown that microorganisms on meats respond in the same way (Ingram 1962). Similarly, Huffman (1974) confirmed that high CO_2 atmospheres significantly reduce microbial growth on fresh pork chops, and established that the inhibition of microbial growth was not simply the result of lowered amounts of O_2 present in the storage atmosphere, since the chops stored in N_2 had counts similar to those stored in air.

King and Nagel (1967) investigated various mechanisms and factors regulating the growth of Pseudomonas spp. that could be influenced by CO_2 levels. The inhibition did not appear to be produced by alterations in O_2 tension, pH or ionic strength of the substrate solutions. It appeared that specific enzymes involved in the catabolism of the various substrates examined were influenced to different degrees by CO_2. More recently (1975), these workers concluded that the

action of CO_2 on Pseudomonas aeruginosa was to limit the rate of growth by a mass action inhibition on certain decarboxylating enzymes, particularly isocitric and malate dehydrogenases. Enfors and Molin (1978) proposed that the mechanism of CO_2 inhibition involves a change in the cell membrane fluidity, which affects its functional properties, membrane permeability or the function of integral membrane proteins. These researchers considered that it may be argued that the inhibition obtained at hyperbaric pressures of CO_2 is due to a decrease in pH and not to any specific effect of CO_2.

Some of the fundamental work related to packaging of fresh meat under CO_2 atmospheres was done by the Canadian workers Clark and Lentz (1969, 1972, 1973), who pointed out three most important facts:

1. The optimum concentration of CO_2 is 15-20%. Higher concentration causes color change on the meat and gives little additional inhibition of growth.

2. There is an interaction of temperature and CO_2. The most pronounced effect is at 0°C, while at temperatures exceeding 5°C, the CO_2 has a very limited effect.

3. The bacteriological counts and especially the physiological conditions of the bacteria are important. If the bacteria have started the growth phase, CO_2 has nearly no effect at all.

These investigators observed that the growth of psychrotolerant, slime-producing bacteria found on the surface of fresh beef was markedly inhibited by CO_2 gas in the atmosphere. Inhibition was manifested mainly by an increase in the lag phase of growth, although rate during the log phase was also affected; the log generation times for 15 and 20% CO_2 were approximately twice that for air.

Christopher et al. (1979) observed in their studies that inhibition of Pseudomonas spp. and predominance of lactic acid bacteria on pork roasts held in 20-50% CO_2 and less than 50% O_2 were likely caused by the inhibitory activity of CO_2 on Gram-negative aerobic flora. In studies done by Blickstad et al. (1981) it was concluded that high partial pressures of CO_2 have a considerable shelflife prolonging effect by; (1) selecting the microflora towards Lactobacillus spp., (2) reducing the growth rate of those Lactobacillus spp. The controlling and growth inhibition effect of CO_2 was promoted by reduced temperatures. According to Enfors et al. (1979), the clear predominance of lactic acid bacteria on CO_2-stored meat probably

reinforces the shelflife prolonging effect of CO_2. Carbon dioxide primarily inhibits the growth of CO_2-sensitive organisms, i.e., Pseudomonas spp., which gives the more CO_2-resistant lactic acid bacteria a chance to develop. By virtue of their antagonistic properties, the lactic acid bacteria then inhibit the development of other CO_2-resistant organisms which may, compared to lactic acid bacteria, have a more deletorious effect on the meat.

Many statements have been made in reference to the effect of CO_2 on meat color. For example, Cutaia and Ordal (1964) considered that one of the principal disadvantages in the use of high CO_2 atmospheres in fresh meat storage is the development of color darkening. Taylor (1982) mentioned that, while adverse effects on meat color may discourage the use of high CO_2 concentrations with beef and lamb, they may have an application with pork where the difference between the three pigment states is not nearly so pronounced. Ordonez and Ledward (1977) proposed that metmyoglobin formation was independent of CO_2 concentration.

Related to rancidity, Lopez, Hernandez, Sanz-Perez, and Ordonez (1980) determined that 20% CO_2 greatly reduced the rate of lipid oxidation of refrigerated ground pork.

Nitrogen

As an inert gas, nitrogen is ideally suited to gas packing. If added to the package after evacuation of the air,

the effect on meat is similar to that of vacuum packaging, but residual O_2 is diluted and formation of metmyoglobin in the meat should be less than with vacuum. Although this improves purity of myoglobin at the meat's surface, the nitrogen also dilutes the carbon dioxide and the concentration required to inhibit spoilage bacteria takes longer to accumulate. This can be a weakness during the first two days of storage. So far, its use for fresh meat retailing seems unlikely (Taylor 1982).

Enfors et al. (1979) noted some retardation of growth on nitrogen stored samples, and they attributed that to the higher CO_2 concentration which developed in the N_2 packages (10% after 10 days) as a consequence of the small head spaces of the bags. Also, Hermansen (1983) considered N_2 as a neutral filler as it influences neither the color of the meat nor its keeping quality.

Acetic and Lactic Acids

Very little pure acetic acid, as such, is used in foods although it is classified by the Food and Drug Administration as a GRAS material (generally recognized as safe). It is the principal component of vinegars, which are produced from cider, grapes (or wine), sucrose, glucose or malt by successive alcoholic and acetous fermentations.

On the other hand, lactic acid is one of the most widely distributed acids in nature and one of the earliest

used in foods. It is a viscous, nonvolatile liquid, and it is included in the FDA list of GRAS additives too. Food-grade DL-lactic acid is available as aqueous solutions, and it is colorless, practically odorless, and very soluble in water. Edible lactic acid is normally produced by the controlled fermentation of highly refined sucrose, although less expensive carbohydrates such as potato starch, and molasses may be used (Gardner 1972).

Gill and Newton (1982) explained that Pseudomonas spp. were essentially unaffected by the pH of normal meat. From the other Gram-negative psychrotrophic organisms they isolated from a meatworks, a large number of strains would not grow on meat of normal pH at chill tmeperatures, unless the pH or the incubation temperature were raised. In this study, they also observed that the Gram-negative psychrotrophic organisms were more severely inhibited by acetic acid than by lactic acid or hydrochloric acid at pH 5.5 and 2°C. They attributed this strong inhibitory action of acetic acid to the undissociated acid, and the inhibitory effect of hydrochloric acid to the decrease in pH. Since the pattern of inhibition produced by lactic acid was very similar to that produced by hydrochloric acid and markedly different from that due to acetic acid, these investigators concluded that the lactic acid in meat exerts any selective effect on Gram-negative flora mainly by reducing the pH. On the contrary, Grau (1980) proposed that the lactic acid present in muscles with low

pH value is mostly in the undissociated form, which he suggested as the most effective form. In this study, lactic acid showed to be more effective in inhibiting the growth of Brochothrix thermosphacta under anaerobic conditions than under aerobic conditions, and that in muscles of pH higher than 5.7, although the concentration of lactic acid will not be sufficient to prevent bacterial growth, it can be sufficient to reduce the rate of growth.

