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Shelf-life of sausage patties made from pre-rigor and post-rigor pork under vacuum and modified atmosphere packaging

Sangam A. Kurade

To the Graduate Council:

I am submitting herewith a dissertation written by Sangam A. Kurade entitled "Shelf-life of sausage patties made from pre-rigor and post-rigor pork under vacuum and modified atmosphere packaging." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science and Technology.

H.O. Jaynes, Major Professor

We have read this dissertation and recommend its acceptance:

M. James Riemann, Sharon L. Melton, Dwight H. Loveday, John P. Hitchcock

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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SHELF-LIFE OF SAUSAGE PATTIES MADE FROM PRE-RIGOR AND POST-RIGOR PORK UNDER VACUUM AND MODIFIED ATMOSPHERE PACKAGING

A DISSERTATION PRESENTED FOR THE

DOCTOR OF PHILOSOPHY DEGREE

THE UNIVERSITY OF TENNESSEE, KNOXVILLE

SANGAM A. KURADE

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DEDICATION

This dissertation is dedicated to my parents, Anand G. Naik-Kurade and Suman A. Naik-Kurade. They have inspired, guided and supported me throughout my life and without which this degree would not have been possible.

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ABSTRACT

This study was conducted to determine the effect of pre-rigor (PRR) and post-rigor (POR) pork on the shelf-life of sausage patties stored under six treatments - vacuum (Trt 1), 100% CO2 (Trt 2), Air (Trt 3), 75% N2 + 25% CO2 (Trt 4), 75% N2 + 15% CO2 + 10% O2 (Trt 5) and 75% N2 + 20% CO2 + 5% O2 (Trt 6).

Six sows were slaughtered and the meat (PRR and POR) was ground (0.48 cm), mixed with seasonings and stuffed into casings. Sausage was crust-frozen in a CO2 batch freezer (-70C) and transferred to a blast freezer (-21C) for 10 days. After frozen storage, the sausages were sliced into patties, packaged under six treatments (Trt 1 through Trt 6) and stored at 4C for 14 days. Analyses were conducted at 0, 3, 7, 10 and 14 day intervals.

PRR had higher ($P \leq 0.001$) TBA value, total bacterial count and lactic acid bacterial count than POR. PRR had higher ($P \leq 0.001$) percent OMb, Hunter 'L' and 'a' values as well as lower ($P \leq 0.001$) percent MMb than POR.

Trt 2 and Trt 4 gave lower ($P \leq 0.001$) TBA values and total bacterial counts, whereas Trt 3, Trt 5 and Trt 6 gave higher ($P \leq 0.001$) percent OMb and lower ($P \leq 0.001$) percent MMb. Trt 2 and Trt 4 are recommended for shelf-life extension in terms of lipid oxidation and microbial growth. For color shelf-life extension, at 50% MMb level, Trt 3, Trt 5 and Trt 6 are recommended.

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CHAPTER 1

INTRODUCTION

The demand for pork sausage has grown steadily in recent years. Fresh sausage production in 1976 totaled 854,782 thousand pounds in the United States (Anon., 1976) and the figure rose to 947,598 thousand pounds in 1987 (Anon., 1988). The total red meat production is also expected to rise from 60,351 million pounds in 1988 to 62,787 million pounds in 1990 (Anon., 1990).

Fresh pork sausages are made from fresh chilled pork trimmings containing approximately 65% lean content, ground and mixed with additional pork fat, seasoned and stuffed in edible casings (Gerrard, 1976). This product is generally packaged in polystyrene trays in the frozen state.

Color evaluation plays a major role in consumers' perception of fresh meat quality. The consumer often utilizes his/her perception of color as an indicator of flavor, juiciness, tenderness and freshness of the meat (Naumann et al., 1957). Therefore, the color of fresh ground pork sausage, as affected by packaging and storage conditions, is an important aspect of successful pork merchandising. The reactions affecting color primarily involve myoglobin - an iron containing, purple colored, protein pigment. Upon exposure to air, oxygenation converts

the purple pigment to red colored oxymyoglobin. Subsequent oxidation results in the formation of metmyoglobin, a brown pigment, which contributes significantly to the appearance of spoiled meat (Govindrajan, 1973; Giddings, 1974). At least three major broad based factors can influence the spoilage rate of fresh red meats: postmortem internal enzymatic and non-enzymatic biochemical reactions, microbial action and, internal chemical and physical changes (Dawn et al., 1971).

As early as 1930, whole chilled beef carcasses were shipped from New Zealand and Australia to Great Britain in modified atmospheres containing elevated concentrations of carbon dioxide (Lawrie, 1979; Silliker and Wolfe, 1980). Studies have shown that vacuum packaging (VP) and modified atmosphere packaging (MAP) are effective ways of extending shelf-life (Ordal, 1962; Baran et al., 1969). VP as a means of extending the storage life of fresh meats at temperatures of 0C-5C has been in use for about twenty years, primarily for cuts of beef (Shay and Egan, 1986). Taste panel studies showed that vacuum packaged pork of normal pH (5.4-5.8) had half the storage life (6 weeks) of similarily packaged beef (12 weeks) at 0C. Taylor (1973) suggested MAP as an alternative to conventional vacuum packaging. Clark and Lentz (1973) reported that optimum meat color was obtained when low concentrations of carbon dioxide (10%-15%) were used in conjunction with high concentrations of oxygen

whereas studies by Seideman et al. (1979) and Christopher et al. (1979) showed the effectiveness of using a carbon dioxide(CO2)-nitrogen(N2) environment for increased storage life of pork. They recommended the use of 20%(CO2):80%(N2) environment as optimum gas mixtures for extending the shelf-life. Huffman (1974) reported that 5% oxygen concentration was necessary to maintain the oxygenated form of myoglobin.

Although some of the pork industries are turning to pre-rigor processing of meat, it is not widespread (Riemann, 1989). A number of investigators have stated the advantages of using pre-rigor meat over post-rigor meat with respect to economics in terms of space and energy savings (Hamm, 1977), higher yield (Schmidt and Keeman, 1974), higher water holding capacity (Cuthbertson, 1980), better emulsifying capacity (Trautman, 1964) and smaller cooking losses (Ray et al., 1980). However, Huffman (1980) explained that pre-rigor muscle had a darker color which could lower the retail acceptance and Mandigo (1968) stated that pre-rigor meat could have a tougher texture as compared to the post-rigor meat. Also, there are difficulties in processing pre-rigor meat during cutting. The meat is wet and slippery, and the warm cuts do not hold their shape well (Riemann, 1989).

Much research has been conducted on beef color (Kropf, 1980; Kropf and Hunt, 1984); however, little work has been reported on the color retention in pork and pork products. Research on pre-rigor processing of meat has hardly been

explored and previous research on the use of vacuum and gas packaging is not extensive and often contradictory. This research was initiated to: (1) evaluate the effects of vacuum packaging and modified atmosphere packaging treatments on the shelf-life of pork sausage patties, (2) assess ways to retain color of fresh pork sausage patties, along with studies on oxidative rancidity and microbial growth during storage, and (3) compare the pre-rigor and post-rigor processing of pork and it's effect on the shelf-life of the patties.

CHAPTER 2

REVIEW OF LITERATURE

I. Shelf-life of fresh meats

It was stated by Dawn et al. (1971) that " shelf-life of meat and meat products is the time during which a prepackaged meat item shall remain saleable, safe and nutritious."

It has also been stated (Dawn et al., 1971; Clark and Lentz, 1973) that at least three major broad based mechanisms can influence the shelf-life of fresh red meats: postmortem internal enzymatic and non-enzymatic biochemical reactions, microbial action and, internal chemical and physical changes. Also, the factors that aid in deterioration of the meat and diminish the acceptability are: color degradation, lipid oxidation and microbial growth (Cole, 1986).

Holland (1980) suggested that extension of shelf-life in red meats can be achieved by reducing the temperature during transit and storage as well as by modifying the atmosphere of the package or the container in which it is held.

II. Low temperature storage of meats

Love (1966) determined that the total volume occupied by ice after freezing of meat at -78C varies with the post-rigor muscle and pre-rigor muscle occupying 60% and 40%, respectively. Kraft and co-workers (1979) found that freezing of meat usually takes place between -10C and -30C. Changes that take place during freezing include expulsion of oxygen and carbon dioxide (Ciobanu et al., 1976), as well as changes in pH, ionic strength, viscosity, surface and interfacial tension, and oxidation-reduction potential (Creigler and Davison, 1968).

Researchers have studied the effects of low temperature storage on lipid oxidation. Awad et al. (1968, 1969) found that maximum autoxidation occurred at -4C to -10C for beef and pork. Caldironi and Bazan (1982) reported that peroxide values and TBA values did not increase significantly if meat was stored between -18C and -32C but at -15C a rapid rate of autoxidation occurred. Some of the factors that influence autoxidation during low temperature storage are: meat grinding (Keskinel et al., 1964), presence of proxidants such as heme compounds (Watts, 1954) and temperature fluctuations (Buckley and Kearney, 1975). Antioxidants (Rousseau et al., 1957) and the presence of an inert atmosphere surrounding the meat (O'Keeffe and Hood, 1981) can help in restricting the oxidation of lipids.

Rate of freezing and conditions under which meat is stored at low temperatures also have effects on meat color. Voyle (1974) and Buckley and Kearney (1975) stated that the color of meat during freezing varies with the rate of freezing. At slower rates of freezing, the meat surface could become translucent due to the formation of large crystals whereas at faster rates of freezing, smaller crystals are formed leading to a lighter appearance of the meat surface. Color deterioration, according to MacDougall (1974), is due to metmyoglobin formation upon exposure to light and packaging in transparent wrapping films. Voyle (1974) reported that maximum discoloration occurred when the meat was stored under fluorescent light at -10C to -12C. Lanier et al. (1977) found that metmyoglobin formation was accelerated under conditions of increased temperature and air velocity.

Temperature fluctuations during low temperature storage of meats can lead to thawing which can result in microbial growth, evaporation losses, faster deterioration reactions and loss of nutrients through the exudate (Fennema, 1968; Calvelo, 1981). Nacito et al. (1973) reported that the damage due to freezing allows the myoglobin pigment to be released more readily by the meat fibers. Therefore, freezing may decrease the overall color acceptability due to excessive thaw exudate and this may markedly affect the raw meat color acceptability.

III. Modified atmosphere storage of meats

As early as 1930, whole chilled beef carcasses were shipped from New Zealand and Australia to Great Britain in modified atmospheres containing elevated concentrations of carbon dioxide (Silliker and Wolfe, 1980; Lawrie, 1979). It has only been recently that the developments in packaging materials, techniques and research in the area made this technology more practical and economical.

Storage or packaging of meats under modified atmospheres is one technique to improve the shelf-life of meats. It involves the packaging of meat and meat products in a high-barrier package under the influence of an atmosphere other than air. It can involve an atmosphere devoid of any gases (vacuum packaging) or an atmosphere of a single gas or combination of gases (modified atmosphere packaging/gas packaging).

Vacuum Packaging

Genigeorgis (1985) defined vacuum packaging (VP) as "the packaging of a product in a high-barrier package from which air is removed to prevent growth of aerobic spoilage organisms, shrinkage, oxidation, and color deterioration." Hintlian and Hotchkiss (1986) stated that vacuum packaging is a form of modified atmosphere packaging because the removal of air is in itself a modification of the

atmosphere. There also is a production of elevated levels of carbon dioxide by the microorganisms on the meat as they consume residual oxygen (Silliker and Wolfe, 1980).

Effect of vacuum packaging on color:

Dean and Ball (1958) advocated the marketing of red meat in vacuum packages but they were concerned about the darkening of color in the packages. Pierson et al. (1970) reported an initial formation of metmyoglobin followed by subsequent reduction to reduced myoglobin, at the surface of vacuum packaged meat within a few hours after packaging. The authors also noted little difference in myoglobin chemical state patterns for semimembranosus muscle kept under aerobic conditions for 2 or 6 hours prior to vacuum packaging. A 24 hour aerobic period caused more metmyoglobin formation which persisted longer while 48 hours of aerobic holding resulted in greater and persistent metmyoglobin level and a slow and incomplete conversion to reduced myoglobin.

Smith et al. (1974) found that retail cuts from vacuum packaged pork loins were scored higher in consumer acceptability and had less surface discoloration than those from pork loins wrapped in traditional parchment paper or in 90 gauge polyvinylchloride film. Harrison et al. (1979) compared subjective and objective color measurements of steaks from 4 muscles cut after a 48 hour carcass chill or cut after an added 21 days of vacuum storage. Steaks from

longissimus, biceps femoris and semimembranosus muscles stored in vacuum 21 days remained brighter through 3 days of display than steaks cut after a 48 hour chill. Vacuum storage prolonged visual acceptability of biceps femoris and semimembranosus steaks an extra day. However, steaks from vacuum packaged muscles were darker after 5 days of display. Percent metmyoglobin exhibited similar trends but the darker color was associated with higher metmyoglobin.

Kropf (1980) reported that meat upon vacuumization remained satisfactory after 21 days of continous display under 100 foot candles of lighting at 2C. The author also stated that a number of attempts have been made to market retail cuts in a reduced myoglobin (purple-red color) state and only some have been reasonably successful. Therefore, he recommended consumer education and promotion to market red meat in vacuum packages.

Effect of vacuum packaging on microbial growth:

During storage of vacuum packaged meat, residual oxygen is converted to carbon dioxide through the respiration of lean tissues and microbial activity (Holland, 1980). This gaseous environment in the package is responsible for the supression of common aerobic spoilage bacteria and favors the growth of facultative anaerobic microorganisms such as lactic acid producing bacteria. Dainty et al. (1979) and Egan (1983) in their research have stated that the presence

of a flora dominated by lactic acid bacteria indicates that the storage life would be maximal. Holland (1977) reported that potential food-poisoning bacteria such as Staphylococcus aureus and Clostridium perfringens did not pose a problem at low refrigeration temperatures but they are capable of surviving at 0C-2C in VP meats.

It has been reported (Taylor, 1971; Taylor and MacDougall, 1973) that with the use of conventional VP, some time may elapse before the carbon dioxide accumulates to a level for bacterial growth inhibition and this time period may permit the spoilage of meats.

Shay and Egan (1986) studied microbial growth in VP, chilled pork. They found that VP pork of normal pH (5.4-5.8) had a shelf-life of about 6 weeks at 0C. In contrast, high pH meat (above 6.0) often spoiled after only 3-4 weeks due to the development of putrefactive off-odors and green color formation. These were produced as a result of an increase in growth of psychrotrophic gram-negative bacteria which occurred due to high pH.

Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) has been defined by Hotchkiss (1989) as "a one-time alteration of gases surrounding a product in a package. The gas mixture will change over time due to absorption by the product or the microorganisms on the product." Holland (1980) defined the

concept as an alternative to VP where artificial atmospheres may be flushed and sealed into a gas impermeable package containing fresh meat.

The atmosphere inside the package flushed with gases is a dynamic one. It has been reported (Ingram, 1962; Seideman et al., 1979) that respiration by the meat occurs at a fast rate. Microbes consume oxygen and at the same time carbon dioxide is given off. Seideman et al. (1979) reported that some of the carbon dioxide may be dissolved into the meat. Ingram (1962) has suggested that the partial pressures of these gases affect the microbiological picture, but besides atmospheric influences, other influences on the product stability exist such as initial microbial contamination and relative humidity.

Several gases have been used for modifying the gas atmosphere around packaged meat. Oxygen, carbon dioxide and nitrogen are the most common gases and each one has a specific function in the extension of shelf-life of meats. Mixtures of two or more gases are often used.

Role of nitrogen in modified atmosphere packaging:

According to Cole (1986), nitrogen gas is essentially used as an inert filler to displace or dilute the presence of other gases and prevents the collapse of the package from carbon dioxide being absorbed into the meat. Huffman (1974) reported that a pure nitrogen atmosphere exhibited no

inhibition of aerobic bacterial growth. However, Smith et al. (1977) reported that nitrogen increased the off-odor and decreased the attractiveness of the subcutaneous fat. Packaging retail cuts of fresh meat in a pure nitrogen atmosphere aids in shelf-life extension because oxidation changes are prevented. Meat stored for a short time in such atmospheres retains the purple-red color of reduced myoglobin during storage and blooms to bright red color upon exposure to air (O'Keeffe and Hood, 1981).

Role of oxygen in modified atmosphere packaging:

The primary function of oxygen is to enhance the bright red color of red meats (Cole, 1986). It has been reported (O'Keeffe and Hood, 1981) that at least 0.1% oxygen level is necessary to prevent discoloration. But other reports suggest that level to be at least 5% (Ledward, 1970; Taylor, 1971; Clark and Lentz, 1973). Clark and Lentz (1973) also reported that as the concentration increases to 50%, there is an increase in color and odor shelf-life.

High oxygen levels lead to oxidative rancidity (Watts, 1954) which causes MAP of meats to have a lower shelf-life (8-14 days) as compared to VP (28-35 days) (Cole, 1986). Another problem associated with the presence of oxygen in MAP is microbial growth. Adams and Huffman (1972) suggested that a low oxygen concentration (less than 5%) is required to inhibit the growth of aerobic bacteria and leads to the

growth of microorganisms which tolerate low oxygen tension. However, Lawrie (1979) pointed out that the inhibition of aerobes is not due to low oxygen levels but rather because of accumulation or high concentration of carbon dioxide.

There is a decrease in oxygen concentration over the storage time in MAP (Taylor, 1971; Taylor and MacDougall, 1973) due to the absorbtion by the meat surface and respiration by the tissue and bacteria. The consumption of oxygen varies with the type of meat in the package, microbial infestation and the method of applying the atmosphere.

The purple-red pigment myoglobin undergoes autoxidation at a faster rate at low oxygen tensions (Saleh and Watts, 1968). Thus, the authors deduced that partial removal of oxygen from the headspace of the package can accelerate the autoxidation of the pigment. Myoglobin autoxidation has been found to be a first order reaction that increases with temperature (Adams and Huffman, 1972). Metmyoglobin, the brown-red pigment responsible for color degradation, can be enzymatically reduced back to myoglobin under anaerobic conditions (Saleh and Watts, 1968). This reduction occurs through a pathway involving the coenzyme nicotinamide adenosine dinucleotide (NADH) in intact meat. Ledward et al. (1977) found that upon grinding the meat, there is a loss of NADH and the ground meat loses its ability to reduce metmyoglobin back to myoglobin.

Role of carbon dioxide in modified atmosphere packaging:

The inhibitory action of carbon dioxide gas on bacterial growth is documented by many researchers (Baran et al., 1969; Clark and Lentz, 1969; Huffman, 1974; Huffman et al., 1975; Enfors et al., 1979; Silliker and Wolfe, 1980; Enfors and Molin, 1984). Researchers have reported that carbon dioxide increased shelf-life primarily by lowering the pH of the meat (King and Nagel, 1967; 1975) by the formation of carbonic acid (Huffman, 1974). However, Huffman et al. (1975) stated that a small pH drop of 0.1 unit could not account for lowered bacterial counts alone. Another postulation by King and Nagel (1967; 1975), suggests the suppression of bacterial enzymatic decarboxylation rates by carbon dioxide, particularily at high concentrations.

Clark and Lentz (1969) proposed that the maximum effect of the gas is achieved during early stages of bacterial growth. Other reseachers (Enfors and Molin, 1984; Grau et al., 1985) have found that gram negative spoilage flora of refrigerated meat are most susceptible to carbon dioxide, whereas the lactic acid bacteria are less affected. Enfors et al. (1979) postulated that antagonistic properties of lactic acid bacteria may inhibit other carbon dioxide resistant bacteria (which may have more degrading effect on meat) and thereby promote increased shelf-life.

The use of high concentrations of carbon dioxide (over 20%) has been found to have detrimental effect on color

acceptability of meat (Clark and Lentz, 1969; Ledward, 1970; O'Keeffe et al., 1975). Other undesirable effects include lipid oxidation and development of sour flavor (Haard and Lee, 1982). Cole (1986) stated that since carbon dioxide decreases pH and muscle myoglobin is oxidized to metmyoglobin more rapidly at lower pH, it would be likely that the reduced surface pH accelerates the discoloration of carbon dioxide flushed meat.

According to Holland (1980), there is considerable debate as to the optimum carbon dioxide concentration for maximal extension of fresh meat shelf-life. Some researchers (Clark and Lentz, 1969; Naumann et al., 1971; Spahl et al., 1981; Bartkowski et al., 1982) have proposed a concentration level of 15-20% carbon dioxide for inhibitory effect on bacterial growth without color fading. However, to inhibit the formation of metmyoglobin, Ledward (1970) suggested the use of 5% oxygen and 20% carbon dioxide.

Role of carbon monoxide in modified atmosphere packaging:

Wolfe et al.(1976) and Wolfe (1980) stated that carbon monoxide inhibits pigment browning by combining with the reduced myoglobin to form the red pigment carboxymyoglobin, which is similar in color to oxymyoglobin, but is stable against oxidation to brown metmyoglobin. It has also been stated (Wolfe et al., 1976) that the gas tends to inhibit rancidity by reducing the concentration of myoglobin

derivatives which are good lipid oxidation catalysts.

