EFFECT OF ATMOSPHERIC GASES AND PACKAGING ON THE MICROBIAL QUALITY, COLOR AND LIPID CONTENT OF BEEF

by

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ABSTRACT

Beef top round steaks were utilized to determine the influence of gaseous atmospheres and packaging on the extent of microbial development, color, lipid composition and lipid content of beef.

Both high and low concentrations of carbon dioxide and vacuum packaging had a significant effect ($P \leq .05$) on discouraging the growth rate of microorganisms. Nitrogen and high levels of oxygen without carbon dioxide promoted microbial growth.

High concentrations of carbon dioxide (100%) significantly increased ($P \leq .05$) the muscle color attribute, hue, whereas low levels of carbon dioxide (15% or less) combined with high levels of oxygen maintained muscle hue and provided an acceptable lean color for a longer period of time.

Elevated levels of oxygen without carbon dioxide had a significant effect ($P \leq .05$) on the increase of monoglycerides, diglycerides and free fatty acids with a corresponding decrease in the triglyceride lipid fraction of the muscle. However, with low levels of carbon dioxide and high levels of oxygen, a significant decrease ($P \leq .05$) occurred in the muscle monoglyceride, diglyceride and free fatty acid lipid fractions.

INTRODUCTION

Fresh meat is one major food product which is still processed and packaged at the retail level. Since the consumer has limited methods to evaluate this product at the retail level, the shelf-life of fresh meat is of short duration.

Many factors which effect shelf-life have been extensively reported in the literature. The influence of light intensity, storage time and temperature, types of packaging materials and microbial contamination have been at least partially elucidated.

It has long been recognized that a bright red color is an essential requirement for successful merchandising of fresh meat. However, with the abundance of research relating to the extension of shelf-life, the retailer can only hope to hold fresh meat for three to four days in the retail case.

Over the last few years, a keen interest has developed in the utilization of gas mixture as a method for extending the shelf-life of fresh meat. It was known as early as 1933 that carbon dioxide provided an inhibitory effect on microbial growth. But only recently was the advantageous effect of combining carbon dioxide and oxygen realized. It has also been realized that this advantage might be most

useful in overseas transit of fresh meat by ship. A few investigators have studied the effect of certain gas mixtures on microorganism growth and color development (Gonzales 1971; Balasundaram 1974 and Huffman 1974). However, this effect on other fresh meat criteria is totally lacking.

This study was designed to determine the effects of gaseous atmospheres and packaging materials on the extent of microbial development, color, lipid composition and lipid content of beef steak. A wide range of carbon dioxide and oxygen concentrations were selected in an effort to obtain a complete analysis of their effects on muscle color, since color is possibly the most important criteria in determining the shelf-life of fresh meat.

LITERATURE REVIEW

General

Fresh meat is one major food product which is still processed and packaged at the retail level. The concept of centralized packaging has been applied in the processing and distribution of cured and cooked meats, but has only recently been applied to fresh meats. Since the consumer has limited methods to evaluate this product at the retail level, the shelf-life of fresh meat is of short duration.

Types of Microorganisms Found on Fresh Meat

A wide variety of different genera of bacteria has been found on fresh meats. More recent studies by numerous investigators (Kirsch et al. 1952; Wolin, Evans and Niven 1957; and Ayres 1960a) have indicated the genue *Pseudomonas* as the major strain of bacteria found on fresh meats. Brown and Weidman (1958) reported that psychrophilic gram-negative bacteria causing degradation of fresh beef were almost all *Pseudomonas*. Ayres (1951, 1960a, b) concluded that the primary spoilage organisms of meat at low temperatures are psychrophilic gram-negative rods of general *Achromobacter* and *Pseudomonas*. Stringer, Bilskie and Naumann (1969) found that *Pseudomonas fragi* and *Pseudomonas geniculata* were the predominate bacteria identified on prepackaged fresh beef

steaks. Wiles (1971) concluded that 22 to 24% of the bacteria growing on fresh meat are gram-negative rods and approximately one-half of these bacteria posses biochemical characteristics similar to those of Pseudomonas and one-third posses biochemical characteristics similar to those of Proteus.

Changes in pH of Fresh Meats During Storage

Although the degree of freshness or spoilage of meat is often evaluated by microorganism numbers, it is known that spoilage is not the result of microorganism numbers per se but rather due to the type of microorganisms present and metabolic products produced by them (Gardner 1965). Shelef and Jay (1970) reported a gradual increase in the production of alkaline substances by the spoilage flora. The range of increase went from pH 5.6 to 5.8 to as much as pH 8.5 when fresh beef becomes putrid.

Huffman (1974) working with beef under gaseous atmospheres reported essentially no increase in pH from 7 to 21 days post slaughter using 100% CO_2 and a gas mixture (70% N_2, 25% CO_2, 5% O_2). However, when using O_2, N_2 and air for storage, the pH of beef followed a typical pattern that would have been expected in that from 7 to 21 days the pH of these samples increased from approximately 5.5 to 5.9.

Compositional Aspects of Muscle Closely Associated with Shelf-Life

Meat Color

Pirko and Ayres (1957) in an attempt to evaluate fresh meat color using a color panel noted inconsistencies in subjective scores from one observation period to the next if there was a substantial time lapse between successive evaluations.

Butler (1962) and Snyder (1965) proposed methods for the quantitative determination of fresh meat colors based on reflectance spectrophotometry. In addition, Steward, Zipser and Watts (1965) successfully used spectrophotometry in measuring the color of fresh meat, while Ockerman and Cahill (1969) using spectrophotometrics relating machine readings to visual color scores of beef noted that the procedure did not lend itself to use under commercial conditions.

Butler (1953) working with fresh boneless beef steaks cut from the Longissimus muscle found a correlation coefficient of 0.81 between the index of color fading and $\log_{10}$ of bacterial counts. The index of color fading was determined by the objective measures of color given by the Macbeth-Munsell colorimeter. Jeremiah (1972) also working with the Macbeth-Munsell colorimeter and consumer preferences of beef loin steaks found visual color scores and scores given by the colorimeter to be significantly related ($P < .01$). Jeremiah (1972) also reported that consumer

preferred steaks were more pale than the "cherry red" previously proposed as "ideal" (Allen, 1968).

Lipid Composition of Various Muscles

The classical lipid component reference has been that of Hilditch (1956). However, much of the bovine lipid composition data reported therein (English and Indian perinephric and American brisket) are not applicable in other than a general way to tissues and animals currently being studied in the United States. As noted by Hilditch, there are many factors that influence the kinds of fats deposited in animals; factors such as the anatomical site of deposition and the climate. Age and sex also affect differences in fat composition, the amount of fat deposited and the distribution of fat deposits. Nutritional influences on fat characteristics are minimal in the bovine (compared to those observed in nonruminants), primarily because of the nature of the rumen and its microbial populations.

In utilizing beef rounds, Awad, Powrie and Fennema (1968) reported cholesterol 1.7%, phospholipid 28.8% and free fatty acids 1.6% as percent of total lipid. Bloor and Snider (1934) reported that muscles follow the general rule observed for other tissues and that increased activity is accompanied by increased phospholipid and cholesterol content. The concentration of these substances appears to depend on the amount and the nature of usage and to be

relatively independent of species and of the location of the muscle. O'Keefe et al. (1968) utilizing silicic acid column chromotograph fractionated bovine intramuscular lipids from the Semitendinosus, Triceps brachii and Longissimus muscles into phospholipids and neutral fats. In spite of the wide range in total fat content at each location, phospholipids were present in all three muscles at a level of approximately 500 mg per 100 g of muscle tissue. This result, coupled with the lower fat content of the Semitendinosus as compared to the other two muscles, indicated a significantly higher percentage of phospholipid material in the total fat from the Semitendinosus as compared to the Triceps brachii or Longissimus.

Thrall and Cramer (1971) utilizing 373 beef cattle including heifers, bulls and steers of the Hereford, Angus and Shorthorn breeds reported the overall means of the Longissimus intramuscular lipids (as percent total lipids) to be 9.4% phospholipid, 2.2% monoglyceride, 2.6% diglyceride, 8.8% cholesterol, 6.2% free fatty acid, 74.4% triglycerides and 1.5% cholesterol esters. Also reported as overall means were the lipid classes of the subcutaneous fat which were 30% phospholipid, 1.7% monoglyceride, 2.8% diglyceride, 3.7% cholesterol, 6.4% free fatty acids, 86.4% triglycerides and 0.3% cholesterol esters. Sex and seasonal effects on lipid classes were also studied. The most significant difference between sex was that heifers maintained

approximately 79% triglycerides; whereas bulls only possessed approximately 60% triglycerides. As a seasonal difference between bulls, the most significant difference was noted in the diglyceride fraction; in the fall the diglyceride level was found to be 6.7% as opposed to the spring where the level was found to be 0.0%. Also noted in this study was that the percent of intramuscular lipid varied not only between breeds and sex but also between animals.

Factors Associated with Microbial Development

Sanitation

Good sanitation is a major concern to those in the meat industry who desire to have an economically successful meat prepackaging operation. The literature is voluminous on this subject and the importance of sanitation has been emphasized by many workers (Jensen and Hess 1941; Kirsch et al. 1952; Drewniak et al. 1954 and Ayers 1955).

Voegeli, Bratzler and Mallman (1953) found that equipment used directly with meat cutting operations offered the greatest source of contamination for prepackaged fresh meat in retail stores. Bratzler (1955) stated that sanitation in the packaging operation is greatly important if there is to be satisfactory color development and maintenance. He lists sanitation, temperature control and the use of correct wrapping material as being the most important factors in fresh meat packaging. Brown and Weidemann (1958)

rated sanitation in the packaging operation as being of paramount importance in maintaining satisfactory color. Sanitation is important to decrease the initial contamination as meat is an ideal culture medium (Briskey et al., 1960). A good sanitation program is necessary in the operation of a centralized fresh meat processing plant (Volz and Marsden, 1963). It was recommended that all equipment and cutting blocks should be cleaned at least once a day to get a desirable shelf-life of fresh meat of two to five days. Frazier (1967) indicated that a sanitation program in the food industry should be concerned with aseptic practices in the preparation, processing and packaging of food products of a plant, the general cleanliness and sanitation of plant and premises, and the health of the employees. Naumann, Stringer and Gould (1965) reported that a shelf-life of six days is possible for prepackaged fresh meat with proper sanitation and refrigeration that will maintain the product at 30 F in a display case. Exposure to spoilage microorganisms and food poisoning organisms occurs during the slaughtering, cutting and processing operations. It is important that product handling based on sound sanitation principles be conducted during all plant operations. They also demonstrated that low microbial counts could be obtained on packaged beef cuts with good sanitation techniques.

Temperature

It has long been known that lower temperatures cause increased storage life of meats. All investigators report that cool temperatures extend the storage life of prepackaged meats, as would be expected from the general delay of growth and metabolism of microorganisms at low temperatures (Ingram, 1962).

Butler (1953) and Rey et al. (1970) are just a few of the many workers who have reported that meat cuts stored at lower temperatures exhibit longer shelf-life and lower bacterial counts. Ayres (1960b), Jaye (1962) and Stringer and Naumann (1966) have reported studies that were in agreement, i. e., the most important factor associated with the shelf-life of prepackaged meats is temperature during storage, processing and displaying. Temperature affects growth of microflora and has a decided effect on increasing enzymatic activity of muscle (Jensen 1945). Both have shown to cause surface discoloration and subsequent shortening of shelf-life (Halleck, Ball and Stier 1958b).

Berry and Morgan (1934) reported that the minimum temperature for psychrotrophic growth was approximately -10 C. This report is supported by Mallman and Churchill (1946) where they found that foods with relatively high microbial population could be held for a considerable period of time if held at relatively low temperatures without loss of quality. Snyder (1962) concluded that the beneficial

effect of low temperature depended on a delay in microbial growth and an increase in partial oxygen pressure resulting from lowering of temperature.

Briskey et al. (1960) found that sliced products at 32 F (0 C) had no significant increase in bacteria after seven days of storage, but sliced pork products stored at 42 F for the same number of days had a 100-fold increase in bacteria. At 52 F there was a 100-fold increase in number of microorganisms after 48 hours, almost a 1,000-fold increase in three days, and a 100,000-fold increase in six days. Gould (1963) reported that round steaks and ground beef stored at 38 F had a significantly shorter shelf-life than when stored at 34 F.

Light

Roth (1967) in displaying meat in a 120 foot candle of light opposed to darkness found that meat discolors more rapidly when stored in lighted areas. This is in agreement with earlier work done by Lane and Bratzler (1962) in which they also reported that meat discolored faster in the presence of light as compared to darkness.

Marriott (1967) working with fresh beef steaks stored for various time periods in a -1 C dark environment and at -1 C lighted environment found that the steaks exposed to light had an increase in color deterioration. It was also reported that under these conditions less bacterial

growth was evident in dark storage as compared to lighted storage; however, whether the presence of light radiation stimulated the growth of bacteria was not determined.

Ramsbottom, Goeser and Shultz (1951) reported that fresh meats become discolored after display for 36 hours under ultra-violet light and that fresh meat was very resistant to color change upon exposure to fluorescent light. Voegeli (1952) studied the color changes in packaged fresh meat. He found that intensities of light up to 215 foot-candles under comparable storage temperatures and indicated that when incandescent light was used, rapid discoloration occurred due to increased surface temperature from heat generated by lights. Kraft and Ayres (1954) reported that soft white fluorescent light intensity was unimportant in influencing the course of discoloration of packaged fresh beef. Also intensity of visible light did not affect development of surface organisms on such meat. From the practical aspect, display of fresh meat in self-service markets could be carried out using high intensities of soft white fluorescent light without the occurance of undue discoloration.

Packaging

A bright red color is an essential requirement for successful merchandising of fresh meat. In order to maintain this desirable color, the package must protect the product against physical damage and microbial contamination

as well as permit the proper rate of oxygen and moisture transmission. For the latter reason, fresh meat should be wrapped in transparent films which have a high oxygen transmission rate and a low moisture vapor transmission. Kraft and Ayres (1952) reported that films that permit oxygenating conditions necessary for maintenance of red color of fresh meat allowed rapid growth of microorganisms on the meat surface.