MATERIALS AND METHODS

Four trials were conducted in this study utilizing a total of four pork loins provided by Tucson Meat Packers Ltd. in Tucson, Arizona. One loin was used for each trial.

Preparation of Samples

Pork loins (only skin removed) were cut into chops approximately 3/4 inch thick at the Tucson Meat Packers processing plant. Fifty two chops were then randomly assigned to one of six treatments and storage periods of 0, 3, 6 or 9 days. The design of the various trials is shown in Table 1.

Trials I and II

These trials were conducted in July and August, 1984. All conditions during preparation, handling and evaluation were identical.

Pork chops were weighed and placed on styrofoam trays (Mobilfoam, size 8" x 5 1/2" x 5/8") and individually packed according to treatment. In each trial, thirteen chops were used. One of the chops was analyzed at Day 0 for microbial counts, color and peroxide value. These values were used as initial data. Three chops served as controls and were overwrapped with gas permeable Borden resinite film (transmission rate for oxygen of 310 - 387.5 cc/sq. cm/24 hr

Table 1. Experimental Design.

Trial	Treatment	Film Type
I-II	1. Overwrap	Borden Resinite Film
	2. Vacuum	Cryovac barrier bags
	3. 15% CO_2 + 85% N_2	Cryovac barrier bags
	4. 15% CO_2 + 40% O_2 + 45% N_2	Cryovac barrier bags
III-IV	1. Overwrap	Borden Resinite Film
	2. Vacuum	Cryovac barrier bags
	3. Vacuum + 1% Lactic Acid sprayed solution	Cryovac barrier bags
	4. Vacuum + 1% Acetic Acid sprayed solution	Cryovac barrier bags

and carbon dioxide transmission rate of 2480 - 2790 cc/sq. cm/24 hr) and subsequently heat sealed. Nine chops were assigned to three different treatments (vacuum, 15% CO_2 + 85% N_2, and 15% CO_2 + 40% O_2 + 45% N_2) and placed in gas-impermeable Cryovac barrier bags (oxygen transmission rate of 0.0035 cc/sq. cm/24 hr and carbon dioxide rate of 0.0250 cc/sq. cm/24 hr). These sample bags were vacuumized and, when required, they were back-flushed with the corresponding gas-mixture by using a Multivac AG 900 unit. Then they were heat sealed.

After packaging, the chops were stored at 4 $\pm$ 1°C until they were ready to be analyzed. An open-display cooler (MasterBilt, Model LMC 1230) was used.

Trials III and IV

These trials were conducted in September and October, 1984. All conditions during preparation, handling and evaluation of samples were identical. Again, 13 chops were used for each trial. The treatments used were: film overwrap, vacuum, vacuum + 1% acetic acid (AA) sprayed solution, and vacuum + 1% lactic acid (LA) sprayed solution. The overwrapping and vacuum packaging were done as in Trials I and II. The chops treated with acetic and lactic acid were sprayed with 1% acetic and 1% lactic acid solutions, respectively. Then, they were agitated to remove excessive liquid, placed on styrofoam trays, weighed and vacuum packaged.

The storage temperature for these packaged chops was also 4 ± 1°C.

Microbiological Evaluation of Samples

Sampling for microbial enumeration was carried out by swabbing two 3.8 sq. cm circular areas of the surface of each sample. A dry, sterile cotton swab was rolled repeatedly across the given area as defined by a sterile aluminum template. The tip of the swab was immersed in 99 ml of a sterile phosphate buffered solution and shaken vigorously. The swab was subsequently removed, rolled across the same area, placed in the dilution blank and broken off below the point of handling. The above process was repeated on a second area on the surface of the same sample. The sample dilution was shaken approximately twenty five times and appropriate dilutions were made. Psychrotrophic bacterial counts were enumerated by use of Plate Count Agar (Difco) with plate incubation at 4 ± 1°C for 10 days in a walk-in cooler, and Lactobacilli by use of MRS broth (Difco) plus 1.5% agar, incubating the plates in a Napco unit (Model 330) at 25 ± 1°C.

Viable counts were calculated as follows:

$$\text{counts/sq. cm} = \frac{\text{number of colonies x dilution factor}}{7.6 \text{ sq. cm}}$$

and the results were reported as $\log_{10}$ numbers.

Color Evaluation

The color of the pork chops was determined by using a Macbeth-Munsell Disk Colorimeter (Macbeth Corporation, Model BBX 320 DC). In this system for color measurement, the color of the sample is matched to a set of Munsell disks, and the CIE color coordinates are obtained. By means of the Munsell-CIE diagrams, those coordinates are converted to Munsell color notations (hue, value and chroma). To determine the color difference between a particular pork chop and the chop measured at Day 0, an index of fading was estimated, using a formula derived by Nickerson (1936). The formula is:

$$I = C/5(2\Delta H) + 6\Delta V + 3\Delta C \quad \text{where}$$

I is the color difference

C is the chroma reading for the Day 0 sample for each trial, and ΔH, ΔV, and ΔC are the absolute differences between the readings of each particular sample and the Day 0 sample.

Moisture Loss

It was calculated as follows:

$$\%ML = \frac{(\text{initial sample weight} - \text{final sample weight}) \times 100}{\text{initial sample weight}}$$

All data were obtained by using an analytical balance (Mettler, P1000).

Fat Oxidation

Two fat samples were obtained from each pork chop, and then the degree of oxidation for each one was determined by using the Peroxide Value Test. This test is described in the Official Methods of the A.O.C.S. (1973) as follows:

Reagents:

1. Acetic acid-chloform solution. Mix 3 parts by volume of glacial acetic acid, reagent grade, with 2 parts by volume of chloform, U.S.P. grade.
2. Potassium iodide solution, saturated solution of KI, A.C.S. grade, in recently boiled distilled water. Make sure the solution remains saturated as indicated by the presence of undissolved crystals. Store in dark. Test daily by adding 2 drops of starch solution to 0.5 ml. of potassium iodide solution in 30 ml. of acetic acid-chloroform solution. If a blue color is formed which requires more than 1 drop of 0.1 N sodium thiosulfate solution to discharge, discard the iodide solution and prepare a fresh solution.
3. Sodium thiosulfate solution, 0.1 N, accurately standardized. Store in dark, at refrigeration temperature.
4. Sodium thiosulfate solution, 0.01 N, accurately standardized. This solution may be prepared by

accurately pipetting 100 ml. of the 0.1 N solution into a 1000 ml. volumetric flask and diluting to volume with recently boiled distilled water.