Although color retention with the use of this gas in MAP is proven to be better than any other gas, concern about possible toxicity prevents carbon monoxide from being popular (Wolfe, 1980). Dawn et al. (1971) pointed out that the meat treated with this gas masks the primary spoilage indicators such as odor and color changes. But this has been refuted by Wolfe et al. (1976). They have shown the presence of visually detectable levels of metmyoglobin in carbon monoxide treated meats at the same time as microbial spoilage.

Wolfe et al. (1976) indicated that several pounds of carbon monoxide treated meat would have to be consumed in order to produce any detectable carbon monoxide level in blood. But because of these concerns of possible toxicity and masking of spoilage indicators, this gas has not been approved for use in MAP (Cole, 1986).

Commercial application of modified atmosphere packaging in the pork industry:

Lugg and Woodruff (1973) stated that most commercial storage of pork and other red meats is done under Tectrol atmosphere, the patent of which is held by Transfresh Corporation. It involves the use of 35-75% carbon dioxide, 21-28% oxygen and the rest nitrogen gas. This system has been successfully used for the shipment of pork; providing

longer color stability, less surface dehydration, less shrinkage and greater resistance to microbial spoilage than packaging under other atmospheres.

Tectrol atmosphere has been researched (Lugg and Woodruff, 1973) for the storage of fresh pork loins and hams at 0C-1C. It was reported that after 21 days of storage in air, the pork was yellowish-green with a very foul and sour odor. On the other hand, after 21 days Tectrol storage, the pork remained fresh red, the fatty tissue remained white and there was no detectable off-odor. Also comparing the air and Tectrol storage after 21 days, the latter resulted in 45% less rancidity, 37% less aerobic plate count and 35% less psychrophillic count.

It has been reported (Anon., 1979) that the major problem with packaging pork is the puncture of bags. By placing pork on a tray inside a bag, which in turn is boxed, and by flushing and sealing the bag, this problem could be solved (Cole, 1986). This technique has been used (Anon., 1979) for packaging pork ribs that were stored under carbon dioxide atmospheres at -2C. The shelf-life has been extended from 5-7 days to 21 days. It has also been noted under high carbon dioxide concentrations when compared with beef, pork did not brown as quickly.

Effect of modified atmosphere packaging on meat color pigments:

Huffman (1980) described the color pigments in fresh red meats as myoglobin which is predominant (65-70% and over of total pigment) and hemoglobin (12-30% of total pigment). Since the two pigments have similar spectral properties, myoglobin alone is used as an index for fresh meat color. Bodwell and McClain (1971) reported the myoglobin content of the muscle tissue in pork to be 1-3 mg of myoglobin per gram of wet tissue.

Myoglobin can exist in three forms: reduced myoglobin (Mb), which is purple, red oxymyoglobin (OMb) and metmyoglobin (MMb), which is brown (Govindrajan, 1973; Huffman, 1980; Cole, 1986). When the conditions in the meat package are completely anaerobic, the color of the meat is purple-red as that of reduced myoglobin. Upon exposure to oxygen, oxygenation of the pigment to form oxymyoglobin takes place making the meat surface bright red. Myoglobin and oxymyoglobin may be oxidized to metmyoglobin, giving the meat a brown appearance, in low oxygen pressures or from the oxidation of iron heme in the myoglobin molecule (Seideman et al., 1984).

The oxygenated form, OMb, extends to a depth of a fraction of a centimeter below the surface (Giddings, 1977) of the meat. According to Taylor (1971), the depth of penetration of oxygen into the meat depends on the partial

pressure of the gas at the surface, the rate of utilization by the tissue and a diffusion constant. If stored at high oxygen partial pressures, a thick bright-red surface of OMb is produced which masks the development of MMb in the underlying tissue (Ogrydziak and Brown, 1982). It has also been mentioned (Clydesdale and Francis, 1971; Seideman et al., 1984) that MMb formation is influenced by a number of factors, and MMb is normally reversible to OMb and Mb based on the oxidation/reduction potential and other factors.

O'Keeffe and Hood (1981) stated that a minimum oxygen level of 0.1% is necessary to maintain the bright red color of the meat under MAP, but Adams and Huffman (1972) indicated the level to be above 5%. Cole (1986) suggested that the retail packaging of fresh red meats under MAP should be accomplished within 15 minutes after fabrication and processing to minimize MMb formation. Exposure to oxygen prior to packaging allows the oxygen to be attached to the surface of the muscle. Upon vacuumization, oxygen is removed from the package, but not necessarily from the surface of the meat. This results in low partial pressure of oxygen, causing the meat surface to brown. The brown color normally subsides after 1-8 hours of packaging (Pierson et al., 1970; Cole, 1986). Formation of brown color MMb immediately after vacuum packaging after prolonged exposure to oxygen was also noted by Ernst (1980).

Studies on the effect of MAP on pork by some researchers have produced differing results. Ordonez and Ledward (1977) found higher oxygen levels to result in lower MMb formation. They exposed pork longissimus and biceps femoris to 80% oxygen and 20% carbon-dioxide and found the MMb levels to be below 30% after 15 days of storage. Lopez-Lorenzo et al. (1980) found that increasing oxygen level in the packages of ground pork from 80% to 100% reduced myoglobin oxidation. High levels of oxygen increased the shelf-life from 4 days for controls to 13 days. Seideman et al. (1980) found the appearance of pork chops under 20% carbon dioxide : 80% nitrogen level to be the same as under vacuum packaging except for long storage (14 and 21 days) and long display (5 days). Partmann et al. (1970a,b) found 1% oxygen and 99% nitrogen levels did not give satisfactory appearance on pork when stored at 3C for 7 days. Increasing oxygen to a 3% level and storing at 7C gave the same results. Seideman et al. (1979) investigated various gas mixtures for boneless pork loin roasts stored at 1C-3C under 970 lux of incandescent light. They found that cuts displayed for 3 days under MAP were more extensively discolored than those displayed under VP.

Effect of modified atmosphere packaging on meat lipids:

The process of lipid oxidation is accepted (Labuza, 1971) to be through free radical formation and the initial

substrate for lipid oxidation is free radical lipids. The radical chain mechanism has been generally accepted (Khayat and Schwall, 1983) as the only process involved in autoxidation. Unsaturated lipids, coming in contact with oxygen, produce lipid peroxy radicals and lipid radicals which again react with oxygen to propagate the oxidation process. The hydroperoxides formed can be converted to free radicals also, which in turn can accelerate the rate of lipid oxidation (Maier and Tappel, 1959). According to Watts (1961a), heme pigments act as catalysts for lipid oxidation and are more active when iron is in the ferric state, as in MMb, than when it is in the ferrous state, as in OMb and Mb. Watts (1954) also found that the promotion of fat oxidation by heme pigments is reciprocal as unsaturated fatty acids, after oxidation, aid in accelerating the destruction of heme pigments. Tappel (1961) reported that the catalytic effect of iron porphyrins on the oxidation of lipids brings about the destruction of pigments as well as oxidation of fat. Obanu et al. (1980) stated that the catalytic effect of heme pigments depends on the fatty acid/pigment ratio.

Liu and Watts (1970) have reported that the process of lipid oxidation in an intact muscle is minimized since the interaction of polyunsaturated fatty acids with heme pigments is limited due to stuctural integrity of the intact muscle cell. The authors found an increase in lipid oxidation in ground meat. It has been noted (Devore and

Solberg, 1974; Ordonez and Ledward, 1977) that lipid oxidation, like pigment degradation, is a surface effect and Devore and Solberg (1974) concluded that in samples of large volume to surface area ratios, the amount of lipid oxidation is relatively unimportant when compared to discoloration and microbial growth.

According to Lopez-Lorenzo et al. (1980), meat develops an amount of MMb which results in the meat being unacceptable before a TBA value within a rancid range is achieved. An unacceptable level of MMb in meat is said to be 40-50% (Greene et al., 1971) and TBA values above 1.0 are considered rancid for meats (Ockermann, 1980b).

Lopez-Lorenzo et al. (1980) used ground pork under MAP and found the use of 20% carbon dioxide reduced lipid oxidation. Wolfe et al. (1976) found the addition of carbon monoxide inhibits rancidity by reducing the concentration of free myoglobin derivatives. Cole (1986) explained that the presence of oxygen in MAP of meats causes the shelf-life to be only 8-14 days as compared to 28-35 days for VP.

Effect of modified atmosphere packaging on microbial growth in meats:

Many researchers (Baran et al., 1969; Clark and Lentz, 1969; Enfors et al., 1979; Silliker and Wolfe, 1980) have stated the effectiveness of MAP on bacterial growth characteristics is dependent on carbon dioxide gas. For

maximum shelf-life extension, early application of this gas is essential (Clark and Lentz, 1969). The effect of nitrogen gas on bacterial development is not well understood. It has been reported, however, that different concentrations of the gas did not significantly alter the microbial quality of meat (Huffman, 1974; Marriott et al., 1977). Spahl et al. (1981) found that the temperature had a much greater effect on psychrotrophic bacteria in an air environment than in a carbon dioxide environment. Erickson and Molin (1981) explained that low temperatures increase the solubility of carbon dioxide gas, making inhibition of microorganisms by this gas more effective during refrigeration.

Clark and Lentz (1969) found the most common spoilage microorganisms of fresh meat to be bacteria of the genera Pseudomonas and Achromobacter, and other gram-negative bacteria which are highly aerobic and generally are surface contaminants. These microbes are inhibited in the presence of high carbon dioxide levels whereas bacteria of the genera Lactobacilli were found to be highly tolerant of the gas (Sander and Soo, 1978). Potential food poisoning microbes such as Staphylococcus aureus, Clostridium and Salmonella did not pose a problem at refrigeration temperatures (Gee and Brown, 1981), and storage of fresh meat under 20% carbon dioxide aided in reducing the population of bacteria such as Salmonella and Enterococci (Silliker and Wolfe, 1980).

IV. Packaging of fresh red meats

Mills and Urbin (1960) stated that "the primary function of a meat package is to present the meat to the consumer in the most attractive manner possible and at the same time protect the product from physical damage, microbial deterioration and chemical change." Taylor (1974) stated that packaging of meat shall aid in handling and marketing of the product.

Packaging films and their effect on red meat

The quality of red meat is judged by the color, as seen through the packaging film. The color is affected by the physical characteristics of the film including opaqueness, translucence, glossiness, matteness and the degree of wrinkling that occurs when the film is used (Mackinney et al., 1966). According to Satterlee and Hansmeyer (1974), the quality of meat is dependent on many factors such as oxygen penetration, microbial growth, fat oxidation and oxygen permeability of the film. The films used in meat packaging vary in their chemical structure and physical properties. These properties include oxygen permeability, moisture vapor transmission rate, heat seal and shrinkage properties (Paul, 1967; Karel et al., 1975). In MAP, the gases present are in a dynamic state and so the permeability of the film is an important characteristic. The permeation coefficient

has been found to be highest for nitrogen, followed by oxygen and carbon dioxide had the lowest coefficient among the gases used in MAP (Rizvi et al., 1980).

MacDougall and Taylor (1975) suggested the use of high permeability films in order to develop a good color in fresh meats. Sacharow (1974) recommended the film oxygen permeability to be 5000cc O2/m2/24 hr/atm. at 24C (with 100% and 52% relative humidity on the inside and outside of the package, respectively) to maintain the red color. Perdue et al. (1975) found that an O2 transmission rate of 50cc/m2/24 hr. at 22.8C was acceptable to prevent the degradation of fresh meat pigments under VP storage up to 28 days at 2.2C.

Pierson et al. (1970) explained the use of high permeability film would aid in good color development, but also would allow the growth of psychrotrophic bacteria. Hess et al. (1980) reported a reduction in the growth of Pseudomonas by the use of films of lower permeabilities but suggested that the cost would make it uneconomical. Seideman et al. (1976) suggested that a tight fitting vacuum package with little residual air could have high carbon dioxide concentration which would have an inhibiting effect on gram negative aerobic spoilage bacteria.

Taylor (1974) suggested that the packaging material must be free of trace elements that could catalyze autoxidation reactions, even though light has a bigger catalytic effect and is a major concern in transparent

materials. Since pork has high fat and unsaturated fatty acids, Keskinel et al. (1964) stated that oxidation is the most critical factor from the packaging point of view of this meat.

Package design should also be considered while packaging meat. Perdue et al. (1975) found that package design had an effect on the amount of purge in vacuum packaged fresh meat. The packages on which the film surface area was equal to the product surface area had approximately 1% lower purge than packages in which the film surface area was significantly greater than the meat product area.

Packaging systems for red meat

Packaging systems for fresh red meat were revolutionized with the introduction of the "boxed beef" concept in 1967 (Cole, 1986). This involved fabricating the meat into primal or sub-primal cuts, vacuum packaging in oxygen barrier material and boxing the cuts. This technique provided a method of extending the shelf-life of the meat during shipment and storage as compared to conventional whole carcass distribution (Seideman and Durland, 1983). By the 1970's the technique was applied to the pork industry (Terlizzi, 1982) and is largely followed in the meat industry today for the wholesale distribution of fresh red meats (Cole, 1986). This concept, also known as central packaging, along with it's numerous advantages such as

longer shelf-life, economical and improved sanitation (Paul, 1967), also has disadvantages. The rate of discoloration is one of them (Webb et al., 1972; Finne, 1982).

Sacharow and Griffin (1980) have explained the different machinery and their individual ways of packaging. Hotchkiss (1989) explained that there are two basic techniques involved for MAP of fresh red meats. One involves a chamber-type machine where the package with meat is placed inside a chamber which is evacuated and refilled with the desired atmosphere. The package is then sealed before the chamber is opened. The author stated that this system is ideal for semi-rigid containers such as trays. Another system involves a tubing or snorkel which evacuates the air within the package and aids in refilling the desired atmosphere. The snorkel is quickly withdrawn and the package sealed. Since this system involves vacuumization prior to refilling of the gas, it has been recommended for meats that can withstand being vacuumed.

Cole (1986) summarized some techniques being used presently in the red meat industry to package fresh red meat for retail display. The author reported that vacuum skin packaging (VSP) was one. It involves a technique similar to vacuum packaging, but uses a heated dome to heat the packaging material to a semi-molten state. Another technique involves MAP under an Atmospack concept, which has a unique gas atmosphere system of high oxygen (50-90%) and low carbon

dioxide (10-50%) levels. The typical thermoforming vacuumization machines with gas flush capabilities are involved. A latest technique involves high permeability/bulk gas flushing. The fresh red meat is placed on polystyrene foam trays, flushed with a selected gas atmosphere and overwrapped in strechable, sealable, high oxygen permeable, flexible packaging films. These overwrapped packages are then placed into a master package (a large impermeable bag or pouch), which is vacuumized, filled with the desired atmosphere and sealed. This system is ideal for marketing and distribution of fresh red meat in retail trays.

V. Pre-rigor meat processing

Physiology and biochemistry of post-mortem meat

The biochemical reactions which occur after slaughtering of the animal have been studied by many investigators. Forrest et al. (1975) explained that upon exsanguination of the animal, the body tries to maintain a homeostatic condition. With the depletion of blood from the body, the oxygen supply becomes limited, and the body converts from an aerobic to anaerobic metabolic pathway or glycolysis. There is a lack of aerobic synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) in the muscle mitochondria, which results in the depletion of glycogen and ATP within a few hours postmortem.

Grinding the muscle tissue immediately after postmortem results in the same changes, but at a faster rate (Hamm, 1977).

Lactic acid build up in the muscles resulted in the lowering of pH (Marsh, 1981). Hoinkel and Hamm (1978) found that the ATP level of 1.0 micromole per gram at pH 5.9 was insufficient to keep the muscles in a relaxed state. The researchers noted that at that ATP level, the calcium was concentrated in the filaments. This caused contraction of the muscle, and led to rigor mortis state. When lactic acid production ceased upon the depletion of glycogen or denaturation of glycolytic enzymes, rigor mortis was at the most severe state. This was found to be at pH of 5.5 and the ATP level was at 0.1 micromole per gram (Newbold and Harris, 1972; Hoinkel and Hamm, 1978). Marsh (1981) stated that at this ATP level, the actin and myosin filaments are cross-bridged to a maximum extent leading to the formation of actomyosin and complete rigor-mortis is reached.

Forrest et al. (1975) stated that aging of the meat leads to a drop in the pH as the cathepsin enzymes are released from the lysosomes of the cell. These enzymes denature the proteins and along with the breakdown of collagen tissue, the meat becomes tender in a post-rigor state.

Use of pre-rigor meat in processing

Advantages:

Reagan (1983) studied the advantages of pre-rigor processing of pork which included improved shelf-life, more uniform quality, creative marketing, less shrinkage and reduced level of purge. The author also reported that the benefits include decreased cooler space, less energy consumption, lower labor requirements and reduced in-plant holding time. Cordray and Huffman (1983) found that a 600 pound carcass required 24% less energy to chill after excess fat and bone were removed. It has also been reported (Henrickson, 1981) that a 600 pound carcass required 31,500 BTU's to cool the temperature from 39C to 0C, whereas 420 pounds of edible portion required only 22,380 BTU's resulting in 30% energy savings.

Hamm (1972; 1982) stated that the processing of meat prior to the onset of rigor mortis provided sausages of excellent quality. Other advantages include higher water holding capacity (Cuthbertson, 1980; Hamm, 1982), better emulsifying capacity (Trautman, 1964) and smaller cooking losses (Ray et al., 1980).

Cross and Tennet (1981) and Cross et al. (1979) mentioned that the fat in pre-rigor vacuum packaged meat was whiter in appearance than post-rigor meat. This was attributed to the discoloration of fat during the chilling period and greater amount of purge in the vacuum packaged

post-rigor meat. Cross et al. (1979) reported that the lean color of vacuum packaged pre-rigor and post-rigor meat did not differ significantly, whereas Lawrie (1979) stated that pre-rigor meat could produce cuts with more uniform lean color. Also pre-rigor ground pork muscle has been reported to have a reduced rate of lipid oxidation during storage at 2C (Judge and Aberle, 1980) and at 0C (Drerup et al., 1981). These workers, along with Hamm (1977) indicated that the addition of salt has a greater pro-oxidant effect in ground pork made from post-rigor muscle than pre-rigor muscle.

Disadvantages:

The pre-rigor meat produces a dark purple color which results in the meat not being well accepted by retail marketers. Huffman (1980) explained that in pre-rigor muscle there exists a higher degree of oxygen utilization in muscle enzymatic systems thereby causing myoglobin to exist in a reduced state. Cornforth and Egbert (1986) gave another hypothesis that oxygen reserves in pre-rigor muscle tissue are used for mitochondrial respiration and this causes the myoglobin to remain in a reduced state.

Rapid chilling in order to save space and refrigeration energy in pre-rigor meat can lead to a cold-shortening problem. This results in a tougher meat for the consumers (Taylor et al., 1981; Raymond, 1982). Other problems include difficulty in handling hot and sticky meat resulting in

distorted meat cuts (Kastner, 1977; Reagan 1983) and the need for redesigning the plant to enable pre-rigor processing (Ferguson, 1981).

Microbial contamination is a major factor for rapid deterioration of pre-rigor meat (Locker et al., 1975). McMillin et al. (1981) indicated the conditions that favored the growth of microorganisms included the high pH of the muscle, high temperature of the meat during processing and the high oxidation-reduction potential of the muscle tissue. Fung et al. (1980) explained the need for temperature control for pre-rigor meat during the first several hours of chilling from a microbial standpoint. Their research indicated that cuts from pre-rigor meat had longer periods of rapid bacterial growth (above 21C) than post-rigor meat and even vacuum packaging for 14 days at 2.2C produced pre-rigor meat cuts with higher mesophilic and psychrotrophic counts. Lin et al. (1979) used pre-rigor pork sausage samples and reported that at 2C-5C there were higher total aerobic mesophilic and lipolytic bacterial counts than on post-rigor pork sausage. This was attributed to a higher pH and higher temperature of pre-rigor pork during processing.

VI. Effect of retail display conditions on red meats

Kropf (1980) defined retail display of red meat as "offerring of the product under lighting and at a

refrigerated temperature, usually pre-packaged." The following factors which affect the retail display and retail consumers have been outlined.

