

COLOR AND PIGMENTS OF PACKAGED REFRIGERATED BEEF

by

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COLOR AND PIGMENTS OF PACKAGED REFRIGERATED BEEF

INTRODUCTION

Consumer selection of pre-packaged meat from the refrigerated show case in today's supermarket is greatly influenced by the overall appearance of the meat and its package. The reason a buyer chooses a particular piece of meat is largely dependent on its color. Bright cherry red is the color which most consumers desire, while meat having a brown color is generally considered unacceptable and gives the impression of spoilage, although this may not be true.

The coloring materials in fresh meat are mainly hematin proteins. The metaloprotein pigments in the animal muscle are myoglobin, hemoglobin and cytochromes. The predominant pigment in tissue is myoglobin, and its derivatives, although it is not the most important in a biological sense. Myoglobin (Mb) has a purple red color, but when oxygenated (blooming) to oxymyoglobin (O_2Mb), a bright red color develops which is responsible for keeping the desirable appearance of fresh meat. Oxidation of myoglobin to metmyoglobin (MMb) yields an unacceptable brown color. The oxidized form of the heme iron is found to be an active catalyst promoting rancidity in the tissue (83,

p. 728-733).

Various packaging materials and methods are applied to the fresh meat to protect it from physical and chemical changes, microbial contamination and to increase the shelf-life as well as to present the meat to the buyer in the most attractive manner. Ball (8, p. 12) indicates that the success in increasing the color life of the packaged fresh meat may move the packaging operation back to the slaughtering plant.

Some investigators used gases such as nitrogen (N_2), carbon dioxide (CO_2), oxygen (O_2) or combination of them under various pressures, in addition to packaging, to develop and maintain the color quality of fresh meat during the period of storage. Carbon monoxide (CO) is briefly mentioned in the literature as a possible gas which might be used to stabilize the fresh meat cuts for a fairly long period. Other investigators have used chemicals especially ascorbic acid, nicotinic acid or other antioxidants for the same reason.

Refrigeration is still the most widely used method, either in the market or home, to preserve fresh meat from microbial and enzymatic changes.

The objectives of this study were to discuss the color of fresh meat, to study the influence of some packaging

materials, methods, and gases on the redness and spectral characteristics of beef cuts during cold storage, and to determine the myoglobin derivatives on the meat surface.

REVIEW OF LITERATURE

Biochemistry of Myoglobin

Myoglobin is a heme protein having a molecular weight of about 17,500. It has one iron porphyrin unit per molecule and reaches its isoelectric point at a pH of 6.8 (45, p. 194-196). Biologically, myoglobin functions as an oxygen storage reservoir for the cell. The myoglobin pigment is actually chemically related to hemoglobin, the oxygen carrying blood pigment. Hemoglobin has four iron atoms per molecule, a molecular weight of 68,000 and an isoelectric point of 6.7-7.1 (32, p. 165-183).

The characteristic properties of myoglobin are due to globin, the protein portion of the molecule. The myoglobin has its sharpest absorption band at 580 millimicrons ($m\mu$). It has its greatest affinity for oxidation at low oxygen pressures. Although the iron of myoglobin in nonexposed meat is in the ferrous state, oxygenation of myoglobin by oxygen to oxymyoglobin does not affect the reduced form of iron. Eliminating the oxygen supply to the pigment causes myoglobin to be formed again and then oxidized to metmyoglobin, in which state the iron is in the ferric form (17, p. 707; 21, p. 65-77; 27, p. 595-622; 33, p. 418-425; 57, p. 479-492). Brooks (17, p. 707) reported that an oxygen pressure of about four millimeters

of mercury was necessary for the maximum rate of metmyoglobin formation at 32°F. He also found that for each mole of metmyoglobin formed, 2.5 moles of oxygen were used. A decrease in the reaction rate of myoglobin to metmyoglobin occurs at pressures above 30 millimeters of mercury at a temperature of 86°F. and a pH of 5.69, according to George and Stratmann (33, p. 418-425). The dissociation of oxy-myoglobin to myoglobin before oxidation to metmyoglobin was observed by Watts (79, p. 1-52). She also reported that this dissociation was increased by decreasing oxygen tension and that metmyoglobin formation was favored thereafter. In addition, Watts stated that any treatment causing denaturation of the globin resulted in a weakening of the bonds between the heme and globin. Whenever this weakening of the bonds occurred, the heme lost its ability to be oxygenated, and instead, was oxidized rapidly to the brown ferric form. Furthermore, she found that the metmyoglobin formation was accelerated by increasing the acidity, salt, certain metals and freezing. This acceleration was accompanied by greenish or faded pigments resulting from breakdown products of the porphyrin ring.

The attraction of myoglobin or oxymyoglobin to carbon monoxide has been reported by many investigators (32, p. 165-183; 46, p. 207-335). The affinity of myoglobin to

carbon monoxide is 28 to 51 times greater than that to oxygen. Consequently, carbon monoxide displaces oxygen from oxymyoglobin to form carboxymyoglobin. Both carboxymyoglobin and the oxygenated form of myoglobin have the iron in the ferrous state and the same kind of bond (covalent) between the iron and the gas. It has been reported by the same authors that the carboxymyoglobin can be dissociated by light to myoglobin and carbon monoxide.

Factors Affecting the Changes of Color and Pigments in Fresh Meat

The reactions responsible for the changes in color and pigments of fresh meat are very complex. The difficulty in controlling the conditions to maintain an acceptable color in packaged meat cuts during storage in the cold cabinet has been discussed extensively by Ball (8, p. 12). Although many factors influence the color and the state of the myoglobin forms of packaged fresh meats, each factor has a critical point at which, with the other factors controlled, the color of meat will be preserved. The factors influencing the fresh meat color can be divided into: anti-mortem factors, post-mortem factors and storage conditions.

Anti-Mortem Factors

Species of Animals. It is well known that the concentration of myoglobin pigments is different in beef than in pork and lamb (3, p. 88). Ginger et al. (34, p. 1037-1040) has reported values of 3.7 and 0.79 milligrams of myoglobin per gram of fresh tissue for beef and pork muscles, respectively. Broumand et al. (21, p. 65-77) found that the relative myoglobin content of beef, lamb, pork and veal were roughly 62%, 37%, 16% and 9%, respectively. Values of 1-3, 4-10, 16-20 and 1-3 milligrams of myoglobin per gram of wet tissue in veal, beef, old beef and pork, respectively, have been reported (3, p. 88). It was noted by Ball (9, p. 193-204) that the pattern of changes in the color of meats from beef, lamb and pork varied when stored under the same conditions.

Age of Animals. It is well recognized that muscles from old animals have more redness and myoglobin than those of younger animals (3, p. 88). Broumand et al. (21, p. 65-77) reported that the relative myoglobin contents of beef and veal were roughly 62% and 9%, respectively. Jacobson and Fenton (40, p. 427-435) noted an appreciable increase in redness in the semimembranosus muscle when the age of the animals increased from 32 to 80 weeks. Such an

increase, however, was not so marked in the longissimus dorsi and psoas major muscles.

Animal Individuality. The degree of variation in the color of lean meat within an individual animal has been reported by Dean and Ball (31, p. 222-227) and Pirko and Ayres (64, p. 461-467). The latter investigators, using meat from one specific muscle of animals of the same age, stated that due to the large variation in their results, such data were not satisfactory for comparative purposes. Ginger et al. (34, p. 1037-1040) observed that dark colored pork muscle had almost twice as much myoglobin as the light colored muscles. Wilson et al. (82, p. 1080-1085) found that the contrast in pigmentation between muscles within the ham was directly related to the myoglobin concentration of the muscles. Giffie (3, p. 88) states that the amount of myoglobin in different muscles appears to be a function of the muscular activity, the blood supply and oxygen availability. As a result of their studies, Dean and Ball (31, p. 222-227) concluded that the variation in color patterns of meat during storage might be the result of differences between animals.

Feeding of Animal. There are some indications that the diet of the animal may have an effect on the redness

of the muscles. Although Shenk et al. (73, p. 741-760) observed that the meat from grass-fed cattle showed more redness than that of grain-fed animals, they thought that the differences might have been due to the greater activity of the grazing animals rather than to the feed. The results by Longwell (49, p. 8) tend to support the above data. However, Jacobson and Fenton (40, p. 427-435) found that the muscles of beef fed on grass or a combination of grass and grain had higher myoglobin contents than the muscles of those fed on grain only. The increase of redness was particularly noticeable in the semimembranosus muscle when the dietary level of total digestible nutrients was increased from 60% to 160% of the standards required for growing dairy heifers.

Post-Mortem Factors

From the results of previous mentioned investigations, it is clear that the redness or the myoglobin content of meat at slaughtering are not the same. It is reasonable to assume that the amount of blood staying in the meat tissue influences the color of meat and this factor is rarely constant. However, the most important factors of meat influencing the color are pH, fat content, fat covering and the enzyme activity of meat.

pH. Hall et al. (37, p. 55-78) have stated that the brightness or darkness of beef is pH dependent. They reported that meat at pH of 5.6 or below had a normal bright red color. At a pH of 5.7, the meat was shady or dull. The meat was dark at pH 6.5 or above. Pirko and Ayres (64, p. 461-467) also noticed that meat started to turn dark at a pH of 5.7. In contrast, Brooks (18, p. 1379-1383; 19, p. 35-50) observed an increase in oxidation of myoglobin by decreasing pH.

The pH of the meat is related to the glucose and glycogen content of the tissues of the animals prior to slaughter (53, p. 40-42). Hall et al. (37, p. 55-78) described the characteristics of dark-cutting beef. This meat failed to brighten when exposed to air, had an abnormally high pH, low glucose levels, only traces or no myoglobin, high inorganic phosphate, low oxidation-reduction potential, sticky and gummy texture.

Ramsbottom et al. (66, p. 84-85) and Wilson (3, p. 259-266) reported that the type of handling the animals received prior to slaughter could materially reduce the pH of meat. Any reduction of pH of meat depends on the presence of lactic acid in the tissue. A more desirable color was gained by feeding and resting the animals prior to slaughter. Wilson also reported that the production

of lactic acid by glycolysis in carcasses of animals fed before slaughtering yielded a value of pH 5.3 in the tissue, while the pH of muscles from fatigued animals ranged between 6.0 to 6.6.

Fat and Fat Cover. Generally, the fat and fat cover are concerned with oxidation-reduction of the lean pigment. Brooks (15, p. 75-78) noticed that the rate of oxidation of myoglobin present in fat, except in local areas, was slower than in the lean. The increase in the rate of reduction of metmyoglobin to myoglobin by fat in the absence of molecular oxygen was reported by Rikert et al. (69, p. 570-572). They observed that the lean surfaces of the samples in contact with a layer of fat were lower in redness than the uncovered surface, but after one week of storage, the surface covered by fat was higher in redness than that in contact with the packaging material.

Watts (79, p. 1-52), however, stated that the contact of myoglobin with unsaturated fat in the presence of oxygen resulted in discoloration of the meat and rancidity of the fat. This reaction was faster in meats having a pH below normal.

Enzymes. Muscle tissues retain a certain respiratory activity after the death of the animal resulting in a

continuous consumption of oxygen (64, p. 461-467). Grant (35, p. 250-253) theorized that the browning of meat was due to the depletion of oxygen from oxymyoglobin by the respiratory enzymes. Pirko and Ayres (64, p. 461-467) recommended the reduction of the enzyme activity to prevent spectral changes of the meat pigments. Grant (35, p. 250-253) and Tappel and Martin (75, p. 280-282) reported that the surviving succinic oxidase system was responsible for the continuous consumption of oxygen by the tissue. Grant also found that the addition of malonic acid provided a bright red color. Tappel and Martin reported that the addition of succinate would serve as an efficient oxygen scavenger if the removal of oxygen from meat was desired.

Storage Conditions

There are many factors affecting the color and the pigment state of meat after slaughtering and cutting. ^{according to the conditions} The color of fresh cut meat is purplish red which is due to a relatively high concentration of reduced myoglobin. Within 30-40 minutes after exposure to air, the meat will bloom to a bright red color due to the formation of oxymyoglobin. Normally, this acceptable color will last for one to two days at refrigerated temperatures as long as there is a continuous supply of oxygen. Controlling the storage conditions helps to prevent the fading after the

blooming period or to maintain high redness throughout a storage period of about 15 days.

Factors affecting the color and myoglobin fractions on the surface of meat after cutting are grouped as follows: oxygen availability, desiccation, temperature, light, bacteria, gases, additives, packaging methods and materials.

Oxygen Availability. Although the color of fresh meat is a function of several factors, oxygen availability is one of special importance (45, p. 194-196). The utilization of oxygen by the muscle tissues after the death of the animal was reported by Pirko and Ayres (64, p. 461-467). Brooks (17, p. 707) found that the maximum depth of oxygen diffusion into the tissue was one centimeter. He concluded that the discoloration of meat was restricted to this relatively thin superficial but penetrable zone. Coleman (24, p. 222-229) and Pirko and Ayres (64, p. 461-467) observed that when slices of beef had been covered by thin glass plates, the middle of the slice showed a purple color with an adjoining zone of brownish color. The purple region was found to contain myoglobin while the brown zone consisted mostly of metmyoglobin.

The oxygenation of myoglobin or blooming of meat occurs within 30 to 40 minutes after being cut and exposed to air (14, p. 62-72; 51, p. 281-286; 64, p. 461-467).

Rikert et al. (71, p. 17-23) stated that the best way to delay the initial discoloration of fresh meat was to keep the meat exposed to a high partial pressure of oxygen. They also observed that oxygen tension higher than that normally found in the air had no significant effect on meat color. Tappel and Martin (75, p. 280-282) noticed that the reduction of the oxygen concentration made myoglobin more labile to oxidation to metmyoglobin.

Using a low pressure of oxygen (vacuum) as a storage atmosphere of meat cuts was studied by Ball (8, p. 12-14), Broumand et al. (21, p. 65-77), and Rikert et al. (70, p. 625-632; 71, p. 17-23). Rikert et al. (70, p. 625-632) observed that when fresh meat was stored under a vacuum of 20 inches or more, the initial loss of redness and its return were faster than in meat stored under less than 20 inches of vacuum. They concluded that vacuum might be necessary for returning redness, but not for the initial discoloration. This conclusion was also reported by Broumand et al. (21, p. 65-77), and in addition, they noticed a brightening in the color of the vacuum packaged samples when exposed to air after the second red color had appeared.

Rikert et al. (71, p. 17-23) studied the color of meat when stored under vacuum in the complete absence of

oxygen. They stated that this might be the best method to overcome the loss of redness, especially during the first two or three days of storage.

These investigations involving the use of vacuum or the complete absence of oxygen to maintain the red color of meat were based on regenerating or keeping the pigment of the meat in the reduced state, myoglobin.

Desiccation. Darkening of meat due to desiccation is believed to be the result of changes of the pigments and their concentration on the surface of the meat (15, p. 75-78; 43, p. 290-295; 77, p. 140-144). Baker and Penrod (6, p. 511-514) observed that an increase in temperature and in air velocity accelerated dehydration and discoloration of beef samples but these changes were reduced by increasing the relative humidity. They also noticed that the discoloration of the meat surface exposed to air was more rapid than total dehydration during the first few hours, after which parallel rates took place. Landrock and Wallace (45, p. 194-196) reported that meat permitted to dehydrate in open air began to turn a dark cherry red, and after eight hours showed distinct rings of blackening which they identified as an intensification of color rather than discoloration. They also found that the meat turned an objectionable reddish brown color after

four hours when moisture was lost rapidly (5-26% loss of weight) and the meat was exposed to an atmosphere of low oxygen. Pirko and Ayres (64, p. 461-467) stated that the desiccation of packaged meat was inversely proportional to the sample thickness and that excessive loss of water resulted in a hardening of the surface of the meat and a simultaneous increase in all reflectance values.

Temperature. The increase of temperature tends to decrease the depth of oxygen penetration in the tissue as well as accelerating the dehydration and consequently causes a rapid darkening to occur near the surface of the meat (17, p. 707; 41, p. 155-159; 63, p. 26-40; 69, p. 567-573). The maximum rate of metmyoglobin formation was found to occur at 20 millimeters of oxygen pressure at 30°C. (57, p. 479-492) while at 0°C. the maximum rate was at 4 millimeters of oxygen pressure (15, p. 75-78).

Reducing the temperature apparently causes an increase in the rate of oxygenation of the meat pigment (1, p. 9; 14, p. 62-72; 51, p. 281-286). Butler et al. (22, p. 397-400) reported that the desirable color of prepackaged meat was maintained better at 32°F. than that at 40°F. in the self-service case. It was stated by Rikert et al. (70, p. 625-632) that both the rate of the initial

darkening and time for return of redness in fresh meat stored under vacuum, were accelerated by increasing the storage temperature. However, Ball (9, p. 194-204) reported that the intensity of red color throughout the storage period was somewhat better for meat samples stored at 41-46°F. than at 32-34°F.

Light. Although light causes severe discoloration of cured meats (3, p. 270), visible light has little or no effect on the color of fresh meat (23, p. 363-373; 43, p. 290-295; 67, p. 222-223; 69, p. 567-573). The application of ultraviolet to self-service display cases for preventing the microbial spoilage of packaged beef, was not effective to prevent microbial growth and resulted in discoloration of the meat because of the oxidation of the myoglobin as well as desiccation of the meat.

Effect of Bacteria. The effect of bacteria on the fresh meat color varies with different microorganisms. Rikert et al. (69, p. 567-573) observed the reduction of metmyoglobin to myoglobin in the presence of anaerobic or facultative anaerobic bacteria. They also reported a significant correlation between the color of the lean meat and concentration of Achromobacter and Lactobacillus organisms on the meat. A complete return of color was

noticed at the time that a maximum bacterial concentration occurred. They postulated that the red-brown color change resulted from the removal of oxygen of oxymyoglobin by respiratory enzymes of meat and bacterial flora. Hewitt (39, p. 48-50) also reported the possibility of bacterial cultures depleting the available oxygen supply, thereby developing reducing conditions. However, Butler et al. (22, p. 397-400) reported that the main effect of bacteria (Pseudomonas species) on the color of prepackaged retail beef cuts was an increase in the rate of metmyoglobin formation, and this effect was greatest during the logarithmic growth phase. It was noticed by Costilow et al. (28, p. 560-563) that the stability of the desirable color of meat could be prolonged more successfully by preventing initial bacterial contamination than by treating highly contaminated steaks with ascorbic acid. Watts (79, p. 12-17) stated that the free hydrogen peroxide of bacterial origin and possibly the catalase activity of the fresh meat might be very effective for promoting discoloration of meat.

Gases. Various gases have been suggested or used as the storage atmosphere for keeping the packaged meat color desirable throughout the storage period. Bayes (11, p.