5. Starch indicator solution, 1.0% of soluble starch in recently boiled distilled water.

Procedure:

1. Weigh 5 ± .05 gr. of sample, and dissolve it in 30 ml. of the acetic acid-chloroform solution. Add 0.5 ml. of saturated potassium iodide.
2. Allow the solution to stand with occasional shaking for exactly 1 minute and then add 30 ml. of distilled water.
3. Titrate with 0.1 N sodium thiosulfate adding it gradually and with constant and vigorous shaking. Continue the titration until the yellow color has almost disappeared. Add 0.5 ml. of starch indicator solution. Continue the titration, shaking the flask vigorously near the end point to literate all the iodine from the chloroform layer. Add the thiosulfate dropwise until the blue color has just disappeared.

   Note: If the titration is less than 0.5 ml., repeat the determination using 0.01 N sodium thiosulfate solution.

4. Conduct a blank determination of the reagents daily. The blank titration must not exceed 0.1 ml. of the 0.1 N sodium thiosulfate solution.

Calculation:

Peroxide value as milliequivalents of peroxide per 1000 gr. of sample:

$$\frac{S \times N \times 1000}{\text{weight of sample}}$$

S: titration of sample (minus titration of the blank)

N: normality of sodium thiosulfate solution

Analysis of Data

Data was evaluated by analysis of variance as indicated in the SPSS Statistical Package (Nie, Hull, Jenkins, Steinbrenner and Bent 1975). Duncan's New Multiple Range Test (Duncan 1955) was used to isolate mean differences with treatments, and times of storage for microbial populations, color attributes and moisture loss.

RESULTS AND DISCUSSION

Trial I

A comparison of packaging treatments within sampling days is presented in Table 2.

Microbial Growth

Psychrotrophic counts in vacuum packaged pork chops were smaller than for any of the other packaging treatments, up to sampling Day 6. Baran et al. (1970) showed that growth of aerobes on vacuum packaged meat was slower than growth on meat packaged in air. On sampling Day 9, the smallest ($P<.05$) aerobic microbial growth was in pork chops packaged in Atmosphere 3 (15% CO_2 + 85% N_2) possibly due to the ability of CO_2 to retard the growth of spoilage flora on meat (Clark and Lentz 1972), and the greatest ($P<.05$) growth was in pork chops packaged in Atmosphere 4 (15% CO_2 + 40% O_2 + 45% N_2). Hermansen (1983) explained that O_2-rich atmospheres provide good growth conditions for spoilage bacteria.

Lactobacilli microbial growth on pork chops packaged under vacuum was the lowest ($P<.05$) for all the sampling days as compared to the other three packaging treatments. Conversely, Baran et al. (1970) observed that growth of anaerobes

Table 2. Means of Microbial Numbers[a], Color Attributes, and Moisture Loss for Packaging Treatments within a Sampling Day, Trial I.

Treatment	Psychro-trophs	Lacto-bacilli	Hue	Value	Chroma	Fading	% Moisture Loss
Day 0							
Initial	2.36	2.01	6.65	4.30	2.10	0.00	0.00
Day 3							
Overwrap	2.46^{cd}	2.17^{d}	7.35^{c}	4.50^{d}	2.50^{c}	3.00	0.80
Vacuum	2.09^{c}	1.27^{c}	8.45^{d}	4.70^{d}	2.10^{c}	4.50	0.90
ATM-3[b]	2.73^{d}	2.41^{de}	8.05^{d}	4.60^{d}	2.10^{c}	3.25	0.70
ATM-4[b]	2.71^{d}	2.61^{e}	7.25^{c}	4.20^{c}	3.25^{d}	4.60	0.70
Day 6							
Overwrap	5.35^{d}	4.88^{d}	8.40	4.70^{d}	2.45	4.90	0.60
Vacuum	3.32^{c}	2.57^{f}	7.90	4.35^{c}	2.40	2.25	0.90
ATM-3[b]	6.34^{f}	6.15^{f}	7.90	4.20^{c}	2.55	3.00	0.80
ATM-4[b]	6.10^{e}	5.83^{e}	7.50	4.40^{c}	3.05	4.20	0.90
Day 9							
Overwrap	7.23^{d}	6.43^{e}	9.25^{d}	4.75	1.90	5.50	1.20
Vacuum	7.35^{d}	3.36^{c}	8.10^{d}	4.50	2.45	3.45	1.10
ATM-3[b]	6.32^{c}	6.26^{d}	8.05^{d}	4.45	2.00	3.30	1.10
ATM-4[b]	7.76^{e}	7.53^{f}	7.85^{c}	4.10	2.50	3.40	2.20

[a] Log_{10} numbers of microorganisms per cm^2 surface area.

[b] ATM-3 = 15% CO_2 + 85% N_2

ATM-4 = 15% CO_2 + 40% O_2 + 45% N_2

[c,d,e,f] Means within the same column for each sampling day or variable which bear unlike superscripts differ significantly ($P < .05$).

occurred earlier in fresh meat packaged under vacuum than in fresh meat packaged in air.

Color

Pork chops packaged in Atmosphere 4 (15% CO_2 + 40% O_2 + 45% N_2) tended to have lower hue and value readings than those chops packaged under other conditions, through the entire sampling period. On the other hand, chroma means were always higher than the means for the other three treatments. According to Haas and Bratzler (1965), chroma increases as the oxygenation of meat increases. No differences were found in the index of fading of pork samples within the various packaging treatments throughout the storage period.

Moisture Loss

Due to the absence of replicates, an analysis of variance for these data could not be done. However, a subjective evaluation showed no differences in moisture loss between the four packaging treatments, on sampling Days 3, and 6. At the end of the storage period, chops packaged under Atmosphere 4 had the greatest weight loss, which could be due to experimental error, since the procedure used for this determination is very unprecise.

A comparison of sampling days within packaging treatments is presented in Table 3.

Table 3. Means for Microbial Numbers[a], Color Attributes, and Moisture Loss for Sampling Days within a Packaging Treatment, Trial I.