Halleck, Ball and Stier (1958a) reported that the packaging material employed did not selectively affect the growth rates of the predominant genera of bacteria (aerobic as well a anaerobic) found on fresh meat. In a later study, Halleck, Ball and Stier (1958b) reported that packaging material did affect total bacterial growth in packaged meats. The workers separated packaging materials into two categories, permeable and nonpermeable packaging materials. It was observed that the permeable films (cellophane, cellophane acetate and polyethylene) permitted growth at optimum rates until dehydration. The nonpermeable packaging materials such as cellulose acetate-pliofilm laminate inhibited bacterial growth by the atmosphere produced in the container.

A recent and increasing trend has been the use of polyvinyl chloride film as a shrink wrap for fresh meats (Sacharow, 1970). Other shrink films used for fresh meats

include polyisoprene, polypropylene and polyvinylidene chloride. The advantages of using shrink films include a contour fit, neat appearance and ease in handling.

Another development extending the shelf-life of fresh meat involves vacuum packaging. The value of an essentially anaerobic environment produced by vacuum packaging had been demonstrated by Warnecke et al. (1966) and Beban, Kraft and Walker (1970). In work done with fresh meat, they noted significantly lower numbers of spoilage bacteria on vacuum-packaged cuts. However, under these conditions, the bright red color of fresh meat changed to dark red or purple which are unacceptable to the consumer who associates freshness and high quality with the bright red colored meats. Therefore, vacuum packaged meats must be repackaged in oxygen permeable films in retail markets.

Gaseous Atmosphere

The literature on the use of carbon dioxide as a preservative has become voluminous and occasionally contradictory. Haines (1933) reported that the inhibitory effect of carbon dioxide upon growth rate was greater than that due to pH alone and also suggested that an explanation of carbon dioxide action may be found in carbon dioxide inhibition of dehydrogenating cellular enzymes.

McCulloch (1946) suggested that the inhibitory and germicidal action of carbon dioxide at low concentration is

due to the hydrogen ion concentration of the substrate and provides conditions unfavorable for the development of some aerobic microorganisms. In high concentration it is believed to act as a protoplasmic poison.

Empey and Vickery (1933) reported that the storage time of beef carcass was increased about 40% by using 10 to 20% carbon dioxide. Scott (1938) extended the life of fresh meat by storage in selected atmospheres, low in oxygen and/or enriched with carbon dioxide. Rikert, Ball and Stier (1958) found that flushing storage atmospheres with carbon dioxide or nitrogen before vacuum storage had a beneficial effect on color for both fresh and cured meats as compared with samples in vacuum without previous flushing.

Price and Schweigert (1971) indicated that, in general, the highly aerobic bacteria and yeasts and molds are selectively inhibited by carbon dioxide. The facultative bacteria may or may not be inhibited, and the lactic acid bacteria and anaerobic bacteria are virtually unaffected. Therefore, surface spoilage of foods as a result of the growth of molds, yeasts, or the aerobic, psychrophilic bacteria should be retarded with appropriate carbon dioxide concentrations.

Gonzales (1971) concluded that packaging steaks in a mixture of carbon dioxide and oxygen reduced significantly the microbial count. In this respect, 15% carbon dioxide was slightly better than 10% carbon dioxide.

Huffman (1974) reported that beef stored in CO_2 had significantly lower bacterial counts than beef stored in other gases. Also reported was that beef stored in the gas mixture (70% N_2, 25% CO_2 and 5% O_2) had significantly lower bacterial counts than beef stored in N_2, O_2 or air and no significant differences were noted in lactic acid or anaerobic counts for any treatment. It was also suggested that the mechanism responsible for increased storage time in high CO_2 concentrations involves the pH of the meat.

Balasundarum (1974) reported that a 15% CO_2 plus 85% O_2 atmosphere reduced significantly ($P < .05$) the microbial count on the surface of prepackaged steaks. This was even more effective when coupled with a 4% acetic acid spray sanitation treatment and a temperature just above freezing.

Factors Influencing the Pigment Changes in Beef

Chemical

The color of meat involves the chemistry of the heme pigments, hemoglobin and myoglobin. In the muscle of living animals both pigments serve to complex oxygen required for metabolic activity. Hemoglobin carries oxygen via the blood to the muscle; myoglobin provides the red color of muscle and serves as a storage site for oxygen in muscle. Myoglobin is a conjugated protein consisting of a heme moiety (iron containing porphyrin compound) attached to globulin type protein (Schweigert, 1956). According to Price and

Schweigert (1971) myoglobin is a complex protein consisting of a protein moiety known specifically as the globin and a nonpeptide portion called the heme. The heme is composed of the iron and a large planar ring, the porphyrin. Lemberg and Legge (1949) provide details of the chemistry of the muscle pigment. The chemical state of iron and the constituents attached to it are responsible for the various colors of the pigment in meat. If denaturation of the protein moiety occurs, the heme is no longer able to complex oxygen; hence, it is not possible to convert to the desirable bright red oxymyoglobin.

When meat is freshly cut the surface is purple owing to the presence of reduced myoglobin, upon exposure to air the ferrous ion in the heme pigment reacts rapidly and reversibly with O_2 to yield the desirable bright red oxymyoglobin. In the presence of O_2 the ferrous ion in the heme tends to oxidize slowly to the ferric state yielding the undesirable brown metmyoglobin (George and Stratmann, 1952a, b). In meat this autoxidation of the myoglobin is pseudo-reversible in that an enzymatic reducing system is present that is capable of converting metmyoglobin back to one of the reduced forms (Steward, Zipser and Watts, 1965).

The relative proportion of myoglobin, oxymyoglobin and/or metmyoglobin in meat is dependent upon two opposing reactions; oxidation of oxymyoglobin or reduced myoglobin to metmyoglobin and the subsequent re-reduction to myoglobin.

This dynamic equilibrium is known to be related to partial pressure of oxygen and enzymatic reducing pathways in meat. Oxymyoglobin will persist as long as oxygen tension is sufficient to supply the need of both enzymatic activity and complexing of oxygen to myoglobin. Early work by Neill and Hastings (1925) suggested that the oxidation of oxyhemoglobin to methemoglobin was correlated with low oxygen pressure. Brooks (1938) found that the low oxygen tension (4 mm Hg) produced a maximum rate of methemoglobin formation. George and Stratmann (1952a, b and 1954) indicated that the maximum rate of oxidation of myoglobin occurred at 1 to 1.4 mm Hg oxygen pressure, but later suggested the rate of maximum conversion may range from 1 to 20 mm Hg of oxygen pressure depending on the pigment, pH and temperature. Similar reaction rates were reported by Grant (1955).

Snyder and Ayres (1961), using enzyme inhibitors, demonstrated that respiratory enzymes in meat, especially succinic dehydrogenase, contributed to metmyoglobin formation by decreasing oxygen tension via oxidative phosphorylation. Urbin and Wilson (1961) also indicated that succinic dehydrogenase was an important competitor for oxygen in postmortem bovine muscle.

Microorganisms

The influence of bacteria on the color of fresh meats has been demonstrated by several investigators, but there

has been some controversy in the literature as to how microorganisms affect meat color. Jensen (1949) indicated that microorganisms, both living and dead, can cause the pigments of fresh meat to become oxidized. Stringer et al. (1969) reported that the growth of microbes on meat is one of the main factors that causes discoloration and spoilage.

The explanations for effects of bacteria on meat color center upon assumed influences of the bacteria on the oxidation-reduction potential of meat (Rïkert et al. 1957). Butler (1953) found that the main bacterial effect on color was exhibited during logarithmic growth phase and there was a close relationship between the number of Pseudomonas species present on beef cuts and the rate of discoloration. Microorganisms decrease case life by causing discoloration due to the oxidation of the meat pigment myoglobin to metmyoglobin (Hewitt 1950). In the logarithmic growth phase, aerobic bacterial cultures will frequently use up all available oxygen and actually cause reducing conditions to develop. Robach and Costilow (1961) concluded that the lowering of oxygen tension in the surface tissue which results in a great increase in reduced myoglobin. Landrock and Wallace (1955) reported that bacteria usually act upon fresh meat by (a) causing discoloration due to an increased rate of metmyoglobin formation, (b) producing off odors and (c) forming slime.

Gaseous Atmospheres

The composition of the gaseous atmosphere surrounding meat influences the color of meat and determines the type and extent of microbiological spoilage during storage. Gases dissolve in meat according to their partial pressures and reactions in the meat may consume or produce gas. A number of studies have indicated that an elevated oxygen level in the fresh meat environment would allow retention of the highly desirable bright red color. Increased oxygen levels might have an accelerating influence on the growth of microorganisms, but when this increased oxygen level is coupled with an elevated carbon dioxide concentration plus other good principles of preservation, it was demonstrated to be an effective means of extending shelf-life of fresh meat products while maintaining color. One of the serious problems here, of course, would be the possibility of fat oxidation resulting in rancidity.

A disadvantage of carbon dioxide is its production of discoloration or loss of "bloom" in fresh meat when used in high concentrations. Brooks (1933) observed that the levels of carbon dioxide above 20% produced discoloration of beef carcasses. This discoloration was ascribed to the acceleration by carbon dioxide of the oxidation of hemoglobin to methemoglobin. According to Oglivy and Ayres (1951) a concentration of 25% carbon dioxide is the maximum at which meat can be kept without incurring color defect.

Gonzales (1971) concluded that packaging steaks in a mixture of carbon dioxide and oxygen reduced significantly microbial count and maintained the desirable bright red color of the meat. In this respect 15% carbon dioxide was slightly better than 10% carbon dioxide.

Huffman (1974) reported that the color of beef stored in CO_2 was less desirable than beef stored in O_2. Color of beef was improved slightly when stored in a gas mixture (70% N_2, 25% CO_2 and 5% O_2).

Factors Associated with Changes in Lipid Composition

Production of Lipase and Lipolysis of Fats by Microorganisms

The production of lipase by Pseudomonas fragi in a synthetic medium was studied by Alford and Pierce (1963). These researchers reported that the failure of Pseudomonas fluorescens to produce appreciable lipase in the synthetic medium, as well as its production of more lipase on a casein digest than on peptone, is added evidence that a single medium is insufficient for the determination of lipolysis by different bacteria. Since Pseudomonas fluorescens is similar to Pseudomonas fragi in its pattern of lipolysis, the differences in nutrient requirements for lipase synthesis probably are not caused by a basic difference in the two enzymes, but rather a difference in synthetic pathway. These data also concluded that lipase production is not a

function of total cell growth. Furthermore, arginine, aspartic acid, glutamic acid and lysine alone accounted for over 90% of the total production of lipase when all 15 amino acids were included in the medium. Since these amino acids or their precursors are found in beef muscle, one would expect beef to be an excellent medium for the growth of some lipolytic microorganisms.

By 1894 many researchers had studied the lipolytic ability of microorganisms by growing them on solid media in which was dispersed olive oil or other fat. No indicator was used but the disappearance of the fat globules from the region surrounding the growth of the bacteria was accepted as evidence of hydrolysis. Since that time, new techniques have been developed for not only the indication of hydrolysis but to identify and isolate the hydrolytic organisms. Collins and Hammer (1933) studied the action of certain bacteria on some simple triglycerides and natural fats. Data from this study support the conclusion that all simple triglycerides were hydrolyzed by lipolytic bacteria if the appropriate pH was provided for their activity except tristearin. Also indicated in this study was that 83.7% of 92 cultures of bacteria on beef fat showed evidence of hydrolysis. It is possible that all 92 cultures would have showed hydrolysis if nile-blue sulfate had not been used since it has been shown to be toxic to many microorganisms (Starr 1941).

Alford and Elliott (1960) reported that decreasing the temperature at which an enzyme was produced caused a slight drop in the optimum pH for lipase activity. In later studies (Alford et al. 1960) it was indicated that this change in optimum pH was not the result of a change in specificity for any particular fatty acid. Also suggested in this study and as would be expected in any enzyme reaction, the rate of fatty acid liberation by bacterial lipase decreases with time. Even so, the decrease appears to be "across the board"; that is, the ratios of the fatty acids liberated remain constant, at least until about one-third of the fatty acids have been liberated.

Since it is generally accepted by many workers that fatty acids are not randomly distributed in fats (Mattson and Lutton 1958 and Quimby, Wille and Lutton 1953), the differences between percentages of fatty acid liberated and percentages in the original fat may be explained as follows: There could be specificity of the lipase for the α position on the triglyceride molecule, irrespective of the fatty acid attached to it. If the fatty acids were not randomly arranged in the fat, a larger percentage of acids esterified at the α position would appear in the free fatty acids than were present in the whole triglyceride. This seems to appear more plausible than the possibility of the lipase being specific for certain fatty acids.

Mattson and Beck (1955) reported, after initial hydrolysis of the fatty acids from the α position by pancreatic lipase, some shifting of the acids from the β to the α position occurred. Nevertheless, there is still doubt that the activity of bacterial lipases is entirely independent of the structure of the fatty acid. It is quite possible that a combination of positional specificity and fatty acid specificity might combine to accentuate or to obscure the real degree of randomness.

Frozen Storage

To date the literature on the changes in lipid classes as influenced by shelf-life conditions is scarce. However, Awad et al. (1968) conducted a study of the changes in three of the seven lipid classes commonly found in the beef muscle under frozen storage. This study concluded that during an eight week storage period of bovine muscle at -4 C there was an increase from 1.6 to 9.1% in the free fatty acid content and a corresponding decrease from 28.8 to 19.5% in the phospholipid fraction. There was no change in the total cholesterol and the total lipid decreased by 0.4% which was probably due to water loss during storage. However, one precaution should be taken into consideration when comparing frozen storage to refrigerated storage. It has been reported in the literature (Alford et al. 1960) that possibly a different group of lipase enzymes are active

below the freezing point as opposed to above the freezing point. Also, since it is known that different lipolytic microorganisms are active at one temperature and not at another, it would be expected that production of lipase would so be affected.

MATERIALS AND METHODS

General

Four experiments were conducted in this study utilizing top round steaks. Experiments I and II were conducted at The University of Arizona Meats Laboratory in a walk-in meat cooler, and Experiments III and IV were conducted on the main campus utilizing two self-contained open meat display cases. With these exceptions, all other conditions and analysis for each experiment were the same.