Color

Color perception is viewed by the consumer as the single criteria which acts as an indicator of flavor, juiciness, freshness and tenderness of the retail meat (Naumann et al., 1957). Francis (1963) stated that color in meats deals with hemoglobin and myoglobin pigments. Clydesdale and Francis (1971) gave the absorption maxima for the three forms of predominant pigment myoglobin. They reported that oxmyoglobin has absorption maxima at 535-545 and 575-588 nm giving the meat a net red color, myoglobin at 555 nm resulting in purple color and metmyoglobin at 505 and 625 nm giving the meat a brown appearance. Besides the meat pigment concentration, the color of meat is also dependent on pH, metallic ions, lighting and temperature.

pH:

Low pH has been found to induce myoglobin oxidation. Chang and Taylor (1975) reported that when pH was lowered, the globin conformation was changed in such a way as to render it less effective in stabilizing the heme-oxygen complex. Adams (1976) stated that any condition which would cause the pH to be lowered to about 5.6 would cause heme

dissociation, unfolding of apoglobin and protonation of bound oxygen resulting in the development of oxymyoglobin and/or metmyoglobin.

Metallic ions:

Certain metallic ions have an effect on the oxidation of oxymyoglobin (Huffman, 1980). Copper is extremely active in promoting the formation of metmyoglobin from oxymyoglobin whereas iron, zinc and aluminum have been found to be less active (Clydesdale and Francis, 1976).

Lighting:

Effect of lighting on meat color has been studied but has provided conflicting reports. Marriott et al. (1967), Santamaria (1970) and Leising (1976) found discloration of fresh meat under lighting, whereas Richert et al. (1957) and Clauss et al. (1957) reported that light versus dark storage had little effect on the color of fresh red meat. Kropf (1980) has postulated that light does affect muscle pigment state and color, but the eye may not be sensitive enough to detect the changes early in display.

Ernst (1980) indicated that the best lighting for reducing changes in meat samples was fluoroscent deluxe warm light supplemented with incandescent spots. Calkins et al. (1986) demonstrated that fluorescent or cool flood and deluxe cool white provided the most desirable color on fresh

pork.

Temperature:

In 1954, Watts reported that every 10C rise in temperature caused a doubling of lipid oxidation rate. This is particularly important in pork sausages which have high fat content. Low temperatures during display and storage depress the enzymatic activity, minimizing the inherent color changes, inhibiting oxidation, reducing the purge and drip loss (Kropf, 1980).

Microbial growth

Lechowich (1971) stated that the most common spoilage found in pork sausages is souring due to the growth of acid-producing bacteria such as Lactobacilli and Leuconostoc. Casings on pork sausages could also develop slime or colored spots due to the growth of Pseudomonas, Achromobacter and Flavobacterium. Ockerman (1980b) indicated that the most common spoilage microbes during low temperature display or storage would be gram-negative rods. He gave the following maximum acceptable levels per gram of boneless meat for pork sausage manufacture and display.

For processing: Coliforms = 7.19 * 10

Yeasts = 7.6

Molds = 7.72

Salmonella= 0.35

For retail display: Total count = 4.59 * 10*5

Coliforms = 8.7 * 10

Yeasts = 9.2

Molds = 9.2

Salmonella = 0

For end of display: Total count = 2.76 * 10*6

Coliforms = 1.58 * 10*2

Yeasts = 1.43 * 10

Molds = 1.43 * 10

Salmonella = 0

Lipid oxidation

Oxidative rancidity results from the oxidative decomposition of unsaturated fats (Watts, 1954). As indicated by Watts (1954), this oxidative decomposition can occur in fatty acids with one double bond. However, the methylene group between two double-bonded carbons are more susceptible to oxidative attack than carbons adjacent to a single double bond. Therefore, pork sausages which are rich in unsaturated fats are highly susceptible to oxidative rancidty. Presence of pro-oxidants accelerates the lipid oxidation (Watts, 1961a).

Ockerman (1980a) gave a TBA value of 1.0 to be an indicator of a rancid and unacceptable product. Watts (1961b) indicated that off-odors due to the formation of aldehydes and ketones could be detected in the range of

0.5-1.0 TBA value.

CHAPTER 3

MATERIALS AND METHODS

I. Preparation, Packaging and Storage

Six sows were processed according to normal slaughtering procedures in the University of Tennessee Meats Laboratory. Half of the carcass was processed into sausage within 4 hours of slaughtering (pre-rigor). The other half was made into sausage after 24 hours of chilling (post-rigor). Fresh pork (pre-rigor and post-rigor) was ground in a stainless steel meat grinder (Model 5424852, The Biro Manufacturing Co., Marblehead, OH) through a 1.9 cm plate. After the incorporation of seasonings and antioxidants (World Spice Company, Knoxville, TN), meat was mixed thoroughly and ground through a 0.48 cm plate. The sausage was then stuffed into cellulose casings with the aid of a hydraulic stuffer. The sausage was then crust frozen in a batch liquid carbon dioxide freezing system (Model BF 300SD, Cryo-chem Inc., Gardena, CA), at -70C for 5 minutes and placed in a blast freezer at -21C for 10 days.

The stuffed casings were then sliced (Model 44, The Biro Manufacturing Co., Marblehead, OH) and the casing film removed from the sausage patties (5.1 cm diameter * 1.3 cm thickness). Two patties were placed per styrofoam tray (13.5

cm * 13.5 cm) and overwrapped with oxygen permeable polyvinyl chloride (PVC) film (Reynolds Metal Co., Richmond, VA) [oxygen transmission rate (O2TR)= 20000 cc/m2/24 h; carbon dioxide transmission rate (CO2TR)= 60000-100000 cc/m2/24 h; nitrogen transmission rate (N2TR)= 6500-8000 cc/m2/24 h]. Each retail tray was placed in a B650 barrier bag [O2TR= 35-50 cc/m2/24 h; CO2TR = 300-500 cc/m2/24 h; N2TR= 15 cc/m2/24 h] (Cryovac Div., W.R. Grace & Co., Duncan, SC) and the treatments applied in an incomplete block design (Table 1). Placement of overwrapped sausage trays in barrier bags was employed to simulate a central bulk-packaging system. Vacuum was created using a Koch Multivac AG4 machine (Koch Supplies Inc., Kansas City, MO) operated at a vacuumizing capacity of 65 mm Hg. Other treatments, applied through the same machine, included the flushing of barrier bags with the following different gas combinations: 100% CO2, Air, 75% N2 + 25% CO2 , 75% N2 + 15% CO2 + 10% O2 and 75% N2 + 20% CO2 + 5% O2 (MG Industries, Morrisville, PA). Packages were then stored at 4C for periods of 0, 3, 7, 10 and 14 days.

II. Chemical and physical measurements

Fat determination

Fat content of the sausage samples was determined using a modification of Babcock method (AOAC, 1984) which

Table 1: Incomplete block design for packaging treatments applied to pre-rigor and post-rigor pork sausage patties.

	Pre-rigor						Post-rigor					
Sow No.	Treatments[a]											
	1	2	3	4	5	6	1	2	3	4	5	6
1	*	*				*			*	*	*	
2			*	*	*		*	*				*
3	*	*				*			*	*	*	
4	*		*	*				*			*	*
5		*	*		*		*			*		*
6				*	*	*	*	*	*			

aTreatment 1 = Vacuum

Treatment 2 = 100% CO_2

Treatment 3 = Air

Treatment 4 = 75% N_2 + 25% CO_2

Treatment 5 = 75% N_2 + 15% CO_2 + 10% O_2

Treatment 6 = 75% N_2 + 20% CO_2 + 5% O_2

eliminated the use of the centrifuge. Nine grams of sample were put into a Paley bottle followed by 10 ml of warm water (55C-60C). After dispersing the sample, 17 ml of conc. sulfuric acid was added and the mix swirled. Care was taken to digest all of the sample. Additional hot water (75C-80C) was added to float the fat into the graduated neck of the Paley bottle. The bottle was placed in a water bath (75C-80C) for five minutes during which it was twice agitated gently to be certain all the fat had floated into the neck of the bottle. After five minutes in the water bath the graduated neck of the bottle was read to determine fat content of the sample.

Moisture determination

Moisture percentage of the sausage patties was determined by placing 2g of powdered sample on a dry pre-weighed Whatman filter paper no.4 and then drying it in a vacuum oven at 70C and 15 mm Hg for a period of 5 hours (AOAC, 1984). The samples were cooled, weighed and percent moisture calculated.

Gas analysis

The relative percentage (by volume) of oxygen, nitrogen and carbon dioxide was determined in the headspace of the packages (Seideman et al., 1980). The analysis was conducted on 0, 3, 7, 10 and 14 day storage periods. Packages were

equilibrated at 4C for 10 minutes before sampling the headspace gas. A rubber tape was applied to the exterior of the barrier bag to facilitate sampling of the gas atmosphere. This provided an area that would not tear when punctured for sampling. Each package was sampled in duplicate for headspace analysis before the package was opened for other analyses. A 2 ml sample of headspace gas was drawn through an 18-gauge needle with a gas-tight syringe inserted into a valve injector and immediately analyzed by gas chromatography. The gas sample withdrawn was within the overwrap film surrounding the patties.

Gas analysis was done using a Hewlett Packard Model 5890 gas chromatograph (GC) equipped with a 1 ml valve injector and a thermal conductivity cell, combined with a Hewlett Packard Model 3393A electronic integrator. Separation of gases was accomplished using a 4.8 M * 0.3 cm i.d. stainless steel column packed with carbosieve B (Supelco Inc., Belefonte, PA). The GC oven temperature was at 50C and the detector, 70C. Carrier gas (Helium) flow rate was kept at 37.5 ml/min.

pH determination

The pH of the sausage patties was determined by blending 10g of sausage in 200 ml of distilled water for 2 minutes (Ockerman, 1972). Two duplications per sample were made and the pH recorded to the nearest 0.01 unit using a

Fisher Accumet pH Meter, Model 600. The electrode was carefully washed with distilled water and wiped between samples.

Lipid oxidation evaluation

The modified distillate method of Rhee (1978) was used for the determination of lipid oxidation. Ten grams of sausage, 5 ml of 0.5% propylgallate (PG), 5 ml of 0.5% ethylenediamine tetra-acetic acid (EDTA) and 20 ml of distilled water were blended for 2 minutes in a Virtis homogenizer at 3000 rpm in a stainless steel container (Virtis, 16 235 1). To this homogenate, 2.5 ml of 4N HCl was added. Diluted homogenate was then transferred to a 800 ml Kjeldahl flask and 47.5 ml of distilled water added to rinse the homogenizing flask. Boiling beads and 1 ml antifoaming agent (Arthur H. Thomas Co. Philadelphia, PA) was added to the Kjeldahl flask. Fifty ml of the distillate was collected in a 100 ml graduated flask within 15 minutes and then transferred to a 50 ml Erlenmeyer flask until analyzed for malonaldehyde (MA) content.

To prepare the standard curve for malonaldehyde (MA) determination, 5 ml of 1*10-4 M tetraethoxypropane (TEP) was mixed with 2.5 ml of 4N HCl and 92.5 ml of distilled water in a Kjeldahl flask, then 50 ml of distillate was collected from this solution in 15 minutes after the onset of boiling. The distillate (1*10-5 M MA) was used to prepare the

solutions of different concentrations of the colored malonaldehyde-2-thiobarbituric acid complex to be used to construct the standard curve. Solutions containing 1*10-5, 2*10-5, 3*10-5, 4*10-5 and 5*10-5 moles MA/10 ml solution were then prepared by pipetting 1, 2, 3, 4 and 5 ml of the distillate from TEP, respectively, into separate test tubes. Distilled water was added to each test tube to bring the total volume to 5 ml. To each tube, 5 ml of 0.02 M 2-thiobarbituric acid (TBA) solution was added, then the tubes were heated for 30 minutes in a water bath (75C-80C) for MA-TBA complex formation. The absorbance (A) of each concentration of MA was determined at 532 nm and the equation of the standard curve of A versus MA concentration determined by linear regression.

Five ml of distillate from each sample was also analyzed in the same manner as the distillate of TEP, but, with the addition of 5ml of 0.02 M TBA. The A of each sample was converted to the concentration of MA by the linear regression equation and TBA-number (mg MA/kg sausage) calculated by the following equation:

mg MA/kg sausage = (sample mmole MA/10 ml soln.)*(10 ml soln/5 ml distillate)*(50 ml distillate /10g meat)*(72 mg MA/mmole MA)*(1000 g meat/kg meat).

Microbial analysis

Aerobic plate counts for total counts (TC), psychrophiles (PC) and lactic acid (LC) bacteria were estimated in the packaged sausage patties at the end of each storage period.

Twenty-five gram portions were blended with 225 ml of sterile peptone (Difco Laboratories) in the Stomacher Lab-Blender 400 (Cook Laboratory Products, Div. Dynatech Labs. Inc., Alexandria, VA) for 2 minutes. Serial dilutions from 10-1 to 10-7 were prepared and used to plate on agar medium (Draughon, 1984). Mesophiles were incubated on Standard Methods Agar (SMA) (BBL Microbiology Systems) for 48 hours at 32C before being counted. Psychrophiles were incubated on SMA but for a period of 10 days at 4C and lactic acid bacteria involved incubation on deMan, Rogosa and Sharpe Agar (MRS) (Difco Laboratories) for 48 hours at 32C before being counted (Draughon, 1984; Kotula et al., 1980). Counts were recorded as Log10 colony forming units per gram of sample (American Public Health Association, 1980; Fung et al., 1980; Kotula et al., 1980).

Color evaluation

Pigment percentage:

The percentage of myoglobin (Mb), oxymyoglobin (OMb) and metmyoglobin (MMb) was estimated by a modified procedure of Snyder and Ayres (1961).

To obtain spectra of Mb, OMb and MMb, phosphate buffer with 0.04 ionic strength and pH 6.8 (Warriss, 1979) was used. Forty mg of crystallized horse skeletal myoglobin (Sigma Chemical Co., St. Louis, MO) was dissolved in 50 ml of phosphate buffer to form MMb and the percent transmission (%T) was determined using an IDL Color-eye Model D-1 (Instrument Development Laboratories, Attleboro, MA) at 20 nm intervals from 400 to 700 nm wavelengths. A small quantity (0.02 g) of sodium hydrosulfite was then added to reduce MMb to Mb and the %T of Mb at the same wavelengths was determined. Care was taken to keep the solution under nitrogen at all times. Reduced Mb was then shaken in air until it became oxygenated to form OMb and the %T from 400 to 700 nm was determined.

From the spectra obtained of pure myoglobin forms, 520 nm (isobestic point for all forms of Mb), 540 nm (wavelength of maximum absorbance for OMb), 560 nm (wavelength of maximum absorbance for Mb) and 640 nm (wavelength of maximum absorbance for MMb) were chosen as analytical wavelengths for measurement of different forms of myoglobin. The absorptivity of each form of myoglobin at each of these wavelengths was determined from the spectra. Solving these simultaneous equations, the following equations were derived to determine the concentration of OMb, Mb and MMb in concentrations of mg/100 ml (Melton, 1990).

For OMb: 13.924*COMb = 4.922*A at 540 - 1.489*A at 520 - 3.561*A at 560,

For Mb: 12.969*CMb + 8.901*COMb = 3.561*A at 560 - 3.063* A at 520,

For MMb: 6.25*CMb + 3.75*COMb + 10.0*CMMb = A at 640, where

A at 520 = Log Ro/%R at 520 nm,

A at 540 = Log Ro/%R at 540 nm,

A at 560 = Log Ro/%R at 560 nm,

A at 640 = Log Ro/%R at 640 nm,

Ro = Percent reflectance of white standard,

%R = Percent reflectance of meat sample and

C = Concentration of the pigment.

Colorimeter values:

Color of sausage patties under different atmospheric treatments was measured using the Hunterlab Color/Difference Meter Model D25-2 and a modification of the method described by Hunter (1958). The Hunter colorimeter was standardized with a pink color standard ('L' = 69.1, 'a' = 22.0, 'b' = 11.9). Measurements were taken that included the 'L' range denoting lightness, the 'a' range denoting redness, and the 'b' range denoting yellowness.

Subjective evaluation

Ten panel members were selected on the basis of their experience in evaluating meat color. A photograph showing

the ideal color for pork sausage patty was used as a standard (Hunter Color/Difference Meter values 'L' = 44.5, 'a' = 12.1, 'b' = 8.9) to which patties in this study were compared. Upon termination of the storage period, trays were removed from the barrier bag, allowed to equilibrate at 4C for 20 minutes and presented to the panelists. The trays were placed under simulated retail display conditions (4C, 60 footcandles of cool white fluorescent light). The panelists evaluated the patties in trays overwrapped with PVC film for surface discoloration (SD), employing a 0-100% scale, and for color (C), employing another scale which had light to normal (0-75 mm) and normal to dark (0-75 mm) demarcations (Appendix A). Patties with coded identity were evaluated in completely random sequence; evaluators had no knowledge regarding the treatment of the sample.

III. Statistical Analysis

An incomplete block design was used to study the effect of vacuum and modified atmosphere packaging and the rigor status on the pH, color, lipid oxidation, microbial condition, surface discoloration and color of pork sausage patties (Table 1) (Sanders and Schneider, 1988). Percent fat, moisture and relative gas concentration was not analysed. A General Linear Mixed Models (GLMM, 1989) procedure was used to identify sources of significant

variation for each variable. Due to the high levels of significance found in the data collected, probability levels of 0.001 ($P \leq 0.001$) were considered significant.

For each variable, contrast statements were written (a total of five contrasts) to compare the different treatments (Trt). These contrast statements are as follows:

Contrast 1 = Trt 1 vs. Trt 3 (comparing vacuum and air effect)

Contrast 2 = Trt 2 vs Trt 4 (comparing effect of 100% & 25% CO2 levels)

Contrast 3 = Trts 2 and 4 vs Trts 3, 5 and 6 (comparing absence and presence of oxygen)

Contrast 4 = Trt 3 vs Trts 5 and 6 (comparing effect of air and two different levels of O2 and CO2)

Contrast 5 = Trt 5 vs Trt 6 (comparing 10% O2 + 15% CO2 and 5% O2 + 20% CO2)

Contrast statements (a total of four contrasts) were also written in order to compare different days (Day) of storage. These contrast statements were as follows:

Contrast 6 = Day 1 vs Day 2 (comparing 0 and 3rd storage day)

Contrast 7 = Day 2 vs Day 3 (comparing 3rd and 7th storage day)

Contrast 8 = Day 3 vs Day 4 (comparing 7th and 10th storage day)

Contrast 9 = Day 4 vs Day 5 (comparing 10th and 14th storage day).

CHAPTER 4

RESULTS AND DISCUSSION

I. Percent fat and moisture in sausage

The incomplete block design of this study prevented the possibility of compositional differences among sows having a strong influence on the dependent variables. Analysis of the data showed state of rigor, packaging treatment, storage days and certain interactions of these independent variables, rather than individual sows, were responsible for the observed variation. Therefore mean fat and moisture composition of the sausage patties used in this study is given with no statistical analysis only to help the reader develop a more complete understanding of the research material.

The percent fat of sausage samples (Table 2) made from pre-rigor and post-rigor sides of different sows was similar except for those from sows two and six. Even though the sausage patties from sows two and six contained a little more fat, the sausage samples were well under the maximum fifty percent allowed for this kind of product.

Moisture content varied from 45.3% to 49.2% in post-rigor samples and from 50.9% to 52.5% in pre-rigor samples (Table 3). It is reasonable for this difference between pre-rigor and post-rigor pork to occur because there is normally two to

Table 2. Percent fat in sausage patties made from pre-rigor and post-rigor pork from six sows.

SOW NO.	PRE-RIGOR	POST-RIGOR
1	35%	34%
2	42%	40.5%
3	30%	32%
4	34%	36%
5	31.5%	34.5%
6	43.5%	41%

Table 3. Percent moisture in sausage patties made from pre-rigor and post-rigor pork from six sows.

SOW NO.	PRE-RIGOR	POST-RIGOR
1	52.5%	48.6%
2	50.9%	45.3%
3	53.1%	49.2%
4	51.9%	48.5%
5	52.4%	48.8%
6	49.5%	45.7%

three percent cooler shrinkage (water loss) of pork carcasses in the University of Tennessee Meat Laboratory coolers during the first 24 hours of chilling. This difference could be due to the pre-rigor sausage samples having higher pH values compared to normal pH values for post-rigor pork sausages being 5.4-5.8 (Shay and Egan, 1986). At lower pH values, as in post-rigor meat, there is a loss of ATP leading to a build up of lactic acid and a consequent formation of actomyosin which leads to a decline in water-holding capacity (Lawrie, 1979).

II. Gas analysis of different packaging treatments

Tables 4, 5 and 6 give the relative percent composition of carbon dioxide, oxygen and nitrogen, respectively, for the packaging treatments applied to sausage patties. The composition of the different gaseous atmospheres was found to be consistent with the gas atmosphere intended. The data was not analysed statistically but is given here to show what changes in atmosphere composition occured during the 14 day storage period.

The vacuum treatment was not completely successful because the package design (placing patties in a styrofoam tray) did not allow all of the air to be expelled from the package while vacuumizing and air pockets existed between the patties (two per tray). The concentration of carbon dioxide (Table 4) increased dramatically over the 14 day period for

Table 4. Percent carbon dioxide found in different packaging treatments of sausage patties stored at 4C over 14 days.