Day	Psychro-trophs	Lacto-bacilli	Hue	Value	Chroma	Fading	% Moisture Loss
Overwrap							
Day 0	2.36^{c}	2.01^{c}	6.05^{c}	4.30^{c}	2.10	0.00^{c}	0.00
Day 3	2.46^{c}	2.17^{c}	7.35^{c}	4.50^{d}	2.50	3.00^{cd}	0.80
Day 6	5.35^{d}	4.88^{d}	8.40^{d}	4.70^{e}	2.45	4.90^{d}	0.60
Day 9	7.23^{e}	6.43^{e}	9.25^{d}	4.75^{e}	1.90	5.50^{d}	1.20
Vacuum							
Day 0	2.36^{d}	2.01^{d}	6.65^{c}	4.30^{c}	2.10	0.00^{c}	0.00
Day 3	2.09^{c}	1.27^{c}	8.45^{d}	4.70^{d}	2.10	4.50^{d}	0.90
Day 6	3.32^{e}	2.57^{e}	7.90^{d}	4.35^{c}	2.40	2.25^{cd}	0.90
Day 9	7.35^{f}	3.36^{f}	8.10^{d}	4.50^{cd}	2.45	3.45^{d}	1.10
ATM-3[b]							
Day 0	2.36^{c}	2.01^{c}	6.65^{c}	4.30^{cd}	2.10	0.00	0.00
Day 3	2.73^{d}	2.41^{c}	8.05^{d}	4.60^{e}	2.10	3.25	0.70
Day 6	6.34^{e}	6.15^{d}	7.90^{d}	4.20^{c}	2.55	3.00	0.80
Day 9	6.32^{e}	6.26^{d}	8.05^{d}	4.45^{de}	2.00	3.30	1.10
ATM-4[b]							
Day 0	2.36^{c}	2.01^{c}	6.65^{c}	4.30^{cd}	2.10^{c}	0.00^{c}	0.00
Day 3	2.71^{d}	2.61^{d}	7.25^{cd}	4.20^{cd}	3.25^{d}	4.60^{d}	0.70
Day 6	6.10^{e}	5.83^{e}	7.50^{cd}	4.40^{d}	3.05^{d}	4.20^{d}	0.90
Day 9	7.76^{f}	7.53^{f}	7.85^{d}	4.10^{c}	2.50^{cd}	3.40^{d}	2.20

[a] LOG_{10} numbers of microorganisms per cm^2 surface area.

[b] ATM-3 = 15% CO_2 + 85% N_2

ATM-4 = 15% CO_2 + 40% O_2 + 45% N_2

[c,d,e,f] Means within the same column for each packaging treatment or variable which bear unlike superscripts differ significantly ($P<.05$).

Microbial Growth

Psychrotrophic counts were always higher than Lactobacilli counts for all of the sampling days within each packaging treatment. Neither one of these two types of flora increased significantly in pork chops overwrapped and vacuum packaged, between sampling Days 0, and 3. The same observation was made for pork chops packaged in Atmosphere 3, but just for the Lactobacilli flora. In addition, these chops in Atmosphere 3 showed no increase in microbial numbers from sampling Day 6 to Day 9, which can be explained by means of the inhibitory effect that has been attributed to CO_2. Significant increases ($P<.05$) in psychrotrophic and Lactobacilli populations were determined in each one of the sampling days for those chops packaged in Atmosphere 4. These data suggest that high O_2 concentrations accelerate the growth of spoilage bacteria of meat.

Color

Hue readings for all of the treatments tended to increase with storage time. This increase was significant ($P<.05$) between sampling Days 0 and 9, for all four treatments. What this increase in hue represents is a change from yellow-red to red color. The value was little affected by storage time and packaging treatments, except for those overwrapped chops, where it increased (though not always significantly) with storage time. This increase in value indicates

an increase in lightness of the color, and could be just a consequence of the subjectivity of the method. Chroma means remained fairly constant throughout the storage period for all the treatments used, with exception of Atmosphere 4, where an increase ($P<.05$) in the intensity of the color was detected on sampling Day 3. Haas and Bratzler (1965) explained that this increase in chroma is produced by oxygenation of myoglobin.

This index of fading estimated for those chops that were overwrapped, vacuum packaged, and treated with Atmosphere 4, showed some differences ($P<.05$) during the storage period. According to Nickerson (1936), these changes represent the color difference between samples (chops) and a standard, in this case the chop measured on Day 9. No significant differences in index of fading due to Atmosphere 3 were observed on sampling Days 0, 3, 6, and 9. These data may suggest that this particular packaging treatment may be suitable for color preservation of pork meat.

Trial II

A comparison of packaging treatments within sampling days is presented in Table 4.

Microbial Growth

Vacuum packaged pork chops had the lowest ($P<.05$) psychrotrophic and Lactobacilli counts on sampling Days 3, 6, and 9. It has been proven that the content of CO_2 in vacuum

Table 4. Means of Microbial Numbers[a], Color Attributes, and Moisture Loss for Packaging Treatments within a Sampling Day, Trial II.

Treatment	Psychro-trophs	Lacto bacilli	Hue	Value	Chroma	Fading	% Moisture Loss
Day 0							
Initial	3.27	1.96	6.95	4.45	2.50	0.00	0.00
Day 3							
Overwrap	5.50^{e}	5.06^{e}	7.80	4.45	2.65	1.30^{c}	0.80
Vacuum	4.12^{c}	3.14^{c}	7.45	4.30	2.60	2.00^{cd}	0.90
ATM-3[b]	4.91^{d}	4.46^{d}	7.45	4.45	2.45	1.25^{c}	0.50
ATM-4[b]	4.83^{d}	4.32^{d}	6.80	4.25	2.95	3.30^{d}	1.00
Day 6							
Overwrap	7.38^{e}	6.77^{d}	7.05	4.70	1.75	3.95	1.50
Vacuum	4.14^{c}	4.17^{c}	7.30	4.75	2.20	3.05	1.40
ATM-3[b]	6.83^{d}	6.71^{d}	7.60	4.60	1.80	3.65	1.50
ATM-4[b]	8.11^{f}	7.85^{e}	7.20	4.50	2.00	2.15	1.50
Day 9							
Overwrap	7.83^{e}	7.43^{e}	8.10^{d}	4.55^{cd}	2.20	2.40^{cd}	2.00
Vacuum	5.21^{c}	5.33^{c}	7.80^{cd}	4.80^{d}	2.05	4.30^{e}	1.40
ATM-3[b]	6.83^{d}	6.47^{d}	6.60^{cd}	4.40^{c}	2.45	1.70^{c}	1.30
ATM-4[b]	8.35^{f}	8.16^{f}	6.15^{c}	4.30^{c}	1.90	3.50^{de}	1.80

[a] LOG_{10} numbers of microorganisms per cm^2 surface area

[b] ATM-3 = 15% CO_2 + 85% N_2

ATM-4 = 15% CO_2 + 40% O_2 + 45% N_2

[c,d,e,f] Means within the same column for each sampling day or variable which bear unlike superscripts differ significantly ($P<.05$).

packages increases during storage due to respiration of the meat and/or microbial activity (Ingram 1962, and Gardner et al. 1967), and that CO_2 inhibits spoilage bacteria (Clark and Lentz 1969). These facts may help to explain how vacuum packaging tends to minimize microbial growth. By sampling Day 3, the maximum bacterial populations (psychrotrophic and Lactobacilli) were attained on the overwrapped chops, whereas by sampling Day 6, by those chops packaged in Atmosphere 4. It can be seen that those packaging treatments with an oxygen atmosphere provide optimum conditions for microbial growth and, as Seideman et al. (1979) stated, they do not appear to be a satisfactory alternative for storage of pork. The fact that overwrapped chops had greater ($P<.05$) counts than chops packaged under Atmosphere 4 on sampling Day 3 could be explained by considering the gas composition of Atmosphere 4 (15% CO_2 + 45% N_2). The high initial CO_2 concentration could have decreased the microbial growth rate.