157) and Grass (36, p. 621-623) stated that nitrogen might be useful for retarding or preventing discoloration due to the action of oxygen. Gas packaging may accomplish the same results as vacuum packaging to prevent oxidative spoilage in meat as well as avoiding the unnatural appearance and structural weakness of a vacuumized package (3, p. 134-136).)

[Kraft and Ayres (44, p. 8-12) observed that] carbon dioxide improved the keeping time when it was part of the atmosphere (25%) in the packages, but when it was used at higher concentrations the meat became discolored. Rikert et al. (71, p. 17-23) noticed that the rate of discoloration of fresh beef varied directly with partial pressures of carbon dioxide (between 0.2-72 centimeters) and nitrogen (59.4 to 74.5 centimeters) while keeping the total pressure of one atmosphere. In a comparison between one atmospheric pressure of air, carbon dioxide and nitrogen as the storage media of the meat samples, the above workers found that the air decreased redness for one day, returning some, but not all, of the original redness by the seventh day and then was discolored up to 11 days. Furthermore, they found that samples held under carbon dioxide or nitrogen rapidly lost redness in one day, the faded color was stable at the 7th day, after which a slight

return of redness was observed. The second return of redness of the samples in nitrogen was slightly more noticeable than those in carbon dioxide.

The same investigators (71, p. 17-23) reported that meats stored under a complete absence of oxygen, by flushing the storage atmospheres with either carbon dioxide or nitrogen before vacuum storage, had a beneficial effect on stabilizing the color of fresh meat as compared to those stored in vacuum.

Pearson (62, p. 37-38) recently mentioned that storing fresh meat under a carbon monoxide atmosphere might be a beneficial method for prolonging the color stability of such meat. Tappel et al. (74, p. 10-16) showed promising results when they flushed samples of freeze-dried beef with carbon monoxide and stored it under an atmosphere of nitrogen. They reported that the color of these samples was acceptable for one year when stored at 100°F. Furthermore, they calculated that the possibility of toxicity of carbon monoxide-treated freeze-dried beef was fairly low if all of this carbon monoxide reached the blood of an average person when fed an average serving of this meat.

Additives. Some chemical compounds have been added to meat to prevent discoloration. It has been reported

that the application of ascorbic acid in small concentrations (about 0.05%) to the surface of small cuts of fresh meat will serve to retain or improve the desired red color of refrigerated meat (25, p. 31788; 26, p. 4374; 28, p. 360-365; 69, p. 567-573; 80, p. 100-108; 81, p. 194-196). These investigators also agreed that using ascorbic acid in high concentrations (more than .1 to .5%) caused discoloration of the meat. Constilow et al. (28, p. 560-563) reported that the direct application of powdered ascorbic acid to the meat resulted in an immediate discoloration.

The effect of ascorbic acid on the color of fresh meat is generally not related to the reduction of pH (34, p. 1037-1040), but for its properties as an antioxidant (80, p. 100-108). The darkening of meat samples treated by a high concentration of ascorbic acid was theorized by Rickert et al. (69, p. 567-573). The great demand of the acid for oxygen causes the removal of oxygen from oxymyoglobin on the surface of the meat, which favors the formation of metmyoglobin.

Coleman (24, p. 222-229) experimented with the use of nicotinic acid and methyl nicotinate to maintain desirable fresh meat color. He concluded that both of these compounds appeared to improve the color of fresh meat, since nicotinic acid increased the acidity while methyl

nicotinate reacted with oxymyoglobin. Maass (50, p. 5709) found that by adding 0.3 gram of nicotinic acid and .05 gram of ascorbic acid to 450 grams of fresh beef, the meat showed good color stability.

Nordihydroguaiaretic acid (NDGA) has been used to inhibit the oxidation of the fresh meat pigments and thereby retard discoloration. It appears that this antioxidant is more effective in the presence of fat. Rikert et al. (69, p. 567-573) found that when NDGA was added at the rate of 0.05% to ground meat containing 35-50% fat, there was a definite improvement of color. In contrast, however, no color effect was noted when this antioxidant was added at the same rate, as above, to ground meat having a low fat content. Clauss et al. (23, p. 363-373) did not observe any color improvement of ground lamb treated with 0.1% NDGA and stored for 2 weeks.

A combination of ascorbic acid, sodium propionate and di-sodium phosphate in low concentrations was found to be effective in maintaining a bright red color of meat when stored at 33-37°F. for 13-16 days (72, p. 14025).

The Effect of Packaging Materials and Methods. The most important characteristics of packaging materials influencing the color of fresh meat are oxygen permeability, moisture proofness, wet strength, flexibility,

greaseproofness, and durability at refrigeration temperature. Factors affecting the package appearance and handling, plus costs and legal requirements, are also important but have no effect on the color of fresh meat. Detailed properties of packaging papers and films are presented in the comprehensive tables of The Modern Packaging Encyclopedia (61, p. 108-109; and p. 149-152).

Allen (2, p. 134-137) reported that for commercial packaging of meats, a film having a water absorptive surface and low water transmission rate should be used. Landrack and Wallace (45, p. 194-196) further added that the film for red meat should have oxygen permeability of at least 5000 milliliters of oxygen per square meter per 24 hours at one atmosphere when used at ambient conditions. Ramsbottom (65, p. 26-29) has reviewed packaging requirements for meat and states that transparency, puncture and tear resistance, heat sealability, tensile strength, moisture proofness and permeability are the factors to consider when choosing packaging materials. Ball (9, p. 193-204) reported that for maintaining color of meat throughout the storage period the packaging films from best to poorest were as follows: 307 x 113 cans, MSAT 80 cellophane (wrapped with coated side out), cellophane-pliofilm laminate, 307 x 306 cans, cellophane-polyethylene

laminate, polyethylene MSAT 80 coated side inside, MSAT 80 cellophane coated side outside and cellulose acetate-pliofilm laminate. Ayres (5, p. 39-51), however, reported that meat packaged with MSAT 80 cellophane developed off-odors more quickly than those stored in a laminate of pliofilm and aluminum. Clauss et al. (23, p. 363-373) also found that better organoleptic quality was noted when ground beef was stored in films having a relatively low oxygen and low water vapor permeability, when compared to samples stored in films with relatively high permeability.

Rikert et al. (71, p. 17-23) observed that fresh meat packaged either in air at atmospheric pressure or under vacuum in a container which was very permeable to gases (cellulose acetate or wet cellophane) kept redness for one to two days then lost redness rapidly and remained discolored. Fresh meat packaged under vacuum in a container slightly permeable to gases (cellophane pliofilm laminate) showed a greater initial loss in redness, but remained fairly constant thereafter. Even though there was some color loss, the residual redness was not considered undesirable at any time. Samples packaged in films slightly permeable to air at atmospheric pressure showed objectionable discoloration within a day of storage, although some samples later showed an increase in redness. Fresh beef stored under vacuum in impermeable containers

(cans) with a large head space showed a rapid loss in redness. These samples became undesirable in color within a day, but from 7 to 14 days of storage at 40°F. redness returned to a higher degree than that which was present at the time of packaging. Fresh meat held in the absence of oxygen in an impermeable container remained purplish or reddish throughout the storage period.

Kraft and Ayres (44, p. 8-12) observed that when fresh beef was wrapped with films, highly impermeable to gases, the myoglobin of this meat was held in the reduced state throughout a storage period of 12 days and blooming of the meat took place upon its subsequent exposure to air.

Dean and Ball (30, p. 468-471; 31, p. 222-227) and Rikert et al. (68, p. 520-525) have recommended the vacuum packaging of fresh beef in a barrier packaging material since they found that such packaged meat maintained good color stability throughout the storage period. These investigators concluded that this color stability was due to the result of stabilization or perhaps, increasing slightly, the concentration of the reduced myoglobin pigment. On the other hand, Urbain (77, p. 140-144) and Bratzler (14, p. 62-72) are of the opinion that vacuum packaging may have a somewhat detrimental effect on the color of fresh beef.

Ball et al. (10, p. 277-283) found that the loss of weight in packaged meat was dependent on the type of packaging material. The superior packages with respect to prevention of weight loss were cans, cellophane-pliofilm laminates (pliofilm inside), cellophane-polyethylene laminates (polyethylene inside), cellulose acetate-pliofilm laminates (pliofilm inside).

Determination of Color and Pigments

Color of Meat

Meat color can be measured either by objective or subjective methods. Little et al. (48, p. 403-409) statistically analyzed data obtained with a series of colored papers on a Beckman DU (non-recording) spectrophotometer with reflectance attachment, three different General Electric recording spectrophotometers, Beckman DK-2 recording spectrophotometer, a Gardner Automatic Color and Color Difference Meter, a Photovolt Tristimulus Photoelectric Colorimeter, and a Color Master Differential Colorimeter. They obtained significant linear relationships between all of the instruments. In addition, Pearson (62, p. 37-38) reported that the Hunter Color and Color Difference Meter or the Munsell Spinning Disks could also be used in measuring the color of fresh meat.

The subjective evaluation of meat color by a panel consisting of several members may produce diversified results due to inherent differences between individual judges and variations in experimental conditions (3, p. 233). This method is used only in very special cases in which the experimental conditions can be rigidly controlled and defined.

Pigments

The only available chemical method for determining the concentration of meat pigments is based on the determination of the total amount of myoglobin, rather than measuring the amount of each derivative of the meat pigments (29, p. 271-273). Hence, this method has very little value in analyzing the color of meat. On the other hand, physical methods are more oftenly used. Both reflectance and absorbeney procedures have been suggested (29, p. 271-273) for determining the percentages of myoglobin fractions of the meat.

Chemical Methods. In reviewing the literature, no chemical method was found for determining the percentages of reduced, oxy-, or metmyoglobin. However, Bowen (12, p. 747-751) proposed a method for the determination of the total amount of myoglobin in fresh meat. Briefly, his procedure involves the extraction of pigments from meat

by water, determining the iron content by a chemical method and dividing the weight of iron by 0.00323 (the fraction of myoglobin which is iron). This method is not effective for measuring color differences of meat.

Physical Methods.

Absorption methods. Austin and Drabkin (4, p. 67-68) extracted the pigments of meat with water and assumed that these solutions contained methemoglobin and oxyhemoglobin only, and measured the absorbency at different wavelengths. By subtracting the extinction coefficient of one of the two pigments from the absorbency of the solution at the same wavelengths, the difference was assumed to be attributed by the other pigment under the conditions of their experiment. Mangel (52, p. 20-21) used the above method to determine the pigments of frozen beef. Due to the large variation in the results, she concluded that a third form of the pigment might be present. Broumand et al. (21, p. 65-74) designed a procedure to determine the relative percentages of all the three myoglobin derivatives by measuring the transmittancy of a water extract of the meat at wavelengths of 473, 507, 593 and 597 millimicrons (the absorbency ratio method).

Reflectance methods. Pirko and Ayres (64, p. 461-467) indicated the change in the amount of reduced, oxy- and metmyoglobin by measuring the reflectance of the meat surface at the absorption maxima of the three pigments (555, 580 and 635 m μ , respectively) as given by Bowen (13, p. 235-245) and Theorell (76, p. 55-63); however, this method does not give the percentages of myoglobin derivatives. Naughton et al. (55, p. 121) used the reflectance method to study myoglobin in fish. They plotted the log of the reciprocal of reflectance (absorbency) and found that the wavelengths of the absorption maxima calculated from reflection data were the same as found in transmission. However, Dean and Ball (29, p. 271-286) reported that the absorbency was not proportional to the pigment concentration. They tried to use the procedure suggested by Brown et al. (21, p. 65-77) to determine the myoglobin fractions on the surface of meat, but the results showed higher values for oxymyoglobin. Hence, they listed the disadvantages of the absorbency method as follows:

1. The possibility of conversion of the reduced myoglobin to oxymyoglobin by atmospheric oxygen during extraction.

2. Naughton et al. (56, p. 933-938) maintained that

methemoglobin and oxyhemoglobin were different in solubility, at least in the case in tuna fish.

3. The slices of meat to be extracted contained some meat pigments which were beneath the surface of the meat.

Furthermore, Broumand et al. (21, p. 65-77) reported that the extraction of the intact pigments from meat for the absorbency ratio determination was not easy and presented many difficulties.

Dean and Ball (29, p. 273-274) proposed a reflectance ratio method using the same basic principles as the absorbency ratio method but depending upon reflectance measurements.

MATERIALS AND METHODS

Meat Samples

Meat used in this experiment was obtained from a local commercial packing plant. The animals producing the meat were Shorthorn steers 30 months of age at the time of slaughter and had been fattened solely on ladino clover for six weeks. After slaughter, the carcasses were aged for seven days in a 36°F. meat cooler. A longissimus dorsi muscle was taken from each of three carcasses of the High Standard grade at the end of the seventh day of aging. These muscles were taken immediately to the laboratory and were placed in a cooler and held at 40°F. On the following day, the external fat was removed from the lean portion before samples, dimensions of 3 inches by $2\frac{1}{2}$ inches by $\frac{1}{2}$ inch, were cut and packed via the various treatments. All equipment coming in contact with the meat was washed with a 0.2% Roccal solution.

Packaging Materials

Beef was packaged in pouches made of three different packaging materials in order to determine the suitability of each of these materials for protecting the color of the meat during storage. Each of these packaging materials possessed varying degrees of permeability in regard to

water vapor and oxygen transmission rates. The barrier saran-mylar coated polyethylene (S-M-P) pouches¹ were considered impermeable to both water vapor and oxygen transmission. The other two types of pouches were made from polyethylene², 2.5 (2.5 polyethylene) and 1.5 mils (1.5 polyethylene). Properties of the polyethylene films, as specified by the supplier, were as follows: the water vapor transmission rate for 100 square inches of film per 24 hours at 100°F. and 95 per cent relative humidity was 0.35 gram and 0.55 gram for the 2.5 and 1.5 mils polyethylene, respectively; while the oxygen transmission rate for 100 square inches of film per 24 hours at 1 atmosphere and at 75°F. was 225 cubic centimeters for the 2.5 mils polyethylene and 300 cubic centimeters for the 1.5 mils polyethylene.

Preparation of Packages

A piece of tygon tubing, two inches in length, was adhered inside one corner of the opening of the pouches in which beef was to be stored under a gaseous atmosphere of carbon monoxide or nitrogen or under high vacuum. A synthetic rubber cement, "Fuller's 911", was used to bond

¹ Manufactured by Minnesota Mining and Mfg. Co. (Scotchpak).

² All polyethylene pouches were supplied through the courtesy of Dobeckman Company, Portland, Oregon.

the tubing to the pouches.

After a meat sample was placed in the bag, the bag was sealed by a hand heat sealer and then flushed by the particular gas or a vacuum was drawn through the tygon tubing. A screw compressor clamp was then inserted over the tygon tubing and clamped into place to give a gas tight closure.

Gases

The following gases were flushed into various pouches containing beef samples to provide specific storage atmospheres in which to determine the effects of these gases in protecting the color of the meat during storage: a gas mixture of 2 per cent carbon monoxide and 98 per cent air (2% carbon monoxide); carbon monoxide, chemically pure grade (100% carbon monoxide); and water pumped nitrogen (N_2).

Packaging and Storage Procedures

An apparatus designed by Ogilvy et al. (60, p. 343-346) was employed to control the gases and/or vacuum inside the packages. All samples were stored in an open-top refrigerated cabinet at 36-38°F. The cabinet was lighted by two 40 watt, cool white, deluxe fluorescence lamps. The intensity of light on the meat was 125 foot candles.

Tests on the Meat Samples

Color and pigment measurements were determined on the surface of the meat samples immediately after treatment and on each day for six days. After the sixth day, measurements were made only on the ninth, twelfth and fifteenth days. Color panel scores were not taken at zero time or at the fifth day of storage.

All samples were held in the refrigerated cabinet for thirty days, after which time they were removed from the pouches for subjective evaluation. Such factors as appearance and odor were observed in this evaluation. In addition, characteristics of the cooked samples of carbon monoxide treated meat were also noted.

Color Measurements

Munsell Notations. A Beckman DK-1 recording spectrophotometer, equipped with reflectance attachment, was used to determine the reflectance spectrum of the meat surface between wavelengths of 400 and 650 millimicrons. The reflectance measurements were taken regularly without changing the storage atmosphere of the various treated samples. This was accomplished by inserting the samples in their respective packages at the open end of the reflectance attachment. The spectrophotometer was standardized with

the standard reference material, magnesium oxide, at 100 per cent reflectance. In standardizing the instrument, magnesium oxide was always covered with the particular packaging material that also was used to store the meat samples.

The wavelengths of the abridged selected ordinates method for standard source C (near daylight) were taken from the samples reflectance curves and were used to determine the tristimulus values X, Y and Z. These values were then used to compute the chromaticity coordinates, x and y, while the values of Y's were obtained from the tables of Judd (50, p. 354-355). After determining the value of Y's and the chromaticity coordinates, x and y, Munsell notations for the samples were obtained from the chromaticity charts of ideal Munsell colors³.

Index of Change. The index of change is a measurement for evaluating the degree of color difference between two different colors. This method was derived from the index of fading introduced by Nickerson (59, p. 509-511). She developed an equation to weight the differences in Munsell color spacings. Her equation is

³ Obtained from Munsell Color Company.

$$I = (c/5)(2\Delta H) + 6\Delta V + 3\Delta C$$

where I = index of fading, c = chroma, Δ = difference, H = hue, and V = value.

Nickerson used the absolute values of the differences between the original color and the faded color in her work on textiles and other colored materials.

In the present study, it was assumed that an increase in shade (hue--within the ten shades of red), brightness (value), or in saturation (chroma) of the red, would be desirable to improve the color of meat when the color of the fresh cut was used as the baseline. On this basis, the actual values of differences between the Munsell notations of the stored samples and the average values of the fresh cut meat, with the exception of that treated by carbon monoxide, were used to measure the color change. Also, this method serves to show the degree and direction of color change, whether improving or fading, of packaged beef during storage. Hence, this measurement is called the index of change ($\pm I$), whereby a positive sign is an indication of fading and a negative sign indicates color improvement.

Color Panel. A panel of seven people, having previous experience in evaluating foods, scored the meat samples for color appearance on a desirability basis. They rated

the meat on a 7 point hedonic ballot which also contained a descriptive word scale: 7, like very much (L.V.M.); 6, like moderately (L.M.); 5, like slightly (L.S.); 4, neutral (N); 3, dislike slightly (D.S.); 2, dislike moderately (D.M.); and 1, dislike very much (D.V.M.). Also, the average per cent panel scores (S) were calculated. Throughout the storage period, the panel judged the meat as it appeared in the refrigerated showcase where the coded samples were distributed at random. The panel members were asked to evaluate the color desirability of the samples on personal preference as to what they considered to be a desirable color for fresh beef.