Selection and Processing of Top Rounds

A total of fifteen top rounds were selected from the Swift Distribution Center at Tucson. Four top rounds were used in Experiment I, three were used in Experiment II and four were used in both Experiments III and IV. In selection of the top rounds three criteria were set:

1. Grade -- All top rounds must have come from carcasses grading choice.

2. Maturity -- All top rounds must have come from carcasses with A- maturity as determined by physiological characteristics.

3. Hot carcass weight -- All top rounds must have come from carcasses with a hot carcass weight between 275 and 300 kg.

Since each experiment was conducted at different times, the top rounds for each experiment were selected independently. However, in each case the top rounds were aged seven to nine days at the Swift and Company Distribution Center at 2 ± 1 C.

After aging, the desired number of top rounds for each experiment were transported to the University Meats Laboratory. The Semimembranosus muscle was excised from each top round and cut transversely into 2.5 cm thick steaks. Each steak was further subdivided into 5 x 5 cm samples. By this method of division, each top round yielded at least sixteen 5 x 5 x 2.5 cm samples.

Allotment of Samples

The allotment of samples to each treatment by experiment was as follows:

It was the objective of Experiment I to study the effect of gaseous atmospheres and time on the shelf-life and microbial growth of beef steak. To accomplish this all samples for each day's analysis or for each sampling period were selected from the same top round. Since there were four sampling periods (Day 0, 3, 9 and 22) and four treatments (Control, 100% CO_2, 100% N_2 and Cryovac), each top round was subdivided into 16 steaks which provided four steaks for each treatment for each day. Experiments II, III and IV also had four steaks assigned to each treatment for each day.

The objective of Experiment II was to study the effect of packaging material and time on the microbial growth and shelf-life of beef steak. To accomplish this objective, the number of sampling intervals was shortened whereby one top round provided all the samples for one treatment throughout all sampling periods. This experiment was composed of three treatments (Control, Nalophan and Cryovac) and four sampling periods (Days 0, 3, 7 and 14).

In Experiment III a completely random design was used to study the effect of time and gaseous atmospheres as they affected the microbial growth and shelf-life of beef steak. To accomplish this objective, each top round was subdivided into at least 16 samples. These 16 samples were then numbered 1 through 16 and assigned numbers from the random numbers' table. Then in increasing order of the numbers from the random numbers' table, the lowest numbered sample was selected for Day 0, Treatment 1 until the highest numbered sample of the first top round was selected for Day 9, Treatment 4. The second, third and fourth top rounds were also handled in this manner. This experiment consisted of four treatments (Control; 95% air, 5% O_2; 90% air, 10% O_2; 85% air, 15% O_2) and four sampling periods (Days 0, 3, 6 and 9).

Experiment IV employed the same design and the same number of sampling periods as Experiment III. The

treatments used were the Control; 90% O_2, 10% CO_2; 85% O_2, 10% CO_2, 5% N_2; 75% O_2, 15% CO_2, 10% N_2.

Packaging and Storage of Samples for each Experiment

Experiment I

All samples were placed in Mobilform TMI-0550 trays with dimensions of 12.7 x 12.7 x 2.54 cm. Borden's Resinite film was used for wrapping the control samples. This was a clear heat sealing film with a gas transmission rate for O_2 of 310 to 387.5 cc/cm^2/24 hrs./atmosphere and for CO_2, 2480 to 2790 cc/100cm^2/24 hrs./atmosphere. Clear, gas impermeable Cryovac bags were used for the carbon dioxide and nitrogen treated samples. The samples were placed in bags, back-flushed with the appropriate gas and then filled with the gas. The bag was then twisted several times, tied with a rubber band, folded back on itself and tied a second time. Cryovac bags were also used for the vacuum treatment. In this case the samples were placed in the bag and a vacuum of 1050 g/cm^2 was pulled on each bag. Each bag was sealed in the same way as the atmospheric gas treatments. The bags were heat shrunk in a water bath at 150 F for ten seconds. For storage these samples were displayed on tables in the walk-in meat cooler. Lighting was furnished by cooler lights, totaling 50 foot candles and was turned on and off

at 12 hour intervals during the experiment. The temperature was held at 4 C ± 1 C for the entire period.

Experiment II

As in Experiment I, the control was over-wrapped with Borden's resinite film and one of the vacuum packages was the same Cryovac bag. In addition a new vacuum package was introduced which carried a trade name "Nalophan". It was obtained from Brechteen and Company, Mount Clements, Michigan. This was a light tan bag in color with a gas transmission rate for O_2 of .008 $cc/cm^2/24$ hrs./atmosphere and for CO_2, .04 $cc/cm^2/24$ hrs./atmosphere. Both the Cryovac and the Nalophan bags were sealed as in Experiment I but heat shrinking was not applied. In Experiment I heat shrinking distorted the color of the sample immediately and since chemical changes under anaerobic conditions were the primary concern, heat shrinking was not used in Experiment II. All storage conditions were the same as in Experiment I.

Experiments III and IV

Both Experiments III and IV utilized the same control as the previous two experiments and Cryovac bags were used for the samples which were exposed to the various gas mixtures. Both experiments utilized gas mixtures as previously identified in the allotment of samples section. These bags were back-flushed, filled and sealed as described in Experiment I.

For display these samples were placed in two Master-Bilt Model LMC 1230 (106.7 x 55.9 x 58.4 cm) self-contained open cases at the main campus laboratory. The temperature was held constant at 4 C ± 0.5 C for the entire storage period. Lighting was furnished by Sylvania Lifeline F96T12-CW flourescent strips, totaling 100 foot candle. A timer was used to turn the lights on and off a 12 hour intervals during the experiments.

Sample Collection

Sample collection was carried out in the same way and under the same conditions in all experiments. For all sampling periods the appropriate samples were selected for analysis. On Day 0 these were non-treated fresh samples which were not packaged. For the other periods the samples were unpackaged and allowed a 30 minute period for color adjustment after exposure to the normal atmosphere and light. After the 30 minute adjustment period, color scores and microorganism samples were taken. After the appropriate scores and samples were taken, the top 0.25 cm of each sample steak was removed using a sanitized Globe meat slicer for lipid extraction.

Analytical Procedures

Several analytical procedures were used in the analysis of the samples. Since some were standard techniques

and some were modifications, all procedures used will be outlined.

Percent Lipid

The samples were analyzed for total extractable intramuscular lipid by the chloroform-methanol ($CHCl_3$-CH_3OH) extraction (Brown, 1969). The modified procedure was as follows:

1. Analytically weigh approximately 10 g of diced and thoroughly mixed muscle tissue.

2. Mix tissue (Omni-Mixer with 300 ml stainless steel adapter) with 90 ml CH_3OH at high speed (speed control = 6.0) for 90 seconds.

3. Add 50 ml $CHCl_3$ and mix for 30 seconds at slow speed (speed control = 2.0).

4. Repeat step 3.

5. Add 50 ml distilled water and mix for 30 seconds at a high speed.

6. Add 0.5 teaspoons (approximately 1.8 g) zinc acetate and mix for 10 seconds at high speed.

7. Filter through two pieces of Whatman No. 42 filter paper in a Buchner funnel. Wet filter paper with distilled water before adding homogenate.

8. Wash mixing jar with approximately 30 ml $CHCl_3$ and transfer contents to Buchner funnel after homogenate has completely filtered.

9. Transfer filtrate to 250 ml graduated cylinder. Rinse flask with 10 ml $CHCl_3$ and add to filtrate.

10. When two phases have clearly separated (allow to set overnight) in the graduated cylinder, read and record the volume of the lower phase ($CHCl_3$). Remove upper phase by suction and discard.

11. Remove a 20 ml aliquot from the lower phase and place in a predried and analytically weighed 50 ml beaker. Evaporate to dryness (overnight) in a vacuum oven at approximately 30 C with a mild vacuum and obtain dried weight analytically. After weighing the lipid was solubilized with $CHCl_3$ and transfered to a two-dram vial.

12. Calculation: % lipid =

$$\frac{\text{aliquot lipid weight} \times \frac{\text{volume of } CHCl_3 \text{ layer}}{20}}{\text{weight of tissue (wet)}} \times 100$$

13. Store sample in a freezer (-18 C) until thin layer chromatography separation.

Thin Layer Chromotography and Charring of Lipids

Thin layer chromotography was used to separate the lipid sample into the six predominate lipid classes of bovine muscle. The procedure used is as follows:

1. Evaporate the sample with N_2 until dry.

2. Take the sample up in the appropriate milliliters of $CHCl_3$ to give 250 to 300 micrograms (μg) of lipid

per 5 microliters (μl) of solution:

Calculation:

$$X \text{ ml} = \frac{5\mu l \times \text{sample lipid wt. in } \mu g}{275\ \mu g} \div 1000$$

3. Activate the strips (Silplate-F-22, Brinkmann Instruments, Incorporated) by heating in a 200 C oven for 30 minutes, then dessicate for 45 minutes. Strips were prepared by cutting the Brinkmann 20 x 20 cm plates into 2.54 cm x 20 cm strips.

4. All strips were washed in a diethyl ether bath for not less than 4 hours to remove contaminates. Immediately after the ether washing, the strips were dried for 5 minutes at 200 C.

5. Spot the strips by applying the 5 μl sample (250 to 300 μg) with a 10 μl syringe.

6. Place strips in the solvent tank containing 200 ml pentane, 25 ml diethyl ether and 5 ml glacial acetic acid for exactly one hour.

7. Remove strips from the solvent tank and allow to dry at room temperature for 15 to 30 minutes.

8. Repeat step 6.

9. Repeat step 7.

10. After strips were allowed to dry, they were dipped as quickly as possible into a 10% (w/v) phosphomolybidic acid ($P_2O_5 \cdot 24MoO_3 \cdot H_2O$) solution and then allowed to drain on an absorbent material. This solution must be

completely yellow and display a cloudy appearance. Once the cloudy appearance has disappeared and the solution is clear, the acid can no longer by used because it has gone through autoxidation.

11. After strips had drained of residual acid, they were placed in an oven at 200 C for exactly 10 minutes. After this time period, the lipid classes were visible as dark gray spots and the strip displayed a light yellow background.

Quantitation of Lipids

Due to the respective amounts of the six lipid classes and the range of accuracy of the densitometer used in this study, it was not possible to determine the individual concentrations of each class by using only one thin layer strip. For these reasons, the phospholipid, monoglyceride, diglyceride, free cholesterol and free fatty acid classes were quantitated on one strip and the triglyceride class on another. As previously stated, 250 to 300 μg of lipid per 5 μl solvent was used as an initial dilution guide. This initial dilution also gave the proper amount of sample for analysis of the phospholipid, monoglyceride, diglyceride, free cholesterol and free fatty acid classes. For the analysis of the triglyceride fraction, a second dilution was made in order to convert the first dilution to one-fourth its original concentration. This provided a

solution with a concentration of approximately 75 μg per 5 μl for application on the strip and quantitation of the triglyceride class. Both strips were treated identically in the chromotographic developing and charring process.

For quantitation the relative areas and densities of the individual lipid classes (dark gray spots) were determined by densitometry (Photovolt Densicord, Model 542). A print-out of the various peaks for each sample was obtained from the densitometer. The peak height of each peak was measured in millimeters and put into a straight-line equation for each respective peak and converted to micrograms of lipid. Data for the straight-line equations was obtained from standard curves made by using lipid standards from Applied Laboratories. Refer to Figure 1 for the standards used and standard curves produced.

Microorganism Determinations

Sterile cotton swabs were used to collect the samples. The swabs were dipped in 100 ml of sterile buffered water (4×10^{-4} M KH_2PO_4) and rolled across a circular six square centimeter area as measured by a sterile overlaying aluminum template. The area was swabbed two times using two separate swabs; after each swabbing the swab was rinsed in the sterile buffered water. The swab was then placed in the sterile buffered water after breaking off the part that had been touched.

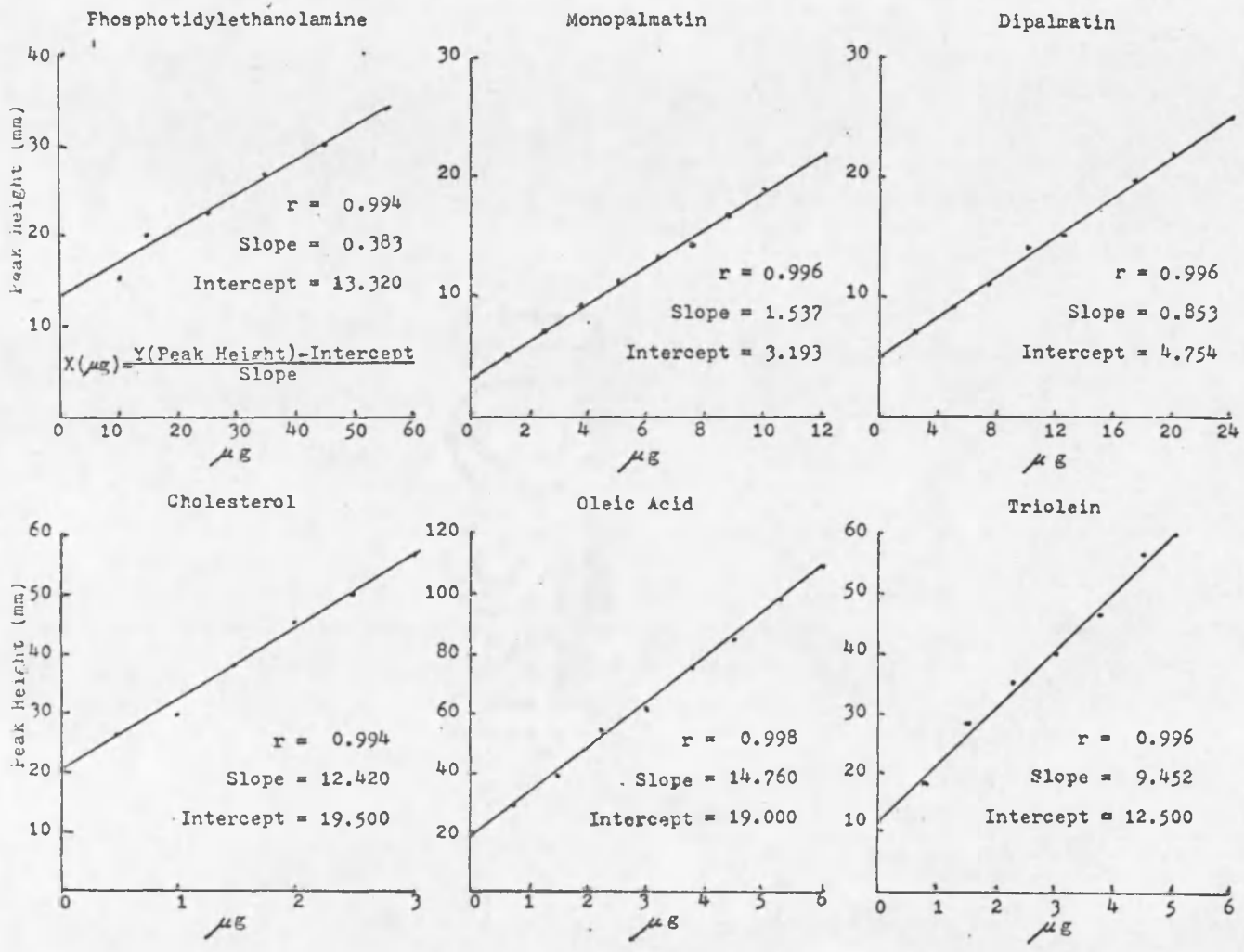

Figure 1. Standard curves and regression equations used in the quantification of the various lipid fractions using densitometry.