Color

The Munsell color renotations were practically unaffected by the different treatments during the entire storage period. However, vacuum packaging and Atmosphere 4 produced the greatest ($P<.05$) differences in color in references to the Day 0 values. According to Butler et al. (1953), this increase in the index of fading can be due to oxidation of myoglobin.

Moisture Loss

As in Trial I, an analysis of variance for these data was not done, due to the lack of replicates. Based on subjective observations, it can be stated that there were not marked effects on moisture loss due to these four different packaging treatments, during the entire storage period.

A comparison of sampling days within packaging treatments is presented in Table 5.

Microbial Growth

Psychrotrophic populations were higher than Lactobacilli populations in all the samples packaged under the four different treatments, throughout the storage period; except in those vacuum packaged chops sampled on Days 6, and 9. Hermansen (1983) considered this dominance of lactic acid bacteria in vacuum packaged meat to be desirable, as most of these bacteria are harmless in respect to spoilage of meat.

The growth of both kinds of flora in chops packaged in permeable film, and under Atmosphere 4 took place faster and to a greater extent than in chops packaged under vacuum and in Atmosphere 3.

Microbial numbers on chops packaged with permeable film, and under Atmosphere 4 increased significantly ($P<.05$) from each sample day to the next, up to the end of the storage time, with exception of Day 9 sample from Atmosphere 4 which did not increase significantly in lactic

Table 5. Means for Microbial Numbers[a], Color Attributes, and Moisture Loss for Sampling Days within a Packaging Treatment, Trial II.

Day	Psychro-trophs	Lacto-bacilli	Hue	Value	Chroma	Fading	% Moisture Loss
Overwrap							
Day 0	3.27^{c}	1.96^{c}	6.95	4.45	2.50	0.00^{c}	0.00
Day 3	5.50^{d}	5.06^{d}	7.80	4.45	2.65	1.30^{d}	0.80
Day 6	7.38^{e}	6.77^{e}	7.05	4.70	1.75	3.95^{f}	1.50
Day 9	7.83^{f}	7.43^{f}	8.10	4.55	2.20	2.40^{e}	2.00
Vacuum							
Day 0	3.27^{c}	1.96^{c}	6.95	4.45^{cd}	2.50	0.00^{e}	0.00
Day 3	4.12^{d}	3.14^{d}	7.45	4.30^{c}	2.60	2.00^{cd}	0.90
Day 6	4.14^{d}	4.17^{e}	7.30	4.75^{de}	2.20	3.05^{d}	1.40
Day 9	5.21^{e}	5.33^{f}	7.80	4.80^{e}	2.05	4.30^{d}	1.40
ATM-3[b]							
Day 0	3.27^{c}	1.96^{c}	6.95	4.45	2.50^{d}	0.00^{c}	0.00
Day 3	4.91^{d}	4.46^{d}	7.45	4.45	2.45^{d}	1.25^{d}	0.50
Day 6	6.83^{e}	6.71^{e}	7.60	4.60	1.80^{c}	3.65^{e}	1.50
Day 9	6.83^{e}	6.57^{e}	6.60	4.40	2.45^{d}	1.70^{d}	1.30
ATM-4[b]							
Day 0	3.27^{c}	1.96^{c}	6.95	4.45^{cd}	2.50^{de}	0.00^{c}	0.00
Day 3	4.83^{d}	4.32^{d}	6.80	4.25^{c}	2.95^{e}	3.30^{d}	1.00
Day 6	8.11^{e}	7.85^{e}	7.20	4.50^{d}	2.00^{cd}	2.15^{d}	1.50
Day 9	8.35^{f}	8.16^{e}	6.15	4.30^{cd}	1.90^{c}	3.50^{d}	1.80

[a] LOG_{10} numbers of microorganisms per cm^2 surface area

[b] ATM-3 = 15% CO_2 + 85% N_2

ATM-4 = 15% CO_2 + 40% O_2 + 45% N_2

[c,d,e,f] Means within the same column for each packaging treatment or variable which bear unlike superscripts differ significantly ($P<.05$).

acid bacteria count. Chops packaged under Atmosphere 4 apparently had a longer lag phase in microbial growth than overwrapped chops, but the former also had the largest ($P<.05$) final populations.

The reason for this longer lag phase could be the high initial CO_2 concentration present in the atmosphere.

Among those chops packaged under vacuum and Atmosphere 3, the latter had the greatest ($P<.05$) psychrotrophic and Lactobacilli counts throughout the storage period. As discussed by Taylor (1982), this could have occurred due to the dilution of CO_2 by the high N_2 concentration present in Atmosphere 3.

Color

Hue means did not show significant differences during the whole storage period within each of the packaging treatments used. At the same time, and inexplicably, value and chroma means showed some differences ($P<.05$) between sampling days in all treatments. However, a tendency of the index of fading to increase with storage time could be observed in all the packaging treatments. As already mentioned, the reason for this change could be an increase in metmyoglobin content of the meat sample (Butler et al. 1953).

Moisture Loss

Subjectively, it is possible to detect an increase in moisture loss during the storage period. It could be

caused by dehydration of the meat surface (Brody 1970). Besides, Taylor (1982) explained that although efficient chilling can be reduce the quantity of exudate, a certain amount will always be present when meat cuts are held for retailing.

Trial III

A comparison of packaging treatments within sampling days is presented in Table 6.

Microbial Growth

Overwrapped pork chops had the greatest ($P<.05$) psychrotrophic and *Lactobacilli* counts on sampling Days 6, and 9. This is clear evidence of the superiority of vacuum packaging treatments in extending shelflife of fresh meat. However, vacuum packaged chops (Treatment 2) had the highest ($P<.05$) microbial counts after Day 3, which may be attributed to a higher initial contamination of that particular sample.

Among the four treatments used, Treatment 5 (vacuum + 1% lactic acid sprayed solution) and Treatment 6 (vacuum + 1% acetic acid sprayed solution) had the lowest ($P<.05$) *Lactobacilli* numbers on sampling Days 3, and 6. The latter of these two packaging treatments, which includes acetic acid, also had significantly smaller ($P<.05$) lactic acid bacteria counts than the other packaging treatments, on sampling Day 9. However, Grau (1980) and Gill and Newton (1982)

Table 6. Means of Microbial Numbers[a], Color Attributes, and Moisture Loss for Packaging Treatments within a Sampling Day, Trial III.