DAY	PRE-RIGOR TREATMENTS						POST-RIGOR TREATMENTS					
	1	2	3	4	5	6	1	2	3	4	5	6
0	32.1	96.4	1.2	23.5	15.2	21.2	30.1	98.0	1.5	26.3	15.3	21.0
3	40.8	98.0	5.6	26.5	21.3	21.1	36.6	95.1	7.1	30.5	18.4	19.0
7	60.0	99.5	12.1	35.2	31.0	28.5	52.1	98.6	9.9	32.6	25.6	23.5
10	68.6	97.3	20.3	48.1	46.6	36.9	60.3	99.6	18.5	48.9	38.2	34.0
14	71.2	99.2	32.6	52.6	62.3	55.8	63.1	99.9	30.8	52.5	55.2	49.3

Treatment 1 = Vacuum

Treatment 2 = 100% CO_2

Treatment 3 = Air

Treatment 4 = 75% N_2 + 25% CO_2

Treatment 5 = 75% N_2 + 15% CO_2 + 10% O_2

Treatment 6 = 75% N_2 + 20% CO_2 + 5% O_2

Table 5. Percent oxygen found in different packaging treatments of sausage patties stored at 4C over 14 days.

D	PRE-RIGOR TREATMENTS						POST-RIGOR TREATMENTS					
A												
Y	1	2	3	4	5	6	1	2	3	4	5	6
0	10.5	1.2	18.8	0.4	9.6	4.9	7.1	1.0	16.3	0.1	10.1	5.0
3	6.3	0.3	13.2	0.0	3.1	1.2	4.9	1.5	15.2	0.3	8.4	3.1
7	2.5	0.1	6.0	0.1	1.1	0.2	3.5	0.3	10.4	0.0	4.9	2.6
10	0.9	0.1	1.2	0.0	0.5	1.0	2.0	0.1	6.2	0.0	1.3	0.3
14	0.4	0.2	1.1	0.3	1.2	0.1	0.8	0.0	2.1	0.0	0.2	0.0

Treatment 1 = Vacuum

Treatment 2 = 100% CO2

Treatment 3 = Air

Treatment 4 = 75% N2 + 25% CO2

Treatment 5 = 75% N2 + 15% CO2 + 10% O2

Treatment 6 = 75% N2 + 20% CO2 + 5% O2

Table 6. Percent nitrogen found in different packaging treatments of sausage patties stored at 4C over 14 days.

D	PRE-RIGOR TREATMENTS						POST-RIGOR TREATMENTS					
A												
Y	1	2	3	4	5	6	1	2	3	4	5	6
0	57.4	2.4	80.0	76.1	75.2	73.9	62.8	1.0	82.2	73.6	74.6	74.0
3	52.9	1.7	87.2	73.5	75.4	77.7	58.5	3.4	77.7	69.2	73.2	77.9
7	37.5	0.4	81.9	64.7	67.9	71.3	44.4	1.1	79.7	67.4	69.5	73.9
10	30.5	2.6	78.5	51.9	52.9	62.1	37.7	0.3	75.3	51.1	60.5	65.7
14	28.4	0.6	66.3	47.1	46.5	44.1	36.1	0.1	67.1	47.5	44.6	50.7

Treatment 1 = Vacuum

Treatment 2 = 100% CO_2

Treatment 3 = Air

Treatment 4 = 75% N_2 + 25% CO_2

Treatment 5 = 75% N_2 + 15% CO_2 + 10% O_2

Treatment 6 = 75% N_2 + 20% CO_2 + 5% O_2

pre-rigor and post-rigor pork sausage. This rise in CO2 was accompanied by a rapid drop of oxygen level in the package atmosphere. The air treatment which had maximum O2 (Table 5) concentration of 18.8% in pre-rigor and 16.3% in post-rigor, showed a final level of 1.1% in pre-rigor and 2.1% in post-rigor, respectively. Oxygen concentrations in different treatments showed a more rapid decline in pre-rigor sausage packages (Table 5).

Reduction in concentration of O2 and subsequent increase in CO2 could be due to the respiration of the meat tissue and the bacteria which may be prevailing at the time of processing, grinding and slicing of the sausage. The meat tissue and the bacteria could convert O2 in the package into CO2 which would explain high CO2 levels. This was supported by the studies conducted by Christopher and co-workers (1979) who also noted that the packages with high concentration (80%) of N2 had smaller amounts of purge at storage intervals of 7 and 14 days at 1C-3C. Purge was not noted in the present study. A more rapid decline in O2 level in the air atmosphere (which had the highest O2 level among the gases studied) indicated a more rapid respiration by the bacteria leading to a very high CO2 level, as compared to the initial level in the air.

Carbon dioxide gas could also dissolve in the meat tissue at the time of packaging and could have been expelled into the package atmosphere over the storage period. This was reported by Taylor and MacDougall (1973) in their studies with meat

packed in mixtures of O2 and CO2.

Christopher et al. (1979) suggested that a high percent concentration of CO2 in the package atmosphere could indicate another source of CO2, other than bacteria and/or meat tissue. The researchers did not theorize the source of CO2.

Nitrogen gas, which acts as a filler, exhibited a gradual reduction in percent concentration and the decline was not large.

III. Chemical and physical analysis

Means of variables studied according to the rigor status, treatments applied and days of storage are tabulated in Tables 7, 8 and 9, respectively.

pH changes in pork sausage

Mean pH values over the 14 day storage period from different treatments, both pre-rigor and post-rigor, are shown in Figure 1. Pre-rigor pH for different treatments varied from 6.5 to 6.8 whereas post-rigor pH varied from 5.7 to 5.9. The mean pH across all treatments for pre-rigor pork sausage was 6.6 which was different ($P \leq 0.001$) than the post-rigor mean pH of 5.8 (Table 7). This difference was due to a lactic acid build up in the post-rigor pork. In pre-rigor meat, there is a high ATP concentration so the meat retains its original pH level; in post-rigor meat there is a loss of ATP and the

Table 7: Means of certain chemical and physical characteristics of pork sausage patties made from pre-rigor and post-rigor pork.

Variable	Pre-rigor	Post-rigor
pH	6.6	5.8
TBA	0.50	0.42
Total bacterial count (Log CFU/gm)	3.8	2.9
Psychrotrophic bacterial count (Log CFU/gm)	2.0	1.8
Lactic acid bacterial count (Log CFU/gm)	1.9	1.6
Oxymyoglobin (%)	24.4	20.4
Myoglobin (%)	39.2	38.4
Metmyoglobin (%)	36.4	41.2
Hunter 'L' value	46.5	44.8
Hunter 'a' value	6.9	5.2
Hunter 'b' value	10.7	9.9
Colora (mm)	31.8	38.1
Surface discoloration[b]	39.8	50.4

a On a color scale of 150 mm, 'normal' is 0 mm, normal to light is 0 to -75 mm and normal to dark is 0 to 75 mm.

b Surface discloration scale of 0 to 100%.

Table 8: Means of certain chemical and physical characteristics of pork sausage patties stored under different packaging treatments.

Variable	Treatmentsc					
	1	2	3	4	5	6
pH	6.1	6.1	6.3	6.2	6.3	6.3
TBA	0.50	0.31	0.60	0.31	0.53	0.51
Total bacterial count(LogCFU/gm)	3.1	2.1	4.9	2.5	4.0	3.6
Psychrotrophic bacterial count (Log CFU/gm)	1.2	1.1	3.0	1.0	2.3	2.8
Lactic acid bacterial count (Log CFU/gm)	1.2	1.4	2.2	1.2	2.2	2.2
Oxymyoglobin (%)	21.7	13.5	30.0	16.6	29.0	23.6
Myoglobin (%)	36.8	37.6	36.0	39.6	39.0	43.8
Metmyoglobin (%)	41.6	49.0	34.0	43.7	32.0	32.5
Hunter 'L' value	44.7	44.3	47.5	44.0	47.1	46.2
Hunter 'a' value	6.2	4.8	6.5	5.1	7.0	6.8
Hunter 'b' value	10.4	10.0	10.7	9.6	10.4	10.5
Colora (mm)	33.5	49.6	27.1	49.8	24.1	25.5
Surface discoloration[b]	48.6	64.2	37.5	63.4	26.0	30.8

[a] On a color scale of 150 mm, 'normal' is 0 mm, normal to light is 0 to -75 mm and normal to dark is 0 to 75 mm.

[b] Surface discloration scale of 0 to 100%.

c Treatment 1 = Vacuum
Treatment 2 = 100% CO2
Treatment 3 = Air
Treatment 4 = 75% N2 + 25% CO2
Treatment 5 = 75% N2 + 15% CO2 + 10% O2
Treatment 6 = 75% N2 + 20% CO2 + 5% O2

Table 9: Means of certain chemical and physical characteristics of pork sausage patties over different storage days.

Variable	Storage days				
	0	3	7	10	14
pH	6.2	6.2	6.2	6.2	6.3
TBA	0.11	0.20	0.48	0.68	0.82
Total bacterial count(LogCFU/gm)	2.4	3.2	3.7	3.9	3.5
Psychrotrophic bacterial count (Log CFU/gm)	1.4	1.5	2.1	2.4	2.2
Lactic acid bacterial count (Log CFU/gm)	1.1	1.7	2.0	2.1	1.8
Oxymyoglobin (%)	30.7	38.1	27.0	12.3	3.9
Myoglobin (%)	47.6	32.3	34.6	40.4	39.2
Metmyoglobin (%)	21.8	29.5	38.4	47.3	56.9
Hunter 'L' value	48.3	48.6	46.2	43.9	41.1
Hunter 'a' value	7.5	7.2	6.1	5.0	4.5
Hunter 'b' value	10.6	10.8	10.5	10.0	9.4
Colora (mm)	27.4	19.3	31.2	45.0	51.8
Surface discolo-rationb	45.4	30.5	37.6	51.1	60.7

[a] On a color scale of 150 mm, 'normal' is 0 mm, normal to light is 0 to -75 mm and normal to dark is 0 to 75 mm.

[b] Surface discloration scale of 0 to 100%.

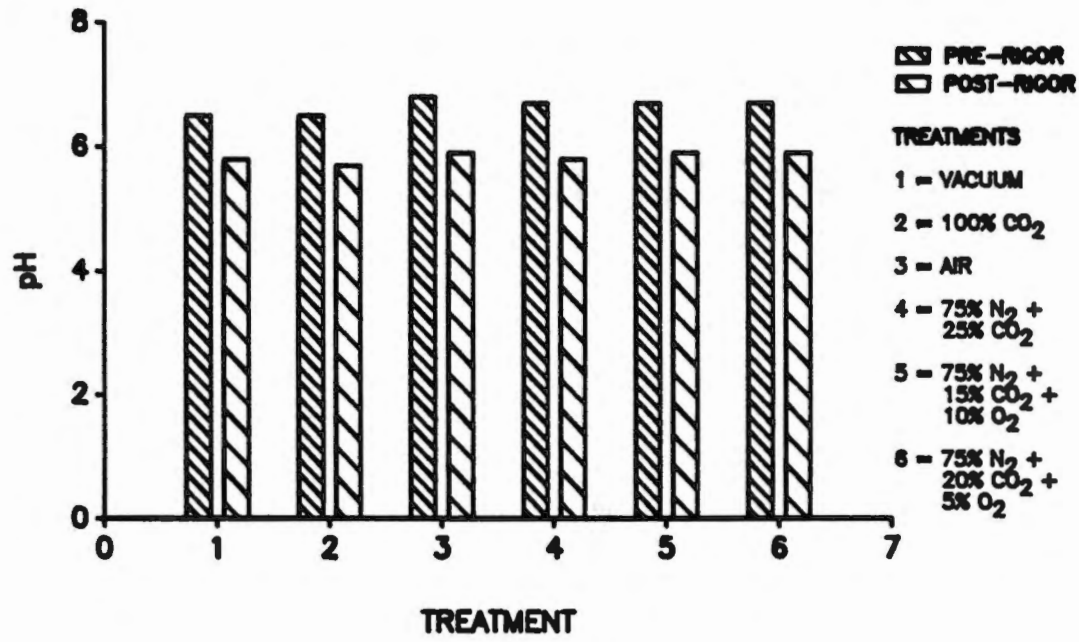

Figure 1. The pH values of pre-rigor and post-rigor pork sausages stored under six different treatments at 4C.

glycogen supply leading to lactic acid formation which lowers the pH into the acidic range (Lin et al., 1979).

Treatment 1 (vacuum) had a lower ($P \leq 0.001$) pH value as compares to treatment 3 (air) (contrast 1) (see Apendix C). While treatment 2 (100% CO2) had a lower ($P \leq 0.001$) pH value than treatment 4 (75% N2 + 25% CO2) (contrast 2), treatments 2 and 4 had lower ($P \leq 0.001$) pH values than treatments 3 (air), 5 (75% N2 + 15% CO2 + 10% O2) and 6 (75% N2 + 20% CO2 + 5% O2) pooled together (contrast 3). Ledward (1970) reported a decrease in pH of meat stored in high CO2-enriched environments due to the activity of lactic acid bacteria and/or dissolution of CO2 in meat fluids. This was supported by Izumimoto et al. (1985) in their study of ground meat. Treatment 1 had approximately 30% CO2 compared to 1.2-1.5% CO2 in treatment 3 which resulted in a lowering of pH. With the increase in CO2 concentration, the lowering effect on pH becomes more prominent as treatment 2 with maximum CO2 concentration had the lowest pH and treatment 3 with minimum CO2 concentration had the highest pH.

There were no differences ($P \leq 0.001$) in pH values across the storage days (Table 9) (contrasts 6, 7, 8 and 9, Appendix D).

Figure 2 shows the changes in pH for different treatments over a 14 day period. Treatments 1 (vacuum) and 2 (100% CO2) show a decrease of 0.2 units over 14 days whereas other treatments undergo an increase in pH. This study showed that treatments containing greater than 25% CO2 levels, without oxygen, had lower ($P \leq 0.001$) pH values than treatments with 25% CO2 and 5-20% O2 when stored at 4C over a 14 day storage period.

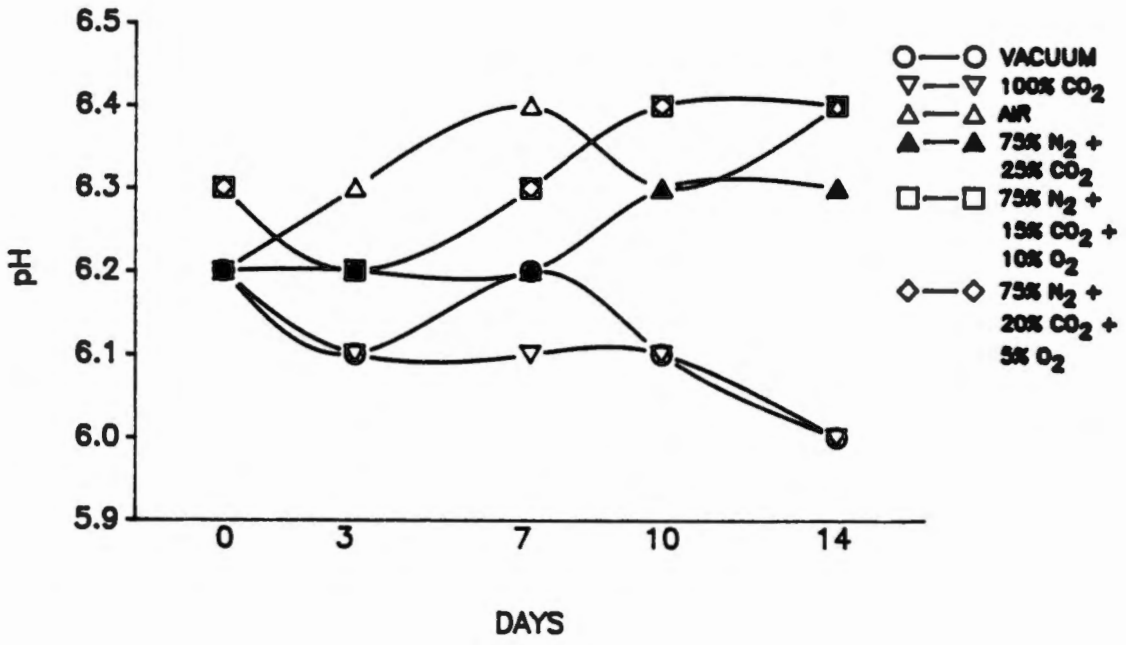

Figure 2. The pH values of sausage patties stored under six different treatments for up to 14 days at 4C.

Lipid oxidation in pork sausage

Greater ($P \leq 0.001$, Appendix B) lipid oxidation (higher TBA value) was observed in pre-rigor as compared to post-rigor sausages when the data were pooled for all treatments and storage times (Table 7). Judge and Aberle (1980) and Drerup et al. (1981) reported greater oxidation in post-rigor than pre-rigor meat. Since salt has a greater pro-oxidation effect in post-rigor pork (Hamm, 1977) and the presence of higher metmyoglobin pigment concentration in post-rigor pork leads to a faster oxidation rate (Watts, 1961a), post-rigor sausage should have had higher TBA values. This study indicated otherwise.

Table 8 shows the mean TBA values for pork sausage stored under six different treatments. Treatment contrasts (see Appendix C) indicate lower ($P \leq 0.001$) TBA values for treatment 1 (vacuum) than for treatment 3 (air) (contrast 1). Treatment 3 had a higher ($P \leq 0.001$) TBA value than treatments 5 (75% N_2 + 15% CO_2 + 10% O_2) and 6 (75% N_2 + 20% CO_2 + 5% O_2) pooled together (contrast 4), which means that treatments with a higher O_2 level had higher TBA values than treatments with lower O_2 levels.

A measure of lipid oxidation (TBA value) over 14 days for pre- and post-rigor pork sausage is shown in Figure 3. Initial values (day 0) indicate oxidation of lipids occurred during the processing. Grinding of meat during processing incorporates air into the meat. Keskinel et al. (1964) and Liu and Watts (1970) noted a higher rate of oxidation in ground meat compared to intact muscle. Storage of meat under freezing temperatures seemed to inhibit any oxidative changes (Caldironi and Bazan, 1982). With the increase of temperatures during display storage,

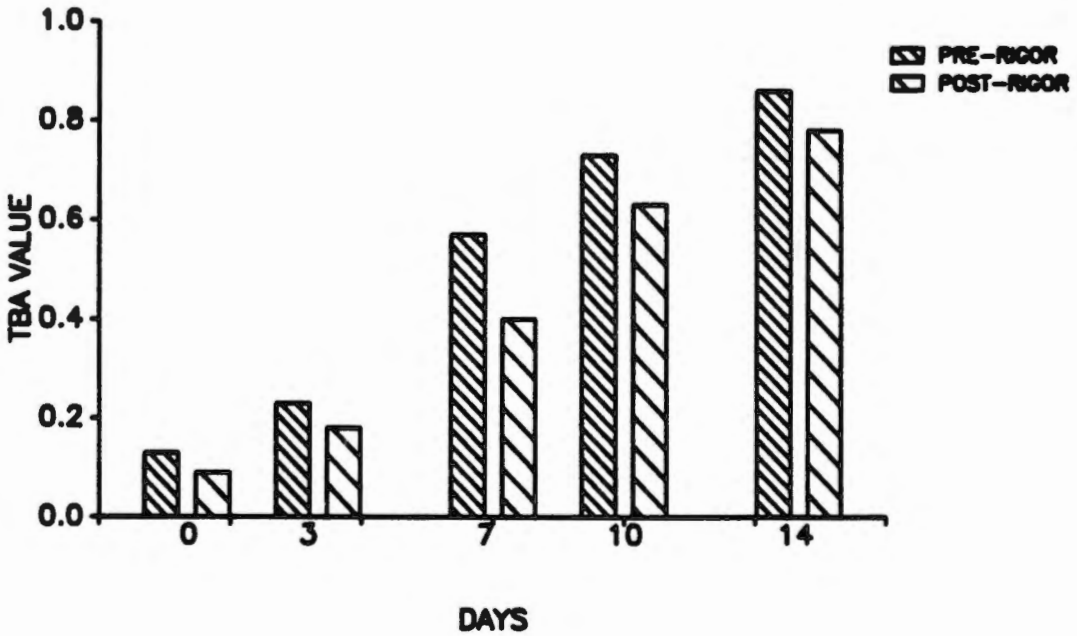

Figure 3. Thiobarbituric acid (TBA) values of pre-rigor and post-rigor sausage stored up to 14 days at 4C.

a rapid rate of oxidation occured over a period of 14 days.

Higher TBA values were found as the days of storage increased (Table 9). Contrasts 6, 7, 8 and 9 were significant ($P \leq 0.001$) (see Appendix D). As days of storage progressed, there was more interaction of sausage lipids with oxygen, leading to higher TBA ($P \leq 0.001$) values.