The samples were then plated using Standard Plate Count Agar. The agar was control number 579720, a dehydrated media of the Difco Manual (1953). Serial dilutions were carried out with the use of sterile 100 ml milk dilution bottles and sterile 1.1 ml pipets.

Mesophilic and psychrophilic microorganisms were both determined. Mesophiles were incubated at 25 C for three days and the psychrophiles were incubated at 4 C for seven days. Total counts were determined as follows:

$$\frac{\text{total counts x dilution factor}}{6\ \text{cm}^2} = \text{counts/cm}^2$$

$$= \text{counts } \log_{10}/\text{cm}^2$$

Detection of Lipolysis

The enumeration of lipolytic microorganisms was used as an estimation of the degree of lipolysis occurring during display (Starr, 1941). Collection of these samples was conducted exactly as with mesophiles and psychrophiles. The only difference was the media used for this enumeration. These samples were plated using Spirit Blue Agar accompanied by a lipodial substrate. The agar was control number 0950-01-9, a dehydrated media of the Difco Manual (1973). The lipase reagent was control number 0431-63-3 distributed in 6 x 20 ml vials of the Difco Manual (1973). These samples were incubated at 25 C for three days. Colonies of lipolytic organisms are recognized by development of a deep blue

color beneath and surrounding the colony. Total counts were determined the same way as with the mesophiles and psychrophiles.

Color Determination

The Macbeth-Munsell Disc Colorimeter was used for the determination of hue, value and chroma of a 5 x 5 cm section of the muscle sample. The method used provided a means for specifying the colors of the lean in terms of the Munsell Color System, a system based on the color-perception attributes hue, lightness and saturation. The basis for the calculations of these attributes was work done by Newhall, Nickerson and Judd (1943).

Statiscal Analysis of Data

All data were tested for significance of interaction by means of the least-squares analysis according to Harvey (1960). After interaction was shown to be significant, all data were tested for significance by means of the least-squares analysis of variance and nested least-squares procedures according to Harvey (1960). The nested variables were treatments and days.

On those effects found to be significant ($P < .05$), the Duncan's New Multiple Range Test according to Li (1964) was applied to isolate these differences. Scattergrams were plotted for the data to study the significance of simple

correlations between days and all other dependent variables according to Hays (1973).

RESULTS AND DISCUSSION

Experiment I

Least-squares means for chemical and physical parameters between treatments within days are presented in Table 1. Both psychrophilic and mesophilic microorganism numbers were found to be significantly different ($P < .05$) within Day 22 between the polyvinyl chloride and cryovac treatments. Kraft and Ayres (1952) reported that films that permit oxygenating conditions necessary for maintenance of red color, such as polyvinyl chloride, allowed rapid growth of microorganisms on the meat surface. Furthermore, the value of an essentially anaerobic environment produced by vacuum packaging, such as cryovac packaging, has been demonstrated by Warnecke et al. (1966) and Beban, Kraft and Walker (1970). Also, these researchers noted a significantly ($P < .05$) lower number of spoilage bacteria on vacuum-packaged cuts.

Highly significant ($P < .01$) positive simple correlations were found between all treatments and days for both psychrophilic and mesophilic microorganisms (Table A-1). Furthermore, significant increases ($P < .05$) were found within all treatments between Day 0 and Day 9 for both psychrophilic and mesophilic microorganisms and then a corresponding significant ($P < .05$) decrease between Day 9 and Day 22 (Table 2). These data point to the fact that regardless of

Table 1. Least-squares means for total microorganism numbers, color parameters, lipid percent and lipid fractions by treatment within days for Experiment I.

Parameter	Day 0	Day 3				Day 9				Day 22			
	Fresh	PVC[a]	CO_2[a]	N_2[a]	Cry.[a]	PVC	CO_2	N_2	Cry.	PVC	CO_2	N_2	Cry.
Microorganisms, logs													
Psychrophiles	4.4	6.8	6.4	6.6	6.2	9.7^{bc}	10.0^{c}	10.0^{bc}	9.5^{b}	9.6^{c}	8.7^{bc}	8.7^{bc}	8.3^{b}
Mesophiles	4.5	6.5	6.4	6.5	6.2	9.9^{bc}	10.6^{c}	10.4^{bc}	9.6^{b}	9.6^{c}	8.7^{bc}	8.9^{bc}	8.3^{b}
Lipotropic	2.4	3.5	2.6	3.1	2.6	5.3^{c}	2.5^{b}	6.1^{c}	3.7^{b}	-	-	-	-
Color													
Hue	9.8	15.2^{bc}	16.8^{c}	16.8^{c}	13.7^{b}	18.8^{c}	18.0^{bc}	16.2^{b}	16.4^{b}	11.5^{b}	18.5^{d}	14.1^{c}	15.3^{c}
Value	6.4	6.5^{c}	6.5^{c}	6.5^{c}	6.4^{b}	6.5^{c}	6.5^{c}	6.4^{b}	6.5^{c}	6.4^{b}	6.5^{c}	6.4^{b}	6.4^{b}
Chroma	7.4	3.9	4.2	4.3	4.5	3.4	3.4	3.5	3.9	5.2^{c}	3.4^{b}	4.3^{bc}	3.7^{b}
Lipid, %	6.7	6.8^{bc}	6.4^{b}	8.2^{c}	7.2^{bc}	6.8^{bc}	7.3^{c}	5.7^{d}	6.2^{bc}	6.8	6.3	5.3	5.9
Lipid Fractions, mg/g													
Phospholipids	142.6	143.5^{b}	140.6^{b}	228.7^{d}	154.3^{e}	184.5^{c}	205.9^{d}	136.6^{b}	266.2^{e}	189.9^{c}	145.0^{b}	182.9^{c}	186.2^{c}
Monoglycerides	9.5	12.7^{bc}	8.5^{b}	15.2^{c}	20.6^{d}	11.9^{b}	20.5^{c}	14.1^{b}	24.7^{c}	59.3^{d}	34.3^{c}	34.0^{c}	22.2^{b}
Diglycerides	52.4	56.2^{c}	41.6^{b}	85.7^{e}	67.8^{d}	79.3^{d}	64.4^{c}	38.9^{b}	60.5^{c}	156.7^{e}	134.7^{e}	126.1^{c}	111.3^{b}
Triglycerides	794.5	781.4^{d}	805.6^{e}	659.7^{b}	750.7^{c}	702.7^{b}	669.5^{c}	787.9^{e}	629.6^{b}	567.2^{b}	674.1^{d}	643.8^{c}	669.2^{d}
Cholesterol	2.4	2.9^{bc}	2.3^{b}	5.3^{c}	2.6^{b}	7.6^{b}	12.6^{c}	8.9^{b}	7.8^{b}	5.8^{d}	5.5^{c}	5.9^{c}	5.3^{b}
Free Fatty Acids	2.4	3.3^{bc}	1.4^{b}	5.2^{c}	4.1^{bc}	14.0^{b}	27.1^{c}	13.7^{b}	11.3^{b}	15.8^{d}	6.4^{c}	7.3^{c}	2.9^{b}

[a]A -- Steaks wrapped in polyvinyl chloride film; B -- Steaks in cryovac bags with CO_2 added; C -- Steaks in cryovac bags with N_2 added; D -- Steaks in cryovac bags which were evacuated.

[b,c,d,e]Values on the same line within each day possessing unlike superscripts are significantly different ($P<.05$).

Table 2. Least-squares means for total microorganism numbers, color parameters, lipid percent and lipid fractions by day within treatments for Experiment I.

Parameter	PVC[a] Day 0	PVC[a] Day 3	PVC[a] Day 9	PVC[a] Day 22	CO_2[a] Day 0	CO_2[a] Day 3	CO_2[a] Day 9	CO_2[a] Day 22	N_2[a] Day 0	N_2[a] Day 3	N_2[a] Day 9	N_2[a] Day 22	Cry.[a] Day 0	Cry.[a] Day 3	Cry.[a] Day 9	Cry.[a] Day 22
Microorganisms, logs																
Psychrophiles	4.4^{b}	6.8^{c}	9.6^{d}	9.6^{d}	4.4^{b}	6.4^{c}	10.0^{e}	8.6^{d}	4.4^{b}	6.6^{c}	9.9^{e}	8.6^{d}	4.4^{b}	6.2^{c}	9.4^{e}	8.3^{d}
Mesophiles	4.5^{b}	6.4^{c}	9.8^{d}	9.6^{d}	4.5^{b}	6.4^{c}	10.5^{e}	8.7^{d}	4.5^{b}	6.4^{c}	10.4^{e}	8.9^{d}	4.5^{b}	6.2^{c}	9.6^{e}	8.3^{d}
Lipotropic	2.4^{b}	3.5^{c}	5.3^{d}	-	2.4	2.6	2.5	-	2.4^{b}	3.1^{c}	6.1^{d}	-	2.4^{b}	2.6^{b}	3.7^{c}	-
Color																
Hue	9.8^{b}	15.2^{d}	15.8^{d}	11.5^{c}	9.8^{b}	16.8^{e}	18.0^{e}	18.5^{e}	9.8^{b}	16.8^{d}	16.2^{d}	14.1^{c}	9.8^{b}	13.6^{c}	16.4^{d}	15.2^{d}
Value	6.4^{b}	6.4^{b}	6.5^{c}	6.4^{b}	6.4^{b}	6.5^{c}	6.4^{b}	6.5^{c}	6.4^{b}	6.5^{c}	6.4^{b}	6.4^{b}	6.4	6.4	6.4	6.4
Chroma	7.4^{d}	3.8^{b}	3.4^{b}	5.2^{c}	7.4^{c}	4.2^{b}	3.4^{b}	3.4^{b}	7.4^{c}	4.3^{b}	3.5^{b}	4.3^{b}	7.4^{d}	4.5^{c}	3.9^{b}	3.6^{b}
Lipid, %	6.7	6.8	6.8	6.8	6.7^{b}	6.4^{b}	7.2^{c}	6.3^{b}	6.7^{c}	8.2^{d}	5.7^{b}	5.2^{b}	6.7^{bc}	7.2^{c}	6.1^{b}	5.8^{b}
Lipid Fractions, mg/g																
Phospholipids	142.6^{b}	143.5^{b}	184.5^{c}	189.8^{c}	142.6^{b}	140.6^{b}	205.9^{c}	145.0^{b}	142.6^{b}	228.7^{d}	136.6^{b}	182.9^{c}	142.6^{b}	154.3^{b}	266.2^{d}	186.2^{c}
Monoglycerides	9.5^{b}	12.6^{c}	11.8^{bc}	59.2^{d}	9.5^{b}	12.6^{b}	11.8^{b}	59.2^{c}	9.5^{b}	15.2^{c}	14.1^{c}	34.0^{c}	9.5^{b}	20.6^{c}	24.6^{d}	22.2^{cd}
Diglycerides	52.4^{b}	56.2^{b}	79.3^{c}	156.6^{d}	52.4^{c}	41.6^{b}	64.4^{d}	134.6^{e}	52.4^{c}	85.7^{d}	39.8^{d}	126.1^{c}	52.4^{b}	67.7^{d}	60.5^{c}	111.2^{e}
Triglycerides	794.5^{d}	781.4^{d}	702.6^{c}	567.2^{b}	794.5^{c}	805.6^{c}	669.4^{b}	674.1^{b}	794.5^{c}	659.8^{b}	787.8^{c}	643.8^{b}	794.5^{e}	750.6^{d}	629.6^{b}	669.2^{c}
Cholesterol	2.4^{b}	2.9^{b}	7.6^{c}	5.8^{c}	2.4^{b}	2.3^{b}	12.6^{d}	5.4^{c}	2.4^{b}	5.3^{c}	8.9^{d}	5.9^{c}	2.4^{b}	2.6^{b}	7.8^{d}	5.7^{c}
Free Fatty Acids	2.4^{b}	3.2^{b}	14.0^{c}	15.8^{c}	2.4^{b}	1.4^{b}	27.1^{d}	6.4^{c}	2.4^{b}	5.2^{c}	13.6^{d}	7.2^{c}	2.4^{b}	4.0^{c}	11.2^{d}	2.9^{b}

[a]A -- Steaks wrapped in polyvinyl chloride film; B -- Steaks in cryovac bags with CO_2 added; C -- Steaks in cryovac bags with N_2 added; D -- Steaks in cryovac bags which were evacuated.

[b,c,d,e]Values on the same line within each day possessing unlike superscripts are significantly different ($P<.05$).

treatment, microorganism growth followed the typical growth curve with a representative lag, logarithmic, stationary and death phase.

Lipolytic microorganisms were observed to be significantly different ($P < .05$) at Day 9 between both the carbon dioxide and cryovac and the nitrogen treatments. Although the actual reason for this difference is not known, some phenomena existed in the carbon dioxide and cryovac treatments to discourage the growth of these microorganisms. The carbon dioxide could have been toxic to these microorganisms as a protoplasmic poison but it is not simply a lack of oxygen in the cryovac treatment that discouraged their growth because the nitrogen treatment supported the growth of these organisms excellently.