Determination of Pigments

The reflectance ratio method developed by Dean and Ball (29, p. 273-274) was used to measure the myoglobin derivatives on the surface of the meat that was not stored under carbon monoxide. Briefly, their procedure is as follows:

1. Measure the per cent reflectance of the meat surface at wavelengths of 473, 507, 573 and 597 millimicrons.
2. Obtain the ratio $\frac{\text{absorption coefficient (K)}}{\text{scattering coefficient (S)}}$ for each value of per cent reflectance from the data of Judd (42, p. 358-362).
3. Calculate the following two ratios

$$\frac{K/S \text{ at } 507 \text{ m}\mu}{K/S \text{ at } 573 \text{ m}\mu}$$

and

$$\frac{K/S \text{ at } 473 \text{ m}\mu}{K/S \text{ at } 597 \text{ m}\mu}$$

4. The value of the first ratio, above, is located on the plot of ratio of absorbency $\frac{507 \text{ m}\mu}{573 \text{ m}\mu}$ versus percentage of metmyoglobin and oxymyoglobin plus myoglobin as given by Broumand et al. (21, p. 67) and is shown in Figure 1. The value of the second ratio is located on the plot of myoglobin and oxymyoglobin plus metmyoglobin as also given by Broumand et al. (21, p. 67) and shown in Figure 2. These two absorbency ratios were then located on the ordinates of the respective plots and the percentages of metmyoglobin and myoglobin were obtained, respectively.

5. By using the following equation, the percentage of oxymyoglobin is then calculated:

$$\begin{aligned} \text{Per cent oxymyoglobin} &= 100 - \text{per cent metmyoglobin} \\ &\quad - \text{per cent myoglobin} \end{aligned}$$

The per cent reflectance of the meat surface, at the previous wavelengths, was taken from the recorded reflectance curve of surface of the meat and this procedure was followed to determine the relative percentages of myoglobin derivatives on the untreated carbon monoxide samples.

Figure 1. CURVE FOR DETERMINATION OF THE RELATIVE CONCENTRATIONS OF MMb AND (O_2 Mb + Mb) AFTER MEASURING THE ABSORBANCIES AT 507 m μ AND 573 m μ (21 : p.67)

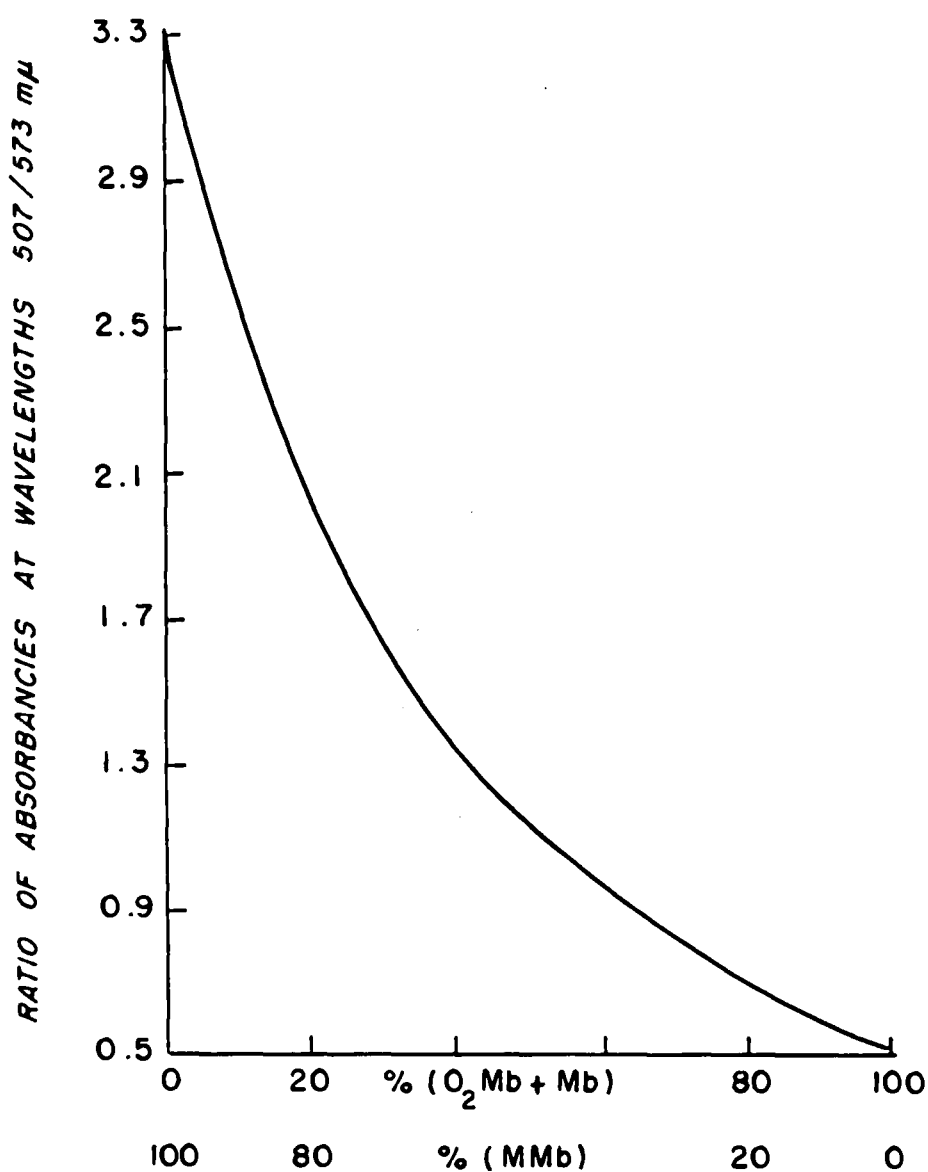
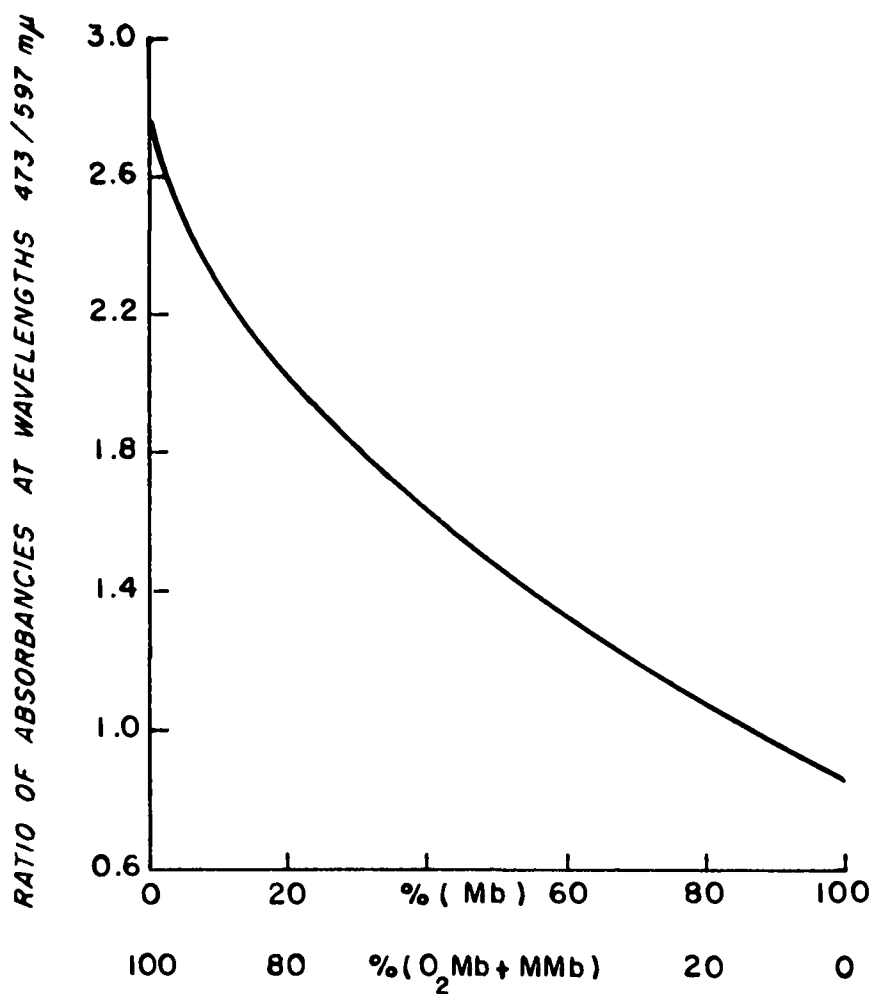


Figure 2. CURVE FOR DETERMINATION OF THE RELATIVE CONCENTRATIONS OF Mb AND (O_2 Mb + MMb) AFTER MEASURING THE ABSORBANCIES AT 473 m μ AND 597 m μ (21 : p. 67)



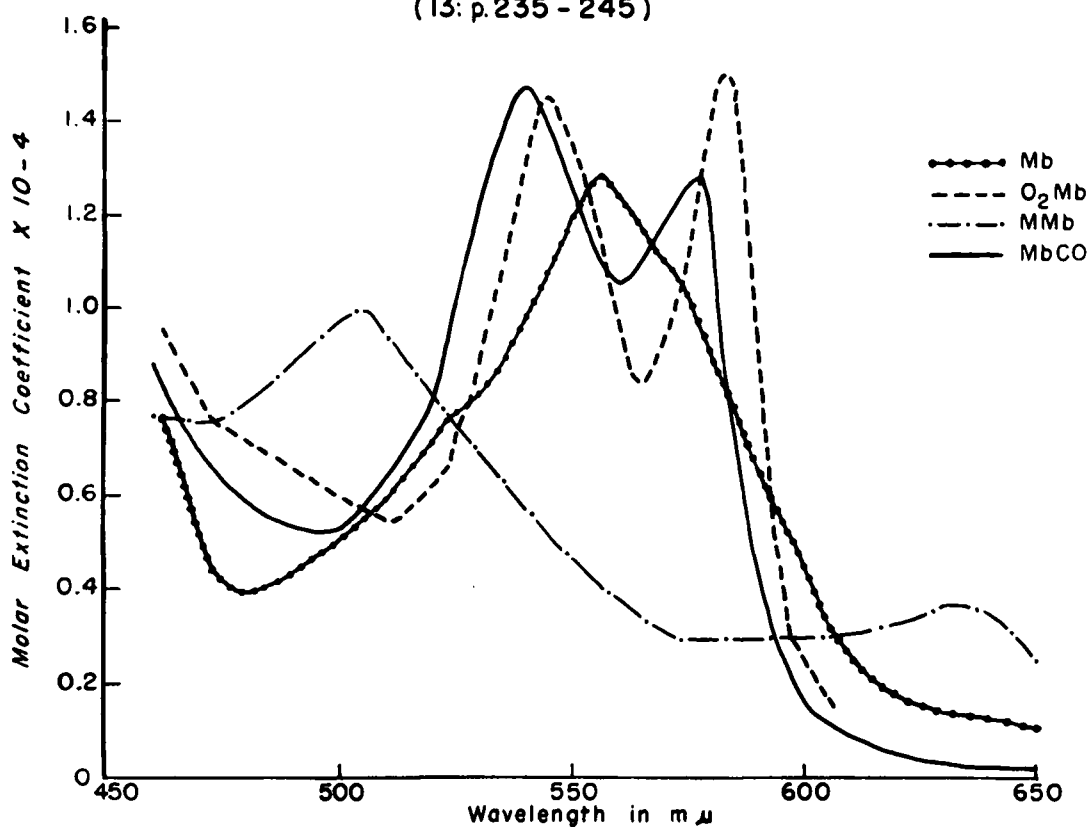
Procedure for the Carbon Monoxide-Treated Meat. A procedure similar to that described above, was developed to measure the pigments of the meat treated by carbon monoxide. Curves for determining the relative concentrations of myoglobin and metmyoglobin plus carboxymyoglobin (Figure 4), and carboxymyoglobin and metmyoglobin plus myoglobin (Figure 5) were developed by using the absorbency ratios at $\frac{466 \text{ m}\mu}{594 \text{ m}\mu}$ and at $\frac{523 \text{ m}\mu}{606 \text{ m}\mu}$, respectively. The relative percent of metmyoglobin was obtained by the equation:

$$\begin{aligned} \text{Per cent of metmyoglobin} &= 100 - \text{per cent of myoglobin} \\ &\quad - \text{per cent of carboxymyoglobin} \end{aligned}$$

In this method, the oxymyoglobin was replaced by the carboxymyoglobin and the wavelengths--at which the per cent reflectance was determined--were replaced by those needed to measure the myoglobin forms when carboxymyoglobin was present. These wavelengths were taken from curves of Bowen (13, p. 235-245), which are shown in Figure 3.

The replacement of oxymyoglobin by carboxymyoglobin was due to the following reasons: (1) No method was found in the literature pertaining to the determination of the four myoglobin derivatives when all are present at the same time. (2) Since myoglobin has an affinity of 28 to 51 times greater for carbon monoxide than for oxygen, the

Figure 3. ABSORPTION SPECTRA OF Mb, O₂Mb, MMb and MbCO
(13: p.235 - 245)



carbon monoxide can displace oxygen from oxymyoglobin (32, p. 177). (3) Lemberg and Legge (46, p. 287-289) stated that the affinity constant (K) for the reaction $\text{Mb} + \text{CO} \longrightarrow \text{MbCO}$ is equal to 9500 as compared to 495 for the reaction $\text{Mb} + \text{O}_2 \longrightarrow \text{O}_2\text{Mb}$ when both reactions were carried out at 20°C. and pH 7.4. (4) In the preliminary work of this study, a procedure similar to that by Dean and Ball (29, p. 273-274) was used to analyze the myoglobin derivatives of carbon monoxide-treated samples by assuming that one of the myoglobin fractions was absent each time. The results showed that it was reasonable to assume that in the presence of carbon monoxide, the oxymyoglobin would be absent. The literature also supports this assumption.

The absorbency ratios used in the preliminary work were as follows:

When O_2Mb was absent:

$$\frac{K/S \text{ at } 523 \text{ m}\mu}{K/S \text{ at } 606 \text{ m}\mu} \text{ to determine } \% \text{ MbCO and } \% \text{ MMb} + \text{Mb}$$

$$\frac{K/S \text{ at } 466 \text{ m}\mu}{K/S \text{ at } 594 \text{ m}\mu} \text{ to determine } \% \text{ Mb and } \% \text{ MMb} + \text{MbCO}$$

When Mb was absent:

$$\frac{K/S \text{ at } 473 \text{ m}\mu}{K/S \text{ at } 597 \text{ m}\mu} \text{ to determine } \% \text{ MbCO and } \% \text{ MMb} + \text{O}_2\text{Mb}$$

$$\frac{K/S \text{ at } 466 \text{ m}\mu}{K/S \text{ at } 520 \text{ m}\mu} \text{ to determine } \% \text{ O}_2\text{Mb and } \% \text{ MMb} + \text{MbCO}$$

When MMb was absent:

$$\frac{K/S \text{ at } 566 \text{ m}}{K/S \text{ at } 583 \text{ m}} \text{ to determine } \% O_2\text{Mb and } \% \text{Mb} + \text{MbCO}$$

$$\frac{K/S \text{ at } 527 \text{ m}}{K/S \text{ at } 593 \text{ m}} \text{ to determine } \% \text{MbCO and } \% \text{Mb} - O_2\text{Mb}$$

The curves for the determination of myoglobin fractions on the meat surface when treated by carbon monoxide with the assumption that oxymyoglobin was absent are presented in Figures 4 and 5, respectively.

Statistical Methods

This study was designed as a randomized block experiment. Nine treatments, three replications in each treatment, were studied. These treatments are shown in Table 1.

TABLE 1
Description of the Treatments

Storage Atmosphere	Packaging Material
Air	S-M-P, 2.5 polyethylene or 1.5 polyethylene
2% CO	S-M-P, 2.5 polyethylene or 1.5 polyethylene
N ₂	S-M-P
28 inches of Vacuum	S-M-P
100% CO + 28 inches of Vacuum	S-M-P

Data of the index of change, after adding twenty to each value to remove the negative sign, if any, for convenience in calculation, were subjected to analysis of

Figure 4. CURVE FOR DETERMINATION OF THE RELATIVE CONCENTRATIONS OF MbCO AND (Mb+MMb) AFTER MEASURING THE ABSORBANCES AT 523 m μ AND 606 m μ .