Figure 4 shows the changes in TBA values for different treatments over time. Initially, all the treatments had similar values but as days of storage progressed, differences were observed. Rate of lipid oxidation increased over storage days (Figure 4) and was found to be dependent upon the presence of oxygen (Table 5) in the atmosphere of the package. Treatments 2 and 4 which had negligible oxygen levels (less than 1%), had lower ($P \leq 0.001$, contrast 3) TBA values as compared to other treatments with higher oxygen levels (5% and higher). In comparing treatments 1 and 3 (contrast 1), the later, which had oxygen had the higher ($P \leq 0.001$) TBA values (Table 8) throughout the study (Appendix C). This was in agreement with Watts (1954) who suggested high lipid oxidation is promoted in the presence of oxygen. Watts (1954) also stated that foods high in unsaturated fatty acids (such as pork sausages) are highly susceptible to oxidative rancidity.

Presence of CO2 also seems to influence lipid oxidation. Treatments 2 and 4 with 25% and 100% CO2 levels, respectively, had less ($P \leq 0.039$) lipid oxidation as compared to treatments 3, 5 and 6 (contrast 3, Appendix C). Between treatments 2 and 4, the difference was not significant ($P \leq 0.001$). The TBA values increased ($P \leq 0.001$, Appendix B) across storage for all treatments, even those in which O2 was 0.3% or lower after 7 days storage (treatments 2 and 4) (Figure 4). This leads

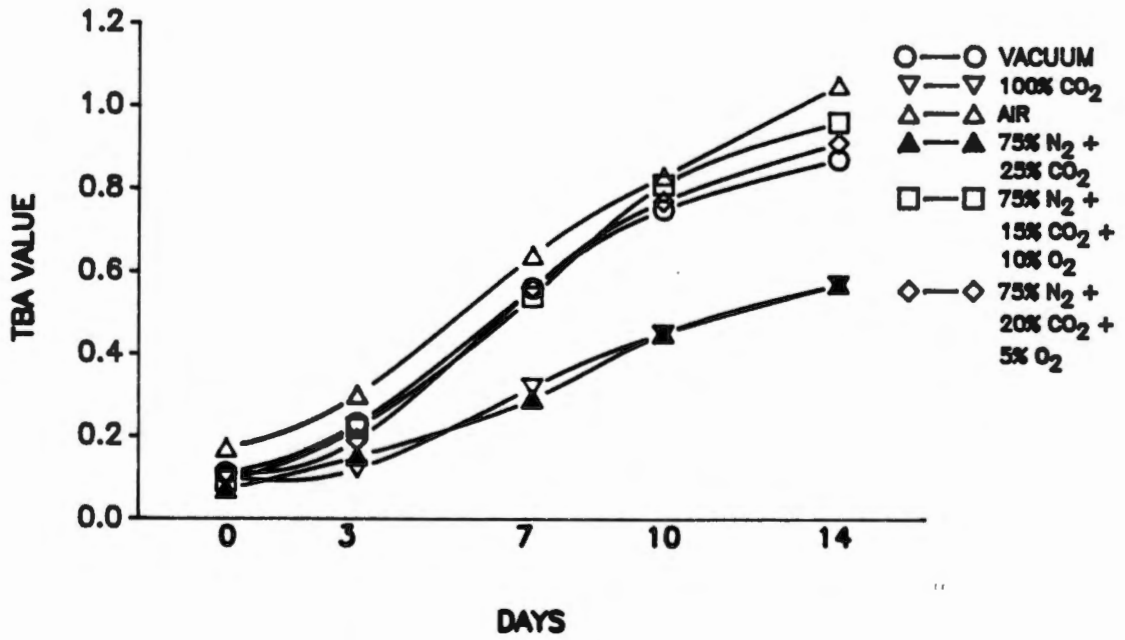

Figure 4. Thiobarbituric acid (TBA) values of sausage stored under six different treatments for up to 14 days at 4C.

to the speculation that either CO2 has the ability to promote oxidation under low O2 concentrations, or simply, that under lower partial pressures of O2, lipid oxidation is promoted. The ability of CO2 to promote lipid oxidation was also indicated by Marriott et al. (1977). The mechanism by which CO2 could promote lipid oxidation is not certain. Similar trends of oxidation under 100% and 25% CO2 in treatments 2 and 4 were seen whereas treatment 1, having a high (30%) CO2 concentration, had a more rapid oxidation rate. Since treatment 1 had high O2 levels (Table 5) as compared to treatments 2 and 4, a higher oxidation rate was observed and CO2 did not have much impact. Contrasts between these treatments, however were not studied. Also, in the absence of O2, a CO2 concentration of 25% or higher will induce similar oxidation rates. A significant difference ($P \leq 0.001$) was observed between treatment 3 and treatments 5 and 6 (contrast 4, Appendix C), suggesting that a difference of approximately 10% O2 concentration could cause a significant increase in lipid oxidation. Such a significant effect was also observed between vacuum and air treatments (contrast 1, Appendix C) and these treatments also had an O2 level differential of approximately 10%.

Ockerman (1980b) suggested a TBA value of 1.0 for maximum shelf-life of meat. After 14 days of storage, treatments 2 and 4 had a TBA value of 0.57 as compared to treatment 3 having a TBA value of 1.05. This suggests treatments with 25% or higher CO2 levels, in the absence of O2, could extend the shelf-life of pork sausages possibly up to 21-28 days. Maximum percent concentration of 5% O2 seems to be the cut-off level in MAP for extending the shelf-life of pork sausages up to 14

days.

Microbial growth in pork sausage

Total aerobic bacterial count:

Means of total bacterial counts (TC) for pre- and post-rigor pork, over 14 days of storage and 6 treatments, indicates pre-rigor sausage had nearly 10*4 colony forming units per gram (CFU/gm) of sausage whereas post-rigor had nearly 10*3 CFU/gm (Table 7). The difference was significant ($P \leq 0.001$, Appendix B). This suggests more favorable growing conditions for bacteria under pre-rigor conditions. These favorable conditions could be the higher pH values of 6.5-6.8, near the neutral range, in pre-rigor pork as compared to 5.7-5.9 in post-rigor pork. Another factor could be higher temperatures in pre-rigor pork as the muscle had been excised shortly after slaughter. Similar findings were reported by Lin et al. (1979) in pork sausage.

The significance levels of contrasts 1 through 5 comparing treatments are given in Appendix C. Treatment 1 (vacuum) had lower ($P \leq 0.001$) counts than treatment 3 (air) (contrast 1). Under treatments of higher O2 levels, treatment 3 (with highest O2 level) had higher ($P \leq 0.001$) counts than treatments 5 (75% N2 + 15% CO2 + 10% O2) and 6 (75% N2 + 20% CO2 + 5% O2) pooled together (contrast 4) (Table 8, Appendix C). Higher O2 levels and lower CO2 levels seem to promote microbial growth.

Significant differences ($P \leq 0.001$) in total bacterial count were found between day 0 and 3 (contrast 6) as well as between day 3 and 7 (contrast 7) but the remaining days of storage did not have a

significant difference in counts (Appendix D). This indicates that the maximal microbial growth took place by day 7 and was followed by the stationary phase, when the growth in bacterial counts was equivalent to the depletion in bacterial counts.

Initial counts, which varied from 10*2 CFU/gm to 10*3 CFU/gm (Figure 5), indicated the presence of bacterial colonies in the meat prior to display. They could have been incorporated into the meat during the processing and handling of pork. Across a 14 day storage period, the lowest values of 10*2 CFU/gm were found in treatments 2 and 4, which had the highest CO2 concentrations, while treatments 3, 5 and 6, with higher O2 levels and lower CO2 levels, showed higher ($P \leq 0.126$) counts at the end of the 14th day. Air treatment with the lowest CO2 level and highest O2 level had the highest counts of 10*6 CFU/gm after 14 days of storage.

The use in MAP of CO2 up to a 25% level, in the absence of O2, can be seen as justifiable for extension of shelf-life. Higher levels of CO2 do not seem to have much additional effectiveness. Clark and Lentz (1969) proposed that a 20% CO2 level was the practical concentration and stated that this level of CO2 was effective at higher temperatures of up to 10C. The inhibitory effectiveness of CO2 on the growth of aerobic bacteria is due to its effect on decarboxylating enzymes such as isocitric and malate dehydrogenase (King and Nagel, 1975). Findings in this study agree with those of Huffman (1974), Huffman et al. (1975) and Shay and Egan (1986) who indicated that CO2 in higher concentrations inhibited the growth of aerobic microorganisms. Huffman et al. (1975) reported that meat stored in N2 had similar counts as meat stored in air, thereby indicating the ineffectiveness of N2 in extending the

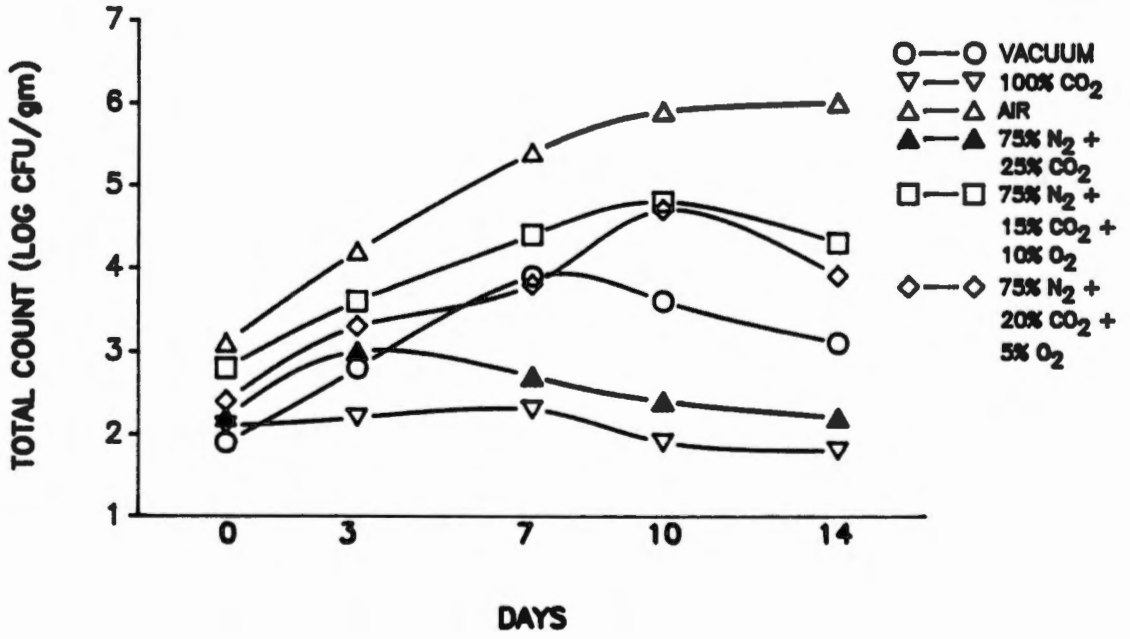

Figure 5. Total bacterial count (Log CFU/gm) of pork sausage stored under six different treatments for up to 14 days at 4C.

shelf-life.

Psychrotrophic bacterial count:

The mean of psychrotrophic bacterial colonies for pre-rigor sausage was found to be 2.0 CFU/gm as compared to 1.8 CFU/gm for post-rigor sausage (Table 7). Though both pre-rigor and post-rigor sausage offer different growth conditions in terms of pH and temperature, at $P \leq 0.001$ level the difference in colony numbers was not significant (Appendix B). Pre-rigor meat with higher temperature has more favorable conditions for bacterial growth, but under low refrigeration temperatures these bacteria may not have proliferated. Thus the bacterial growth was inhibited.

A difference ($P \leq 0.001$) was found between treatments 1 (vacuum) and 3 (air) (contrast 1, appendix C). The air treatment, with higher O2 level, had higher ($P \leq 0.001$) counts than the vacuum treatment, which had lower O2 and higher CO2 levels (Table 8). While differences ($P \leq 0.001$) were not observed between treatment 2 (100% CO2) and 4 (75% N2 + 25% CO2) (contrast 2, Appendix C), differences ($P \leq 0.005$) were found between treatments without oxygen (2 and 4) and treatments with oxygen (3, 5 and 6) (contrast 3). Higher ($P \leq 0.004$) count was found in sausages stored under treatment 3 (with higher O2 level) than treatments 5 and 6 (Table 8) (contrast 4, Appendix C). There was a distinct difference in CFU/gm with treatments 1 (vacuum), 2 (100% CO2) and 4 (75% N2 + 25% CO2) having lower counts and treatments 3 (air), 5 (75% N2 + 15% CO2 + 10% O2) and 6 (75% N2 + 20% CO2 + 5% O2) having higher counts. A contrast statement between these two sets of treatments was not made.

Contrasting storage days 3 and day 7 (contrast 7, Appendix D) indicates a difference ($P \leq 0.001$) in psychrotrophic bacterial count and shows that maximum proliferation of these bacteria, across treatments, took place during that storage period. Packages coming out of frozen storage did not have high counts until day 3 (Table 9). After the 7th day, the effect of gas could have had an impact on the growth in bacterial counts, leading to the stationary or decline phase. Therefore, differences were not ($P \geq 0.001$) observed in contrasts 8 and 9.

The interaction of six treatments and five storage periods was significant ($P \leq 0.001$, Appendix B) and the changes over storage days for psychrotrophic bacterial counts under different treatments are shown in Figure 6. The counts under treatments 1, 2 and 4 remained virtually the same over a 14 day period, but there was an increase of approximately 10*2 CFU/gm in the other treatments. Psychrotrophic counts under the air treatment showed the largest increase, followed by treatments 6 and 5, respectively. The stationary phase of the growth pattern for the counts under treatments 3, 5 and 6 was observed on the 10th day.

The decrease in psychrotrophic counts for sausage stored under treatments 1, 2 and 4 reflects the effect of CO2 on the aerobic microflora. Lower counts under these treatments (1, 2 and 4) at 0 day indicates inhibitory effectiveness of CO2 on the surface micoflora even under frozen storage. Psychrotrophic counts for the vacuum treatment followed the trend of the treatments under 100% and 25% CO2 due to the presence of high CO2 levels (30%). The same was not observed for total bacterial counts under vacuum treatment (Figure 5). Either the high amount of CO2 in the vacuum packages affected psychrotrophs and not

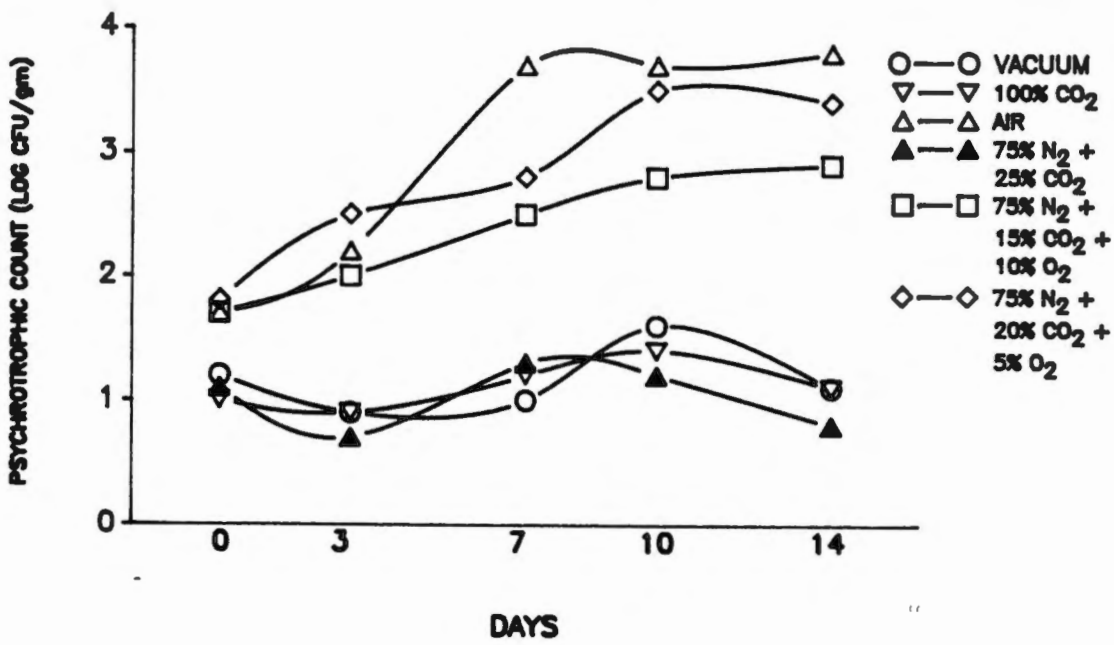

Figure 6. Psychrotrophic bacterial count (Log CFU/gm) of pork sausage stored under six different treatments for up to 14 days at 4C.

mesophilles, or at low temperatures, O2 availability to the bacterial cell was reduced in the presence of high CO2 levels. A significant difference ($P \leq 0.001$) in psychrotrophic bacterial growth was found between the vacuum and air treatments (contrast 1, Appendix C) but no difference was found between the 25% CO2 and 100% CO2 treatments (contrast 2, Appendix C). This study indicates that using CO2 at a 25% level inhibits the growth of psychrotrophic bacteria and extends the shelf-life. Higher levels of CO2 did not seem to have any further inhibitory effect.

Christopher et al. (1980) reported lower psychrotrophic bacterial counts in CO2-containing atmospheres as compared to vacuum treatment. Such a distinction was not observed in this study due to high CO2 levels in the vacuum treatment. As mentioned earlier, the inhibitory effect of CO2 on the aerobic microflora in pork sausages is probably due to the activity of decarboxylating enzymes (King and Nagel, 1975). Enfors and Molin (1978) proposed that CO2 affects the cell membrane fluidity and hence the funtional properties of the bacterial cell.

The effectiveness of O2 and N2 gases in promoting the growth of psychrotrophic bacteria is observed with treatments 3, 5 and 6 (Figure 6). Presence of O2 at more than a 5% level and N2 at more than a 70% level, aided in the growth of these microflora. Since treatment 1 (vacuum) also had higher O2 levels but did not promote growth, the effectiveness of N2 gas in promoting the growth is a valid suggestion. Huffman et al. (1975) reported the microbial counts in steaks held under pure N2 gas were similar to the counts in steaks held under air treatment. The difference in counts between treatments 3 and treatments

5 and 6 was not significant (contrast 4, Appendix C). In this study, since treatments 5 and 6 had over 70% N2 (see Table 6), there is a possibility that psychrotrophic bacteria under these treatments could have behaved similarly to those under air (treatment 3).

Lactic bacterial counts:

Counts for lactic acid producing bacteria (Table 7) over the 14 day period for pre-rigor and post-rigor pork sausage were significantly different ($P \leq 0.001$, Appendix B) and are shown in Figure 7. Pre-rigor samples had higher pH and higher temperature at the time of cutting of meat and provided a more suitable growth medium for lactic acid bacteria as evidenced by higher counts.

Treatment 3 (air) had higher ($P \leq 0.001$) counts than treatment 1 (vacuum) (Table 8) (contrast 1, Appendix C). This was due to air treatment having higher O2 and lower CO2 levels than vacuum treatment. Counts under treatments 3, 5 and 6 (Table 8) were found to be the same ($P=0.941$) (contrast 5, Appendix C), indicating that oxygen levels of the range of 5% to 20% did not lead to differences in lactic acid bacterial counts.

At all storage periods, except 0 day, pre-rigor samples had higher counts (Appendix B). Contrasts 6 and 7 indicate differences ($P \leq 0.001$) in lactic bacterial counts between day 0 and day 3 as well as between day 3 and day 7 (Appendix D). The logarithmic phase of the bacterial growth started on day 0, due to favorable temperature and pH. No difference ($P \geq 0.001$) (contrast 8) was observed between counts on day 7 and day 10 due to unfavorable growing conditions, high concentration of CO2 being

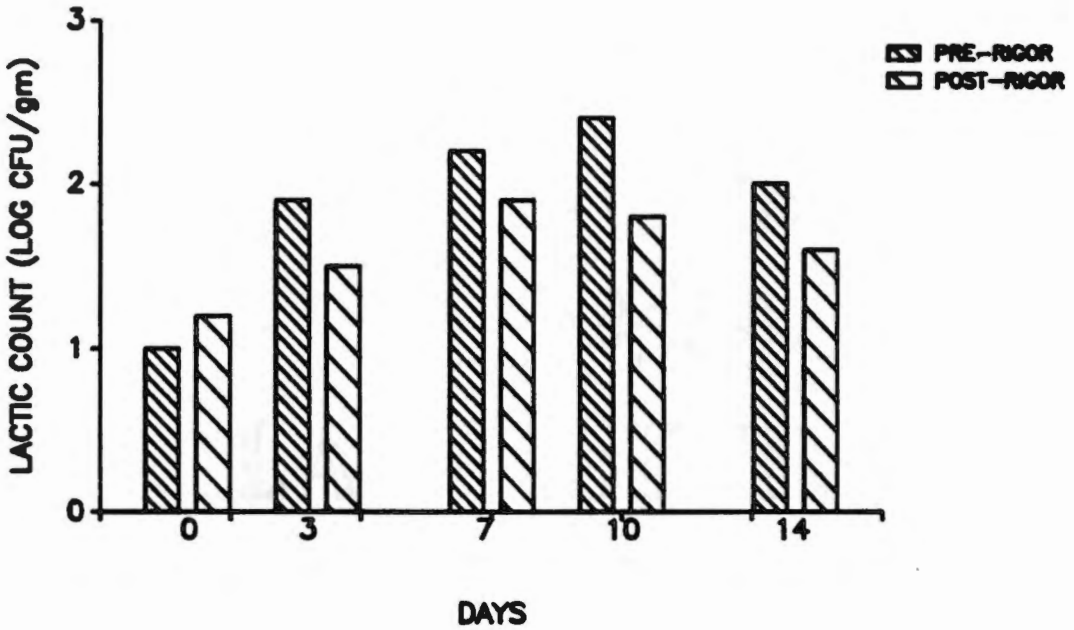

Figure 7. Lactic acid bacterial count (Log CFU/gm) of pre-rigor and post-rigor pork sausages stored for up to 14 days at 4C.

the main factor responsible. Lactic acid bacterial count declined after day 10, but contrast 9 (Appendix D) indicates the decrease in count as significant at $P \leq 0.003$ level.