Also presented in Table 1 are the color data by treatments within days for Experiment I. The three terms used to evaluate color were hue, value and chroma. Definitions of these terms as discussed by Newhall, Nickerson and Judd (1943) are: hue refers to the shade or tint of the color; value refers to the lightness or darkness of the color; and chroma refers to the intensity of the color found in the muscle.

A significant increase ($P < .05$) in hue was found between Day 0 and Day 3 within all treatments (Table 2). Since hue refers to the shade or tint of the color, it was apparent at Day 3 that all samples had darkened within each

treatment. Visual observations found these samples to be darkened to the point that they were no longer acceptable by the consumer. Also, a significant difference ($P < .05$) was noted between the cryovac treatment and both the carbon dioxide and nitrogen treatment for Day 3 for hue (Table 1). This difference can be accounted for by the fact that in an anaerobic condition, such as cryovac, a significantly ($P < .05$) lower microorganism number is maintained (Table 1). Butler (1953) reported that bacteria commonly found on meat caused discoloration by an increase in the rate of metmyoglobin formation during the logarithmic growth phase. The largest increase in hue was found within the carbon dioxide treatment at Day 22 (Table 2).

By employing slope ratios as a measure of relative effectiveness, carbon dioxide was proved to be 177 percent less efficient than polyvinyl chloride in controlling the increase in hue (Table B-1). Thus, a disadvantage of carbon dioxide was its production of discoloration or loss of "bloom" in fresh meat when used in high concentrations (above 20%). Brooks (1933) observed that the levels of carbon dioxide above 20% produced discoloration of beef carcasses. This discoloration was ascribed to the acceleration by carbon dioxide of the oxidation of hemoglobin to methemoglobin.

Furthermore, hue and days within the carbon dioxide treatment were found to be highly correlated ($P < .01$) with a

correlation coefficient of 0.59 (Table A-1). Also hue and value were highly correlated ($P < .05$) with a correlation coefficient of 0.62 (Table A-2), indicating that an increase in hue with a corresponding change in shade or tint darkens the sample which results in a change in value.

A significant decrease ($P < .05$) in the triglyceride class was observed between Day 0 and Day 22 within all treatments (Table 2). Also observed in Table 2 are the significant increases ($P < .05$) between Day 0 and Day 22 in the monoglyceride and diglyceride classes within all treatments. These data indicate that appreciable hydrolysis of the triglyceride class was taking place. Collins and Hammer (1933) studied the action of certain bacteria on some simple triglycerides and neutral fats. Data from this study support the conclusion that all triglycerides were hydrolyzed by lipolytic bacteria if the appropriate pH was provided. Also indicated in this study was that 83.7% of the 92 cultures of bacteria on beef fat showed evidence of a certain ability to cause hydrolysis. In further support of this conclusion, a significant increase ($P < .05$) was noted in the free fatty acid class between Day 0 and Day 9 within all treatments (Table 2). The reason for the significant decrease in the free fatty acid class between Day 9 and Day 22 (Table 2) within all treatments except the polyvinyl chloride treatment is attributed to two factors. First, the lipolytic microorganisms which are capable of lipolysis declined in

number after Day 9 and secondly, it is postulated that microorganisms have the ability to utilize free fatty acids as energy in the stationary phase of their growth cycle.

A highly significant ($P < .01$) correlation coefficient of 0.63 was found between the free fatty acid class and days within the polyvinyl chloride treatment (Table A-1). This may explain why the significant decrease ($P < .05$) in the free fatty acid class between Day 9 and Day 22. The free fatty acid class was less in concentration for both Day 9 and Day 22 for the cryovac treatment as compared to the other three treatments (Table 2). The reason for this is possibly due to the fact that on Day 9 and Day 22 both the mesophilic and psychrophilic microorganisms, which also have the capacity of lipolysis, were lower in number than those for the other treatments. In further support, the data in Table B-1 provide a relative efficiency of 84% for the cryovac treatment over the polyvinyl chloride treatment in controlling the increase of the free fatty acid class. Also, the data in Table B-1 proved the cryovac treatment to be 28% less efficient than the polyvinyl chloride treatment in controlling the decrease in the triglyceride class.

Experiment II

Least-squares means for chemical and physical parameters between treatments within days are presented in Table 3. Both psychrophilic and mesophilic microorganism

Table 3. Least-squares means for total microorganism numbers, color parameters, lipid percent and lipid fractions by treatment within days for Experiment II.

Parameter	Day 0	Day 3			Day 7			Day 14		
	Fresh	PVC[a]	Cry.[a]	Nal.[a]	PVC	Cry.	Nal.	PVC	Cry.	Nal.
Microorganisms, logs										
Psychrophiles	4.3	5.3^{bc}	4.1^{b}	5.3^{c}	7.7^{c}	7.4^{c}	4.6^{b}	9.9^{c}	7.1^{b}	8.8^{c}
Mesophiles	4.6	5.1^{bc}	4.2^{b}	5.4^{c}	7.7^{c}	7.6^{c}	5.8^{b}	9.4	8.8	9.6
Lipotropic	1.8	2.7^{bc}	2.0^{b}	3.5^{c}	3.1	2.8	2.4	2.8	2.5	2.0
Color										
Hue	9.7	9.4^{b}	13.1^{c}	11.5^{bc}	16.0^{c}	13.0^{b}	13.2^{b}	13.8^{bc}	15.8^{c}	13.0^{b}
Value	6.4	6.4^{c}	6.4^{b}	6.4^{bc}	6.5^{c}	6.5^{c}	6.4^{b}	6.5^{c}	6.5^{c}	6.4^{b}
Chroma	6.7	6.9^{c}	4.9^{b}	5.8^{bc}	4.0	5.2	4.7	4.9	4.5	4.9
Lipid, %	5.8	6.2	5.8	6.7	6.5	4.2	5.2	8.0^{c}	4.9^{b}	5.3^{b}
Lipid Fractions, mg/g										
Phospholipids	218.0	207.2^{b}	232.5^{c}	206.3^{b}	143.4^{b}	186.4^{c}	272.4^{d}	153.3^{b}	235.8^{d}	184.6^{c}
Monoglycerides	40.4	36.0^{bc}	41.0^{c}	32.9^{b}	28.6^{b}	61.6^{d}	39.2^{c}	29.9^{b}	40.2^{c}	29.5^{b}
Diglycerides	136.5	94.2^{b}	91.5^{b}	110.7^{c}	93.8^{b}	142.8^{d}	116.9^{c}	101.3^{b}	118.6^{c}	105.8^{b}
Triglycerides	587.4	646.9^{c}	621.3^{b}	634.0^{bc}	725.5^{d}	579.2^{c}	551.5^{b}	690.7^{d}	580.8^{b}	664.5^{c}
Cholesterol	9.6	11.2	11.7	10.5	3.9^{b}	16.4^{c}	13.4^{c}	7.5	10.7	10.4
Free Fatty Acids	7.9	4.4	2.0	5.5	4.7^{b}	13.6^{c}	6.6^{b}	17.6^{c}	13.9^{c}	5.2^{b}

[a]PVC -- Steaks wrapped in polyvinyl chloride film; Cry. -- Steaks in evacuated cryovac bags; Nal. -- Steaks in nalophan bags (nylon material).

[bcd]Values on the same line within each day possessing unlike superscripts are significantly different ($P < .05$).

numbers were found to be significantly different ($P < .05$) between the nalophan and cryovac treatments at Day 7. Both these treatments provided an anaerobic atmosphere and the reason for this difference is not completely understood. All treatments displayed a significant increase ($P < .05$) in psychrophilic and mesophilic microorganism numbers between Day 0 and Day 14 (Table 4). This same trend was also found in Experiment I. Lipolytic organisms increased ($P < .05$) between Day 0 and Day 7 within all treatments and then decreased ($P < .05$) between Day 7 and Day 14 (Table 4). The reason for this is most probably due to the fact that the lipolytic organisms reached the death phase of their growth cycle at some point between Day 7 and Day 14. This decline occurred between Day 9 and Day 22 in Experiment I (Table 2). Also, significant differences ($P < .05$) between the nalophan and cryovac treatments within both Day 7 and Day 14 for lipolytic microorganisms are shown in Table 3. The reason for this may again be due to the difference in oxygen permeability between the two films.

Also found in Table 3 were the color data by treatments within days for Experiment II. The terms used to evaluate color have previously been defined in Experiment I of this section. A significant increase ($P < .05$) was noted between Day 0 and Day 3 within all three treatments for hue (Table 4). This same trend was apparent in Experiment I. Furthermore, significant differences ($P < .05$) between the

Table 4. Least-squares means for total microorganism numbers, color parameters, lipid percent and lipid fractions by day within treatments for Experiment II.

Parameter	PVC[a]				Cry.[a]				Nal.[a]			
	Day 0	Day 3	Day 7	Day 14	Day 0	Day 3	Day 7	Day 14	Day 0	Day 3	Day 7	Day 14
Microorganisms, logs												
Psychrophiles	4.3^{b}	5.3^{c}	7.7^{d}	9.9^{e}	4.3^{b}	4.1^{b}	7.4^{c}	7.1^{c}	4.3^{b}	5.3^{b}	4.6^{b}	8.8^{c}
Mesophiles	4.6^{b}	5.1^{b}	7.7^{c}	9.4^{d}	4.6^{b}	4.2^{b}	7.6^{c}	8.8^{d}	4.6^{b}	5.4^{b}	5.8^{b}	9.6^{c}
Lipotropic	1.8^{b}	2.7^{c}	3.1^{c}	2.8^{c}	1.8^{b}	2.0^{b}	2.8^{c}	2.5^{bc}	1.8^{d}	3.5^{c}	2.4^{b}	2.0^{b}
Color												
Hue	9.7^{b}	9.4^{b}	16.0^{d}	13.8^{c}	9.7^{b}	13.1^{c}	12.9^{c}	15.8^{d}	9.7^{b}	11.5^{c}	13.2^{c}	13.0^{c}
Value	6.4^{b}	6.4^{b}	6.5^{c}	6.5^{c}	6.4^{b}	6.5^{c}	6.5^{c}	6.5^{c}	6.4	6.4^{bc}	6.4	6.4
Chroma	6.7^{c}	6.9^{c}	4.0^{b}	4.9^{b}	6.7^{c}	4.9^{b}	5.2^{bc}	4.5^{b}	6.7^{c}	5.8^{bc}	4.7^{b}	4.9^{b}
Lipid, %	5.8^{b}	6.2^{b}	6.5^{b}	7.9^{c}	5.8^{c}	5.8^{c}	4.2^{b}	4.9^{bc}	5.8	6.7	5.2	5.3
Lipid Fractions, mg/g												
Phospholipids	218.0^{c}	207.2^{c}	143.4^{b}	153.3^{b}	218.0^{c}	232.5^{d}	186.4^{b}	235.8^{d}	218.0^{c}	206.3^{c}	272.4^{d}	184.6^{b}
Monoglycerides	40.4^{c}	36.0^{c}	28.6^{b}	29.9^{b}	40.4^{b}	41.0^{b}	61.6^{c}	40.2^{b}	40.4^{c}	32.9^{b}	39.2^{c}	29.5^{b}
Diglycerides	136.5^{c}	94.2^{b}	93.9^{b}	101.3^{b}	136.5^{d}	91.5^{b}	142.8^{d}	118.6^{c}	136.5^{d}	110.7^{bc}	116.9^{c}	105.7^{b}
Triglycerides	587.4^{b}	646.9^{c}	725.5^{e}	690.7^{d}	587.4^{c}	621.3^{d}	579.2^{b}	580.8^{b}	587.4^{c}	634.0^{d}	551.5^{b}	664.5^{c}
Cholesterol	9.6^{cd}	11.2^{d}	3.9^{b}	7.5^{c}	9.6^{b}	11.7^{b}	16.4^{c}	10.7^{b}	9.6^{b}	10.5^{b}	13.4^{c}	10.4^{b}
Free Fatty Acids	7.9^{c}	4.4^{b}	4.7^{b}	17.6^{d}	7.9^{c}	2.1^{b}	13.6^{d}	13.9^{d}	7.9^{c}	5.5^{b}	6.6^{bc}	5.2^{b}

[a]PVC -- Steaks wrapped in polyvinyl chloride film; Cry. -- Steaks in evacuated cryovac bags; Nal. -- Steaks in nalophan bags (nylon material).

[b,c,d,e]Values on the same line within each day possessing unlike superscripts are significantly different ($P<.05$).

polyvinyl chloride treatment and both the cryovac and nalophan treatments within Day 3 indicate that the polyvinyl chloride treatment was most effective in maintaining a lower hue score (Table 3). This indicated that the polyvinyl chloride treatment maintained a brighter tint or shade and consequently a more desirable fresh meat color. The reason for this being the increased amount of oxygen present for the maintenance of oxymyoglobin as compared to the vacuum treatment.

Trends in lipid classes found in Experiment I were not established as such in Experiment II. However, significant differences ($P \leq .05$) were noted between the cryovac and nalophan treatments for both Day 7 and Day 14 for the free fatty acid classes (Table 3). In both cases, nalophan had the lowest concentration of free fatty acids. The reason for this may be again due to the fact that nalophan had a lower oxygen transmission rate than did the cryovac film. The decreased available oxygen to the nalophan treatments decreased the number of lipolytic microorganisms and consequently the degree of hydrolysis naturally occurring. However, without the trends displayed in Experiment I, it is impossible to determine where this decrease in hydrolysis occurred. The decrease in the free fatty acid class should have helped to maintain a more desirable palatability in that fewer fatty acids are available to be degraded to the

short chain fatty acids, ketones, and aldehydes commonly found in off flavor and odor in spoiled fresh meat.

Experiment III

Least-squares means for chemical and physical parameters between treatments within days are presented in Table 5. Differences between treatments within both Day 3 and Day 6 were not significant ($P \leq .05$) for psychrophilic, mesophilic and lipolytic microorganisms. A significant difference (P .05) was found between Treatment C (90% air and 10% O_2) and Treatment D (85% air and 15% O_2) within Day 9 for mesophilic and lipolytic microorganisms and a non-significant difference ($P \leq .05$) between Treatments C and D within Day 9 for psychrophiles, indicating that microorganisms on samples in Treatment D were entering the death phase of their growth cycle. Furthermore, employing slope ratios as estimates of relative effectiveness, the data indicate that Treatment D was 23, 64 and 20% more effective than the other treatments in discouraging the growth of psychrophilic, mesophilic and lipotropic microorganisms, respectively (Table B-3).