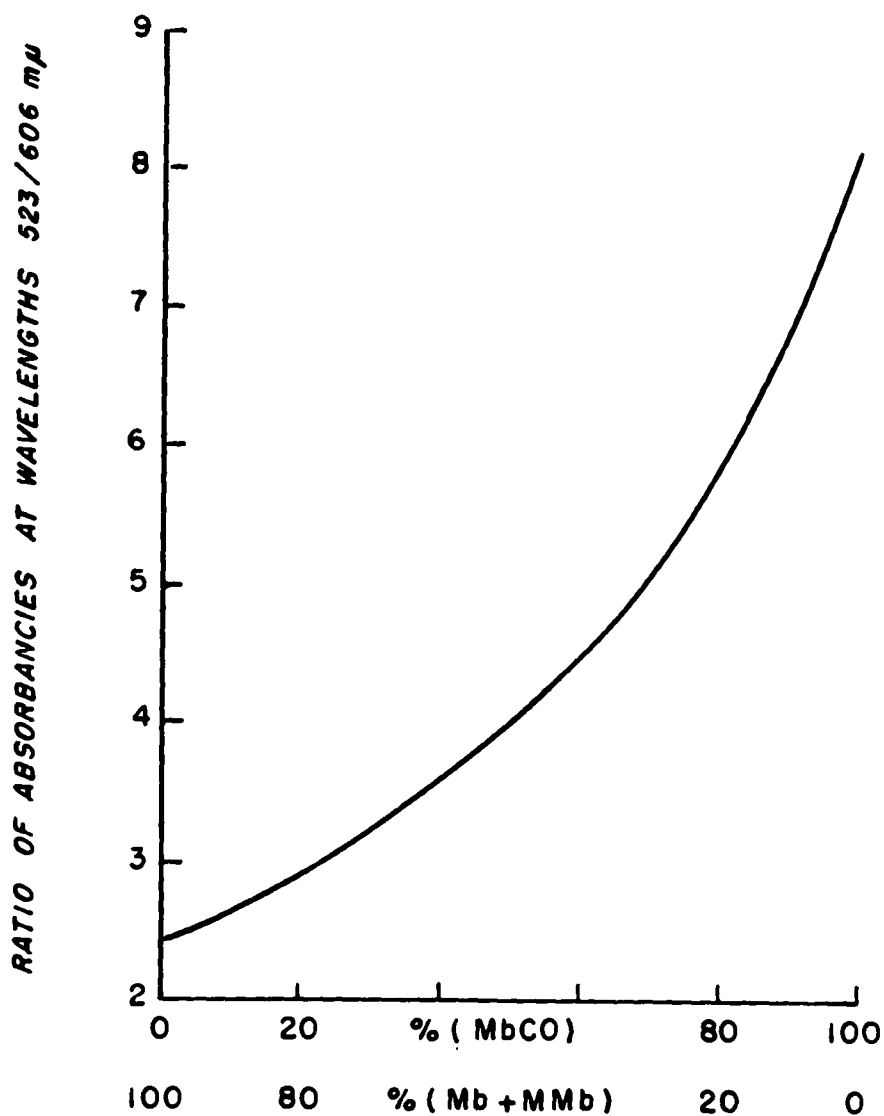
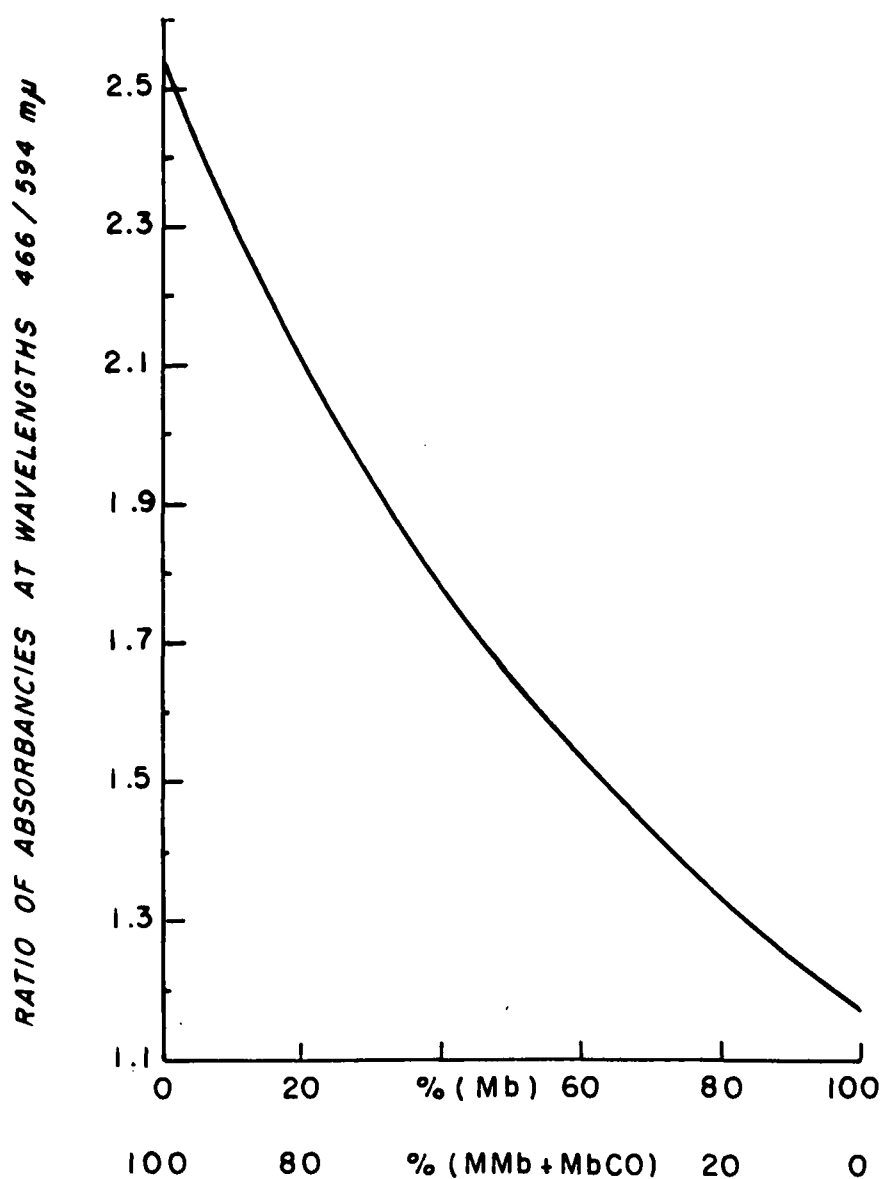


Figure 5. CURVE FOR DETERMINATION OF THE RELATIVE CONCENTRATIONS OF Mb AND (MMb+MbCO) AFTER MEASURING THE ABSORBANCIES AT 466 m μ AND 606 m μ



variance. The various hypotheses were then tested by the individual degree of freedom method (47, p. 226-233).

Tests of linear regression (L.R.) and deviation from linearity (D.F.L.) were applied to each treatment to test the variation of the index of change as a function of storage, Li (47, p. 295-298).

RESULTS AND DISCUSSION

The results of various treatments are presented in tables and figures in this section. The tables contain the Munsell notations, index of change, relative percentages of myoglobin derivatives and per cent panel score. Munsell notations are included in the tables only to indicate the actual colors of the meat samples. Graphically, the index of change is used to show color change since it is practically impossible to discuss changes in color by the three Munsell notations of hue, value/chroma.

Air Treatments

Data of the color measurements and pigment determinations of meat samples packaged and stored in pouches of saran-mylar-polyethylene, 2.5 mils polyethylene and 1.5 mils polyethylene under an air atmosphere are given in Tables 2, 3 and 4 and are plotted in Figures 6, 7 and 8, respectively.

Index of Change

Although the general shape of the curves indicate changes in the index of change as a function of storage, they do not exactly look alike. The statistical analysis, Table 5, of the data does not show a significant difference between index of change of the samples in the three

TABLE 2

Munsell Notations, Index of Change, Relative Percentage of Mb, O₂Mb and MMb and Average Per Cent Panel Score of Triplicate Samples of Beef Packaged in Saran-Mylar Coated Polyethylene Under an Atmosphere of Air and Stored for 15 Days at 36-38°F.

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score
	Ave.	Value/Croma		MMb	Mb	O ₂ Mb	
0	3.98 R	4.16/3.61	- .55	10.0	52.0	38.0	--
	4.21 R	4.10/3.10	+ .81	21.0	55.8	23.2	--
	4.50 R	3.73/4.23	- .96	0.0	52.0	48.0	--
		Mean	- .23	10.3	53.3	36.4	--
1	4.09 R	4.18/5.25	- 6.07	7.0	3.3	89.7	75.5
	5.08 R	3.91/3.56	- .71	18.5	23.5	58.0	53.1
	4.41 R	4.68/4.27	- 6.63	13.3	15.4	71.3	85.7
		Mean	- 4.47	12.9	14.1	73.0	71.4
2	6.67 R	3.95/5.50	- 11.13	3.0	0.0	97.0	69.6
	6.60 R	2.81/4.82	- 1.38	13.5	0.0	86.5	55.3
	5.78 R	4.10/4.45	- 6.16	6.3	9.3	84.4	69.6
		Mean	- 6.22	2.6	3.1	89.3	64.8
3	4.47 R	4.32/5.36	- 8.06	12.0	0.0	88.0	50.0
	5.86 R	4.31/1.74	+ 2.62	32.0	53.2	14.8	26.8
	4.99 R	4.04/4.48	- 4.50	7.5	6.0	86.5	35.7
		Mean	- 3.31	17.2	19.7	63.1	37.5
4	8.37 R	4.08/2.21	+ 0.01	42.0	45.3	12.7	42.8
	9.79 R	4.17/1.07	+ 2.55	44.2	55.8	0.0	17.8
	8.38 R	3.88/2.91	- 2.13	25.5	26.2	48.3	28.6
		Mean	+ 0.14	37.3	42.4	20.3	29.7
5	5.00 R	4.15/3.39	- 1.46	26.0	21.0	53.0	--
	2.65 YR	3.71/1.87	+ .65	53.7	46.3	0.0	--
	8.33 R	4.12/2.08	+ .42	48.1	51.9	0.0	--
		Mean	- .13	42.6	39.7	17.7	--
6	2.33 YR	4.20/1.58	- .21	51.8	48.2	0.0	20.4
	5.00 R	4.08/1.40	+ 5.76	37.2	62.8	0.0	20.4
	7.29 R	4.24/2.29	- .34	40.2	59.8	0.0	28.6
		Mean	+ 1.74	43.1	56.9	0.0	23.1
9	2.50 R	3.94/2.87	+ 4.45	26.7	73.3	0.0	33.9
	5.58 R	4.06/1.72	+ 4.38	26.8	73.2	0.0	23.2
	2.16 R	4.15/1.72	+ 6.19	24.1	75.9	0.0	28.6
		Mean	+ 5.00	25.9	74.1	0.0	28.6

TABLE 2, continued

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score	
	Ave.	Value/Croma		MMb	Mb	O ₂ Mb		
12	2.63 R	4.11/2.88	+	3.25	27.6	72.4	0.0	30.6
	0.70 R	3.82/2.33	+	8.15	20.6	79.4	0.0	26.5
	1.88 R	4.12/2.80	+	4.23	18.1	81.9	0.0	26.5
		Mean	+	5.21	22.1	77.9	0.0	27.9
15	1.89 R	4.23/3.08	+	2.95	18.6	81.4	0.0	35.7
	.92 R	3.46/2.41	+	9.96	18.0	82.0	0.0	23.2
	9.44 RP	4.22/2.67	+	6.52	20.3	79.7	0.0	21.4
		Mean	+	6.48	19.0	81.0	0.0	26.8

Figure 6. MEAN OF INDEX OF CHANGE AND OF PERCENT PANEL SCORE (PART I) AND MEAN PERCENTAGES OF MMb, Mb AND O₂Mb (PART II) OF TRIPLICATE SAMPLES PACKAGED IN SARAN-MYLAR-POLYETHYLENE IN AIR

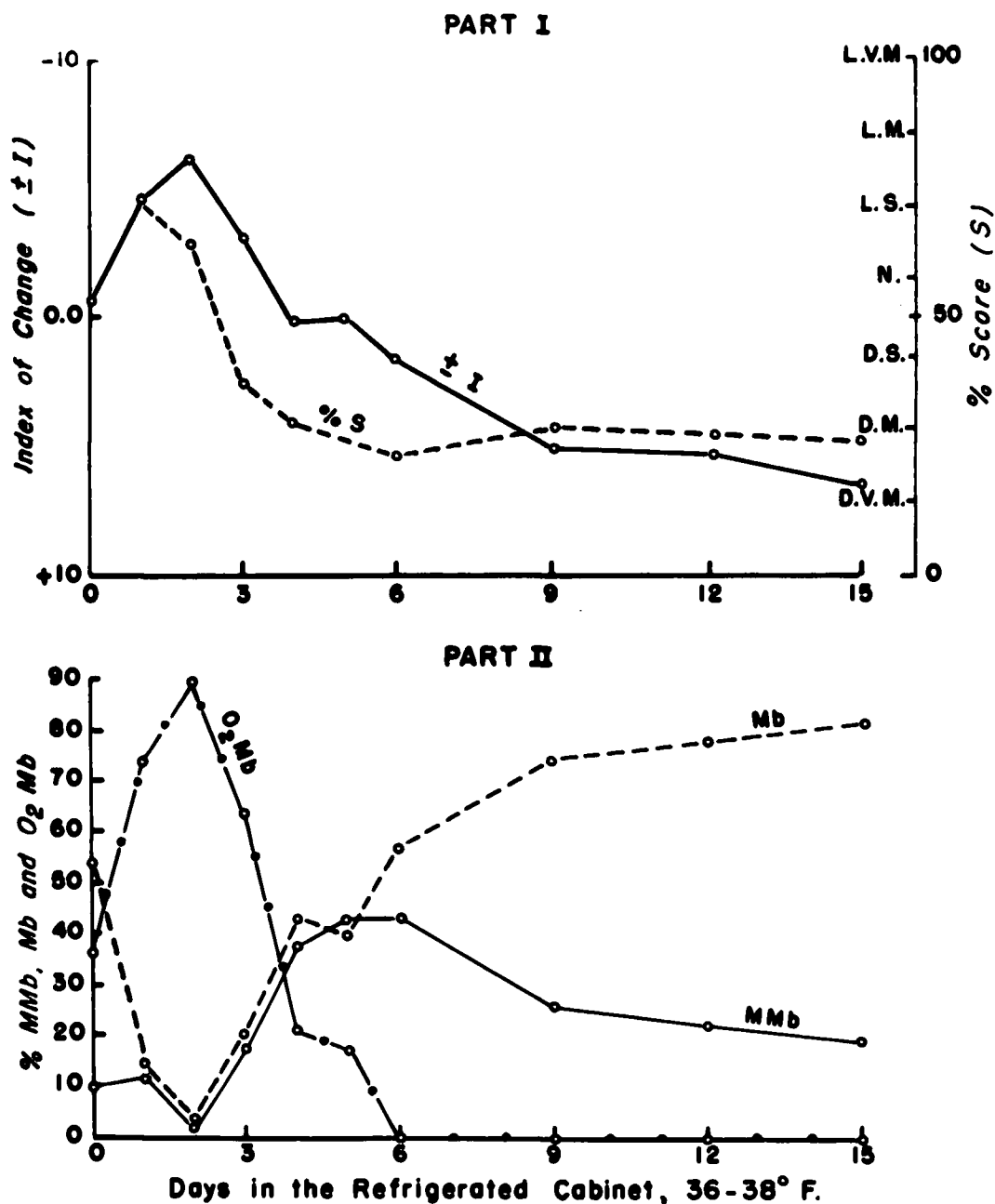


TABLE 3

Munsell Notations, Index of Change, Relative Percentage of Mb, O₂Mb and MMb and Average Per Cent Panel Score of Triplicate Samples of Beef Packaged in 2.5 Mils Polyethylene Under an Atmosphere of Air and Stored for 15 Days at 36-38°F.

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score
	Ave.	Value/Chroma		MMb	Mb	O ₂ Mb	
0	4.16 R	3.63/4.57	- .83	0.0	44.0	56.0	--
	4.79 R	3.92/3.64	- .62	16.0	47.2	36.8	--
	5.00 R	4.23/3.94	- 3.75	8.5	46.6	44.9	--
		Mean	- 1.73	8.2	45.9	45.9	--
1	4.80 R	4.05/5.32	- 7.01	3.0	0.5	96.5	79.6
	5.19 R	3.70/3.51	+ .57	17.0	19.4	63.6	30.6
	5.95 R	3.98/5.60	- 10.10	0.0	0.0	100.0	85.7
		Mean	- 5.51	6.7	6.6	86.7	65.3
2	5.77 R	3.48/5.77	- 7.33	0.0	0.0	100.0	75.0
	7.50 R	3.44/2.70	+ 2.46	31.0	29.8	39.2	19.6
	3.83 R	4.13/4.14	- 1.96	11.0	20.0	69.0	75.0
		Mean	- 2.28	14.0	16.6	69.4	56.5
3	4.78 R	4.02/4.80	- 5.06	3.5	0.0	96.5	67.8
	5.36 YR	3.60/1.45	+ 2.45	56.6	43.4	0.0	14.3
	5.08 R	4.08/5.26	- 7.58	0.0	0.0	100.0	60.7
		Mean	- 3.40	20.0	14.5	65.5	47.6
4	5.30 R	3.95/3.67	- 1.65	15.4	17.3	67.3	51.8
	2.11 YR	3.78/1.91	+ .28	56.7	43.3	0.0	16.1
	1.68 R	3.94/2.99	+ 5.14	15.0	50.5	34.5	39.3
		Mean	+ 1.26	29.1	37.0	33.9	35.7
5	.37 YR	3.84/2.31	- .87	40.0	34.2	25.8	--
	6.72 R	3.37/2.12	+ 6.10	41.4	59.6	0.0	--
	2.79 YR	4.53/1.98	- 5.09	55.3	44.7	0.0	--
		Mean	+ .05	45.6	46.2	8.6	--
6	3.35 YR	4.32/1.28	+ .45	50.8	49.2	0.0	22.4
	5.93 R	4.26/1.54	+ 3.62	35.7	64.3	0.0	16.3
	9.84 R	4.42/1.06	+ 2.82	38.2	61.8	0.0	26.5
		Mean	+ 2.30	41.6	58.4	0.0	21.7
9	5.07 R	3.89/2.64	+ 2.59	36.1	63.9	0.0	26.8
	5.56 R	4.09/3.29	- 1.49	33.1	66.9	0.0	19.6
	1.94 R	4.45/2.42	+ 3.01	31.7	68.3	0.0	26.8
		Mean	+ 1.37	33.6	66.4	0.0	24.4

TABLE 3, continued

Days in Storage	Munsell Ave.	Notations Value/Croma	Index of Change	% Myoglobin Derivatives			% Panel Score
				MMb	Mb	O ₂ Mb	
12	1.56 R	3.72/3.16	+ 6.26	17.3	82.7	0.0	30.6
	1.00 R	3.39/3.05	+ 9.14	19.3	80.7	0.0	22.4
	1.82 R	3.90/3.16	+ 4.85	19.3	80.7	0.0	30.6
		Mean	+ 6.75	18.7	81.3	0.0	27.9
15	1.75 R	3.57/3.74	+ 5.69	12.7	87.3	0.0	25.0
	4.29 R	3.61/3.30	+ 3.03	16.6	83.4	0.0	19.6
	1.56 R	3.74/3.43	+ 5.59	16.6	83.4	0.0	25.0
		Mean	+ 4.77	15.3	84.7	0.0	23.2

Figure 7. MEAN OF INDEX OF CHANGE AND OF PERCENT PANEL SCORE (PART I) AND MEAN PERCENTAGES OF MMb, Mb AND O₂Mb (PART II) OF TRIPLICATE SAMPLES PACKAGED IN 2.5 MILS POLYETHYLENE IN AIR