Figure 8 illustrates the trends followed by lactic acid bacterial counts with each packaging treatment over the 14 day storage period. At 0 day, all treatments had higher counts as compared to the vacuum treatment. The same was seen on the 3rd day. On the 7th day, the counts for treatments 2 and 4 were the lowest, whereas the counts for other treatments were higher. Christopher et al. (1979) found higher counts of bacteria for beef roasts stored under vacuum treatment as compared with six other treatments. This study on pork sausage found lower counts in vacuum and high CO2 treatments as compared with treatments having the higher levels of O2. Like psychrotrophs, lactic acid bacteria are inhibited with presence of over 25% CO2 and the absence of O2. Treatments 1, 2 and 4 can therefore be recommended for extending the shelf-life of pork sausages, in terms of lactic acid bacteria, to over 14 days of storage.

Roth and Clark (1975) reported that final counts of lactic acid bacteria in beef were similar to samples held in air and vacuum packages. Shaw and Nicol (1969) stated that lactic acid bacteria are not affected by either CO2 or O2. This study on pork sausages does not agree with either study.

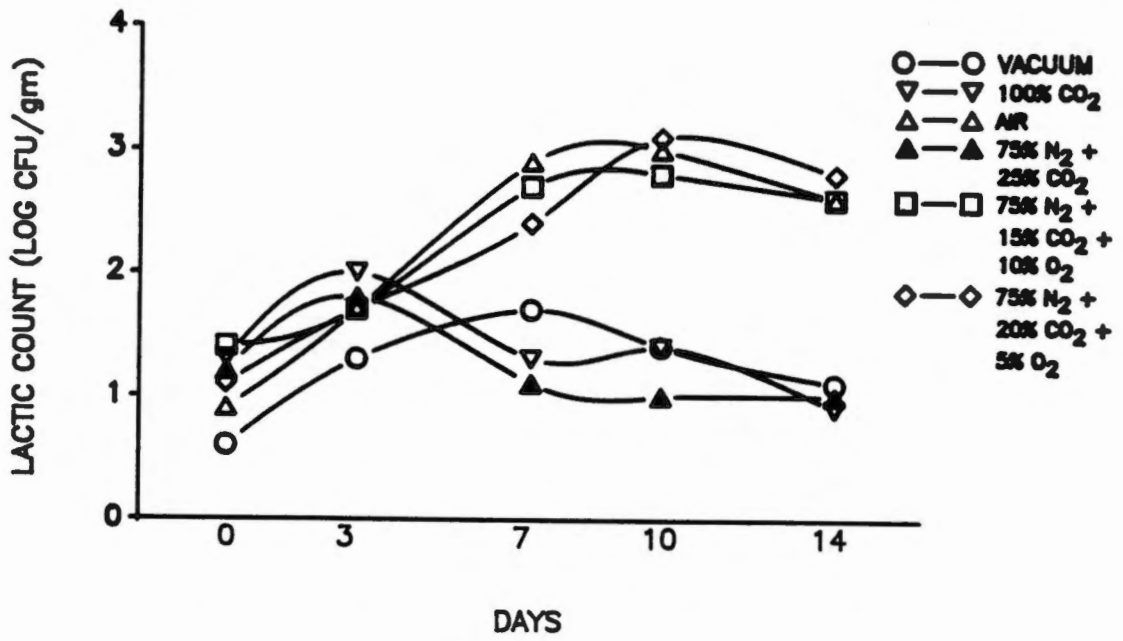

Figure 8. Lactic acid bacterial count (Log CFU/gm) of pork sausages stored under six different treatments for up to 14 days at 4C.

Objective color analysis

Relative pigment concentration:

The mean OMb% of pre-rigor (24.4%) sausage was higher ($P \leq 0.001$) than that of post-rigor (20.4%) sausage (Table 7). Pre-rigor muscle, when intact, has a higher degree of oxygen utilization by the muscle enzymatic system (Huffman, 1980) and the oxygen reserves in the tissue are used for mitochondrial respiration (Cornforth and Egbert, 1985), thus causing myoglobin (Mb) to remain in a reduced state and appear darker. But in sausages, the muscle cell is broken due to the grinding procedure thereby enabling Mb to be oxygenated readily to form OMb and/or oxidized to form metmyoglobin (MMb). Pisula (1981) stated that there was an increased incidence of pigment oxidation reactions in pre-rigor meat.

One hypothesis for pre-rigor sausages having higher OMb% could have been suggested by Watts (1954) who found that unsaturated fatty acids, after oxidation, aided in accelerating the destruction of heme pigments. Since one found higher TBA values in pre-rigor meat (Figure 3), one could suggest lipid oxidation promoted pigment degradation and thus enabled pre-rigor meat to a have higher OMb concentration. Another hypothesis deals with microorganisms promoting oxygenation of myoglobin pigment. Richert et al. (1957) found that inoculating ground pork with Achromobacter bacteria improved the color of meat. This leads to the speculation that higher microbial population could lead to an improved color in meat. In this study, pre-rigor pork sausage had higher pH values thereby enabling a greater bacterial population. Higher microbial count means greater O2 utilization by the aerobic microflora on the

surface of the sausage patties which could have led to the formation of higher OMb% in pre-rigor pork sausage.

Contrasts 1, 4 and 5 (Appendix C) were significant ($P \leq 0.001$) for treatment differences. Treatment 1 (vacuum) had lower ($P \leq 0.001$) percent OMb than treatment 3 (air) (contrast 1). The OMb percentage was higher ($P \leq 0.001$) in treatment 3 than in treatments 5 (75% N2 + 15% CO2 + 10% O2) and 6 (75% N2 + 20% CO2 + 5% O2) which contained less O2 (contrast 4) and treatment 5 had higher ($P \leq 0.001$) percent OMb than treatment 6 (contrast 5). Air treatment had 20% O2 whereas treatments 5 and 6 had 10% and 5% O2, respectively. Thus, a difference of 10% O2 led to a significant difference in OMb% between the air treatment and treatments 5 and 6 (contrast 4, Appendix C). The percent OMb under the vacuum treatment was lower ($P \leq 0.001$) than the air treatment due to the CO2 level being higher and O2 level being lower in the vacuum treatment. In general, the level of OMb in each treatment was dependent upon the O2 level in the treatments (see Table 4). The more abundant the O2 in the treatment across storage, the higher the percent OMb. Treatments 2 (100% CO2) and 4 (75% N2 + 25% CO2) had lowest levels of OMb and the lowest levels of O2. Treatments 3, 5 and 6 had the highest levels of O2 and percent OMb (Table 8).

Percent OMb across all storage days was found to be significant ($P \leq 0.001$, Appendix D). There was an increase ($P \leq 0.001$) in percent OMb on day 3, followed by a decrease ($P \leq 0.001$) on day 7, 10 and 14 (Table 9, Appendix D) which points out the sensitivity of the pigment to degradation. Oxymyoglobin (OMb) concentration was higher on the 3rd day for both pre- and post-rigor samples (Figure 9). Similar OMb% were found

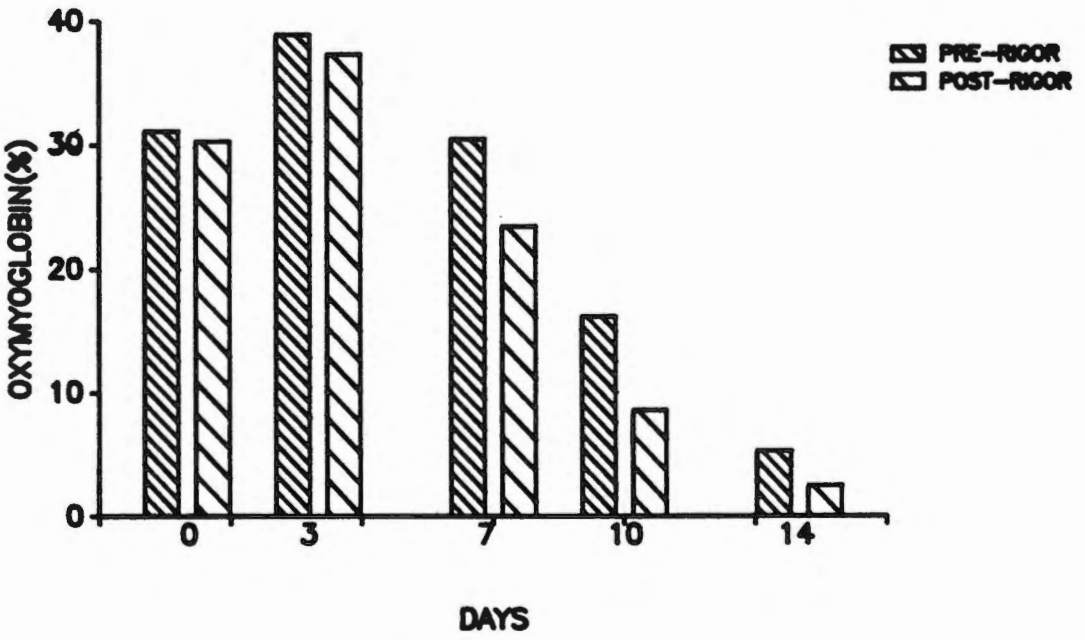

Figure 9. Percent oxymyoglobin in pre-rigor and post-rigor pork sausages stored for up to 14 days at 4C.

on day 0 and day 3 between the two rigors and in pre-rigor sausages, the original bloom, in terms of OMb%, was found to remain until the 7th day. Percent OMb in pre-rigor pork sausage was consistently higher than post-rigor pork sausage.

Percent OMb for the packaging treatments compared over the 14 days of storage was significantly different ($P \leq 0.001$, Appendix C) (Figure 10). No statistical analysis was done to determine differences between treatments on specific storage days. Treatment 2 (with lowest O2%) had the lowest initial percent OMb and treatment 3 (with highest O2%) had the highest initial percent OMb. On the 14th day, treatments 2 and 4 had the lowest values followed by treatment 1 with treatments 3, 5 and 6 (treatments with O2) having the highest percent OMb (Table 8). Thus, depending on the O2 concentration, percent OMb varied with time. OMb concentration for all treatments increased on the 3rd day followed by a decline over the rest of the storage period. The vacuum treatment had a high O2 concentration which enabled the patties to have similar OMb% values as patties under treatment 6, on the 3rd day. A higher concentration of CO2 in the package atmosphere caused a rapid decline in OMb% after the 3rd day.

Percent myoglobin in pre-rigor sausage was found to be 39.2% and in post-rigor was 38.4%. Difference in percent Mb between the rigors was not significant ($P \geq 0.001$, appendix B) (Table 7).

Treatments 2 (100% CO2) and 4 (75% N2 + 25% CO2) were found to have different ($P \leq 0.001$) percent Mb than treatments 3 (air), 5 (75% N2 + 15% CO2 + 10% O2) and 6 (75% N2 + 20% CO2 + 5% O2) (contrast 3, appendix C). Treatments 3, 5 and 6 had higher O2 levels which caused a rapid

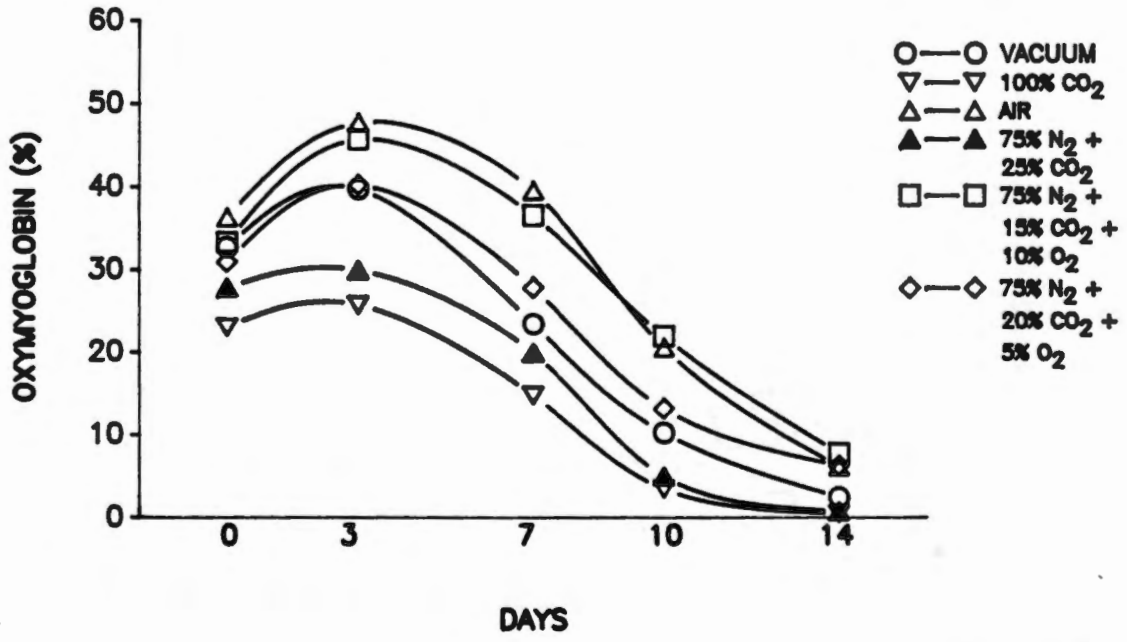

Figure 10. Percent oxymyoglobin in pork sausages stored under six different treatments for up to 14 days at 4C.

formation of OMb from Mb, whereas treatments 2 and 4 with higher CO2 levels caused oxidation reaction converting Mb to MMb, thereby leading to different ($P \leq 0.001$) percent Mb. Contrasts 1, 2, 4 and 5 were found to be non-significant ($P \geq 0.001$).

Contrasts 6 (between day 0 and day 3) and 8 (between day 7 and day 10) showed differences ($P \leq 0.001$) between the respective days of storage (Appendix D). Myoglobin concentration for all treatments decreased drastically after day 0 (Figure 11) due to the rapid formation of OMb causing a reduction in Mb%. Percent Mb in the sausage leveled off between the 3rd and 7th days, probably due to the reversible nature of the reaction converting Mb to OMb and to some extent the oxidation of Mb to MMb. Metmyoglobin gets converted to Mb until a 40% MMb level is reached, which seems to occur around the 7th to 10th day (Figure 12). Until the 7th day, Mb concentration (Figure 11) for the air treatment was the lowest among all treatments, but on the 14th day, the treatment reached the highest level. Treatments under lowest O2 levels (2 and 4) had the lowest concentration of Mb and were significantly different ($P \leq 0.001$) from treatments with higher O2 levels (3, 5, and 6). Contrary findings were observed on the 3rd day when treatments 2 and 4 had the highest Mb% levels as compared to other treatments. Also, the contrast analysis of treatments 2 and 4 with treatments 3, 5 and 6 (contrast 3, Appendix C) shows significant difference ($P \leq 0.001$). This could be due to the absence of oxygen in treatments 2 and 4, which did not allow Mb to be oxygenated to OMb, but oxidized Mb to MMb (as seen in Figure 12). High pigment oxygenation is also seen under high O2 concentrations in the rapid OMb formation under the air treatment, as seen in Figure 10.

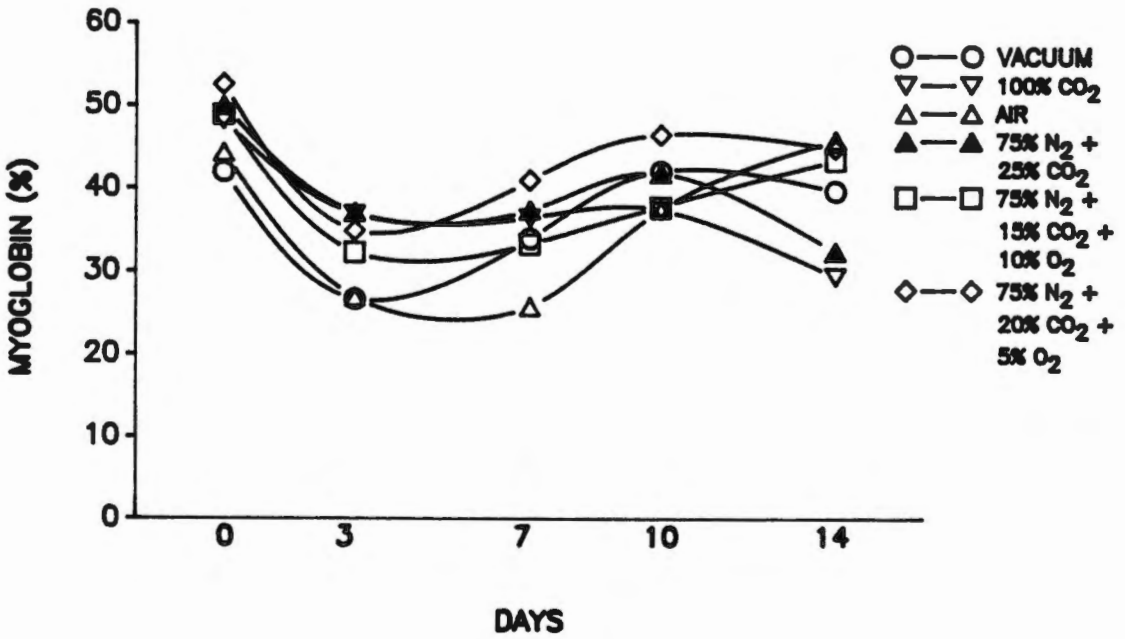

Figure 11. Percent myoglobin in pork sausages stored under six different treatments for up to 14 days at 4C.

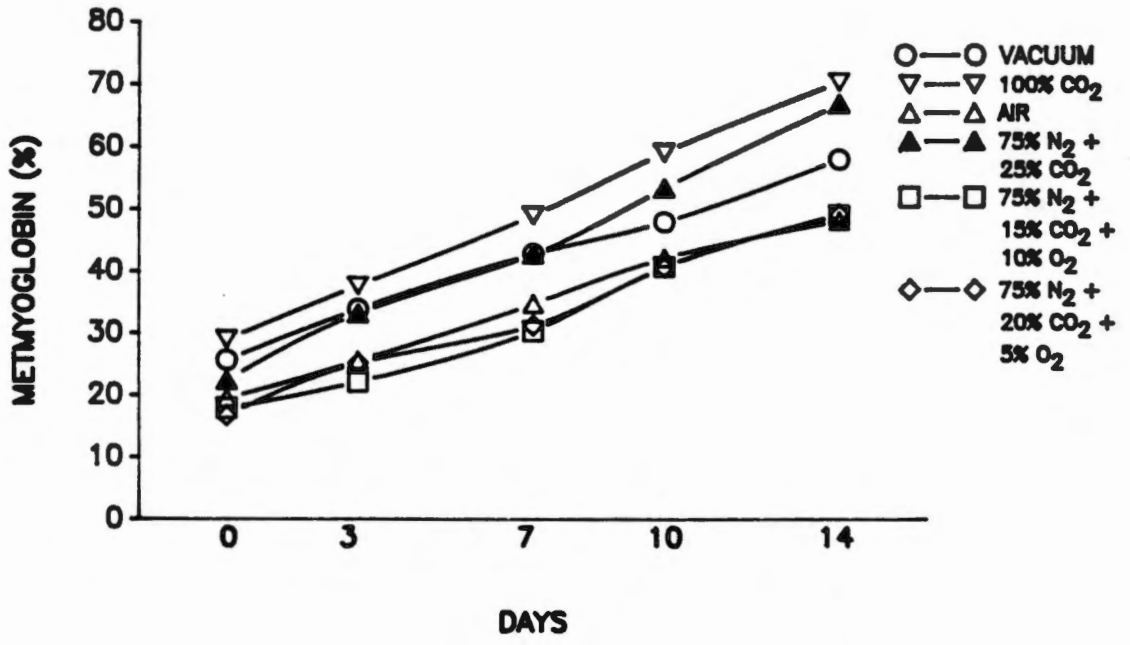

Figure 12. Percent metmyoglobin in pork sausages stored under six different treatments for up to 14 days at 4C.