As in the previous two experiments, Table 6 provides data which show significant increases ($P \leq .05$) in psychrophile, mesophile and lipotropic microorganism numbers

Table 5. Least-squares means for total microorganism numbers, color parameters, lipid percent and lipid fractions by treatment within days for Experiment III.

Parameter	Day 0 Fresh	Day 3 A[a]	Day 3 B[a]	Day 3 C[a]	Day 3 D[a]	Day 6 A	Day 6 B	Day 6 C	Day 6 D	Day 9 A	Day 9 B	Day 9 C	Day 9 D
Microorganisms, logs													
Psychrophiles	3.4	4.5	5.2	5.4	5.5	7.4	6.6	6.4	6.7	8.3_{c}	8.6_{c}	8.0_{c}	7.5_{b}
Mesophiles	3.8	6.5	5.7	6.3	5.9	7.5	6.2	7.2	6.6	8.3_{bc}	8.6_{bc}	9.4_{c}	5.5_{b}
Lipotropic	3.2	3.9	4.8	4.4	5.1	5.2	5.6	5.6	5.9	7.3_{bc}	7.4_{bc}	7.8_{c}	6.6_{b}
Color													
Hue	7.6	10.4^{bc}	9.0^{b}	11.3^{c}	10.9^{bc}	13.3_{b}	12.2_{c}	12.6_{b}	13.1_{b}	12.8^{b}	14.1^{bc}	16.6^{c}	13.5^{bc}
Value	6.1	6.4	6.4	6.4	6.4	6.4_{b}	6.5_{c}	6.4_{b}	6.4_{b}	6.5	6.5	6.5	6.5
Chroma													5.4_{c}
Lipid, %	3.8	4.9	4.6	5.0	5.6	4.7	4.9	4.6	5.5	5.5	5.6	4.9	5.3
Lipid Fractions, mg/g													
Phospholipids	250.5	274.3^{d}	207.3^{b}	264.2^{d}	247.3^{c}	295.5_{c}	269.1_{b}	263.5_{b}	261.3_{b}	261.1_{c}	243.2_{b}	313.2_{d}	257.7_{c}
Monoglycerides	20.5	25.8	22.4	23.6	22.9	26.4_{c}	25.2_{c}	13.0_{b}	22.9_{c}	24.5_{b}	35.0_{c}	43.8_{d}	25.4_{b}
Diglycerides	71.3	87.2^{c}	89.1^{c}	79.8^{b}	86.5^{bc}	87.3_{b}	70.9_{b}	72.0_{b}	45.1_{d}	95.1_{b}	113.6_{d}	142.4_{e}	102.7_{c}
Triglycerides	643.9	544.4^{b}	663.8^{d}	618.6^{c}	632.0^{c}	585.4_{b}	594.7_{b}	636.4_{d}	622.4_{c}	602.6_{d}	586.9_{c}	481.1_{b}	595.0_{cd}
Cholesterol	9.4	9.6^{bc}	11.5^{c}	9.3^{bc}	7.8^{b}	14.3_{c}	15.0_{c}	9.8_{b}	14.3_{c}	6.8_{b}	12.3_{c}	11.0_{c}	10.7_{c}
Free Fatty Acids	3.1	8.6^{c}	5.9^{bc}	4.5^{b}	3.4^{b}	9.8_{c}	9.0_{bc}	6.3_{b}	7.8_{bc}	9.9	8.9	8.3	11.1

[a]A -- Steaks wrapped in polyvinyl chloride film; B -- Cryovac bag with an atmosphere of 95% air and 5% oxygen; C -- Cryovac bag with an atmosphere of 90% air and 10% oxygen; D -- Cryovac bag with an atmosphere of 85% air and 15% oxygen.

[b,c,d,e]Values on the same line within each day possessing unlike superscripts are significantly different ($P < .05$).

Table 6. Least-squares means for total microorganism numbers, color parameters, lipid percent and lipid fractions by day within treatments for Experiment III.

Parameter	A[a] Day 0	A[a] Day 3	A[a] Day 6	A[a] Day 9	B[a] Day 0	B[a] Day 3	B[a] Day 6	B[a] Day 9	C[a] Day 0	C[a] Day 3	C[a] Day 6	C[a] Day 9	D[a] Day 0	D[a] Day 3	D[a] Day 6	D[a] Day 9
Microorganisms, logs																
Psychrophiles	3.4^{b}	4.5^{b}	7.4^{c}	8.3^{c}	3.4^{b}	5.2^{c}	6.6^{c}	8.6^{d}	3.4^{b}	5.4^{c}	6.4^{c}	8.0^{d}	3.4^{b}	5.4^{c}	6.7^{cd}	7.5^{d}
Mesophiles	3.8^{b}	6.5^{c}	7.5^{c}	8.3^{c}	3.8^{b}	5.7^{c}	6.2^{c}	8.6^{d}	3.7^{b}	6.3^{c}	7.2^{c}	9.4^{d}	3.8^{b}	5.9^{c}	6.4^{c}	5.6^{bc}
Lipotropic	3.2^{b}	3.9^{b}	5.2^{c}	7.3^{d}	3.2^{b}	4.8^{c}	5.6^{c}	7.4^{d}	3.2^{b}	4.4^{c}	5.6^{cd}	7.8^{d}	3.2^{b}	5.1^{c}	5.9^{cd}	6.6^{d}
Color																
Hue	7.6^{b}	10.4^{c}	13.3^{d}	12.8^{d}	7.6^{b}	9.0^{b}	12.2^{c}	14.1^{c}	7.6^{b}	11.3^{c}	12.6^{c}	16.6^{d}	7.6^{b}	10.9^{c}	13.1^{d}	13.5^{d}
Value	6.1^{b}	6.4^{c}	6.5^{d}	6.5^{d}	6.1^{b}	6.4^{c}	6.5^{d}	6.5^{d}	6.1^{d}	6.4^{c}	6.4^{c}	6.5^{d}	6.1^{b}	6.4^{c}	6.1^{c}	6.3^{d}
Chroma	8.7^{d}	6.9^{c}	4.8^{b}	5.6^{bc}	8.7^{d}	7.3^{c}	5.5^{b}	5.6^{d}	8.7^{d}	6.4^{c}	4.3^{b}	4.1^{b}	8.7^{d}	6.0^{c}	4.4^{b}	5.4^{bc}
Lipid, %	3.8	4.9	4.1	5.5	3.8	4.6	4.7	5.6	3.8	5.0	4.9	4.9	3.8	5.6	4.6	5.3
Lipid Fractions, mg/g																
Phospholipids	250.5^{b}	274.3^{d}	295.5^{e}	261.1^{c}	250.5^{c}	207.3^{b}	269.1^{d}	243.2^{c}	250.5^{b}	264.2^{c}	263.5^{c}	313.2^{d}	250.5^{b}	247.3^{b}	261.3^{c}	257.5^{bc}
Monoglycerides	20.5^{b}	25.8^{c}	26.4^{c}	24.5^{c}	20.5^{b}	22.4^{b}	25.2^{c}	35.1^{d}	20.5^{b}	23.6^{c}	13.0^{b}	43.8^{d}	20.5^{b}	22.9	22.9	25.4
Diglycerides	71.3^{b}	87.2^{c}	76.1^{b}	95.1^{d}	71.3^{b}	89.1^{c}	87.3^{c}	113.6^{d}	71.3^{b}	79.8^{c}	70.9^{b}	142.4^{d}	71.3^{b}	86.5^{c}	72.0^{b}	102.7^{d}
Triglycerides	643.9^{e}	544.4^{b}	585.4^{c}	602.6^{d}	643.9^{c}	663.8^{d}	594.7^{b}	586.9^{b}	643.9^{c}	618.6^{c}	636.4^{c}	481.1^{b}	643.9^{d}	632.0^{c}	622.4^{c}	595.0^{b}
Cholesterol	9.4^{c}	9.6^{c}	14.3^{d}	6.8^{b}	9.4^{b}	11.5^{b}	15.0^{d}	12.3^{b}	9.4^{b}	9.3^{c}	9.8^{c}	11.1^{d}	9.4^{c}	7.8^{b}	14.3^{d}	10.7^{c}
Free Fatty Acids	3.1^{b}	8.6^{c}	9.8^{c}	9.9^{c}	3.1^{b}	5.9^{c}	9.0^{d}	8.9^{cd}	3.1^{b}	4.5^{c}	6.3^{cd}	9.3^{d}	3.1^{b}	3.4^{b}	7.8^{c}	11.1^{d}

[a] A -- Steaks wrapped in polyvinyl chloride film; B -- Cryovac bag with an atmosphere of 95% air and and 5% oxygen; C -- Cryovac bag with an atmosphere of 90% air and 10% oxygen; D -- Cryovac bag with an atmosphere of 85% air and 15% oxygen.

[b,c,d,e] Values on the same line within each day possessing unlike superscripts are significantly different ($P < .05$).

between Day 0 and Day 9 for all treatments. This indicated that all treatments encouraged microbial growth.

The color data by days within each treatment for Experiment III are listed in Table 6. As previously indicated in the preceding experiments, a significant increase ($P \leq .05$) was observed between Day 0 and Day 9 for all treatments. This, of course, changes the shade or tint of the meat surface and consequently effects the consumer's acceptance of the product. Noted also was a highly significant ($P \leq .05$) correlation coefficient of .80 between hue and chroma (Table A-6). The highly significant ($P \leq .01$) positive correlation coefficients between hue and days within all treatments and the highly significant ($P \leq .01$) negative correlation coefficients between chroma and days within all treatments in Table A-5 supports the significant increase ($P \leq .05$) in hue and the significant decrease ($P \leq .05$) in chroma as display time increased.

A significant decrease ($P \leq .05$) in the triglyceride class was observed between Day 0 and Day 9 for all treatments (Table 6). Significant increases ($P \leq .05$) between Day 0 and Day 9 in the monoglyceride, diglyceride and free fatty acid classes within all treatments were also noted. These data indicate appreciable hydrolysis of the triglyceride class contributing to the increase in concentration of the previously mentioned classes. The increase in oxygen content significantly increased ($P \leq .05$) the lipolytic

microorganisms between Day 0 and Day 9 for all treatments (Table 6). The increase in lipolytic numbers would enhance the lipase enzymes which are responsible for lipolysis. No significant differences ($P \leq .05$) between treatments within Day 9 were noted for the free fatty acid class. However, both the monoglyceride and diglyceride classes were significantly higher ($P \leq .05$) in concentration for Treatment C than the remaining three treatments. Furthermore, the triglyceride class was significantly lower ($P \leq .05$) in concentration for Treatment C than the remaining three treatments. This is explained by the fact that the slope ratio showed that Treatment C was 987% less efficient than the polyvinyl chloride treatment in discouraging the decrease in the triglyceride class (Table B-3). Also, the estimated percent relative effectiveness was respectively 54 and 435% less effective than the polyvinyl chloride treatment in discouraging the increase in the concentration of monoglyceride and diglyceride classes.

Experiment IV

Least-squares means for chemical and physical parameters by treatments within days are presented in Table 4. Treatments B (90% O_2, 10% CO_2), C (85% O_2, 10% CO_2, 5% N_2) and D (75% O_2, 15% CO_2, 10% N_2) were found to be significantly different ($P < .05$) than Treatment A (polyvinyl chloride) within Day 6 for psychrophiles, mesophiles and

lipotropic microorganisms. The reason for focusing in on Day 6 is due to the fact that Treatments B, C and D were the first and only treatments in this study to provide an environment for acceptable shelf-life up to six days of storage. This fact will be dealt with further when the color data is discussed.

The reason for the significant decrease ($P < .05$) in microorganism numbers as compared to Treatment A was due to the carbon dioxide present in Treatments B, C and D at levels of 10, 10 and 15%, respectively. Empey and Vickery (1933) reported that storage time of beef carcasses was increased about 50% by using 10 to 20% carbon dioxide. Scott (1938) extended the shelf-life of fresh meat by storage in selected atmospheres low in oxygen and/or enriched with carbon dioxide. Furthermore, Gonzales (1971) concluded that packaging steaks in a mixture of carbon dioxide and oxygen reduced significantly the microbial count. In this respect, 15% carbon dioxide was slightly better than 10%. These data support the decrease in numbers for all three organisms in Treatment D (15% carbon dioxide) as compared to Treatments B and C (both 10% carbon dioxide) for Day 3, Day 6 and Day 9 (Table 7). Huffman (1974) reported that beef stored in a gas mixture composed of 70% N_2, 25% CO_2 and 5% O_2 had significantly ($P < .05$) lower bacterial counts than beef stored in either N_2, O_2 or air. Also, Balasundaram (1974) reported that a 15% CO_2 plus 85% O_2 atmosphere reduced significantly

Table 7. Least-squares means for total microorganism numbers, color parameters, lipid percent and lipid fractions by treatment within days for Experiment IV.