Treatment	Psychro-trophs	Lacto-bacilli	Hue	Value	Chroma	Fading	% Moisture Loss
Day 0							
Initial	4.46	3.64	9.15	4.45	2.55	0.00	0.00
Day 3							
Overwrap	3.47^{d}	3.22^{e}	8.30	4.55	2.50	1.90	1.30
Vacuum	4.55^{e}	4.01^{f}	8.65	4.50	2.45	1.15	3.80
TMT-5[b]	3.20^{d}	2.85^{d}	8.75	4.65	2.55	2.95	4.10
TMT-6[b]	2.05^{c}	1.27^{c}	8.50	4.65	2.30	2.65	5.10
Day 6							
Overwrap	6.25^{f}	6.17^{e}	8.60	4.65^{cd}	1.90^{c}	3.75	2.20
Vacuum	4.69^{e}	5.46^{d}	7.90	4.50^{cd}	2.25^{cd}	3.05	5.70
TMT-5[b]	3.69^{c}	5.09^{c}	7.80	4.45^{c}	2.45^{d}	2.25	6.10
TMT-6[b]	3.93^{d}	5.08^{c}	7.95	4.70^{d}	2.15^{cd}	3.95	6.60
Day 9							
Overwrap	8.03^{f}	7.23^{f}	9.05	4.55	1.85	3.40	2.80
Vacuum	3.31^{c}	5.43^{d}	8.40	4.75	2.40	3.30	4.10
TMT-5[b]	5.31^{e}	6.32^{e}	8.40	4.55	2.45	2.55	6.40
TMT-6[b]	4.73^{d}	5.84^{c}	8.50	4.85	2.55	4.85	6.30

[a] LOG_{10} numbers of microorganisms per cm^2 surface area

[b] TMT-5 = vacuum + 1% Lactic Acid sprayed solution

TMT-6 = vacuum + 1% Acetic Acid sprayed solution

[c,d,e,f] Means within the same column for each sampling day or variable which bear unlike superscripts differ significantly ($P<.05$).

reported this inhibitory effect of acetic and lactic acid on Gram-negative psychrotrophs, rather than on lactic acid bacteria.

Color

On sampling Days 3, and 9, the Munsell color renotations and the index of fading did not show significant differences between the packaging treatments. The meat sample packaged under Treatment 5 (vacuum + lactic acid solution) had a value reading lower ($P<.05$) than that of Treatment 6 (vacuum + acetic acid), and a chroma reading higher ($P<.05$) than that of the overwrapped sample, on sample Day 6. This represents a more intense or saturated color of the chop under Treatment 5, at that particular time.

Moisture Loss

It is not possible to do a real comparison of these data because of the nature of Treatment 5 and Treatment 6, in which it was required to spray the samples with an acid solution, 1% lactic acid, and 1% acetic acid, respectively. Although the samples were all agitated to remove excess liquid before being placed on styrofoam trays, a great deal of the added water affected the initial weight, and did not show up in the final weight, because most of it remained in the package.

A comparison of sampling days within packaging treatments is presented in Table 7.

Table 7. Means for Microbial Numbers[a], Color Attributes, and Moisture Loss for Sampling Days within a Packaging Treatment, Trial III.

Day	Psychro-trophs	Lacto bacilli	Hue	Value	Chroma	Fading	% Moisture Loss
Overwrap							
Day 0	4.46^{d}	3.64^{d}	9.15	4.45	2.55	0.00^{c}	0.00
Day 3	3.47^{e}	3.22^{c}	8.30	4.55	2.50	1.90^{d}	1.30
Day 6	6.25^{e}	6.17^{e}	8.60	4.65	1.90	3.75^{d}	2.20
Day 9	8.03^{f}	7.23^{f}	9.05	4.55	1.85	3.40^{d}	2.80
Vacuum							
Day 0	4.46^{d}	3.64^{c}	9.15	4.45^{c}	2.55	0.00^{c}	0.00
Day 3	4.55^{e}	4.01^{d}	8.56	4.50^{c}	2.45	1.15^{cd}	3.80
Day 6	4.69^{f}	5.46^{e}	7.90	4.50^{c}	2.25	3.05^{d}	5.70
Day 9	3.31^{c}	5.93^{f}	8.40	4.75^{d}	2.40	3.30^{d}	4.10
TMT-5[b]							
Day 0	4.46^{e}	3.64^{d}	9.15	4.45	2.55	0.00^{c}	0.00
Day 3	3.20^{c}	2.85^{c}	8.75	4.65	2.55	2.95^{d}	4.10
Day 6	3.69^{d}	5.09^{e}	7.80	4.45	2.45	2.25^{cd}	6.10
Day 9	5.31^{f}	6.32^{f}	8.40	4.55	2.45	2.55^{cd}	6.40
TMT-6[b]							
Day 0	4.46^{e}	3.64^{d}	9.15^{d}	4.45^{c}	2.55	0.00^{c}	0.00
Day 3	2.05^{c}	1.27^{c}	8.50^{cd}	4.65^{cd}	2.30	2.65^{cd}	5.10
Day 6	3.93^{d}	5.08^{e}	7.95^{c}	4.70^{cd}	2.15	3.95^{d}	6.60
Day 9	4.73^{e}	5.84^{f}	8.50^{cd}	4.85^{d}	2.55	4.85^{d}	6.30

[a] LOG_{10} numbers of microorganisms per cm^2 surface area.

[b] TMT-5 = Vacuum + 1% Lactic Acid sprayed solution

TMT-6 = Vacuum + 1% Acetic Acid sprayed solution

^{c,d,e,f}Means within the same column for each packaging treatment or variable which bear unlike superscripts differ significantly ($P<.05$).

Microbial Growth

Bacterial counts on sampling Day 0 were higher than normal probably due to a high initial contamination of that particular sample. Psychrotrophic and Lactobacilli populations present on the overwrapped chops increased significantly ($P<.05$) from Day 3 through Days 6, and 9; and the psychrotrophic flora always dominated. Igbinedion et al. (1983) determined that the total plate counts from conventionally overwrapped fresh meat accelerated linearly throughout the storage time. Studies conducted by Baran et al. (1970) showed that growth of aerobes on meat packaged in air was faster than growth on vacuum packaged meat.

Meat samples packaged under vacuum, Treatment 5 (vacuum + LA), and Treatment 6 (vacuum + AA), had psychrotrophic and Lactobacilli counts that increased significantly ($P<.05$) from Day 0 through Days 3, 6, and 9, with one exception, the psychrotrophic count for the vacuum packaged pork chop on Day 9. In spite of this accelerated growth rate, microbial numbers for both types of flora from chops packaged under any of these three different vacuum treatments were much smaller than those from overwrapped chops on the last two sampling days.

The psychrotrophic and Lactobacilli flora from all the samples packaged under vacuum, Treatment 5, and Treatment 6, developed as it was expected. The former dominated on Day 3, whereas the latter dominated on sampling Days 6, and 9.

Taylor (1982) explained why these changes take place. He pointed out that the small volume of residual oxygen left in the vacuum package is quickly consumed by tissue respiration, and its concentration falls to less than 0.5% within 2 or 3 days. During this time, CO_2 accumulates to well above 30% and remains at this level during storage. The combination of CO_2 and low O_2 concentration inhibits the Pseudomonas species normally responsible for meat spoilage, and the developing microflora is mainly composed by Lactobacilli. Similarly, Hermansen (1983) proposed that the inhibitory effect of vacuum packaging to spoilage bacteria is not obtained before the concentration of O_2 is very low. He also mentioned that this condition will occur within a few days after packaging, due to the respiration of lean tissue and microbial activity, which results in conversion of O_2 to CO_2. This new gaseous environment is responsible for the development of facultative anaerobes such as lactic acid bacteria.