Formation of MMb in treatments under high O2 concentrations is slower and until 40% MMb is reached, most of OMb and some MMb gets converted to Mb. But under high CO2 treatments, there is a rapid formation of MMb which is not reversible, leading to a rapid decline in Mb and OMb levels.

A significant difference ($P \leq 0.001$, Appendix B) in MMb% was found between pre-rigor (36.4%) and post-rigor (41.2%) sausages indicating a more rapid pigment degradation in post-rigor sausage (Table 8). Contrast statements indicate significant differences ($P \leq 0.001$) between the vacuum (with 41.6% MMb) and air (34% MMb) treatments (contrast 1) as well as between treatments with oxygen (3, 5 & 6) (with 34%, 32% and 32.5% MMb) and without oxygen (2 & 4) (with 49% and 43.7% MMb) (contrast 3). A difference (P=0.003) in MMb% was also found between treatments 2 and 4. Higher O2 levels in the gas mixtures helps in keeping lower MMb pigment level in pork sausages and thus prolongs the color shelf-life. The mean values for storage days (Table 9) across all the treatments and both the rigors gave values of 21.7% for day 0, 30% for day 3, 38.4% for day 7, 47.3% for day 10 and 56.9% for day 14. A significant difference ($P \leq 0.001$) was observed for the contrasts 6 (between day 0 and day 3), 7 (between day 3 and day 7), 8 (between day 7 and day 10) and 9 (between day 10 and day 14) as shown in appendix D. The rate of pigment degradation is rapid and is caused by rapid oxidation of Mb and OMb pigments.

Izumimoto et al. (1985) reported that in ground meat held under a CO2 atmosphere, color fading was observed and the percentage of metmyoglobin formed increased with storage. They reported a value of

over 90% MMb after 16 days of storage in a 40% CO2 atmosphere. This study found a level of 65% to 70% after 14 days of storage under 100% CO2, in the absence of oxygen. Ledward (1970) and Uebersax et al. (1977, 1978) also reported color deterioration of meat stored under high CO2 concentrations. Hood (1980) stated that after a certain level of MMb formation is reached, the oxidation reaction of the pigment becomes irreversible and the meat remains discolored. This level was previously reported to be 40% to 50% (Dawn et al.,1971) for beef and Greene et al. (1971) proposed this level to be 40% for pork. This study indicates that the 40% level of MMb, an indicator of maximum color shelf-life, was reached under high O2 treatments (3, 5 and 6) on the 10th day. Under the 100% CO2 (treatment 2), 3 days and under 25% CO2 (treatment 4) and vacuum (treatment 1), a color shelf-life of around 5 days was obtained. But, a look at Figure 10 for air (treatment 3) and 10% O2 (treatment 5) indicates that the original bloom, in terms of 20% OMb, was retained until the 7th day. Even on the 10th day, around 20% OMb was found in treatments 5 (with 10% O2) and 3 (with 20% O2), indicating a fresh color. Therefore, from the results of this study, the author proposes that 50% MMb be a cut-off value for maximum shelf-life in terms of color pigment concentration for ground pork sausages. An atmosphere of over 20% O2 is recommended for extending the shelf-life in terms of color pigment concentration.

Hunter lab analysis:

Higher ($P \leq 0.001$) Hunter color 'L' values were found for pre-rigor than post-rigor sausages (Table 7, Appendix B). This indicates more

lightness in pre-rigor sausages which could be due to greater oxymyoglobin (OMb) concentration which gives a light pinkish-red color in pork, as compared to darker colors of myoglobin (Mb) (purplish-red) and metmyoglobin (MMb) (brown).

Higher ($P \leq 0.001$) 'L' values under air (treatment 3) than vacuum (treatment 1) could be due to higher OMb% and lower Mb% and MMb% in pork sausages under air treatment (contrast 1, Appendix C). Lower ($P \leq 0.001$) values were found for treatments 2 (100% CO2) and 4 (75% N2 + 25% CO2) than treatments 3 (air), 5 (75% N2 + 15% CO2 + 10% O2) and 6 (75% N2 + 20% CO2 + 5% O2) (contrast 3). Higher O2 levels in treatments 3, 5 and 6 could have led to lower Mb% and MMb% and causing lower'L' values.

Significant differences ($P \leq 0.001$) were observed for the lightness values for all contrasts for days of storage, except between day 0 and day 3 (contrast 6). Low levels of MMb% and a reduction in Mb% until day 3 could have caused 'L' value to remain the same (48.3 on day 0 and 48.6 on day 3). After the 3rd day, the decline in OMb% and increase in MMb% and Mb% could have caused the rapid decline in lightness values, thereby causing differences in 'L' values (contrasts 7, 8 and 9, Appendix D).

Figure 13 illustrates the changes in Hunter 'L' values over the 14 day period for the packaging treatments. Treatments 2 and 4 had lower Hunter 'L' values when contrasted (Appendix C) with treatments 3, 5 and 6, and treatment 1 (vacuum) had lower Hunter 'L' values ($P \leq 0.001$) when contrasted with treatment 3 (air). Sausages under treatments 2 and 4 had a steady decrease in values throughout the storage period due to a low percent OMb (see Figure 10). Calkins et al. (1986) studied lightness in pork chops and found Hunter 'L' values of around 41.0 on day 0 under the

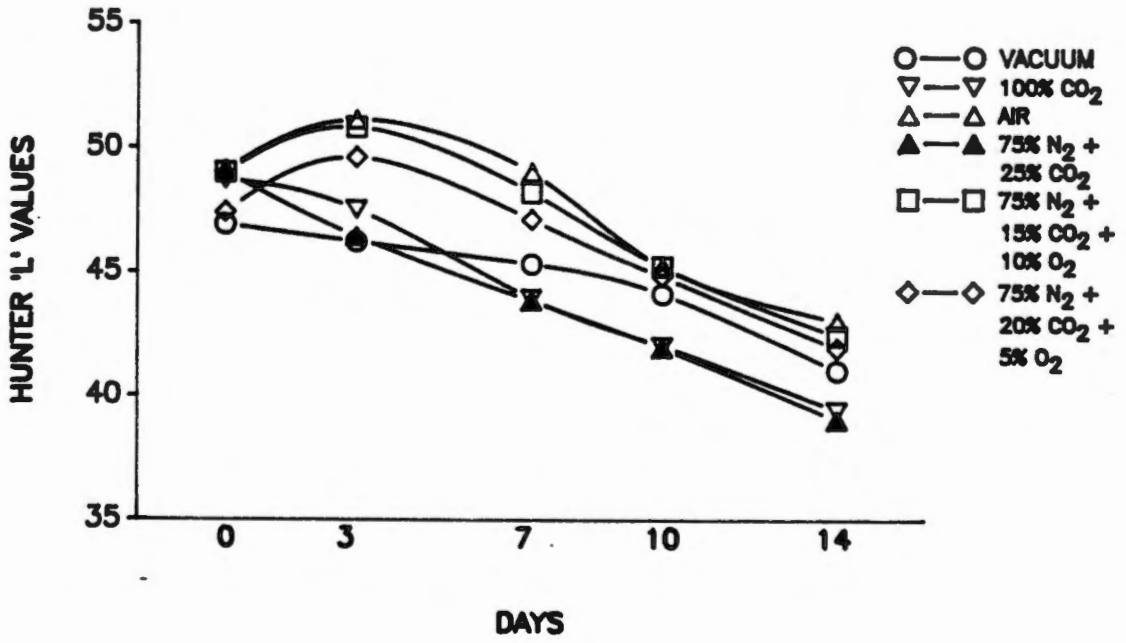

Figure 13. Hunter color 'L' values of pork sausages stored under six six different treatments for up to 14 days at 4C.

air treatment in retail display. The photograph of a 'normal' patty showed an 'L' value of 44.5. This study on pork sausage provides values in excess of 47.0 and the air packaging treatment had a 49.1 value. Higher values in this study could be due to high fat (see Table 2) content in the sausage which gives a lighter appearance.

The mean redness values of sausage patties, measured by Hunter 'a' values were found to be much higher ($P \leq 0.001$) in pre-rigor patties as compared to post-rigor patties (Table 7, appendix B), over the 14 days of storage indicating the desirability of using pre-rigor pork for sausage manufacturing (Figure 14). The difference between the Hunter 'a' values for pre- and post-rigor samples decreased over the 14 day period. Extending the storage period beyond 14 days may eliminate the benefit of utilizing pre-rigor pork. Pre-rigor patties had a Hunter 'a' value of 4.8 on the 14th day of storage whereas a 4.2 value was attained for post-rigor patties (Figure 14). This again proved pre-rigor pork to have longer shelf-life in terms of Hunter 'a' value over post-rigor pork. A significant difference ($P \leq 0.001$) was found when treatments 2 and 4, with Hunter 'a' values of 4.8 and 5.1, respectively, were contrasted with treatments 3, 5 and 6, with Hunter 'a' values of 6.5, 7.0 and 6.8, respectively (contrast 3). Differences in oxygen level between the two sets of treatments led to differences in color which was detected by the colorimeter. Other contrasts did not show significant differences at ($P > 0.001$).

The mean Hunter 'a' values for days of storage were significant ($P \leq 0.001$, Appendix B). These values were found to be 7.5 (day 0), 7.2

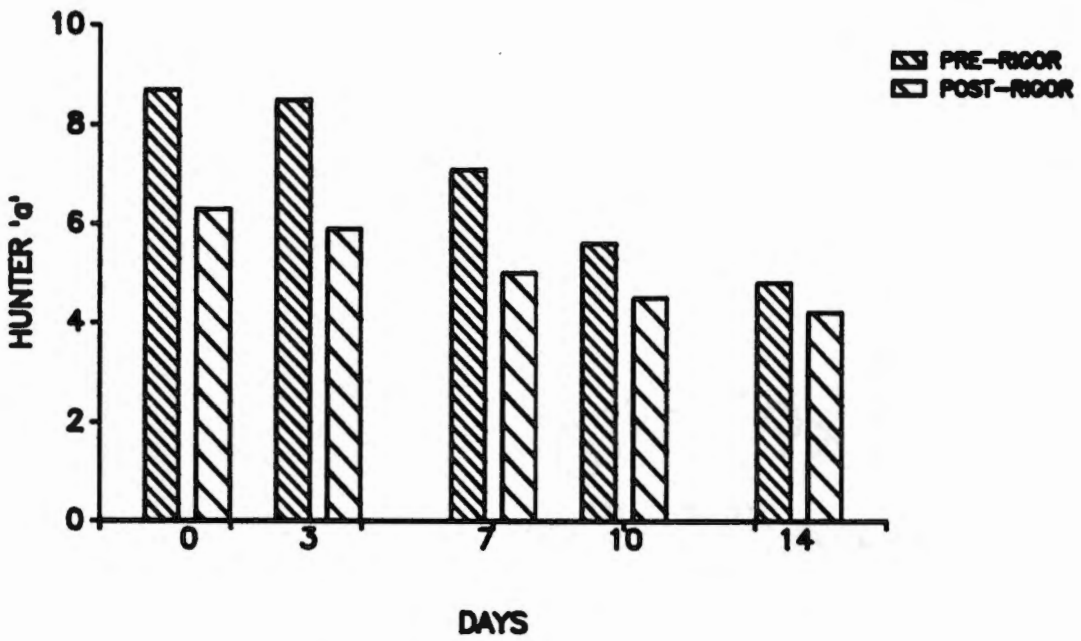

Figure 14. Hunter color 'a' values for pre-rigor and post-rigor pork sausages stored for up to 14 days at 4C.

(day 3), 6.2 (day 7), 5.0 (day 10) and 4.5 (day 14) (Appendix D). Significant difference ($P \leq 0.001$) in Hunter 'a' values were observed in contrasts 7 (between day 3 and day 7), 8 (between day 7 and day 10) and 9 (between day 10 and day 14) whereas in contrast 6, the difference between day 0 and day 3 values, were not significant ($P > 0.001$). This is in accordance with the OMb% (Figure 10), which increased slightly on the 3rd day then decreased. Days 0 and 3 had the greatest redness (Table 9) which indicates that redness can persist until the 3rd day. OMb levels increased on the 3rd day which should have led to higher Hunter 'a' values but there was a corresponding increase in MMb too (see Figure 12). On the following days, the OMb levels declined and there was a rise in MMb levels leading to a decline in redness.

Calkins et al. (1986) found the initial Hunter 'a' values to be around 7.0 to 8.0 for pork chops in retail display. The values peaked on the 3rd day and on the following days a decline was observed. The researchers did not indicate the rigor status of the meat. The values obtained in this study were much lower in post-rigor pork sausages while pre-rigor values were comparable. One would have expected higher values in this study as compared with the ones found by Calkins et al. (1986) since sausage, being ground meat, would have more redness. As indicated by the researchers, the values obtained would depend on the lighting used in the study.

Values for yellowness, measured by Hunter 'b' values, were higher ($P \leq 0.001$) for pre-rigor than post-rigor pork sausage patties at 10.7 and 9.9, respectively (Table 7, Appendix B). The values were much higher than those found by Calkins et al. (1986). This could be due to high

surface fat content (see Table 2) in sausages as compared to the lean surface of pork chops. Lower ($P \leq 0.001$) values were found for treatments 2 (100% CO2) and 4 (75% N2 + 25% CO2) as compared to treatments 3 (air), 5 (75% N2 + 15% CO2 + 10% O2) and 6 (75% N2 + 20% CO2 + 5% O2) (contrast 3, Appendix C). Higher O2 levels in these treatments (3, 5 and 6) caused lipid oxidation to take place at a higher rate and thereby may have caused a greater yellowish tinge. Yellowness increased on day 3 and then declined on day 7, 10 and 14 (Table 9). But contrasts 6 (between day 0 and day 3), 7 (between day 3 and day 7), 8 (between day 7 and day 10) and 9 (between day 10 and day 14) did not indicate differences ($P > 0.001$, Appendix D).

Subjective evaluation

Color evaluation:

A 150 mm scale was used to evaluate the color of the sausage patties. The middle of the scale represented the color of a 'normal' patty. The left half of the scale represented colors lighter than normal and the right half represented colors darker than normal. None of the panelists (n=10) indicated the patty color to be on the light side of the scale. All of the panelists scored the patties on a scale of normal to dark colored (0 to 75 mm). A high value for a patty indicated a darker color.

Sensory panelists scored pre-rigor and post-rigor patties darker than the normal patty over the storage times but the pre-rigor patties showed less ($P \leq 0.001$) darkening than the post-rigor patties. In addition, panelists scored the patties darker ($P \leq 0.001$) with each

successive storage time.

Figure 15 illustrates the color values for pre-rigor and post-rigor sausage patties with different treatments. All treatments, except 100% CO2, had the patties made from post-rigor pork scored darker than pre-rigor pork. A mean value of 31.8 mm for pre-rigor and 38.1 mm for post-rigor pork was found (Table 7). Lower values of redness as well as lower lightness values indicate post-rigor sausage had a darker color ($P \leq 0.001$).

Contrast 3 (Appendix C) and Table 8 show the panelists judged patties of high O2 treatments (3, 5 and 6) to have lower ($P \leq 0.001$) scores and therefore be more 'normal' in color than patties under treatments 100% (2) and 25% (4) CO2. The panelists did not differentiate between treatments 3, 5 and 6 in terms of color which probably indicates that the experiment should have been designed with a bigger differential between the treatments, in terms of relative O2 and CO2 percentages. Contrasting vacuum and air treatments (contrast 1) (mean values of 33.5mm and 27.1mm, respectively), a significant difference ($P \leq 0.001$) between the two treatments was detected by the panelists. The panelists also detected a significant difference ($P \leq 0.001$) between treatments with (3, 5 and 6) and without (2 and 4) oxygen, on the basis of color of sausage patties (contrast 3, appendix C). Treatments 2, 4, 5 and 6 had color scores (Table 8) of 49.6mm, 49.8mm, 24.1mm and 25.5mm, respectively.

The panelists scored sausage patties darker in color over the 14 day storage period (contrasts 6 through 9, appendix D) (Figure 16). Higher ($P \leq 0.001$) O day scores (27.4mm) as compared to lower 3rd day

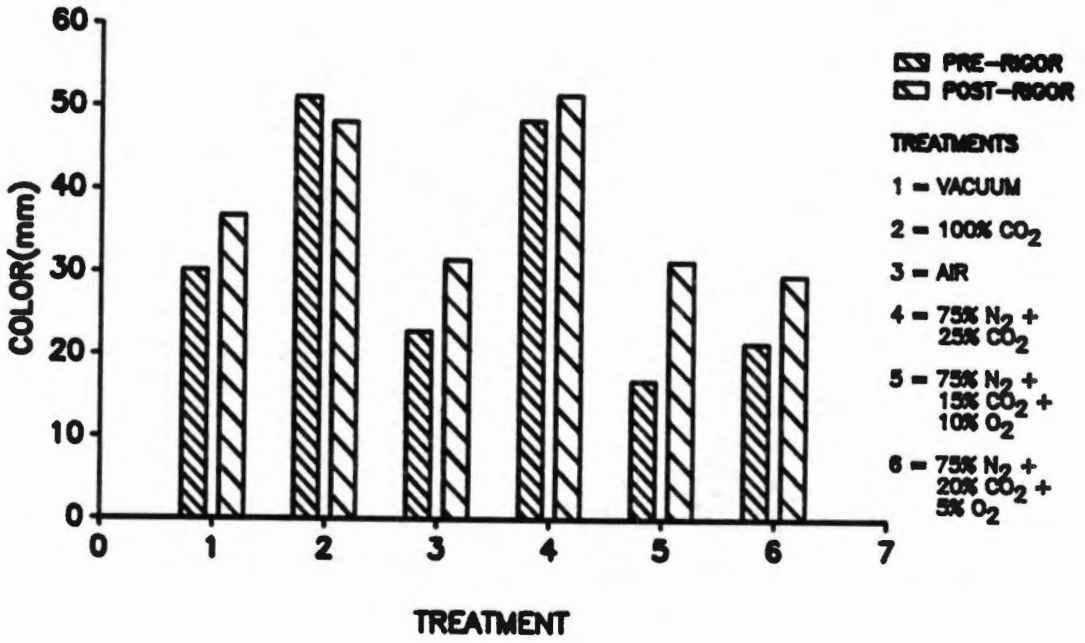

Figure 15. Subjective color scores of pre-rigor and post-rigor pork sausages stored under six different treatments at 4C.

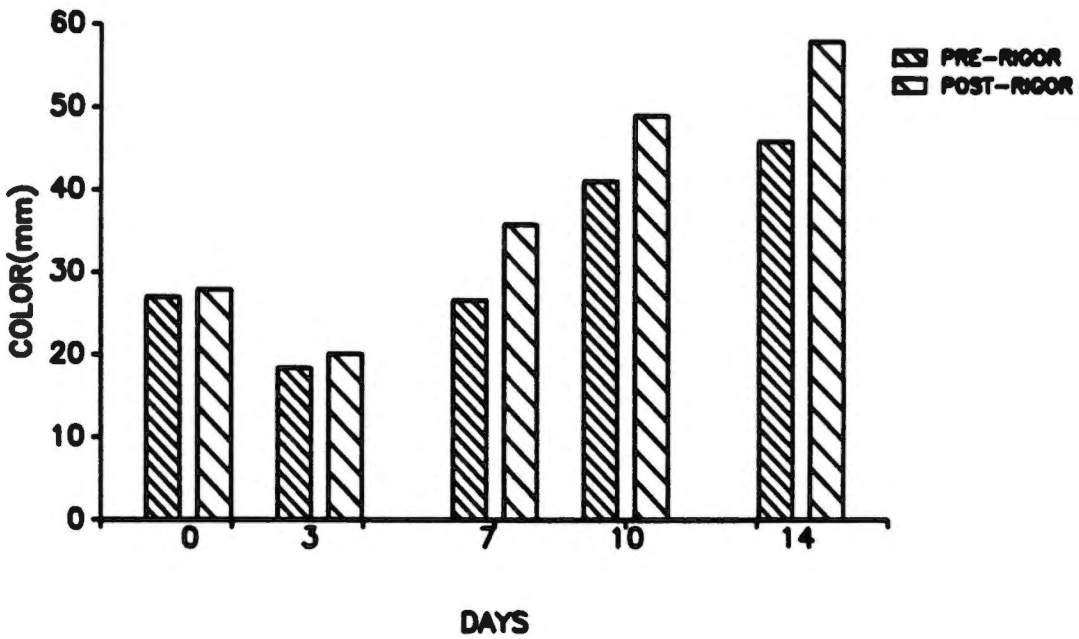

Figure 16. Subjective color scores of pre-rigor and post-rigor pork sausage stored for up to 14 days at 4C.

scores (19.3mm) could be due to factors such as lighting, wrinkles on the overwrap film and frost accumulation on day 0. More 'normal' scores of sausage patties on day 3 as compared to day 0 (contrast 6, appendix D) is due to higher OMb% and Hunter 'L' and 'a' values on day 3. Perhaps, immediately after the frozen period, the bloom time of 20 minutes was not adequate and the patty did not attain a consistent bloom.