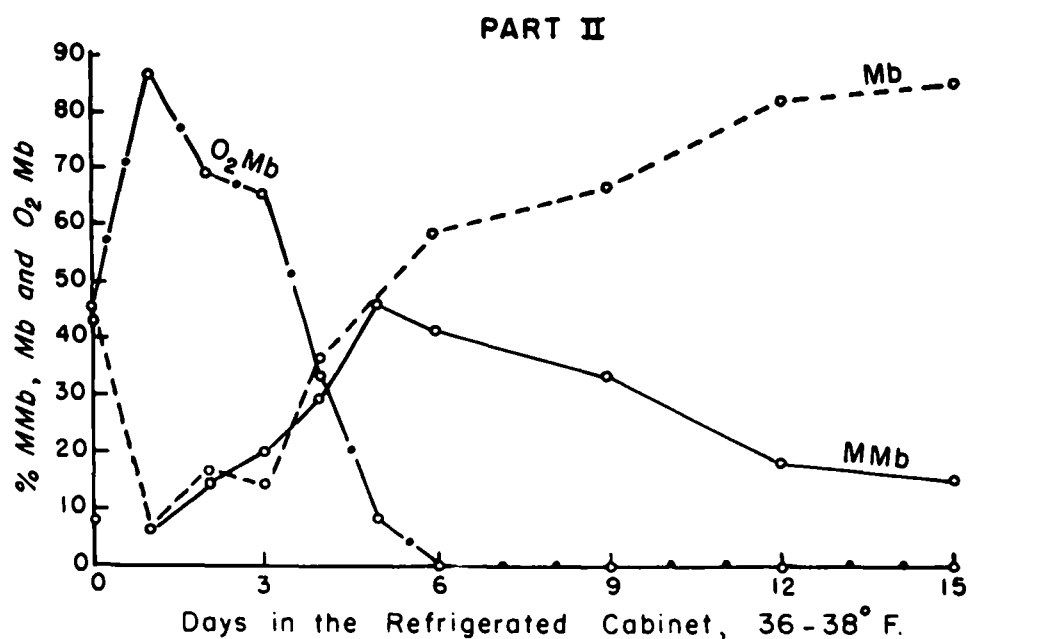
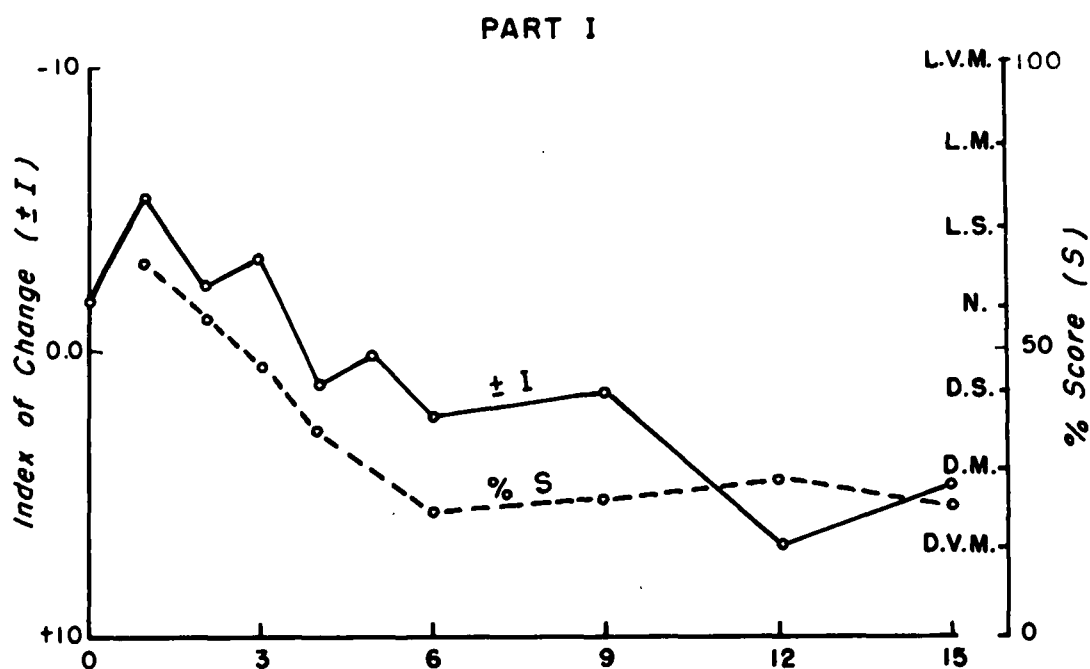


TABLE 4

Munsell Notations, Index of Change, Relative Percentage of Mb, O₂Mb and MMb and Average Per Cent Panel Score of Triplicate Samples of Beef Packaged in 1.5 Mils Polyethylene Under an Atmosphere of Air and Stored for 15 Days at 36-38°F.

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score
	Ave.	Value/Chroma		MMb	Mb	O ₂ Mb	
0	2.63 R	3.47/4.54	+	3.00	0.0	62.2	37.8
	4.50 R	3.74/3.49	+	.55	8.5	47.4	44.1
	5.00 R	4.00/4.26	-	3.52	4.5	46.6	48.9
		Mean	+	.01	4.3	52.1	43.6
1	4.95 R	4.14/4.00	-	3.40	15.0	23.0	62.0
	4.23 R	3.93/2.89	+	2.46	24.0	40.0	36.0
	5.64 R	3.91/5.64	-	9.17	0.0	0.0	100.0
		Mean	-	3.37	13.0	21.0	66.0
2	5.36 R	3.45/5.42	-	4.96	2.5	0.0	97.5
	5.85 R	3.24/5.98	-	6.87	7.5	0.0	92.5
	6.23 R	3.58/6.03	-	9.96	0.0	0.0	100.0
		Mean	-	7.26	3.3	0.0	96.7
3	4.56 R	4.07/3.71	-	1.41	19.5	19.0	61.5
	3.94 R	3.75/2.62	+	4.68	26.0	42.0	31.8
	5.27 R	3.88/4.69	-	4.78	7.5	0.0	92.5
		Mean	-	.50	17.7	20.0	61.9
4	4.92 R	3.79/4.35	-	2.43	13.5	3.3	83.2
	6.07 R	3.32/4.10	-	.65	23.5	0.0	76.5
	6.41 R	3.39/3.55	+	.56	22.0	14.5	63.5
		Mean	-	.84	19.7	5.9	74.4
5	5.32 R	3.40/3.65	+	2.23	18.5	13.0	68.7
	9.27 R	3.37/2.58	+	1.58	47.0	23.0	30.0
	3.44 YR	4.06/2.04	-	3.20	64.5	35.5	0.0
		Mean	+	.20	43.3	23.8	32.9
6	0.86 YR	3.65/2.23	+	.27	42.5	34.9	22.6
	3.14 YR	3.97/0.78	+	5.99	43.9	56.1	0.0
	1.75 YR	3.53/2.22	+	.26	58.8	41.2	0.0
		Mean	+	2.17	48.4	44.1	7.5

TABLE 4, continued

Munsell Notations			Index of Change	% Myoglobin Derivatives			% Panel Score	
Days in Storage	Ave.	Value/Chroma		MMb	Mb	O ₂ Mb		
9	7.04 R	3.55/2.22	+	4.32	44.4	55.6	0.0	26.8
	2.24 YR	3.85/1.76	+	.81	45.7	54.3	0.0	19.6
	2.58 R	3.75/4.21	+	2.22	30.5	69.5	0.0	33.9
		Mean	+	2.45	40.2	59.8	0.0	26.8
12	2.03 R	3.58/3.44	+	5.88	16.3	83.7	0.0	34.7
	6.23 R	4.03/3.10	-	1.27	29.8	70.2	0.0	22.4
	4.32 R	3.56/3.12	+	3.85	14.5	85.5	0.0	34.7
		Mean	+	2.82	20.2	79.8	0.0	30.6
15	2.58 R	3.57/3.29	+	5.55	19.7	81.3	0.0	25.0
	2.95 R	3.60/2.63	+	6.59	24.5	75.5	0.0	25.0
	1.89 R	3.33/3.57	+	7.29	10.7	89.3	0.0	35.7
		Mean	+	6.48	18.0	82.0	0.0	28.6

Figure 8. MEAN OF INDEX OF CHANGE AND OF PERCENT PANEL SCORE (PART I) AND MEAN PERCENTAGES OF MMb, Mb AND O₂Mb (PART II) OF TRIPPLICATE SAMPLES PACKAGED IN 1.5 MILS POLYETHYLENE IN AIR

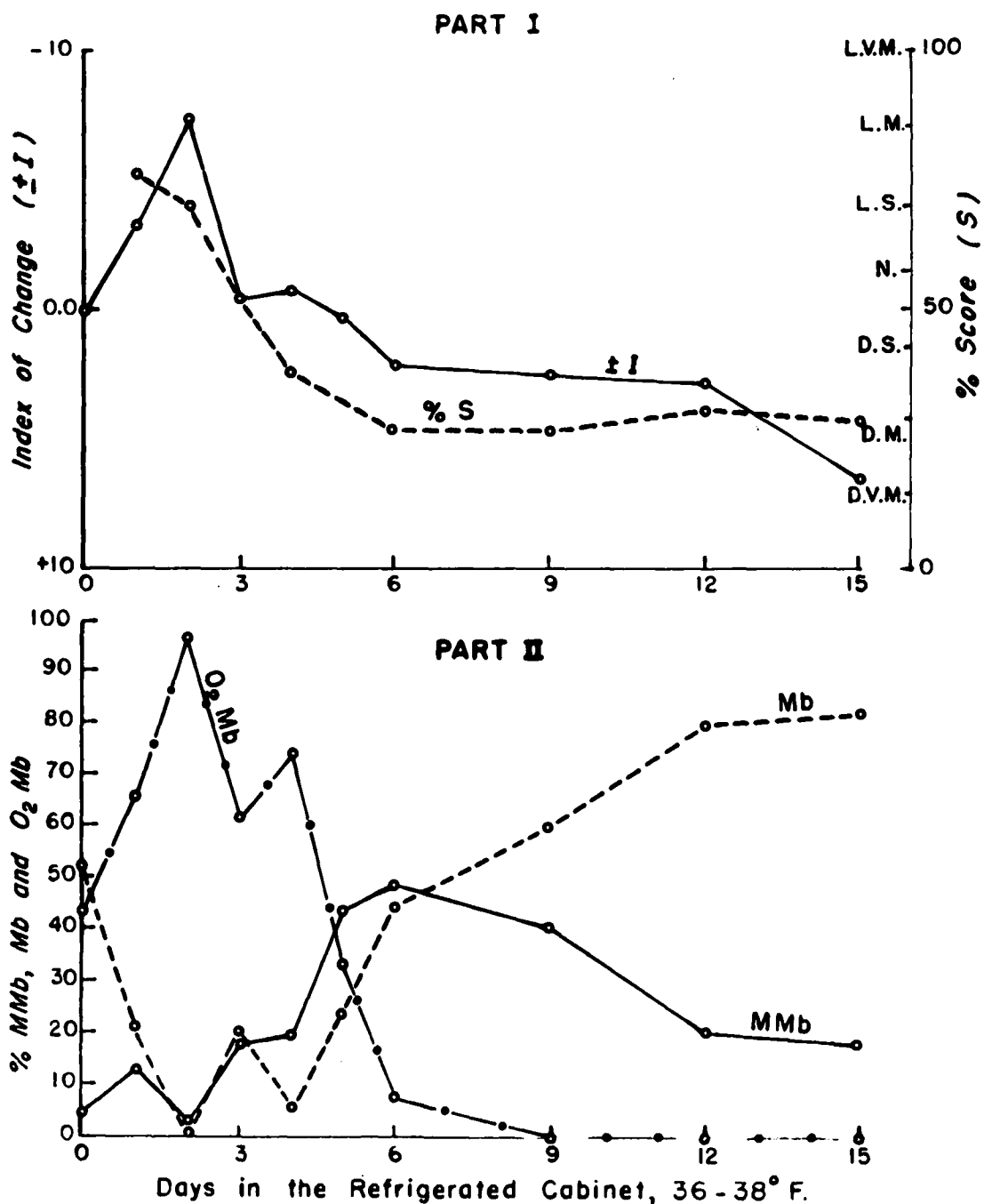


TABLE 5
Analysis of Variance on the Index of Change^x

Source of Variation	Degrees of Freedom	Mean Square	F	Significance at 5%
Replications	2	485.22	40.88	S
Treatments	8	616.74	51.95	S
2% CO vs. Air	1	1,541.65	129.85	S
28 inch Vacuum vs. N ₂	1	50.78	4.28	S
28 inch Vacuum vs. 28 inch Vacuum plus 100% CO	1	1,717.03	144.62	S
2% CO vs. N ₂	1	1,759.44	148.19	S
2% CO vs. 28 inch Vacuum	1	1,408.06	202.83	S
2% CO in S-M-P vs. 2% CO in 2.5 and 1.5 Polyethylene	1	42.85	3.61	NS
2% CO in 2.5 Polyethylene vs. 2% CO in 1.5 Polyethylene	1	122.24	10.30	S
Air in S-M-P vs. Air in 2.5 and 1.5 Polyethylene	1	.36	.03	NS
Air in 2.5 Polyethylene vs. Air in 1.5 Polyethylene	1	.30	.02	NS
Storage	9	71.98	6.06	S
Treatments x Storage	72	21.08	1.78	S
Air in S-M-P: L.R.*	1	374.47	31.54	S
D.F.L.**	8	15.12	1.27	NS
2% CO in S-M-P:				
L.R.*	1	41.34	3.48	NS
D.F.L.**	8	12.74	1.07	NS

TABLE 5, continued

Source of Variation		Degrees of Freedom	Mean Square	F	Significance at 5%
Air in 2.5					
Polyethylene:	L.R.	1	285.13	24.02	S
	D.F.L.	8	11.57	.97	NS
2% CO in 2.5					
Polyethylene:	L.R.	1	7.72	.65	NS
	D.F.L.	8	18.45	1.55	NS
Air in 1.5					
Polyethylene:	L.R.	1	242.02	20.38	S
	D.F.L.	8	16.72	1.41	NS
2% CO in 1.5					
Polyethylene:	L.R.	1	29.10	2.45	NS
	D.F.L.	8	10.15	.85	NS
N ₂ in S-M-P:					
	L.R.	1	.28	.02	NS
	D.F.L.	8	9.75	.82	NS
28 inch Vacuum in S-M-P:					
	L.R.	1	29.59	2.49	NS
	D.F.L.	8	15.40	1.30	NS
100% CO Under 28 inch Vacuum in S-M-P;					
	L.R.	1	11.66	.98	NS
	D.F.L.	8	35.44	2.98	S
Error		177	11.87		
Total		268	37.95		

* Calculations were carried out to four decimal places but were rounded off to two decimal places.

* L.R. = Linear Regression

** D.F.L. = Deviation From Linearity

packaging materials. Each of these curves can be considered as a straight line with a negative slope when tested for linear regression and deviation from linearity at the 5 per cent significance level. In other words, the rate of fading was constant for the storage period and was the same for each packaging material used.

Color Panel

The average panel score indicates that the color of all samples, regardless of packaging material, was slightly undesirable at the end of the first three days of storage. After the fifth day and up to the fifteenth day, the color panel noted the samples as moderately undesirable.

Pigments

The mean of the index of change followed the per cent of oxymyoglobin for the three packaging materials.

The peak level of oxymyoglobin was reached on the second day on the samples packaged in saran-mylar-polyethylene and 1.5 polyethylene films. The relative percentage of this pigment was higher for the beef cuts stored in the 1.5 polyethylene than those held in the saran-mylar-polyethylene pouches. The samples stored in 2.5 polyethylene had the highest concentration of oxymyoglobin after one day of storage.

The pattern of changes of the three meat pigments during storage was fairly similar in each of the three packages. After six days of storage, oxymyoglobin was not detected in the samples packaged in saran-mylar-polyethylene or 2.5 mils polyethylene pouches whereas the pigment disappearance was delayed to the ninth day of storage in those samples packaged in 1.5 mils polyethylene. After the ninth day, no improvement in the color was shown by index of change, but a very slight increase was indicated by the color panel score.

2% Carbon Monoxide Treatments

Results for the samples packaged in saran-mylar-polyethylene, 2.5 and 1.5 mils polyethylene pouches under an atmosphere containing a mixture of 2% carbon monoxide and 98% air are tabulated in Tables 6, 7 and 8 and illustrated in Figure 9, 10 and 11.

Index of Change

The analysis of variance calculations, Table 5, of the index of change indicate no significant difference between the color of the samples packaged in saran-mylar-polyethylene and those packaged in 2.5 polyethylene and 1.5 polyethylene; however, there was significant difference in the color between samples packaged in 2.5 polyethylene

TABLE 6

Munsell Notations, Index of Change, Relative Percentage of Mb, MbCO and MMb and Average Per Cent Panel Score of Triplicate Samples of Beef Packaged in Saran-Mylar-Polyethylene Under an Atmosphere of 2% Carbon Monoxide and 98% Air and Stored for 15 Days at 36-38°F.

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score
	Ave.	Value/Chroma		MMb	Mb	MbCO	
0	3.04 R	4.02/4.21	- .17	12.5	69.0	18.5	--
	5.02 R	3.97/4.32	- 3.58	36.1	41.3	22.6	--
	3.05 R	4.01/4.01	+ .40	7.0	80.0	13.0	--
		Mean	- 1.12	18.5	63.4	18.1	--
1	3.25 R	4.23/6.55	- 8.13	27.0	7.8	65.2	81.6
	3.45 R	3.88/4.76	- 1.56	41.6	28.1	30.3	85.7
	3.24 R	4.30/5.27	- 5.06	33.0	19.5	47.5	79.6
		Mean	- 4.92	33.9	18.5	47.6	82.3
2	3.29 R	4.34/7.71	- 12.22	10.5	0.0	89.5	85.7
	4.60 R	3.63/5.01	- 3.06	35.2	12.8	52.0	78.6
	3.85 R	4.13/6.21	- 8.10	31.2	1.0	67.8	82.1
		Mean	- 7.79	25.6	4.6	69.8	82.1
3	3.15 R	4.36/6.91	- 9.61	36.0	0.0	64.0	83.9
	2.49 R	4.52/4.51	- 2.97	20.3	60.5	19.2	78.6
	2.95 R	4.28/5.93	- 6.04	29.0	12.5	58.5	80.3
		Mean	- 6.21	28.4	24.3	47.3	80.9
4	3.23 R	4.36/6.53	- 8.81	22.5	8.2	69.3	83.9
	2.43 R	3.60/6.72	- 2.61	23.0	19.5	57.5	76.8
	2.96 R	4.26/6.48	- 7.53	17.2	7.8	75.0	83.9
		Mean	- 6.32	20.9	11.8	67.3	81.5
5	3.52 R	4.07/5.75	- 8.23	49.0	0.0	51.0	--
	3.33 R	4.00/6.70	- 7.38	26.8	10.0	63.2	--
	2.87 R	4.40/6.31	- 7.54	36.8	0.0	63.2	--
		Mean	- 7.72	37.6	3.3	59.1	--
6	3.69 R	4.28/6.33	- 8.95	29.2	5.8	65.0	57.1
	2.63 R	4.00/6.70	- 7.82	27.5	19.3	53.2	73.5
	3.01 R	4.36/7.09	- 9.70	16.0	9.2	74.8	73.5
		Mean	- 8.82	24.3	11.4	64.3	68.0

TABLE 6, continued

Days in Storage	Munsell Notations Ave.	Value/Chroma	Index of Change	% Myoglobin Derivatives			Panel Score
				MMb	Mb	MbCO	
9	4.96 R	4.17/5.30	- 8.01	46.0	1.0	53.0	55.3
	2.67 R	4.37/6.17	- 6.51	18.1	33.9	48.0	76.8
	2.83 R	4.35/7.19	- 9.38	18.3	3.7	78.0	80.3
		Mean	- 7.97	27.5	12.8	59.7	70.8
12	3.19 R	4.45/4.92	- 4.92	3.7	65.3	31.0	55.1
	3.19 R	4.22/7.06	- 9.29	22.8	7.2	70.0	77.5
	3.91 R	3.85/7.91	- 11.63	3.0	0.0	97.0	79.6
		Mean	- 8.61	9.8	24.2	66.0	70.7
15	2.28 R	4.30/5.37	- 3.27	0.0	66.3	33.7	53.6
	2.58 R	4.52/5.98	- 6.73	0.0	73.8	26.2	76.8
	2.80 R	4.12/6.64	- 6.52	12.3	21.6	66.1	78.6
		Mean	- 5.51	4.1	53.9	42.0	69.7