Parameter	Day 0	Day 3				Day 6				Day 9			
	Fresh	A[a]	B[a]	C[a]	D[a]	A	B	C	D	A	B	C	D
Microorganisms, logs													
Psychrophiles	2.4	3.2	2.8^{bc}	2.3	1.8^{b}	6.9^{c}	2.8^{b}	3.8^{b}	3.4^{b}	8.7^{c}	7.2^{cd}	7.6^{c}	5.3^{b}
Mesophiles	2.6	4.0^{c}	3.6^{bc}	4.7^{c}	2.5^{b}	6.9^{c}	3.4^{b}	4.0^{b}	3.4^{b}	8.8^{d}	7.2^{cd}	5.6^{b}	5.6^{b}
Lipotropic	1.8	3.0	2.8	2.8	2.2	5.6^{c}	2.5^{b}	3.6^{b}	2.6^{b}	7.4	7.4	6.8	6.6
Color													
Hue	8.9	8.4	7.6	8.4	7.8	11.9^{c}	9.4^{bc}	8.6^{b}	9.6^{bc}	12.2^{b}	16.5^{c}	14.4^{bc}	13.9^{b}
Value	6.4	6.4	6.4	6.4	6.4	6.4^{b}	6.4^{c}	6.4^{c}	6.4^{c}	6.5^{c}	5.5^{b}	6.5^{bc}	6.5^{bc}
Chroma	7.9	8.3	8.8	8.9	8.6	4.8^{b}	8.2^{c}	8.4^{c}	7.6^{c}	6.2^{c}	4.2^{b}	4.9^{bc}	4.6^{bc}
Lipid, %	5.8	6.5^{cd}	7.8^{d}	4.2^{b}	4.6^{bc}	5.7^{bc}	4.4^{b}	6.0^{bc}	6.6^{c}	4.6	5.6	6.0	5.2
Lipid Fractions, mg/g													
Phospholipids	192.6	246.2^{c}	230.6^{b}	259.3^{d}	271.0^{e}	318.0^{c}	274.9^{d}	213.2^{b}	245.6^{c}	260.3^{d}	241.7^{c}	183.1^{b}	240.4^{c}
Monoglycerides	35.5	23.6^{c}	22.9^{c}	20.8^{c}	16.4^{b}	16.2^{b}	19.4^{bc}	25.4^{d}	21.2^{c}	35.3^{d}	19.2^{b}	27.2^{c}	23.6^{c}
Diglycerides	101.5	73.1^{d}	85.2^{e}	52.8^{c}	43.2^{b}	68.9^{c}	61.2^{bc}	57.7^{b}	73.4^{d}	96.2^{d}	67.0^{b}	84.7^{c}	86.5^{c}
Triglycerides	658.1	641.8^{c}	526.5^{b}	653.1^{cd}	656.6^{d}	582.3^{b}	633.6^{c}	689.6^{d}	645.2^{c}	585.2^{b}	652.4^{d}	690.6^{e}	633.8^{c}
Cholesterol	7.6	9.0	8.7	11.4	9.3	10.0^{cd}	9.0^{b}	11.3^{bc}	11.4^{d}	12.2^{c}	10.6^{b}	10.6^{b}	11.6
Free Fatty Acids	4.6	1.2	0.7	0.9	3.0	4.8^{cd}	1.8^{b}	2.6^{bc}	6.4^{d}	13.2^{c}	3.1^{b}	3.6^{b}	4.0

[a]A -- Polyvinyl chloride film overwrap; B -- Cryovac bag with an atmosphere of 90% oxygen and 10% carbon dioxide; C -- Cryovac bag with an atmosphere of 85% oxygen, 10% carbon dioxide and 5% nitrogen; D -- Cryovac bag with an atmosphere of 75% oxygen, 15% carbon dioxide and 10% nitrogen.

[b,c,d,e]Values on the same line within each day possessing unlike superscripts are significantly different ($P<.05$).

($P \leq .05$) the microbial count on the surface of prepackaged steaks. Employing slope efficiency ratios (Table B-4), Treatments B, C and D were found to be more effective in discouraging the growth of all microorganisms than Treatment A.

In observing the color data for Experiment IV by days within treatments (Table 8), a significant increase ($P \leq .05$) in hue was noted between Day 0 and Day 6 within Treatment A. However, there was no significant increase ($P \leq .05$) between Day 0 and Day 6 within Treatments B, C and D. These data indicate a lesser change in the tint or shade of the steak surface and consequently a more desirable color was maintained. The reason for this more desirable color is two fold: First, the decrease in microbial numbers found for Treatments B, C and D as compared to Treatment A; second, the added oxygen in the environment. Gonzales (1971) concluded that prepackaged steaks in mixtures of carbon dioxide and oxygen reduced the microbial count and maintained a desirable bright red color of meat. Huffman (1973) reported the color of hue was improved significantly ($P \leq .05$) when stored in a gas mixture of 70% N_2, 25% CO_2 and 5% O_2.

Least-squares means for chemical and physical parameters by days within treatments for Experiment IV are found in Table 8. Although definite trends do not exist, it is of interest to note the significant decrease ($P \leq .05$) in the monoglyceride, diglyceride and free fatty acid classes

Table 8. Least-squares means for total microorganism numbers, color parameters, lipid percent and lipid fractions by day within treatments for Experiment IV.

Parameter	A[a] Day 0	A[a] Day 3	A[a] Day 6	A[a] Day 9	B[a] Day 0	B[a] Day 3	B[a] Day 6	B[a] Day 9	C[a] Day 0	C[a] Day 3	C[a] Day 6	C[a] Day 9	D[a] Day 0	D[a] Day 3	D[a] Day 6	D[a] Day 9
Microorganisms, logs																
Psychrophiles	2.4^{b}	3.2^{b}	6.9^{c}	8.7^{d}	2.4^{b}	2.8^{b}	2.8^{b}	7.3^{c}	2.4^{b}	2.3^{b}	3.9^{c}	7.6^{d}	2.4^{bc}	1.9^{b}	3.5^{c}	5.3^{d}
Mesophiles	2.6^{b}	4.0^{c}	7.4^{d}	8.8^{e}	2.6^{b}	3.6^{b}	3.4^{b}	7.2^{c}	2.6^{b}	4.7^{c}	4.1^{c}	7.6^{d}	2.6^{b}	2.5^{b}	3.2^{b}	5.6^{c}
Lipotropic	1.8^{b}	3.0^{b}	5.7^{c}	7.4^{d}	1.8^{b}	2.8^{b}	2.5^{b}	7.4^{c}	1.8^{b}	2.8^{bc}	3.7^{c}	6.9^{d}	1.8^{b}	2.2^{b}	2.7^{b}	6.6^{c}
Color																
Hue	8.9^{b}	8.4^{b}	12.0^{c}	12.2^{c}	8.9^{b}	7.6^{b}	9.5^{b}	16.5^{c}	8.9^{b}	8.4^{b}	8.6^{b}	14.5^{c}	8.9^{b}	7.9^{b}	9.6^{b}	13.9^{c}
Value	6.4^{b}	6.4^{b}	6.4^{b}	6.5^{c}	6.4^{c}	6.3^{b}	6.4^{c}	6.5^{d}	6.4^{b}	6.4^{b}	6.4^{b}	6.5^{b}	6.4^{b}	6.4^{b}	6.4^{b}	6.5^{b}
Chroma	7.9^{bc}	8.3^{c}	4.8^{b}	6.3^{b}	7.9^{c}	8.9^{c}	8.3^{c}	4.3^{b}	7.9^{c}	8.9^{c}	8.4^{c}	4.9^{b}	7.9^{c}	8.7^{c}	7.6^{c}	4.6^{b}
Lipid, %	5.8	6.5	5.7	4.6	5.8^{b}	7.9^{c}	4.4^{b}	5.6^{b}	5.8	4.2	6.0	6.0	5.8	4.7	6.6	5.2
Lipid Fractions, Mg/g																
Phospholipids	192.6^{b}	246.2^{c}	318.0^{e}	260.4^{d}	192.6^{b}	230.6^{c}	275.0^{d}	241.7^{c}	192.6^{c}	259.3^{d}	213.3^{cd}	183.1^{b}	192.6^{b}	271.0^{d}	245.7^{c}	240.5^{c}
Monoglycerides	35.5^{d}	23.6^{c}	16.2^{b}	35.3^{d}	35.5^{d}	22.9^{c}	19.5^{b}	19.3^{b}	35.5^{d}	20.9^{b}	25.5^{c}	27.3^{c}	35.5^{d}	16.5^{b}	21.2^{c}	23.6^{c}
Diglycerides	101.5^{c}	73.1^{b}	68.9^{b}	96.2^{c}	101.5^{d}	85.2^{c}	61.2^{b}	67.1^{b}	101.5^{d}	52.8^{b}	57.7^{b}	84.7^{c}	101.5^{e}	43.2^{b}	73.4^{c}	86.5^{d}
Triglycerides	658.1^{c}	641.9^{c}	582.2^{b}	585.2^{b}	658.1^{d}	526.5^{b}	633.7^{cd}	652.4^{d}	658.1^{b}	653.1^{b}	689.6^{c}	690.7^{c}	658.1^{c}	656.7^{c}	645.2^{bc}	633.6^{b}
Cholesterol	7.6^{b}	9.0^{b}	10.0^{bc}	12.2^{c}	7.6	8.7	9.1^{bc}	10.7^{bc}	7.6^{b}	11.4^{c}	11.3^{c}	10.6^{bc}	7.6^{b}	9.3^{bc}	11.4^{c}	11.6^{c}
Free Fatty Acids	4.6^{c}	1.2^{b}	4.9^{c}	13.2^{d}	4.6^{c}	0.7^{b}	1.9^{bc}	3.2^{bc}	4.6^{c}	1.0^{b}	2.6^{bc}	3.6^{bc}	4.6^{bc}	3.0^{b}	6.4^{c}	4.1^{bc}

[a] A -- Polyvinyl chloride film overwrap; B -- Cryovac bag with an atmosphere of 90% oxygen and 10% carbon dioxide; C -- Cryovac bag with an atmosphere of 85% oxygen, 10% carbon dioxide and 5% nitrogen; D -- Cryovac bag with an atmosphere of 75% oxygen, 15% carbon dioxide and 10% nitrogen.

[b,c,d,e] Values on the same line within each day possessing unlike superscripts are significantly different ($P < .05$).

between Day 0 and Day 9 within Treatments B, C and D. Also noted were the small changes in concentration of the triglyceride class between Day 0 and Day 9 within Treatments B, C and D as compared to Treatment A and all other treatments previously mentioned in this study. The reason for this is of course the decrease in psychrophilic, mesophilic and especially lipotropic microorganism numbers between Treatments B, C and D and Treatment A (Table 7). Furthermore, the steaks from Treatments B, C and D would be most palatable because of the low concentration of free fatty acids.

CONCLUSION

Under the conditions of this study, the following conclusions are valid:

1. Anaerobic environments and both high and low levels of carbon dioxide suppressed the normal growth rate of all microorganisms.

2. Anaerobic environments, high levels of carbon dioxide and low levels of carbon dioxide combined with high levels of oxygen were most effective in controlling the increase of monoglycerides, diglycerides and free fatty acids; consequently, the most effective in controlling the decrease is the triglyceride fraction.

3. Anaerobic environments, both high and low levels of carbon dioxide and nitrogen did not maintain a desirable red meat color for an extended period of time.

4. High levels of oxygen without carbon dioxide promoted the growth of all microorganisms excellently. Also, these treatments encouraged the increase of monoglyceride, diglyceride and free fatty acid concentrations. These treatments did not maintain a desirable red meat color beyond four days.

5. Only the low levels of carbon dioxide and elevated levels of oxygen maintained a desirable color for an extended period of time.

6. It is suggested that further work should be done to better realize the advantage of elevated oxygen levels in combination with carbon dioxide in extending fresh meat shelf-life.

SUMMARY

This study was conducted to determine the effect of gaseous atmospheres and packaging on the retail shelf-life of beef top round steaks.

Top rounds used in this study were from carcasses of choice grade exhibiting A- maturity. The Semimembranosus muscle was excised from each top round and subdivided into (5 x 5 x 2.5 cm) steaks. A total of 240 steaks were used and packaged according to each experiment. Samples in Experiments I and II were stored at 4 ± 1 C in the University Meats Laboratory in a walk-in cooler. Lighting was furnished by regular cooler lamps totaling 50 candle foot. Experiments III and IV were stored at 4 ± .5 C in open top display cases at the main campus laboratory. Lighting was furnished by flourescent strips, totaling 100 candle foot.

Samples were collected at various time intervals depending on experiment. These samples were unpackaged and allowed to equilibrate with the normal atmosphere for 30 minutes. After this time period had elapsed, color evaluations were made, microorganism swab samples were taken for total microorganism counts and the top 0.25 cm of each sample was removed for lipid analysis.

High levels of carbon dioxide and vacuum packaging had a significant ($P < .05$) effect on discouraging the growth

of all microorganisms. These treatments were also found to have a significant ($P \leq .05$) effect on controlling the increase of monoglyceride, diglyceride and free fatty acid fractions as well as controlling the decrease of the triglyceride fraction when compared to the polyvinyl chloride or nitrogen treatments. However, these treatments significantly ($P \leq .05$) increased the score for hue and consequently were undesirable for usage in maintaining bright fresh meat color.

High levels of oxygen without carbon dioxide significantly ($P \leq .05$) encouraged the growth of all microorganisms. Elevated oxygen levels also promoted the increase of monoglyceride, diglyceride and free fatty acids concentrations. Furthermore, with the superior microorganism growth a desirable color was not maintained.

When using high levels of oxygen and low levels of carbon dioxide, microorganism growth was retarded and a desirable color maintained for approximately seven days. Also these treatments discouraged the increase in concentration of the monoglyceride, diglyceride and free fatty acid fractions.

In general, increased length of display reduced the acceptability of beef steak except with the high level of oxygen and low level of carbon dioxide combinations. It is apparent that a definite advantage exists with these treatments in extending the shelf-life of beef.

APPENDIX A

SIMPLE CORRELATION COEFFICIENTS

Table A-1. Simple correlation coefficients of total microorganisms, color parameters, lipid percent and lipid fractions with days within treatments for Experiment I.

Parameter	Treatments			
	Polyvinyl Chloride	CO_2	N_2	Cryovac
Microorganisms, logs				
Psychrophiles	.74**	.63**	.64**	.63**
Mesophiles	.72**	.62**	.66**	.59**
Lipotropic	.84**	.19	.77**	.51**
Color				
Hue	.16	.59**	.23	.25
Value	.02	.29	-.19	-.39*
Chroma	-.32*	-.51**	-.47**	-.47**
Lipid, %	.08	-.03	-.10	-.25
Lipid Fractions, mg/g				
Phospholipids	-.20	-.29	-.16	.14
Monoglycerides	.74**	.55**	.31	.40*
Diglycerides	.67**	.71**	.50**	.62**
Triglycerides	-.25	-.12	-.13	-.41**
Cholesterol	-.21	-.01	-.16	.12
Free Fatty Acids	.63**	.12	.28	.18

*($P < .05$).

**($P < .01$).

Table A-2. Simple correlation coefficients of total microorganism numbers, color parameters, lipid percent and lipid fractions with total microorganism numbers, color parameters, lipid percent and lipid fractions for Experiment I.