Color evaluation scores throughout the storage period increased as a result of increased MMb concentration on the sausage patty surface and the loss of Mb reducing activity (Figure 17). From day 0 to day 3 storage, there was an increase in OMb% but the panelists rated the patties on day 3 to be darker than day 0. The difference in the color score was found to be significant ($P \leq 0.001$). Perhaps the use of a photograph of a 'normal' patty needs to be questioned. The glossiness of the photograph could make the patty in the picture look redder and affect the scoring by the panelists. Use of fresh sausage patty, from day 0 and allowed to bloom for 1 hour, could be an alternative to the use of a photograph for a normal sausage color.

Surface discoloration:

Pre-rigor patties were judged by the panelists to have less ($P \leq 0.001$) surface discoloration than post-rigor patties (Table 7, appendix B). Mean surface discoloration values of 39.8mm and 50.4mm for pre-rigor and post-rigor patties, repectively, were found.

The trend exhibited by the surface discoloration scores (Figure 18) was similar to the subjective color scores. Patties were rated

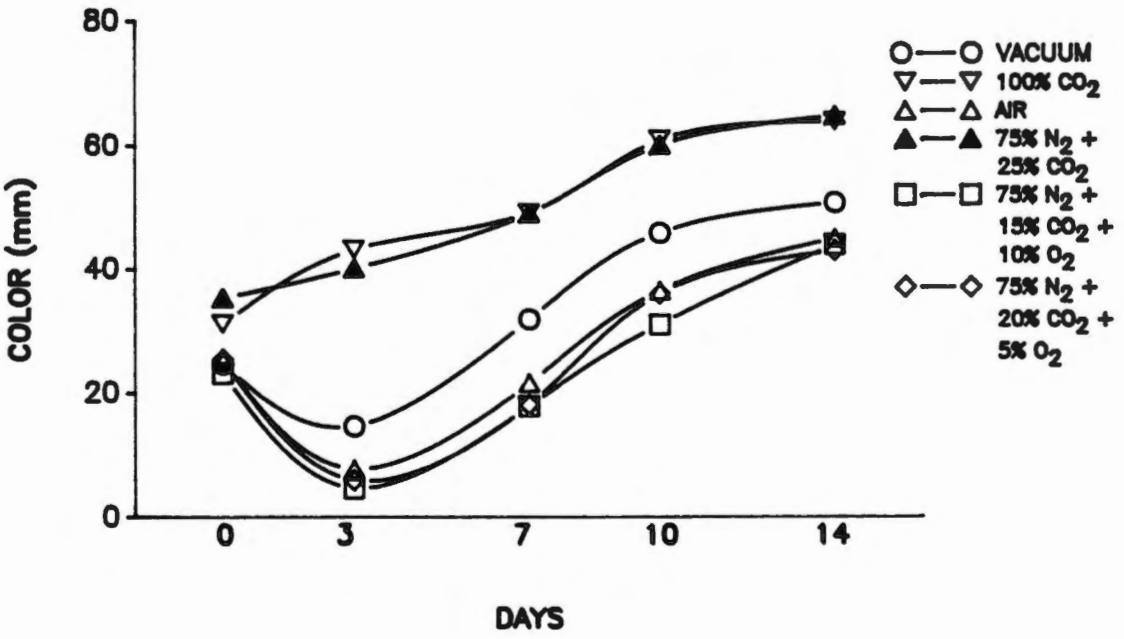

Figure 17. Subjective color scores of pork sausages stored under six different treatments for up to 14 days at 4C.

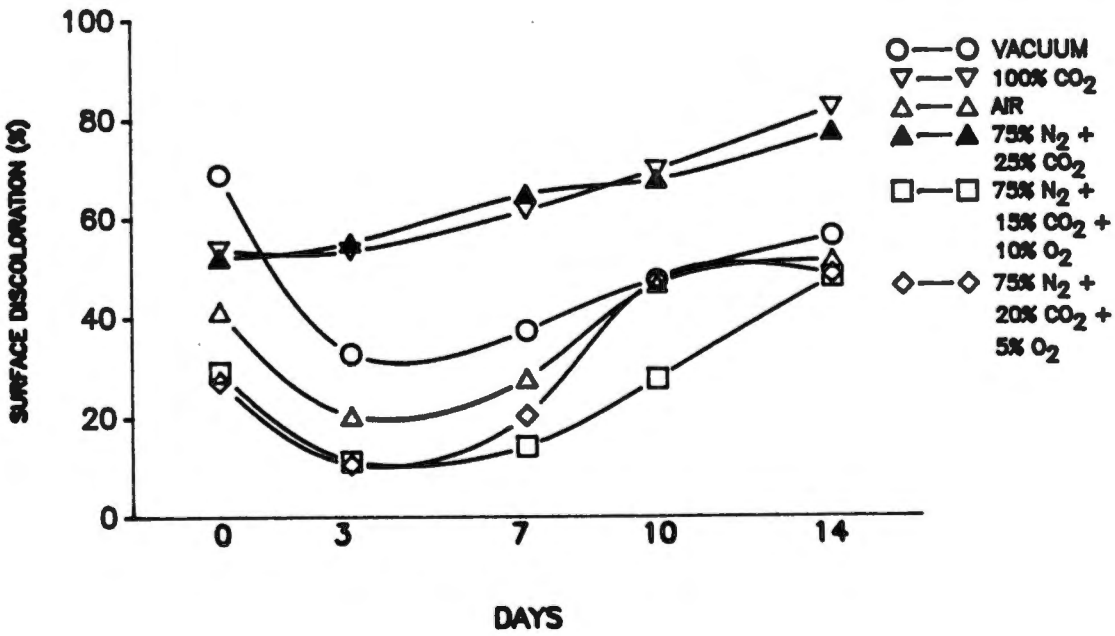

Figure 18. Subjective surface discoloration scores of pork sausages stored under six different treatments for up to 14 days at 4C.

higher in surface discoloration on day 0 for all treatments probably due to the frozen condition of the patty as well as the frost accumulation on the patty surface. Vacuum packaged patties were judged to be 70% discolored on the surface on 0 day. This was probably due to the dark purple colored Mb remaining in the reduced form. Vacuum packaged patties showed less discoloration ($P \leq 0.001$) when contrasted with air treatment patties. Patties from treatments with oxygen (3, 5 and 6) were judged to have less ($P \leq 0.001$) surface discoloration than patties from treatments without oxygen (2 and 4). The panelists detected a significant difference between air treatment and treatments with lower oxygen levels, whereas when contrasting treatments with lower O2 levels (treatments 5 and 6), the difference was not detected ($P \leq 0.001$). This indicates the ability of the panelists to detect differences in surface discoloration between treatments with 10% O2 level differential but they could not detect differences with a 5% O2 level differential.

Significant differences ($P \leq 0.001$) in surface discoloration on patties were found between the storage days (contrasts 6, 7, 8 and 9, appendix D). The panelists also indicated a difference ($P \leq 0.001$) in surface discoloration across all storage days which agrees with the high ($P \leq 0.001$) percent metmyoglobin formation on the patties over 14 storage days (Appendix D). Due to an increase in MMb% on days 3, 7, 10 and 14, the panelists detected an increase ($P \leq 0.001$) in surface discoloration on the respective days. Contrast 6 through 9 were therefore found to be significant ($P \leq 0.001$).

Seideman et al. (1979) reported surface discoloration of beef roasts stored under high O2-containing atmospheres (above 50%) to be

significantly higher than vacuum packaged roasts after 7 and 14 days of storage. Gas analysis during this period was not given. Panelists, in this study, rated patties under treatments with up to 20% initial O2 to have less surface discoloration than vacuum packaged sausages. Since increasing the O2 concentration increases the MMb%, O2 concentration of 50% and above could induce faster surface discoloration than under vacuum treatment.

While treatments with high O2 levels had less surface discoloration, the same treatments induced higher TBA values and higher microbial counts, than the treatments with high CO2 levels. Hence one can conclude that the change in bacterial counts and lipid oxidation does not seem to be related to the changes in surface discoloration.

CHAPTER 5

CONCLUSION

The objectives of this study were to determine the effect of six packaging treatments - vacuum, 100% CO2, air, 75% N2 + 25% CO2, 75% N2 + 15% CO2 + 10% O2 and 75% N2 + 20% CO2 + 5% O2 on the shelf-life of sausage patties made from pre-rigor and post-rigor pork. Shelf-life of the sausage patties was studied over a 14 day storage period by evaluating lipid oxidation, microbial growth and color (objective and subjective).

Higher pH for pre-rigor sausages as compared to post-rigor sausages was found due to lactic acid build up in post-rigor meat leading to a lowering of pH. Lipid oxidation took place more vigorously in pre-rigor sausages which was contrary to other findings. Higher pH along with higher initial count (due to higher meat temperatures at the time of cutting) enabled pre-rigor sausages to have higher total and lactic acid bacterial counts as compared to post-rigor sausage. At low temperatures, no differences were found between psychrotrophic counts on pre- and post-rigor sausage.

Higher OMb% was found in pre-rigor sausages over all storage days which also led to higher Hunter 'L' values. This suggests the role of microbial action and lipid oxidation in the oxygenation of the myoglobin pigment. No differences were found in Mb% between pre- and post-rigor sausage, whereas post-rigor sausage had greater pigment degradation in the form of higher MMb%. The benefit of utilizing pre-rigor pork for

sausage manufacture, due to higher redness values, may be lost by extending the storage period beyond 14 days. Higher yellowness (Hunter 'b') values in pre-rigor sausage could be due to a higher degree of lipid oxidation. Panelists judged post-rigor patties as darker and more discolored than pre-rigor patties.

Storage of sausages under vacuum and 100% CO2 atmosphere led to a decrease in pH, whereas an increase of pH was observed under other treatments. In the absence of O2, over 25% CO2 was found to lower the pH. Concentration of O2 in the package atmosphere was found to have a greater impact on the TBA values, than CO2 or N2. Treatments with 25% CO2 and 100% CO2 had the lowest TBA values whereas air treatment, with highest O2 and lowest CO2 concentration, had the highest TBA value with a shelf-life of less than 14 days. No difference was found between TBA values for treatments with 100% or 25% CO2, which suggestsd the practicality of using 25% CO2 for maximum shelf-life extension, under the treatments studied. Treatments 1 (vacuum), 2 (100% CO2) and 4 (75% N2 + 25% CO2) had lower total bacterial counts as compared with counts under treatments 3 (air), 5 (75% N2 + 15% CO2 + 10% O2) and 6 (75% N2 + 20% CO2 + 5% O2) at the end of 14 day storage. Sausages under vacuum treatment had significantly lower counts than sausages under air treatment, which had the highest total count of more than 10*6 CFU/gm of sausage and was not acceptable for retail display after 14 days. Differences in counts were not found under 25% and 100% CO2 treatments. Therefore a minimum of 25% CO2 and a maximum of 10% O2 is suggested for shelf-life extension in terms of bacterial counts for pork sausages.

At the end of the storage period, treatments 2 and 4 (without O2) had lower OMb% than treatments 3, 5 and 6 (with O2). Higher O2 levels promote OMb formation and therefore over 20% O2 concentration, in the gas atmosphere, is recommended for modified atmosphere packaging. Treatments 2 and 4 had lowest Mb% and were different than treatments 3, 5 and 6. It suggests a greater formation of MMb in treatments with 25% or more CO2. Through this study, 50% MMb concentration is proposed to be the cut-off value for pigment degradation in ground pork. At 50% MMb concentration, treatments 2 and 4 had a shelf-life of about 10 days, followed by vacuum treatment with 14 days. Treatments 3, 5 and 6 were still acceptable after 14 days of storage. Original lightness was retained until day 7 under treatments 3, 5 and 6, whose patties had the highest values. Therefore, a treatment of at least 5% O2 coupled with less than 25% CO2 aided in improving the lightness scores. The same was found for redness values. Under treatments 3, 5 and 6, the patties had more 'normal' color than patties under treatments 2 and 4 and air treatment patties were more 'normal' than vacuum treatment patties. Increase in storage days, after the 3rd day, led to higher color and surface discoloration scores, which is due to the accumulation of MMb on the patty surface, Microbial growth and lipid oxidation do not seem to related to surface discoloraton.

Shelf-life extension of pork sausages in terms of lipid oxidation could be achieved with use of post-rigor meat, under a modified atmosphere containing a maximum of 25% CO2 and 5% O2, along with the rest of the gas mixture being N2. From the point of view of microbial growth, shelf-life extension of up to 14 could be achieved with use of

post-rigor pork stored under a gas mixture having a minimum 25% CO2 and a maximum 10% O2. Color shelf-life could be extended with use of minimum 20% O2 and maximum 20% CO2, though in this study, air treatment gave the best results.

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APPENDICES

APPENDIX A

SUBJECTIVE EVALUATION SCORE-CARD

NAME _____ DATE _____

You are to evaluate color and surface discoloration of fresh pork sausage patties. Each patty is to be evaluated separately. For each sample there is a scale for color 'C' and a scale for surface discoloration 'SD'. Mark a vertical line across the horizontal scale at the point which best describes your impression of the attribute in the sausage patty. Thank You.

CODE NO. PATTY NO.1

C	Light			Normal			Dark
SD	0%	20%	40%	50%	60%	80%	100%

PATTY NO.2

C	Light			Normal			Dark
SD	0%	20%	40%	50%	60%	80%	100%

CODE NO. PATTY NO.1

C	Light			Normal			Dark
SD	0%	20%	40%	50%	60%	80%	100%

PATTY NO.2

C	Light			Normal			Dark
SD	0%	20%	40%	50%	60%	80%	100%

APPENDIX B

ANALYSIS OF VARIANCE OF VARIABLES STUDIED

Analysis of variance for pH of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.001
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.001
Rigor*Day	4	0.228
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.148
Error	20	
Total	79	

Analysis of variance for TBA of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.001
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.374
Rigor*Day	4	0.001
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.031
Error	20	
Total	79	

Analysis of variance for total bacterial count of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.001
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.623
Rigor*Day	4	0.374
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.215
Error	20	
Total	79	

Analysis of variance for psychrotrophic bacterial count of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.079
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.109
Rigor*Day	4	0.666
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.481
Error	20	
Total	79	

Analysis of variance for lactic bacterial count of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.042
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.065
Rigor*Day	4	0.001
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.164
Error	20	
Total	79	

Analysis of variance for percent oxymyoglobin of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.001
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.065
Rigor*Day	4	0.001
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.312
Error	20	
Total	79	

Analysis of variance for percent myoglobin of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.593
Treatment	5	0.044
Day	4	0.001
Rigor*Treatment	5	0.046
Rigor*Day	4	0.009
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.058
Error	20	
Total	79	

Analysis of variance for percent metmyoglobin of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.001
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.008
Rigor*Day	4	0.024
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.048
Error	20	
Total	79	

Analysis of variance for Hunter color 'L' of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.001
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.006
Rigor*Day	4	0.009
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.005
Error	20	
Total	79	

Analysis of variance for Hunter color 'a' of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.001
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.010
Rigor*Day	4	0.001
Treatment*Day	20	0.011
Rigor*Treatment*Day	20	0.008
Error	20	
Total	79	

Analysis of variance for Hunter color 'b' of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.001
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.200
Rigor*Day	4	0.018
Treatment*Day	20	0.032
Rigor*Treatment*Day	20	0.002
Error	20	
Total	79	

Analysis of variance for subjective color of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.001
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.001
Rigor*Day	4	0.001
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.001
Error	20	
Total	79	

Analysis of variance for subjective surface discoloration of sausage made from pre-rigor and post-rigor pork, packaged under vacuum and modified atmosphere and stored for 14 days.

Source	Degrees of freedom	Significance level
Rigor	1	0.001
Treatment	5	0.001
Day	4	0.001
Rigor*Treatment	5	0.087
Rigor*Day	4	0.110
Treatment*Day	20	0.001
Rigor*Treatment*Day	20	0.026
Error	20	
Total	79	

APPENDIX C

SIGNIFICANCE LEVELS FOR TREATMENT CONTRASTS

Significance levels for contrasts between six treatments for different variables studied - pH, TBA value (TBA), total bacterial count (TC), psychrotrophic bacterial count (PC), lactic acid bacterial count (LC), percent oxymyoglobin (OMb), percent myoglobin (Mb), percent metmyoglobin (MMb), Hunter color 'L' value (L), Hunter color 'a' value (a), Hunter color 'b' value (b), subjective color (C) and subjective surface discoloration (SD).

Variable	Contrastsa				
	1	2	3	4	5
pH	0.001	0.001	0.001	0.352	0.341
TBA	0.001	0.814	0.039	0.001	0.411
TC	0.001	0.049	0.126	0.001	0.118
PC	0.001	0.548	0.005	0.004	0.016
LC	0.001	0.267	0.030	0.941	0.860
OMb	0.001	0.012	0.057	0.001	0.001
Mb	0.734	0.039	0.001	0.011	0.049
MMb	0.001	0.003	0.001	0.229	0.731
L	0.001	0.642	0.001	0.058	0.063
a	0.368	0.312	0.001	0.124	0.528
b	0.162	0.053	0.001	0.093	0.638
C	0.001	0.874	0.001	0.024	0.232
SD	0.001	0.776	0.001	0.001	0.058

awhere 1 = contrast between vacuum treatment and air treatment,

2 = contrast between 100% CO2 treatment and 75% N2 + 25% CO2 treatment,

3 = contrast between 100% CO2 and 75% N2 + 25% CO2 treatments and

air, 75% N_2 + 15% CO_2 + 10% O_2 and 75% N_2 + 20% CO_2 + 5% O_2 treatments,

4 = contrast between air treatment and 75% N_2 + 15% CO_2 + 10% O_2 and 75% N_2 + 20% CO_2 + 5% O_2 treatments,

5 = contrast between 75% N_2 + 15% CO_2 + 10% O_2 treatment and 75% N_2 + 20% CO_2 + 5% O_2 treatment.

APPENDIX D

SIGNIFICANCE LEVELS FOR STORAGE DAY CONTRASTS

Significance levels for contrasts between five storage days for different variables studied - pH, TBA value (TBA), total bacterial count (TC), psychrotrophic bacterial count (PC), lactic acid bacterial count (LC), percent oxymyoglobin (OMb), percent myoglobin (Mb), percent metmyoglobin (MMb), Hunter color 'L' value (L), Hunter color 'a' value (a), Hunter color 'b' value (b), subjective color (C) and subjective surface discoloration (SD).

Variable	Contrasts[a]			
	6	7	8	9
pH	0.098	0.202	0.124	0.111
TBA	0.001	0.001	0.001	0.001
TC	0.001	0.001	0.283	0.008
PC	0.418	0.001	0.059	0.169
LC	0.001	0.001	0.347	0.003
OMb	0.001	0.001	0.001	0.001
Mb	0.001	0.068	0.001	0.037
MMb	0.001	0.001	0.001	0.001
L	0.217	0.001	0.001	0.001
a	0.048	0.001	0.001	0.001
b	0.040	0.011	0.246	0.111
C	0.001	0.001	0.001	0.001
SD	0.001	0.001	0.001	0.001

[a]where 6 = contrast between day 0 and day 3,

7 = contrast between day 3 and day 7,

8 = contrast between day 7 and day 10,

9 = contrast between day 10 and day 14.

VITA

Sangam A. Kurade was born on November 11th, 1962 to Dr. Anand G. Naik-Kurade and Mrs. Suman A. Naik-Kurade. He graduated from Delhi Public School, New Delhi, India in May, 1980 and proceeded for his undergraduate degree in Food Science & Technology from Marathawada Agricultural University, Parbhani, India. As a part of his coursework, he received training at Brooke Bond India Ltd., Hindustan Cocoa Products (Cadbury's) Ltd. and Modern Food Industries (India) Ltd. He received his Bachelors degree in August, 1984. The same year, (September 1984) he entered the University of Hawaii at Honolulu as a graduate student in Food Science. He was the recipient of Hawaii State Scholarship and worked as a Graduate Research Assistant at the department. Upon receiving his Masters degree in August 1985, he entered The University of Tennessee in Knoxville in order to pursue his Doctorate degree. He worked at a variety of jobs in order to attain his educational goals and was the recipient of Elsie L. Crenshaw Scholarship twice. While working for his doctorate, he also worked toward the degree of Master of Business Administration which he received from Pacific Western University, Los Angeles, in December 1987. He is a member of The Institute of Food Technologists, Phi Tau Sigma and Gamma Sigma Delta. He received his Doctor of Philosophy degree in Food Technology and Science in August 1990.