Figure 9. MEAN OF INDEX OF CHANGE AND OF PERCENT PANEL SCORE (PART I) AND MEAN PERCENTAGES OF MMb, Mb AND MbCO (PART II) OF TRIPPLICATE SAMPLES PACKAGED IN SARAN - MYLAR - POLYETHYLENE IN A MIXTURE OF 2% CO AND AIR

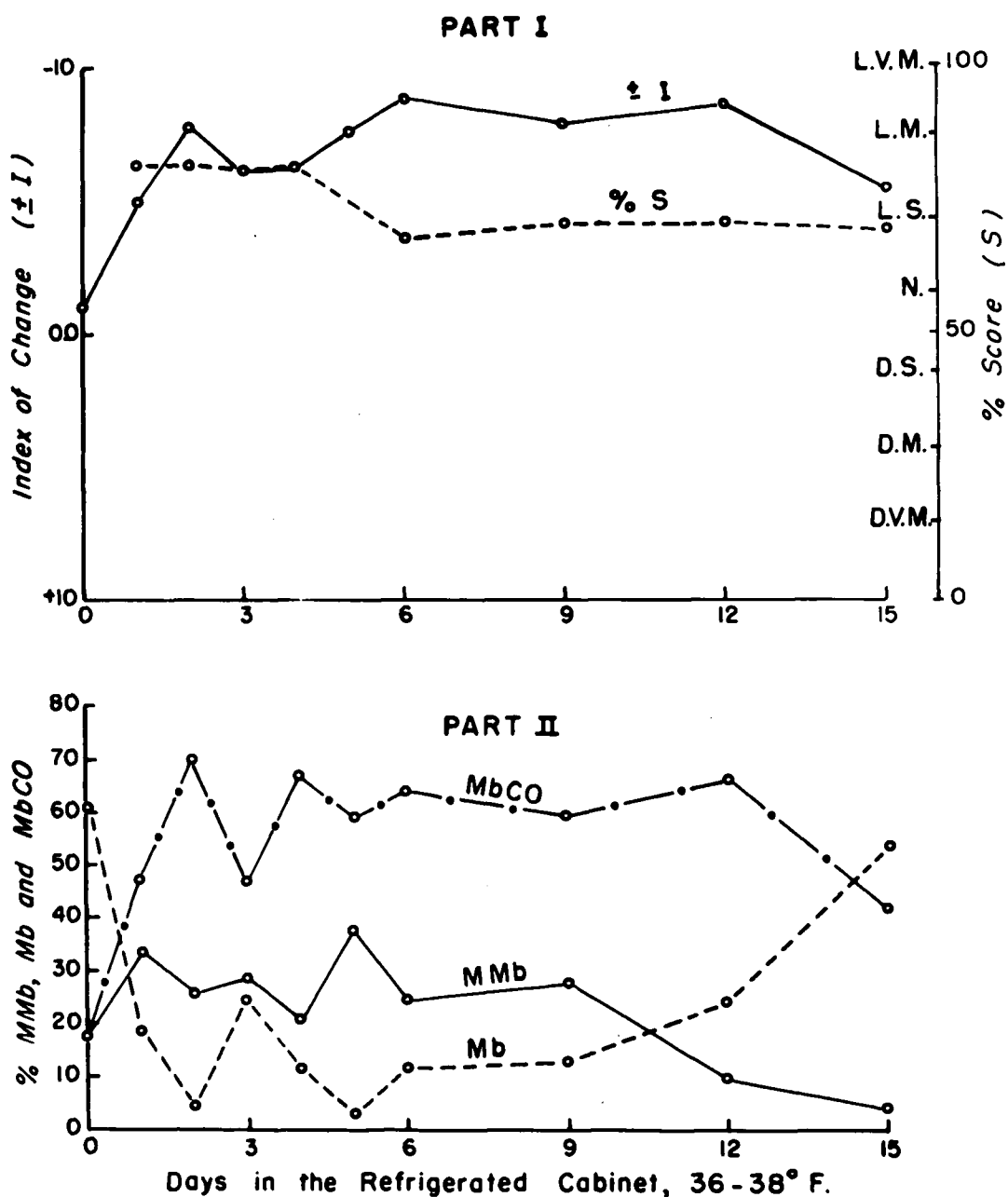


TABLE 7

Munsell Notations, Index of Change, Relative Percentage of Mb, MbCO and MMb and Average Per Cent Panel Score of Triplicate Samples of Beef Packaged in 2.5 Mils Polyethylene Under an Atmosphere of 2% Carbon Monoxide and 98% Air and Stored for 15 Days at 36-38°F.

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score
	Ave.	Value/Croma		MMb	Mb	MbCO	
0	3.91 R	3.88/4.88	- 2.85	8.0	49.0	43.0	--
	5.11 R	3.85/3.95	- 1.74	27.5	60.2	12.3	--
	6.66 R	4.29/4.68	- 9.81	24.0	40.8	35.3	--
		Mean	- 4.80	19.8	50.0	30.2	--
1	3.64 R	4.00/7.00	- 9.15	19.3	7.2	73.5	85.7
	1.29 R	3.92/3.84	+ 4.09	35.0	65.0	0.0	79.6
	5.19 R	4.39/6.15	- 12.78	33.4	0.0	66.6	77.5
		Mean	- 5.95	29.2	24.1	46.7	80.9
2	4.39 R	3.29/8.77	- 17.43	0.0	0.0	100.0	92.8
	4.41 R	4.58/4.41	- 6.48	31.4	35.1	33.5	75.0
	5.90 R	4.03/6.25	- 12.74	21.2	0.0	78.8	67.8
		Mean	- 12.22	17.5	11.2	70.8	78.5
3	3.66 R	3.91/8.02	- 11.52	4.5	0.0	95.5	85.7
	2.92 R	4.16/4.67	- 1.94	33.2	36.3	30.5	78.6
	5.07 R	3.88/4.78	- 4.71	45.2	7.8	47.0	39.3
		Mean	- 6.06	27.6	14.7	57.7	67.9
4	3.85 R	4.10/7.94	- 13.03	5.5	0.0	94.5	85.7
	3.35 R	3.57/5.44	- 1.38	27.0	24.0	49.0	82.1
	1.25 YR	4.25/2.43	- 4.86	45.0	55.0	0.0	41.1
		Mean	- 6.42	25.9	26.3	47.8	69.6
5	3.36 R	4.22/8.44	- 13.57	0.0	0.0	100.0	--
	0.47 R	4.18/4.32	+ 3.02	34.0	63.0	3.0	--
	7.02 R	3.77/2.89	+ .19	36.0	64.0	0.0	--
		Mean	- 3.45	23.3	42.3	34.4	--
6	4.10 R	4.38/8.00	- 15.69	5.2	0.0	94.8	81.6
	1.91 R	4.13/5.90	- 2.61	21.7	29.8	48.5	73.5
	8.69 R	4.31/3.00	- 5.51	30.0	70.0	0.0	46.9
		Mean	- 7.94	18.9	33.3	47.8	67.3

TABLE 7, continued

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score
	Ave.	Value/Chroma		MMb	Mb	MbCO	
9	3.23 R	4.00/8.00	- 10.62	8.7	5.3	86.0	83.9
	3.00 R	4.20/7.69	- 10.28	14.0	8.5	77.5	75.0
	3.89 R	4.00/4.00	- .86	0.0	87.3	12.7	51.8
		Mean	- 7.25	7.6	33.7	58.7	70.2
12	3.35 R	4.35/8.03	- 13.19	7.0	0.0	93.0	81.6
	2.93 R	3.72/7.47	- 6.61	3.7	20.0	76.3	75.5
	2.27 R	3.84/4.21	+ 2.20	0.0	87.3	12.7	42.8
		Mean	- 5.87	3.5	35.8	60.7	66.6
15	3.35 R	3.96/7.93	- 10.57	2.5	8.5	89.0	80.4
	2.46 R	4.06/6.66	- 5.31	10.2	29.5	60.2	76.8
	2.31 R	3.75/4.54	+ 1.90	0.0	82.6	17.4	51.8
		Mean	- 4.66	4.3	40.2	55.5	69.7

Figure 10. MEAN OF INDEX OF CHANGE AND OF PERCENT PANEL SCORE (PART I) AND MEAN PERCENTAGES OF MMb, Mb AND MbCO (PART II) OF TRIPPLICATE SAMPLES PACKAGED IN 2.5 MILS POLYETHYLENE IN A MIXTURE OF 2% CO AND AIR

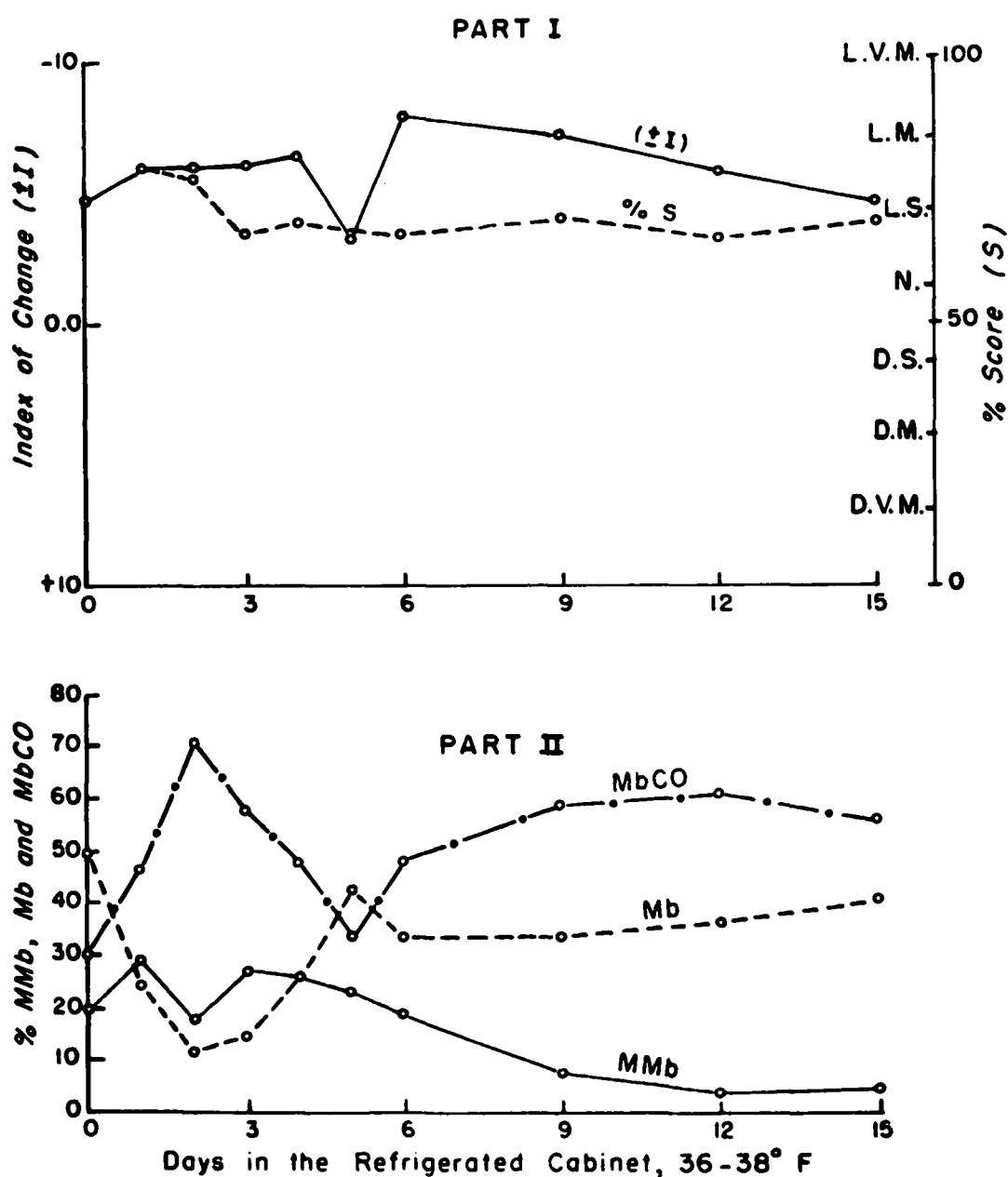


TABLE 8

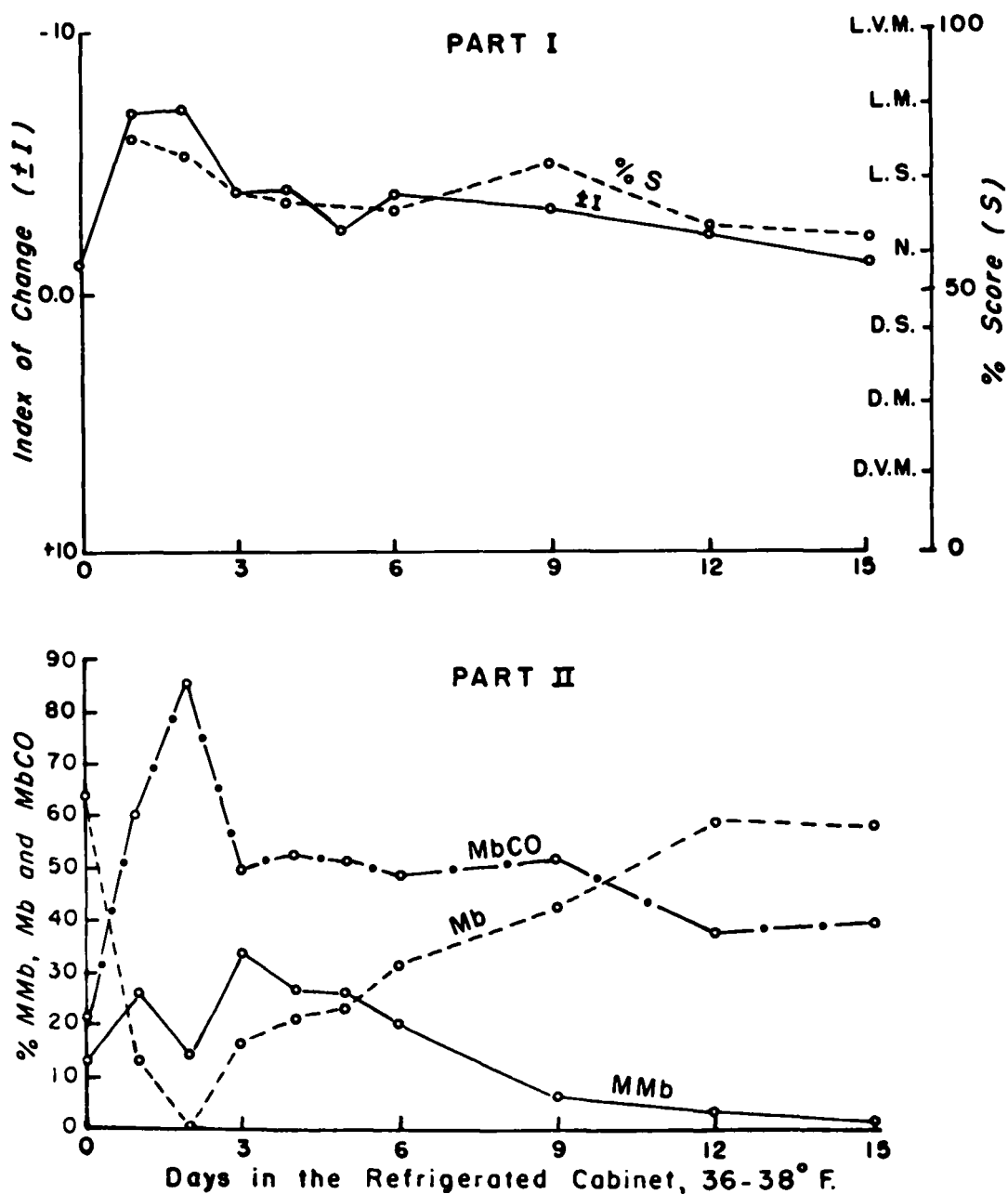
Munsell Notations, Index of Change, Relative Percentage of Mb, MbCO and MMb and Average Per Cent Panel Score of Triplicate Samples of Beef Packaged in 1.5 Mills Polyethylene Under an Atmosphere of 2% Carbon Monoxide and 98% Air and Stored for 15 Days at 36-38°F.

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score
	Ave.	Value/Croma		MMb	Mb	MbCO	
0	4.11 R	3.72/4.23	- .24	15.3	58.5	26.2	--
	3.31 R	4.19/3.17	- .48	21.0	79.0	0.0	--
	4.08 R	4.03/4.49	- 2.84	8.0	54.0	38.0	--
		Mean	- 1.18	14.8	63.8	21.4	--
1	4.11 R	3.88/7.06	- 9.84	24.6	0.0	75.4	79.6
	3.78 R	3.73/4.45	- .38	33.7	41.3	25.0	79.6
	4.70 R	3.95/6.59	-10.38	19.6	0.0	80.4	79.6
		Mean	- 6.87	26.0	13.4	60.3	79.6
2	4.34 R	3.36/7.68	- 9.32	4.5	0.0	95.5	80.3
	5.03 R	2.99/5.34	- 1.21	37.0	0.0	63.0	69.6
	4.36 R	3.57/7.66	-10.58	2.8	0.0	97.2	80.3
		Mean	- 7.04	14.8	0.0	85.2	76.7
3	4.19 R	3.85/5.92	- 6.37	24.5	11.0	64.5	80.3
	4.33 R	3.70/3.50	+ 1.81	49.8	37.2	13.0	42.8
	3.27 R	4.06/6.54	- 7.14	26.5	2.5	71.0	83.9
		Mean	- 3.90	33.6	16.9	49.5	69.0
4	4.02 R	3.97/6.65	- 8.89	16.5	1.5	82.0	83.9
	3.67 R	3.63/3.87	+ 2.08	35.8	40.7	23.5	39.3
	3.68 R	4.11/5.36	- 5.11	27.1	21.7	51.2	80.3
		Mean	- 3.97	26.5	21.3	52.2	67.8
5	3.71 R	3.53/7.16	- 6.91	12.6	0.0	87.4	--
	1.98 R	3.71/3.80	+ 4.37	38.5	55.0	6.5	--
	3.27 R	4.00/5.92	- 5.09	26.5	14.2	59.3	--
		Mean	- 2.54	25.8	23.1	51.1	--
6	3.81 R	3.75/6.30	- 5.99	17.0	14.0	69.0	71.4
	2.70 R	4.17/4.30	- .73	22.4	52.6	25.0	57.1
	3.33 R	4.00/5.67	- 4.55	23.0	27.0	50.0	69.4
		Mean	- 3.76	20.8	31.2	48.0	66.0

TABLE 8, continued

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score
	Ave.	Value/Chroma		MMb	Mb	MbCO	
9	3.58 R	3.71/6.99	- 7.13	8.4	14.3	77.3	82.1
	2.78 R	3.45/4.47	+ 3.03	3.8	72.2	24.0	62.5
	3.60 R	4.07/5.65	- 5.52	8.3	39.9	51.8	78.6
		Mean	- 3.21	6.9	42.1	51.0	74.4
12	3.32 R	4.12/6.52	- 7.58	11.2	25.8	63.0	81.6
	1.89 R	3.59/4.44	+ 3.84	0.0	82.3	17.7	51.0
	3.14 R	3.83/5.64	- 3.02	0.0	66.8	33.2	55.1
		Mean	- 2.25	3.7	58.3	38.0	62.6
15	2.90 R	3.81/6.37	- 4.23	6.3	27.5	66.2	76.8
	2.89 R	3.51/4.27	+ 2.98	0.0	85.5	14.5	48.2
	3.14 R	3.82/5.57	- 2.77	0.0	61.6	38.4	57.1
		Mean	- 1.34	2.1	58.2	39.7	60.7

Figure 11. MEAN OF INDEX OF CHANGE AND OF PERCENT PANEL SCORE (PART I) AND MEAN PERCENTAGES OF MMb, Mb AND MbCO (PART II) OF TRIPPLICATE SAMPLES PACKAGED IN 1.5 MILS POLYETHYLENE IN A MIXTURE OF 2% CO AND AIR



and 1.5 polyethylene at the 5% significant level. The curves in the graphs representing the index of change for the samples packaged in the three different films were considered, statistically, as horizontal lines to the storage axis when tested at the 5% significance level. These results indicate that the index of change for the samples packaged with each of the three materials and the subsequent color of the beef treated by 2% carbon monoxide were stable for the 15 days of storage.