Color

Storage time did not seem to have an effect on the Munsell color renotations in any of the packaging treatments. However, as in Trial II, a tendency of the index of fading to increase with storage time was noted in all the packaging treatments.

Trial IV

A comparison of packaging treatments within sampling days in presented in Table 8.

Microbial Growth

Overwrapped pork chops had the greatest ($P<.05$) amount of psychrotrophic and Lactobacilli growth throughout the storage period. These results may be supported by many investigators, who have described the effectiveness of vacuum packaging in controlling the shelflife of fresh meat (Ingram 1962, Gardner et al. 1967, Baran et al. 1970, Brody 1970). The lowest ($P<.05$) psychotrophic counts through the entire storage time were achieved by chops packaged under vacuum and under vacuum (AA). Furthermore, these chops treated with acetic acid also had the lowest Lactobacilli populations during storage time, although not significantly ($P<.05$) different from the population of the vacuum packaged chop on sampling Day 3.

From these data, and the data from Trial III, it is possible to suggest that acetic acid not only affects aerobic spoilage bacteria, but it also affects lactic acid bacteria. On the other hand, vacuum packaging combined with a 1% lactic acid sprayed solution did not show any advantage over conventional vacuum packaging. This observation was also made on the last sampling day of Trial III. It might be

Table 8. Means of Microbial Numbers[a], Color Attributes, and Moisture Loss for Packaging Treatments within a Sampling Day, Trial IV.

Treatment	Psychro-trophs	Lacto-bacilli	Hue	Value	Chroma	Fading	% Moisture Loss
Day 0							
Initial	3.60	2.63	8.50	4.55	2.95	0.00	0.00
Day 3							
Overwrap	3.76^{f}	3.75^{e}	8.45	4.80^{c}	2.40	3.20^{cd}	1.30
Vacuum	2.70^{c}	2.12^{cd}	8.15	5.10^{d}	2.05	4.90^{d}	3.20
TMT-5[b]	3.43^{e}	2.43^{d}	8.35	4.65^{c}	2.45	2.25^{c}	4.30
TMT-6[b]	3.19^{d}	1.93^{c}	8.40	4.85^{c}	2.45	3.50^{cd}	5.10
Day 6							
Overwrap	8.25^{f}	7.03^{f}	8.50	4.70^{c}	1.80^{c}	3.05^{c}	2.70
Vacuum	3.94^{d}	3.87^{d}	8.05	5.50^{d}	2.45^{d}	8.75^{d}	4.50
TMT-5[b]	4.61^{e}	4.46^{e}	6.50	5.20^{d}	2.25^{cd}	8.35^{d}	5.30
TMT-6[b]	2.65^{c}	2.24^{c}	7.55	4.75^{c}	2.45^{d}	3.80^{c}	7.60
Day 9							
Overwrap	7.85^{e}	7.59^{e}	7.55^{c}	5.05	2.10^{c}	6.65^{d}	3.20
Vacuum	4.73^{c}	5.74^{d}	7.70^{c}	4.90	2.70^{d}	3.75^{c}	4.70
TMT-5[b]	5.85^{d}	5.84^{d}	9.05^{d}	4.80	2.55^{cd}	3.35^{c}	7.30
TMT-6[b]	4.81^{c}	4.62^{c}	8.30^{cd}	4.85	2.90^{d}	2.55^{c}	7.20

[a] LOG_{10} Numbers of Microorganisms per cm^2 surface area.

[b] TMT-5 = Vacuum + 1% Lactic Acid sprayed solution

TMT-6 = Vacuum + 1% Acetic Acid sprayed solution

[c,d,e,f] Means within the same column for each sampling day or variable which bear unlike superscripts differ significantly ($P<.01$).

that the lactic acid concentration was not strong enough to inhibit aerobic spoilage bacteria, and that somehow it improves the initial environment for the growth of the lactic acid bacteria.

Color

Some significant ($P<.05$) differences for hue, value, chroma, and index of fading readings appeared between packaging treatments on sampling Days 3, 6, and 9. This variability of the data without following any trend, could be originated due to the presence of excessive intramuscular fat in the samples analyzed. Govindarajan (1973) considered the presence of intramuscular fat an important factor interfering with the color measurement.

Moisture Loss

Once again, no comparisons of moisture loss were made due to the bias introduced by the addition of a sprayed solution in Treatments 5, and 6.

A comparison of sampling days within packaging treatments is presented in Table 9.

Microbial Growth

Psychrotrophic counts on chops packaged under the four different packaging treatments were always higher than Lactobacilli counts throughout the storage period, with exception of that from the vacuum packaged chop on sampling

Table 9. Means for Microbial Numbers[a], Color Attributes and Moisture Loss for Sampling Days within a Packaging Treatment, Trial IV.

Day	Psychro-trophs	Lacto-bacilli	Hue	Value	Chroma	Fading	% Moisture Loss
Overwrap							
Day 0	3.60^{c}	2.63^{c}	8.50^{d}	4.55^{c}	2.95^{e}	0.00^{c}	0.00
Day 3	3.76^{d}	3.75^{d}	8.45^{d}	4.80^{d}	2.40^{d}	3.20^{d}	1.30
Day 6	8.25^{f}	7.03^{e}	8.50^{d}	4.70^{cd}	1.80^{c}	3.05^{d}	2.70
Day 9	7.85^{e}	7.59^{f}	7.55^{c}	5.05^{e}	2.10^{cd}	6.65^{e}	3.20
Vacuum							
Day 0	3.60^{d}	2.63^{d}	8.50	4.55^{c}	2.95^{d}	0.00^{c}	0.00
Day 3	2.70^{c}	2.12^{c}	8.15	5.10^{cd}	2.05^{c}	4.90^{d}	3.20
Day 6	3.94^{e}	3.87^{e}	8.05	5.50^{d}	2.45^{cd}	8.75^{e}	4.50
Day 9	4.73^{f}	5.74^{f}	7.70	4.90^{c}	2.70^{cd}	3.75^{d}	4.70
TMT-5[b]							
Day 0	3.60^{d}	2.63^{d}	8.50^{d}	4.55^{c}	2.95^{d}	0.00^{c}	0.00
Day 3	3.43^{c}	2.43^{c}	8.35^{d}	4.65^{c}	2.45^{cd}	2.25^{d}	4.30
Day 6	4.61^{e}	4.46^{e}	6.50^{c}	5.20^{e}	2.25^{c}	8.35^{e}	5.30
Day 9	5.85^{f}	5.84^{f}	9.05^{d}	4.80^{d}	2.55^{cd}	3.35^{d}	7.30
TMT-6[b]							
Day 0	3.60^{e}	2.63^{e}	8.50^{d}	4.55^{c}	2.95	0.00^{c}	0.00
Day 3	3.19^{d}	1.93^{c}	8.40^{d}	4.85^{d}	2.45	3.50^{e}	5.10
Day 6	2.65^{c}	2.24^{d}	7.55^{c}	4.75^{d}	2.45	3.80^{e}	7.60
Day 9	4.81^{f}	4.62^{f}	8.30^{cd}	4.85^{d}	2.90	2.55^{d}	7.20

[a] LOG_{10} numbers of microorganisms per cm^2 surface area

[b] TMT-5 = Vacuum + 1% Lactic Acid sprayed solution

TMT-6 = Vacuum + 1% Acetic Acid sprayed solution

[c,d,e,f] Means within the same column for each packaging treatment or variable which bear unlike superscripts differ significantly ($P<.05$).