	Mesophiles	Lipotropic	Hue	Value	Chroma	Phospho-lipids	Monoglyc-erides	Diglyc-erides	Cholesterol	Free Fatty Acids	Triglyc-erides	Lipid
Microorganism, logs												
Psychrophiles	.34*	.25*	.27*	.18*	.22*	.12	.07	-.09	.10	.10	-.06	-.32*
Mesophiles		.18*	.20*	.27*	-.01	.06	.00	.04	-.04	-.10	-.05	-.05
Lipotropic			.19	.13	-.17	-.06	-.01	-.05	-.03	.07	.08	-.04
Color												
Hue				.62*	-.36*	.09	-.18*	-.13	-.02	.10	.02	-.15
Value					.22*	.00	-.02	.06	-.02	-.03	-.02	.05
Chroma						-.08	.09	.18*	.00	-.14	.03	.08
Lipid Fractions, mg/g												
Phospholipids							.14	.10	.28	.17	-.84*	-.29*
Monoglycerides								.46*	.58*	.20	-.48*	-.16
Diglycerides									.39*	-.01	.50*	.02
Cholesterol										.35*	-.54*	-.29*
Free Fatty Acids											-.24*	-.26*
Triglycerides												.27*

*($P < .05$).

Table A-3. Simple correlation coefficients of total microorganisms, color parameters, lipid percent and lipid fractions with days within treatments for Experiment II.

Parameters	Treatments		
	Polyvinyl Chloride	Cryovac	Nalophan
Microorganisms, logs			
Psychrophiles	.98**	.83**	.77**
Mesophiles	.97**	.93**	.83**
Lipotropic	.58**	.64**	-.55*
Color			
Hue	.60*	.85**	.66**
Value	.49*	.70**	.11
Chroma	-.57*	-.64**	-.70**
Lipid, %	-.03	.09	-.08
Lipid Fractions, mg/g			
Phospholipids	-.39	-.14	-.06
Monoglycerides	-.39	-.17	-.01
Diglycerides	-.39	.14	-.21
Triglycerides	.44	.06	.10
Cholesterol	-.20	-.31	.39
Free Fatty Acids	.62**	-.34	.05

*($P < .05$).

**($P < .01$).

Table A-4. Simple correlation coefficients of total microorganism numbers, color parameters, lipid percent and lipid fractions with total microorganism numbers, color parameters, lipid percent and lipid fractions for Experiment II.

	Mesophiles	Lipotropic	Hue	Value	Chroma	Phospholipids	Monoglycerides	Diglycerides	Cholesterol	Free Fatty Acids	Triglycerides	Lipid
Microorganism, logs												
Psychrophiles	.72*	.08	.26	.29	-.17	.10	.05	-.12	.08	-.01	-.03	-.01
Mesophiles		.11	.23	.11	-.22	.18	-.07	-.20	.11	.07	-.04	-.23
Lipotropic			-.08	-.10	.01	.03	-.18	-.25	.24	-.12	.11	.06
Color												
Hue				.42*	-.78*	.30	.10	-.17	.13	.06	-.16	-.16
Value					.05	-.07	.06	-.12	.03	-.22	.10	.06
Chroma						-.27	.02	.18	.05	-.20	.13	.16
Lipid Fractions, mg/g												
Phospholipids							.03	.17	.53*	.62*	-.83*	-.20
Monoglycerides								.67*	.31	.17	-.48*	-.04
Diglycerides									.34*	.14	-.66*	.21
Cholesterol										.49*	-.63*	-.26
Free Fatty Acids											-.60*	-.51*
Triglycerides												.10

*($P < .05$).

Table A-5. Simple correlation coefficients of total microorganisms, color parameters, lipid percent and lipid fractions with days within treatments for Experiment III.

Parameters	Treatments			
	A[a]	B[a]	C[a]	D[a]
Microorganisms, logs				
Psychrophiles	.93**	.96**	.90**	.88**
Mesophiles	.89**	.90**	.94**	.30
Lipotropic	.90**	.93**	.96**	.84**
Color				
Hue	.72**	.90**	.91**	.85**
Value	.72**	.44*	.86**	.89**
Chroma	-.78**	-.90**	-.88**	-.78**
Lipid, %	.37	.70**	.23	.36
Lipid Fractions, mg/g				
Phospholipids	.03	.19	.55*	-.02
Monoglycerides	.34	.43	.39	.46
Diglycerides	.25	.62**	.71**	.32
Triglycerides	-.08	-.52*	-.67**	-.26
Cholesterol	.08	.62**	.71**	.54*
Free Fatty Acids	.56*	.48*	.44	.80**

[a]A -- Polyvinyl chloride film overwrap; B -- Cryovac bag with an atmosphere of 95% air and 5% oxygen; C -- Cryovac bag with an atmosphere of 90% air and 10% oxygen; D -- Cryovac bag with an atmosphere of 85% air and 15% oxygen.

*($P < .05$).

**($P < .01$).

Table A-6. Simple correlation coefficients of total microorganism numbers, color parameters, lipid percent and lipid fractions with total microorganism numbers, color parameters, lipid percent and lipid fractions for Experiment III.

	Mesophiles	Lipotropic	Hue	Value	Chroma	Phospholipids	Monoglycerides	Diglycerides	Cholesterol	Free Fatty Acids	Triglycerides	Lipid
Microorganism, logs												
Psychrophiles	.42*	.32*	.19	.05	-.19	.08	-.03	-.19	.16	-.16	-.04	.18
Mesophiles		.33*	-.15	.03	.16	-.01	.09	.02	.13	-.04	-.04	.05
Lipotropic			.16	.13	-.25	-.01	-.10	.17	.04	-.38*	.13	.44*
Color												
Hue				.08	-.80*	.00	-.05	-.02	-.11	.14	.08	-.04
Value					-.07	-.02	-.07	-.04	.10	-.04	.04	-.02
Chroma						-.10	.14	-.05	.17	-.12	-.01	.01
Lipid Fractions, mg/g												
Phospholipids							.03	.25	-.04	.22	-.77*	-.23
Monoglycerides								.33*	.57*	.01	-.34*	-.13
Diglycerides									.18	.00	-.61	.08
Cholesterol										-.13	-.19	-.09
Free Fatty Acids											-.27	-.33*
Triglycerides												.29*

*($P < .05$).

Table A-7. Simple correlation coefficients of total microorganisms, color parameters, lipid percent and lipid fractions with days within treatments for Experiment IV.

Parameters	Treatments			
	A[a]	B[a]	C[a]	D[a]
Microorganisms, logs				
Psychrophiles	.96**	.72**	.82**	.80**
Mesophiles	.99**	.79**	.80**	.76**
Lipotropic	.97**	.80**	.93**	.84**
Color				
Hue	.48*	.74**	.67**	.65**
Value	.42	.71**	.63**	.47
Chroma	-.54*	-.66**	-.60**	-.68**
Lipid, %	-.16	-.16	.06	-.17
Lipid Fractions, mg/g				
Phospholipids	.56*	.23	-.12	.29
Monoglycerides	-.05	-.77**	-.28	-.38
Diglycerides	-.05	-.65**	-.18	-.02
Triglycerides	-.61**	.18	.25	-.26
Cholesterol	.55*	.14	.35	.54*
Free Fatty Acids	.58*	.04	-.11	.02

[a]A -- Polyvinyl chloride film overwrap; B -- Cryovac bag with an atmosphere of 90% oxygen and 10% carbon dioxide; C -- Cryovac bag with an atmosphere of 85% oxygen, 10% carbon dioxide and 5% nitrogen; D -- Cryovac bag with an atmosphere of 75% oxygen, 15% carbon dioxide and 10% nitrogen.

*($P < .05$).

**($P < .01$).

Table A-8. Simple correlation coefficients of total microorganism numbers, color parameters, lipid percent and lipid fractions with total microorganism numbers, color parameters, lipid percent and lipid fractions for Experiment IV.

	Mesophiles	Lipotropic	Hue	Value	Chroma	Phospholipids	Monoglycerides	Diglycerides	Cholesterol	Free Fatty Acids	Triglycerides	Lipid
Microorganism, logs												
Psychrophiles	.78*	.60*	.26	.22	-.14	-.22	.11	.28*	-.01	-.01	.33*	.10
Mesophiles		.49*	.18	.14	-.25	-.26	.07	.20	-.14	-.16	.15	.13
Lipotropic			.21	.18	-.35*	-.25	.05	.28*	.06	-.14	.32*	.26
Color												
Hue				.80*	-.46*	-.04	.09	.27	-.11	.21	.02	.21
Value					-.14	-.03	.03	.27	-.17	.20	-.01	.22
Chroma						.24	-.04	-.15	.13	-.07	-.27	-.29*
Lipid Fractions, mg/g												
Phospholipids							-.02	-.24	.62*	.24	-.57*	.10
Monoglycerides								.21	.19	.30*	-.25	.16
Diglycerides									-.02	.13	.01	.00
Cholesterol										.02	-.37*	.05
Free Fatty Acids											-.23	.03
Triglycerides												-.12

*($P < .05$).

APPENDIX B

ESTIMATES OF RELATIVE EFFECTIVENESS USING SLOPE RATIOS

Table B-1. Estimate of percent relative effectiveness employing slope ratios for Experiment I.

Parameters	Polyvinyl Chloride	Treatments					
		CO_2		N_2		Cryovac	
	Slope	Slope	% Relative Effectiveness	Slope	% Relative Effectiveness	Slope	% Relative Effectiveness
Microorganisms, logs							
Psychrophiles	.25	.20	+20	.19	+24	.20	+20
Mesophiles	.25	.20	+20	.21	+16	.19	+24
Lipotropic	.40	.05	+88	.47	-18	.21	+48
Color							
Hue	.09	.25	-177	.01	+89	.08	+12
Value	.01	.01	0	-.01	+100	-.01	+100
Chroma	-.07	-.10	-43	-.07	0	-.08	-15
Lipid, %	.02	-.01	+50	-.02	0	-.04	+200
Lipid Fractions, mg/g							
Phospholipids	-1.90	-2.91	-53	-1.29	+32	1.17	+62
Monoglycerides	1.80	.92	+49	.56	+69	.45	+75
Diglycerides	3.39	3.29	+03	2.20	+35	2.74	+20
Triglycerides	-3.45	-1.55	+56	-1.49	+57	-4.41	-28
Cholesterol	-.12	-.01	+92	-.10	+17	.06	+50
Free Fatty Acids	.56	.14	+75	.23	+59	.09	+84

Table B-2. Estimate of percent relative effectiveness employing slope ratios for Experiment II.

Parameters	Treatments				
	Polyvinyl Chloride	Cryovac		Nalophan	
	Slope	Slope	% Relative Effectiveness	Slope	% Relative Effectiveness
Microorganisms, logs					
Psychrophiles	.44	.27	+39	.25	+44
Mesophiles	.40	.37	+08	.29	+28
Lipotropic	.08	.06	+25	.07	+13
Color					
Hue	.33	.36	-09	.27	+19
Value	.01	.01	0	.01	0
Chroma	-.14	-.12	+16	-.16	-14
Lipid, %	-.01	.02	+200	-.02	-200
Lipid Fractions, mg/g					
Phospholipids	-4.16	-1.24	+71	-.72	+83
Monoglycerides	-.75	-.44	+42	.01	+99
Diglycerides	-3.29	.76	+77	-.99	+70
Triglycerides	7.52	.73	+91	1.42	+82
Cholesterol	-.17	-.19	-12	.25	-47
Free Fatty Acids	.86	.29	+55	.04	+96

Table B-3. Estimate of percent relative effectiveness employing slope ratios for Experiment III.

Parameters	Treatments A[a] Slope	B[a] Slope	B[a] % Relative Effectiveness	C[a] Slope	C[a] % Relative Effectiveness	D[a] Slope	D[a] % Relative Effectiveness
Microorganisms, logs							
Psychrophiles	.57	.58	-2	.50	+12	.44	+23
Mesophiles	.48	.50	-4	.60	-.25	.18	+63
Lipotropic	.41	.49	-20	.54	-32	.33	+20
Color							
Hue	.62	.76	-23	.95	-53	.67	-8
Value	.02	.10	-400	.02	0	.02	0
Chroma	-.38	-.38	0	-.53	-40	-.38	0
Lipid, %	.10	.22	-220	.07	+30	.15	-50
Lipid Fractions, mg/g							
Phospholipids	.38	2.60	-584	7.51	-1876	-.20	+52
Monoglycerides	.96	1.05	-9	1.48	-54	1.03	-7
Diglycerides	1.39	4.80	-245	7.44	-435	2.04	-46
Triglycerides	-1.71	-9.21	-538	-16.88	-987	-4.01	-234
Cholesterol	.08	.23	-287	.01	+88	.54	-575
Free Fatty Acids	.82	.55	+33	.44	+66	1.04	-26

[a]A -- Polyvinyl chloride film overwrap; B -- Cryovac bag with an atmosphere of 95% air and 5% oxygen; C -- Cryovac bag with an atmosphere of 90% air and 10% oxygen; D -- Cryovac bag with an atmosphere of 85% air and 15% oxygen.

Table B-4. Estimate of percent relative effectiveness employing slope ratios for Experiment IV.

Parameters	Treatments A[a]	B[a]		C[a]		D[a]	
	Slope	Slope	% Relative Effectiveness	Slope	% Relative Effectiveness	Slope	% Relative Effectiveness
Microorganisms, logs							
Psychrophiles	.76	.47	+39	.56	+27	.35	+54
Mesophiles	.73	.44	+40	.46	+37	.35	+53
Lipotropic	.65	.54	+17	.53	+19	.50	+24
Color							
Hue	.44	.82	-86	.56	-27	.56	-27
Value	.01	.01	0	.01	0	.01	0
Chroma	-.28	-.38	-36	-.32	-14	-.37	-32
Lipid, %	-.06	.08	-33	.04	+34	-.08	-33
Lipid Fractions, mg/g							
Phospholipids	10.00	3.84	+62	-1.65	+84	4.78	+53
Monoglycerides	-.16	-2.07	-1193	-.57	-256	-.93	-481
Diglycerides	-.38	-5.09	-1239	-1.22	-221	-.20	+48
Triglycerides	-9.98	6.74	+46	3.22	+70	-4.06	+62
Cholesterol	.55	.12	+79	.35	+37	.53	+4
Free Fatty Acids	.94	.02	+98	-.08	+91	.02	+98

[a]A -- Polyvinyl chloride film overwrap; B -- Cryovac bag with an atmosphere of 90% oxygen and 10% carbon dioxide; C -- Cryovac bag with an atmosphere of 85% oxygen, 10% carbon dioxide and 5% nitrogen; D -- Cryovac bag with an atmosphere of 75% oxygen, 15% carbon dioxide and 10% nitrogen.

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