Color Panel

The mean color panel score for all samples, regardless of packaging material, ranged from like slightly to like moderately during the 15 day storage period. The panel score was highest during the first 2 to 4 days of storage, declining somewhat thereafter. Color score for the samples packaged in saran-mylar-polyethylene film did not decline until the fourth day of storage, whereas samples stored in 2.5 polyethylene and 1.5 polyethylene showed a color loss after 3 and 2 days of storage, respectively.

Pigments

As shown in Figures 9, 10 and 11, curves for the index of change followed those shown for the per cent of carboxymyoglobin concentration. This pigment increased

in all samples during the first 2 days of storage, after which there was a sharp decrease in the concentration of the samples packaged in both saran-mylar-polyethylene and 1.5 polyethylene, while such a decrease was not noted in the samples sealed in 2.5 mils polyethylene until after the fifth day of storage.

The percentage of carboxymyoglobin of the samples packaged in saran-mylar-polyethylene film increased about 20 points between the third and the fourth day of storage and maintained this level fairly well for the remainder of the storage period. After the fifth day of storage, the per cent of MbCO of the samples sealed in 2.5 polyethylene increased from 35 to 60% by the ninth day, remaining somewhat stable for the rest of the storage period. The decrease of carboxymyoglobin in the 1.5 polyethylene stored samples stopped after the third day of storage and the percentage of the pigment remained fairly constant up to the ninth day at which time it gradually declined until the end of the storage period. The relative percentages of metmyoglobin and reduced myoglobin appeared to have little or no effect on either the index of change or the color panel score when carboxymyoglobin was present.

Nitrogen Treatment

Nitrogen gas, an inert gas, was not used in the permeable-to-gas films. The barrier saran-mylar-polyethylene was used to pack the meat samples stored under an atmosphere of nitrogen. The color measurements and per cent myoglobin fractions on the surface of this treated beef are presented in Table 9 and plotted in Figure 12.

Index of Change

The data of the index of change were tested for linearity at the 5% significant level. The statistical results, presented in Table 5, show that no significant changes occurred in the color of the beef during the storage period. Although the color of the meat was stabilized by the nitrogen treatment, the color was very faded.

Color Panel

Results of the color panel scores indicate that the color of the beef sealed in the saran-mylar-polyethylene pouches under a nitrogen atmosphere was rated at the dislike moderately level throughout the storage period.

TABLE 9

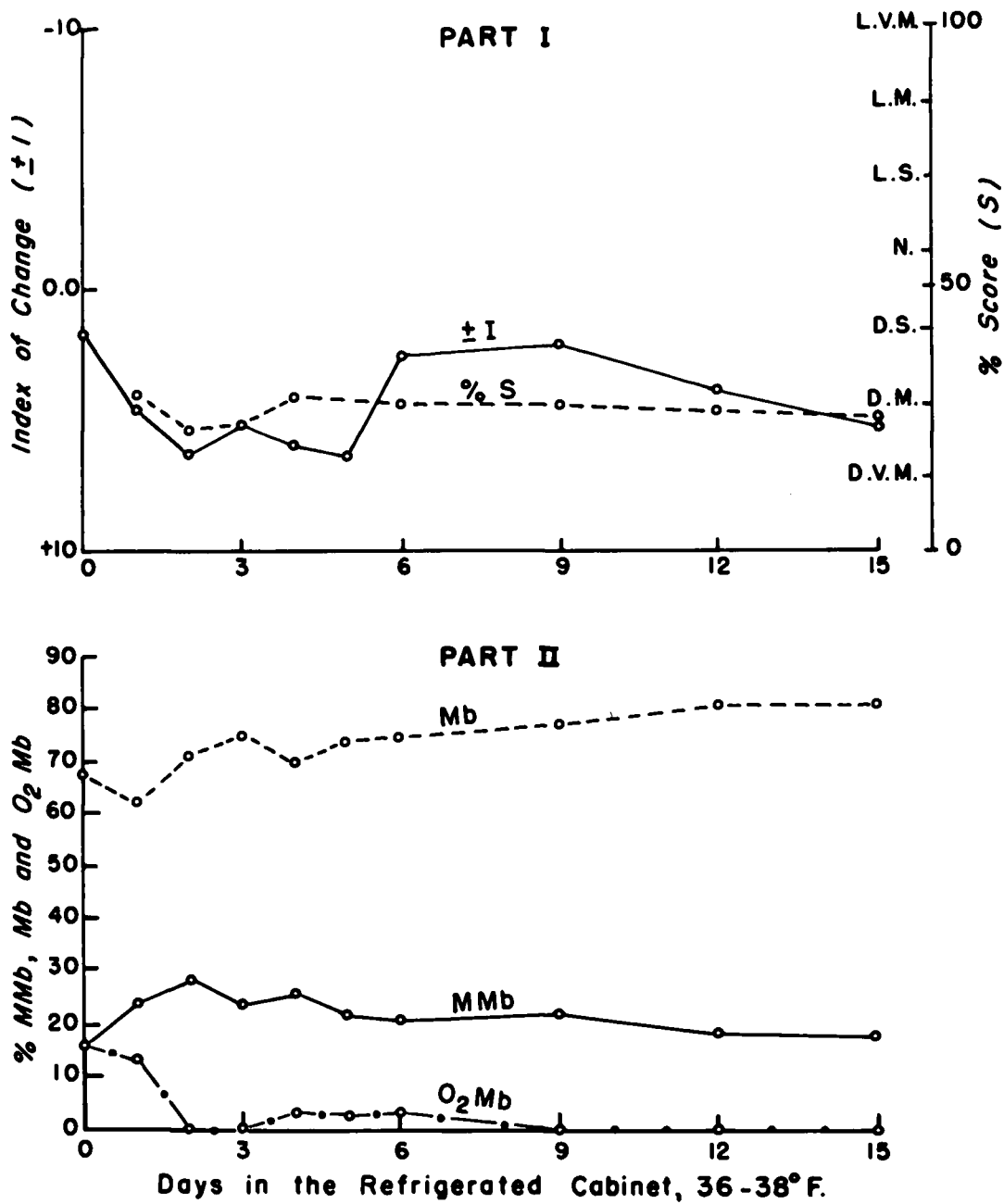
Munsell Notations, Index of Change, Relative Percentage of Mb, O₂Mb and MMb and Average Per Cent Panel Score of Triplicate Samples of Beef Packaged in Saran-Mylar-Polyethylene Under an Atmosphere of Nitrogen and Stored for 15 days at 36-38°F.

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score
	Ave.	Value/Chroma		MMb	Mb	O ₂ Mb	
0	3.41 R	4.10/3.65	+ .28	15.5	61.0	23.5	--
	2.72 R	3.78/3.30	+ 4.08	19.5	73.0	7.5	--
	3.38 R	4.06/3.52	+ .92	13.0	69.0	18.5	--
		Mean	+ 1.76	16.0	67.7	16.3	--
1	1.69 R	3.88/2.98	+ 5.51	21.3	78.7	0.0	24.5
	4.57 R	3.23/3.04	+ 5.78	19.4	41.0	39.6	38.8
	3.61 R	3.88/3.25	+ 2.45	31.8	68.2	0.0	26.5
		Mean	+ 4.58	24.2	62.6	13.2	29.9
2	2.15 R	3.46/3.48	+ 6.34	23.6	76.4	0.0	25.0
	6.05 R	2.45/3.17	+ 8.20	37.0	63.0	0.0	21.4
	3.44 R	4.09/2.17	+ 4.42	24.8	75.2	0.0	23.2
		Mean	+ 6.23	28.5	71.5	0.0	23.2
3	1.37 R	3.97/3.19	+ 4.94	21.6	78.3	0.0	23.2
	3.42 R	2.87/3.63	+ 7.70	26.2	73.8	0.0	16.1
	2.46 R	4.06/2.17	+ 2.72	25.0	75.0	0.0	35.7
		Mean	+ 5.12	24.3	75.7	0.0	25.0
4	1.80 R	3.96/3.14	+ 4.55	23.7	66.0	10.3	26.8
	1.29 R	3.55/3.00	+ 7.93	31.3	68.7	0.0	23.2
	0.37 R	3.89/3.88	+ 4.96	23.8	76.2	0.0	39.3
		Mean	+ 5.81	26.3	70.3	3.4	29.8
5	2.65 R	3.76/3.69	+ 3.33	22.5	66.6	10.9	--
	1.83 R	3.56/2.76	+ 7.73	28.5	71.5	0.0	--
	1.62 R	3.48/3.11	+ 7.72	15.3	84.7	0.0	--
		Mean	+ 6.26	22.1	74.3	3.6	--
6	2.90 R	3.91/3.44	+ 2.71	22.0	67.6	10.4	30.6
	2.50 R	3.91/2.93	+ 4.48	23.5	76.5	0.0	26.5
	3.76 R	3.99/3.68	+ .34	18.2	81.8	0.0	28.6
		Mean	+ 2.51	21.2	75.3	3.5	28.6

TABLE 9, continued

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score
	Ave.	Value/Chroma		MMb	Mb	O ₂ Mb	
9	4.64 R	3.91/3.60	- .21	26.5	73.5	0.0	28.6
	1.10 R	3.60/3.62	+ 6.71	19.0	81.0	0.0	28.6
	3.40 R	4.10/3.79	- .10	22.0	78.0	0.0	28.6
		Mean	+ 2.13	22.5	77.5	0.0	28.6
12	1.17 R	3.80/3.16	+ 6.27	21.4	78.6	0.0	26.5
	2.15 R	3.96/3.30	+ 3.75	21.6	78.4	0.0	26.5
	3.71 R	3.78/3.78	+ 1.38	13.3	86.7	0.0	28.6
		Mean	+ 3.80	18.8	81.2	0.0	27.2
15	1.21 R	4.05/3.17	+ 4.70	19.8	80.2	0.0	25.0
	2.47 R	4.06/2.75	+ 4.05	18.3	81.7	0.0	25.0
	0.45 R	4.06/2.71	+ 6.34	17.4	82.6	0.0	26.8
		Mean	+ 5.03	18.5	81.5	0.0	25.6

Figure 12. MEAN OF INDEX OF CHANGE AND OF PERCENT PANEL SCORE (PART I) AND MEAN PERCENTAGES OF MMb, Mb AND O₂ Mb (PART II) OF TRIPPLICATE SAMPLES PACKAGED IN SARAN - MYLAR - POLYETHYLENE IN NITROGEN GAS



Pigments

Pigment concentrations of the nitrogen treated samples showed very little fluctuation after the first day of storage. During the entire storage period, the oxymyoglobin content was between 0 and 3 per cent and the amount of metmyoglobin ranged between 20 and 30 per cent while the myoglobin concentration stayed around 75%.

High Vacuum Treatments

Results of two experiments, one in which beef was packaged and stored under 28 inches of vacuum and the other in which the samples were first flushed with an atmosphere of 100 per cent carbon monoxide then sealed under vacuum of 28 inches, are tabulated in Tables 10 and 11. Also, the plottings of these data are illustrated in Figures 13 and 14.

Index of Change

As shown in Table 5, the relationship between the color and the storage of the high vacuum treated meat can be considered, statistically, as a straight line function with a slope equal to zero. The index of change was positive, indicating that the color was faded.

The relationship between color and storage of samples undergoing the 100% carbon monoxide flushing followed by

TABLE 10

Munsell Notations, Index of Change, Relative Percentage of Mb, O₂Mb and MMb and Average Per Cent Panel Score of Triplicate Samples of Beef Packaged in Saran-Mylar-Polyethylene Under 28 Inches of Vacuum and Stored for 15 Days at 36-38°F.

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score	
	Ave.	Value/Croma		MMb	Mb	O ₂ Mb		
0	3.06 R	4.00/3.41	+	2.02	12.0	66.0	22.0	--
	3.27 R	3.54/4.41	+	1.77	10.0	41.5	48.5	--
	5.00 R	4.14/4.16	-	4.02	4.5	35.3	60.2	--
		Mean	-	.08	8.8	47.6	43.6	--
1	2.07 R	3.47/3.38	+	6.62	16.4	83.6	0.0	24.5
	1.28 R	2.74/3.21	+	12.39	17.3	82.7	0.0	28.6
	2.33 R	3.55/4.10	+	4.10	19.4	80.6	0.0	34.7
		Mean	+	7.70	17.7	82.3	0.0	29.3
2	2.14 R	3.51/3.49	+	6.03	13.2	86.8	0.0	25.0
	3.99 R	2.49/2.98	+	10.89	33.2	66.8	0.0	19.6
	3.62 R	2.73/4.81	+	4.87	17.4	82.6	0.0	26.8
		Mean	+	7.26	21.2	78.7	0.0	23.8
3	2.51 R	3.98/3.48	+	2.72	20.7	79.3	0.0	23.2
	--	---	+	7.47*	--	--	-- ^x	17.8
	2.01 R	3.94/3.44	+	3.76	21.6	78.4	0.0	33.9
		Mean						25.0
4	1.90 R	3.58/3.27	+	6.43	23.1	76.9	0.0	26.8
	1.43 R	3.42/2.58	+	9.37	30.2	69.8	0.0	23.2
	3.26 R	3.19/3.87	+	5.36	25.4	74.6	0.0	33.9
		Mean	+	7.05	26.2	73.8	0.0	28.0
5	1.49 R	3.77/3.78	+	4.80	20.0	80.0	0.0	--
	1.21 R	3.52/2.43	+	9.28	26.5	73.6	0.0	---
	4.38 R	3.75/2.46	+	4.73	23.0	77.0	0.0	--
		Mean	+	6.27	23.1	76.9	0.0	--
6	2.18 R	3.77/2.98	+	5.58	20.8	79.2	0.0	30.6
	2.06 R	3.13/3.16	+	9.16	22.4	77.6	0.0	28.6
	2.75 R	3.66/2.96	+	5.61	21.5	78.5	0.0	34.7
		Mean	+	6.78	21.6	78.4	0.0	31.3

TABLE 10, continued

Munsell Notations			Index of Change	% Myoglobin Derivatives			% Panel Score	
Days in Storage	Ave.	Value/Croma		MMb	Mb	O ₂ Mb		
9	2.20 R	3.14/3.66	+	7.78	18.2	81.8	0.0	28.6
	1.53 R	3.24/2.97	+	9.56	24.7	75.3	0.0	23.2
	2.88 R	3.43/3.09	+	6.51	29.6	70.4	0.0	33.9
		Mean	+	7.95	24.2	75.8	0.0	28.6
12	0.83 R	3.70/3.20	+	7.23	18.3	81.7	0.0	26.5
	0.96 R	3.68/2.86	+	7.91	22.0	78.0	0.0	24.5
	1.37 R	3.75/2.92	+	6.79	21.9	78.1	0.0	26.5
		Mean	+	7.31	20.7	79.3	0.0	25.8
15	2.83 R	4.04/3.29	+	2.40	21.6	78.4	0.0	25.0
	9.77 RP	3.47/2.52	+	11.86	20.6	79.4	0.0	23.2
	1.54 R	3.67/3.35	+	6.20	16.9	83.1	0.0	32.1
		Mean	+	6.82	20.0	80.0	0.0	26.8

* not measured

x a dummy

Figure 13. MEAN OF INDEX OF CHANGE AND OF PERCENT PANEL SCORE (PART I) AND MEAN PERCENTAGES OF MMb, Mb AND O₂ Mb (PART II) OF TRIPPLICATE SAMPLES PACKAGED IN SARAN - MYLAR - POLYETHYLENE UNDER 28 INCHES OF VACUUM