Day 9. Its flora was dominated by lactic acid bacteria. In addition, all the samples from all the different packaging treatments had Lactobacilli populations that increased significantly ($P<.05$) on sampling Days 6, and 9. These populations were much lower ($P<.05$) in chops packaged under vacuum, Treatment 5 (vacuum + LA), and Treatment 6 (vacuum + AA) than in overwrapped samples. Blickstad et al. (1981) proposed that the CO_2 present in a package not only selects the microflora towards Lactobacillus spp., but also reduces the growth rate of these Lactobacillus spp.

Color

As mentioned before, the excessive amount of intramuscular fat present in this particular pork loin made it very difficult to measure the color of the chops cut. However, the same tendency of the index of fading to increase with storage time that was detected in Trials II and III was present in this trial. The index of fading for those overwrapped chops increased (though not always significantly) during the entire storage time, whereas for those chops packaged under vacuum, Treatment 5, and Treatment 6, it only increased ($P<.05$) up to sampling Day 6. What this increase in color difference represents is a deterioration of the color of meat, as compared with the color of the initial sample.

Trials I, II, III, and IV

Oxidative Rancidity

Fat samples were obtained from every chop for each one of the four trials. They were analyzed by means of the peroxide value test, in order to detect the degree of oxidation and be able to compare the effect of the packaging treatments upon fat. However, an increase in oxidation products (peroxides) was not detected in any of the chops, probably due to very small changes in peroxides concentration which were difficult to quantitate. In many occasions, fat samples were taken from the treated chops, especially from those with a high degree of spoilage. Then, they were rendered in a drying oven at approximately 100°C, and subjectively evaluated. In all cases, the rendered fat did not show organoleptic deterioration as determined by odor.

These results suggest that pork fat oxidation is not a problem during this storage time (9 days), and at this refrigeration temperature, 4°C. Conversely, Ordonez and Ledward (1977) claimed that lipid oxidation, rather than bacterial spoilage or metmyoglobin formation, may be the limiting factor in the use of O_2-containing atmospheres for storage of pork.

CONCLUSIONS

The use of controlled atmospheres for retail packaging of fresh meat has become of increasing interest in the last few years, and the reason seems to be its potential for prolonging the shelflife of centralized packaged meat.

This packaging system influences the meat's behavior mainly by changing its gaseous atmosphere. These gases present in the package, mostly CO_2 and O_2, determine the extent and type of microbiological spoilage during storage, and affect other quality indices of meat such as color, shrinkage, and oxidative rancidity.

Results from this study suggest that microbiological spoilage may be the limiting factor in the use of modified atmospheres for storage of pork. The use of CO_2-N_2 and CO_2-O_2N_2 atmospheres did not show any advantage over vacuum packaging. In fact, the highest psychrotrophic and Lactobacilli populations were obtained from chops packaged in CO_2-O_2-N_2 atmospheres, whereas the lowest populations of the same types of flora were obtained from vacuum packaged meat. The inhibitory effect expected from CO_2-enriched atmospheres could have been minimized due to the dilution of the gas by the high concentration of N_2.

The samples packaged under the different vacuum packaging treatments (vacuum, vacuum-LA, and vacuum-AA) supported much lower microbial growth than those over-wrapped samples.

Apparently neither overwrapping nor O_2-rich atmospheres seem to be a suitable alternative for centralized packaging of fresh meat. They supported and enhanced the growth of spoilage bacteria during the entire storage period.

Vacuum packaging can still be considered as one of the most effective and simplest ways of extending the shelflife of fresh meat. In this experiment, vacuumized samples happened to have the lowest microbial growth. By spraying the chops with a 1% acetic acid solution before being vacuum packaged, a significant reduction in the Lactobacilli counts was attained. This effect was not obtained when using a 1% lactic acid solution.

Surface discoloration does not seem to be a problem in pork as it has been reported to be in beef or lamb. The reason may be the lighter color of pork meat, due to the lower myoglobin content. Furthermore, based on the data obtained, lipid oxidation did not take place at 4°C, or if it did, it proceeded so slowly that could not be detected during the 9 days storage period.

Additional research needs to be done to identify optimal gas mixtures for modified gas atmosphere packaging,

and to determine if high CO_2 concentrations inhibit microbial growth by lowering the pH on meat.

SUMMARY

The purpose of this study was to compare the effects of different packaging treatments upon several quality attributes of fresh pork stored for 9 days at 4°C.

Four trials were conducted. Pork loins (only skin removed) were cut into chops approximately 3/4 inch thick at a meat processing plant. Chops were randomly assigned to one of six packaging treatments and held under simulated retail conditions. Samples were evaluated for psychrotrophic counts, Lactobacilli counts, color, shrinkage, and oxidative rancidity after 0, 3, 6, and 9 days of storage.

In Trials I and II, chops packaged in O_2-rich atmospheres usually had the greatest psychrotrophic and Lactobacilli counts throughout the storage period, whereas vacuum packaged chops, with one exception, had the smallest counts. In Trials III and IV, overwrapping proved to be less effective as a packaging treatment compared to vacuum, vacuum-LA, and vacuum-AA packaging. Overwrapped samples supported the largest ($P<.05$) growth of psychrotrophic and Lactobacilli bacteria, except once. Conversely, chops packaged under vacuum-AA always attained the lowest Lactobacilli populations.

Although there were not suggesting changes in the Munsell color notations, the index of fading estimated in Trials II, III, and IV indicated a certain increase in meat discoloration with storage time.

The effect of packaging treatments on moisture loss still remains unclear. Because of the experimental design and the nature of some of the packaging treatments used, it was not possible to do objective comparisons. The different packaging treatments did not show to have an effect on the oxidative rancidity of pork fat, during storage time.

Commercialization of fresh meat through a centralized packaging system could be feasible if good sanitation, good handling practices, temperature control, and relative humidity control were integrated.

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