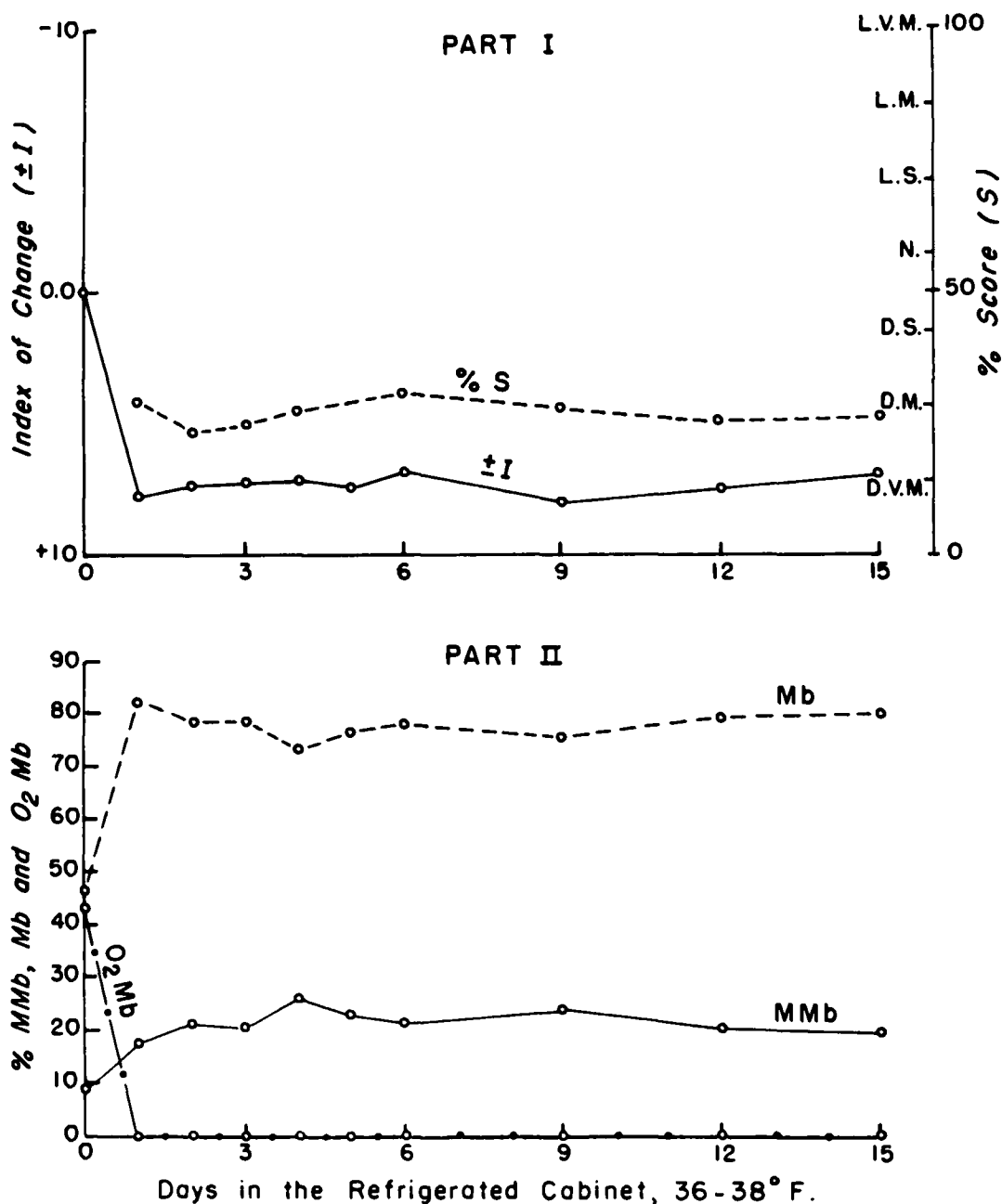


TABLE 11

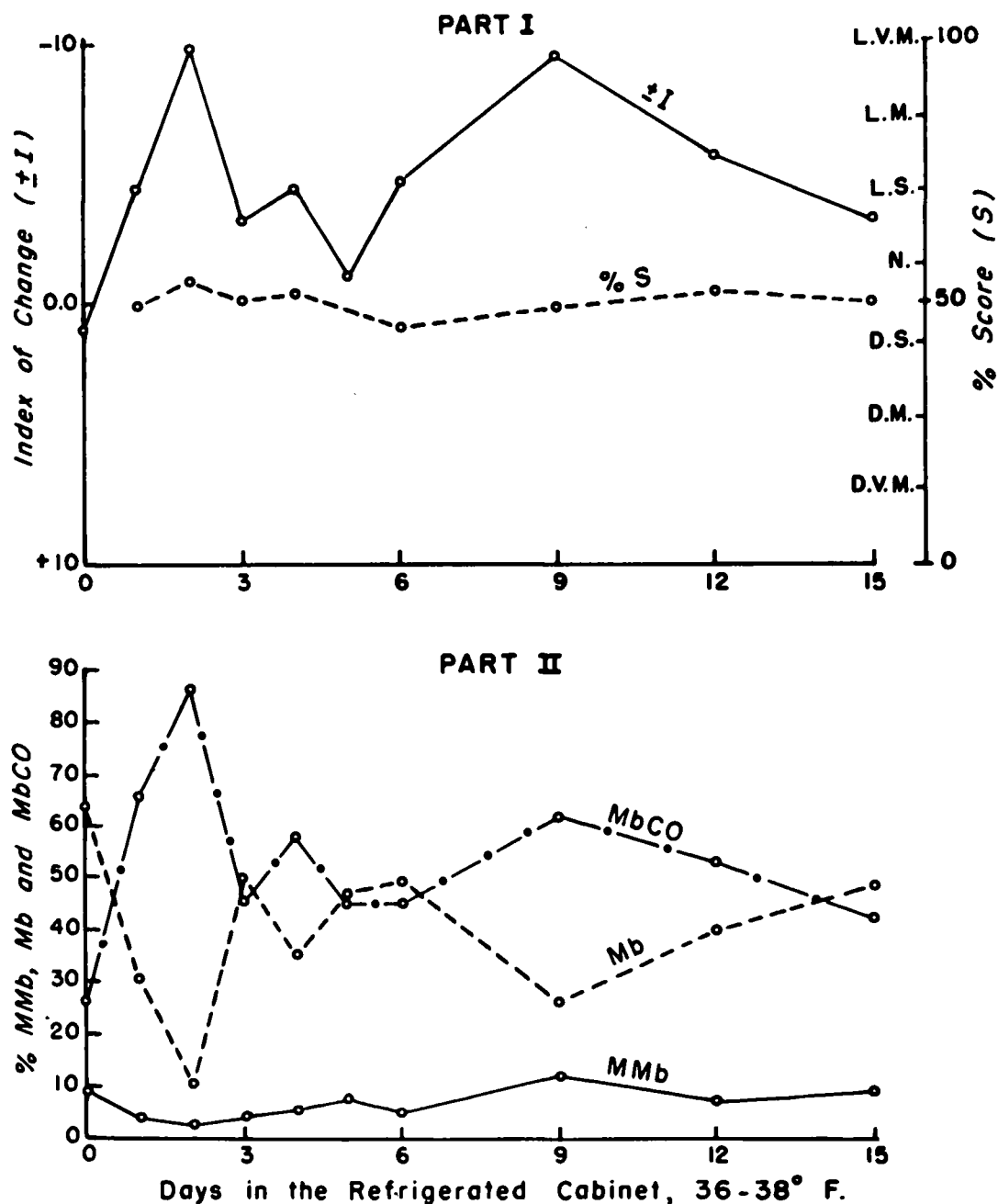
Munsell Notations, Index of Change, Relative Percentage of Mb, MbCO and MMb and Average Per Cent Panel Score of Triplicate Samples of Beef Packaged in Saran-Mylar-Polyethylene Flushed by 100% Carbon Monoxide, Sealed Under 28 Inches of Vacuum and Stored for 15 Days at 36-38°F.

Days in Storage	Munsell Notations		Index of Change	% Myoglobin Derivatives			% Panel Score	
	Ave.	Value/Chroma		MMb	Mb	MbCO		
0	3.10 R	3.78/4.14	+	1.35	6.7	72.3	21.0	--
	3.65 R	2.38/3.94	+	3.41	8.5	73.5	18.0	--
	3.08 R	3.97/4.96	-	1.92	13.5	46.0	40.5	--
		Mean	+	.95	9.6	63.9	26.5	--
1	3.15 R	4.23/6.37	-	7.39	11.7	16.8	71.5	34.7
	3.27 R	3.28/4.57	+	2.84	0.0	66.2	33.8	61.2
	3.76 R	3.71/7.47	-	8.97	0.0	8.0	92.0	53.1
		Mean	-	4.51	3.9	30.3	65.8	49.7
2	3.81 R	4.02/7.96	-	12.48	9.7	0.0	90.3	44.6
	4.08 R	3.40/6.83	-	6.21	0.0	23.3	76.7	51.8
	4.21 R	3.43/8.09	-	10.62	0.0	7.4	92.6	66.1
		Mean	-	9.77	3.2	10.2	86.6	54.2
3	3.61 R	4.16/7.08	-	10.17	10.4	12.3	77.3	37.5
	3.16 R	3.64/4.09	+	2.22	2.0	85.5	12.5	48.2
	2.57 R	3.67/6.02	-	1.70	0.0	52.9	47.1	67.8
		Mean	-	3.22	4.2	50.2	45.6	51.2
4	3.77 R	3.90/7.64	-	10.70	13.0	0.0	87.0	32.1
	3.64 R	3.81/4.64	-	2.24	4.9	61.1	34.0	57.1
	2.68 R	3.57/5.73	-	.64	0.0	45.6	54.4	67.8
		Mean	-	4.53	5.9	35.6	58.5	52.3
5	3.67 R	3.96/6.81	-	8.36	18.0	15.0	67.0	--
	2.21 R	3.58/4.51	+	3.17	5.0	77.0	18.0	--
	2.74 R	3.18/5.64	+	1.90	0.0	49.0	51.0	--
		Mean	-	1.10	7.7	47.0	45.3	--
6	4.02 R	3.82/5.84	-	5.55	5.2	40.8	54.0	36.7
	2.29 R	3.75/4.12	+	2.92	4.5	86.5	9.0	36.7
	4.23 R	4.57/6.21	-	11.69	6.6	21.1	72.3	63.3
		Mean	-	4.77	5.4	49.5	45.1	45.6

TABLE 11, continued

Days in Storage	Munsell Notations Ave.	Value/Chroma	Index of Change	% Myoglobin Derivatives			% Panel Score
				MMb	Mb	MbCO	
9	4.04 R	3.92/6.46	- 8.37	19.5	7.3	73.2	32.1
	3.92 R	3.81/6.25	- 6.48	16.4	23.6	60.0	51.8
	0.42 YR	4.55/3.81	- 13.55	0.0	47.5	52.5	64.3
		Mean	- 9.47	12.0	26.1	61.9	49.4
12	3.33 R	4.15/5.42	- 4.76	11.5	46.0	42.5	36.7
	2.72 R	3.94/8.97	- 11.06	9.5	0.0	90.5	59.2
	2.08 R	4.17/4.95	- 1.12	0.0	73.6	26.4	61.2
		Mean	- 5.65	7.0	39.9	53.1	52.4
15	3.49 R	3.79/6.11	- 4.89	13.0	25.8	61.2	33.9
	3.33 R	3.88/5.82	- 4.24	15.0	40.0	45.0	53.6
	2.51 R	3.94/4.91	- 0.49	0.0	79.7	20.3	64.3
		Mean	- 3.21	9.3	48.5	42.2	50.6

Figure 14. MEAN OF INDEX OF CHANGE AND OF PERCENT PANEL SCORE (PART I) AND MEAN PERCENTAGES OF MMb, Mb AND MbCO (PART II) OF TRIPLICATE SAMPLES PACKAGED IN SARAN - MYLAR - POLYETHYLENE IN 100 % CO UNDER 28 INCHES OF VACUUM



vacuum sealing did not show a linear relationship, nor was linear regression significant. The index of change for this treatment was always represented by a negative sign, although a large variation existed from one day to another.

Color Panel

Panel scores for the samples subjected to the high vacuum treatments were stable throughout the storage time. These scores, on the average, ranged between dislike slightly to dislike moderately for the samples stored under high vacuum, while those treated by carbon monoxide flushing and then sealed under high vacuum fluctuated between dislike slightly to neither like nor dislike. The panel members objected to the samples of the later treatment because of the dots of dark red surrounded by areas of bright red color which gave the meat an unnatural appearance. This might be the reason for the deviation in the index of change and also the low panel scores.

Pigments

Beef held under high vacuum showed only a slight increase in myoglobin content during the storage period, while no change was noted in the metmyoglobin concentration. Oxymyoglobin decreased during the first 2 days, after which it disappeared completely.

Percentage of carboxymyoglobin fluctuated considerably during the storage period in the samples undergoing the carbon monoxide and high vacuum treatment. Again, the index of fading followed the variations of the carboxymyoglobin concentrations. Metmyoglobin contents were stable, but the myoglobin levels varied inversely with the color improvement in these samples. These variations could well be the result of the lack of uniformity of the surface color on each sample.

Effects of Packaging Methods

Packaging of the fresh beef in air was shown to improve the color for about 3 days, after which the color faded to undesirable shades. The blooming of the fresh beef was caused by an increase in the amount of oxymyoglobin during the early period of storage. This is well known as indicated in the literature by numerous investigators, Allen (1, p. 9), Bratzler (14, p. 62-65), Mackintosh and Hall (51, p. 281-286), Meyer (54, p. 191-193), Neil and Hastings (57, p. 479-492) and Rikert et al. (71, p. 17-23).

The increase in the amount of reduced myoglobin and the decrease of metmyoglobin concentration after blooming in a gas impermeable film were observed by Dean and Ball

(29, p. 273-286), Kraft and Ayres (44, p. 8-12), and Pirko and Ayres (64, p. 461-467). As a result of this increase in myoglobin and a decrease in metmyoglobin, they stated that their samples returned to a color which they considered as not undesirable. In the present study, the pattern of changes in the pigments was similar to those mentioned above, but the color was not considered desirable either by the panel scores or by the index of change. This contradiction in results might be caused by their use of the Hunter a_L value to describe color or the inherent differences existing between humans in their ability to agree in evaluating, subjectively, the color of packaged fresh beef.

The storage study results of the 2% carbon monoxide treated samples, regardless of packaging material used, showed that the color was significantly higher than those stored in air, nitrogen or under high vacuum. Moreover, the color for 2% carbon monoxide treatments was found to be stable, statistically, throughout the 15 day storage period at 36°-38°F. These results agree with the recommendations advocated by Pearson (62, p. 37-38). The amount of carboxymyoglobin pigment was likely to be stable or slightly decreasing, when myoglobin was increasing slowly and metmyoglobin was decreasing as the storage period proceeded. The change in the amount of each pigment

in these directions was dependent on the nature of the package material.

Packaging of beef in 100% nitrogen gas in an impermeable film caused quick fading, followed by stabilization of the color at an undesirable shade. These results were also shown by Rikert et al. (71, p. 17-23). In the present study, the myoglobin fractions were stabilized after the first day at the following levels: oxymyoglobin at 0%; metmyoglobin at 20 to 25 %; and myoglobin around 80%.

Packaging of beef under 28 inches of vacuum in an impermeable to air film in order to preserve the color of meat for 12 to 14 days has been recommended by several investigators, Broumand et al. (21, p. 65-77), Dean and Ball (30, p. 468-471), (31, p. 222-227), Rikert et al. (70, p. 625-632; and 71, p. 17-23); however, the success of such treatment depends upon the return in redness caused by an increase in the amount of reduced myoglobin. Although the results of the present study indicate an increase in percentage of myoglobin and some return of redness, the color of the samples was undesirable as indicated by both the panel score and the positive value of the index of change. Furthermore, results concerning the myoglobin formation and return of some color are in agreement with the results of the investigations listed

above. In terms of color desirability, however, the present findings do not agree with those of the above workers but do agree with those of Bratzler (14, p. 62-72) and Urbain (77, p. 140-144). Although there was some return of color in the beef packaged in saran-mylar-polyethylene under 28 inches of vacuum, the increase was not statistically significant as tested by linear regression.

In preliminary experiments where glass jars were used as the storage means, a combination of flushing the storage atmosphere with 100 per cent carbon monoxide prior to drawing vacuum of 28 inches appeared to offer beneficial treatment for maintaining the color of stored fresh beef. When saran-mylar-polyethylene was used to preserve meat, the color of the meat samples was not homogenous. Areas of dark red color were surrounded by a bright red color. Although attempts were made to obtain spectrophotometric data on the same part of the sample, this was not always possible due to the fluctuations in the color and pigment patterns. Hence, these results were probably influenced by the variability of these two factors. Members of the panel were divided in their opinion, either they liked or disliked the meat according to the following factors:

1. The heterogeneity in the color pattern.

2. The abnormally bright red color of these samples gave the meat a somewhat artificial color.

Although there was considerable variation in the color, analysis of variance of the data (Table 5) indicated that samples treated by a combination of carbon monoxide and high vacuum had a significant effect in improving the color when compared to those stored under high vacuum.

Effect of Packaging Materials

There was no significant effect between different packaging materials in either the air or the 2% carbon monoxide treatments except that in the later treatment, the 2.5 polyethylene was more effective in improving the color than 1.5 polyethylene. The packaging materials used had no or low water vapor transmission rates. Apparently, differences in gas permeability were not enough to show significant differences in the air treatments. In the 2% carbon monoxide treatments, the difference between the permeability of the two polyethylene films may have been great enough to allow more carbon monoxide to diffuse out of the 1.5 mil polyethylene packages than out of those made of the 2.5 mils polyethylene. Since carboxymyoglobin can be dissociated by light to form myoglobin and free carbon monoxide (32, p. 177; 46, p. 335), it appears quite probable that the carboxymyoglobin pigment was maintained in a dynamic status, rather than in a steady state, under

the conditions of this experiment.

Observations On the Samples After 30 Days

After 30 days storage at 36-38°F., samples sealed under an atmosphere of air exhibited a dark brownish red color. The meat stored in saran-mylar-polyethylene for 30 days gave off an odor characteristic of putrefaction. Also, considerable swelling of the package was observed. A faint malty odor was noted in the meat stored in 2.5 and 1.5 polyethylene.

The 2% carbon monoxide treated samples showed fairly good color but had a malty odor. The samples held in saran-mylar-polyethylene also had a putrifactive odor.

Samples stored under 28 inches of vacuum or nitrogen had a dark red color, malty odor and were slimy. A greenish fluid was observed in the packages.

The beef treated by 100% carbon monoxide and stored under 28 inches vacuum were of good appearance, but having a malty odor and red fluid was observed.

Four of the carbon monoxide treated samples were broiled for 15 minutes and the color of the samples, both external and internal, was observed. Two of the samples showed the ordinary cooked meat color. The internal color of the other two samples was a faint pinkish color which faded very quickly upon exposure to air while the meat was hot.

SUMMARY AND CONCLUSIONS

1. Index of change, a modification of the index of fading, was used to measure the degree and direction of meat color change.
2. A method for determining the myoglobin fractions on the surface of meat treated by carbon monoxide was presented. This method was based upon the principles of the reflectivity ratio method.
3. The index of change followed the amount of oxymyoglobin or carboxymyoglobin, depending on which was present.
4. Saran-mylar-polyethylene, 2.5 mils polyethylene or 1.5 mils polyethylene had no significant effect on the color or the pigments on the surface of packaged refrigerated beef when air was used as the storage atmosphere.
5. Using a mixture of 2% carbon monoxide and 98% air to flush the packaged beef before sealing the package was very effective in preserving and stabilizing the color for 15 days at 36-38°F.
6. Saran-mylar-polyethylene and 2.5 mils polyethylene were found to be the better packaging films tested to maintain the color when 2% carbon monoxide gas was used.
7. Storing under a nitrogen atmosphere or under 28 inches vacuum were not effective methods in preserving

desirable color of fresh beef during a 15 day storage period.

8. Using a combination of 100% carbon monoxide and high vacuum to store the fresh meat was effective to improve the color but not in a uniform manner.
9. When beef was treated by carbon monoxide and cooked after 30 days storage at 36-38°F., it had an ordinary cooked meat color on the surface but, sometimes, a slightly pink color inside which would fade quickly to a brown color when exposed to air.

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