

THE EFFECT OF NATURALLY FERMENTED VEGETABLE NITRITES ON THE COLOR
OF VACUUM PACKAGED FRESH PORK

by

JOHN SUMMERFIELD

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Major Professor
Terry Houser

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Abstract

The objective of this research was to evaluate the effect of natural nitrites on objective color of vacuum packaged fresh pork. Sections of *longissimus dorsi* muscle (approximately 18 cm) were injected with solutions containing 0, 3, 6, 9 or 12 ppm of natural nitrite. Sections were sliced into chops (2.54 cm) and individually vacuum packaged. Raw chop surface L*, a* and b* values were measured at 1, 5, 15 and 30 days post packaging. At 1, 15 and 30 days post packaging chops were cooked and surface L*, a* and b* values were measured. Hue and Chroma values were calculated for all color measurements. Linear and quadratic contrasts were evaluated on treatments for all measured and calculated color values. A linear ($P < 0.05$) increase was detected on the L* values for days of vacuum storage treatment, all other raw color measurements and calculations for levels of natural nitrite and days of vacuum package storage were found to be quadratic ($P < 0.05$). Cooked L* and Hue values for days of vacuum storage were found to decrease linearly ($P < 0.05$), all other days of storage and levels of nitrite treatments were found to be quadratic ($P < 0.05$) in relationship to the measured and calculated cooked color values. All raw chops containing nitrite had higher a* and Chroma values at all evaluation days than those containing no added nitrite. Raw chops containing nitrite had lower L*, higher b* and Hue values than the 0 ppm chops ($P < 0.05$). Raw chops containing natural nitrite were darker, redder, more yellow and more intense in color than those without nitrite. The longer the chops were vacuum packaged and then cooked, the lower the L* values were ($P < 0.05$). Cooked chops containing nitrite were redder, less yellow and lower in Hue and Chroma values than cooked chops with no added nitrite ($P < 0.05$). These results indicate that low levels of nitrite can alter fresh and cooked pork color during vacuum storage. To balance the increased redness and darkness of the raw chops with the increased redness of the cooked chops, 3 ppm of natural nitrite was found to be the optimal treatment.

Keywords: color, natural nitrite, pork, vacuum package

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Dedication

I would like to dedicate this work to my wife, Lori and my children, Bo, Chloe and Nicole. It has been a balancing act to try and finish my masters while working full time and still trying to be a good husband and father. My hope is that my children have come to understand and appreciate the importance of education while watching me work on my school work.

Education is a tool that can open opportunities in life. The more effort that is put into education, the better the results and the more doors are typically available to be opened. These doors are not only in one's career but also in one's personal life; because they can not be separated as personal income is typically dependent on career income in most cases

Chapter 1 - INTRODUCTION

Color is the most important sensory attribute consumers use to evaluate fresh pork acceptability (Lee et al., 2008; Rosenvold and Anderson, 2003). Stabilized bloomed color gives fresh pork the best appearance from the consumer's perspective (Brewer et al., 2006). Myoglobin bound with oxygen is referred to as a bloomed color or oxymyoglobin.

Fresh pork color is determined by the chemical state of the myoglobin. Myoglobin is a sarcoplasmic protein in the muscle that contains a heme ring with an iron atom attached (Seideman et al., 1984). Hemoglobin carries oxygen to the muscle cells, myoglobin transfers the oxygen from the blood hemoglobin to the mitochondria within the cell, a non-ending cycle as long as the muscle is living.

Very low levels of nitrites, parts per million, can be used in an oxygen free environment to stabilize pink color in fresh pork (Pockat et al., 2006). If pork contains too high of nitrite levels it can have a pink cured color when cooked (MacDougall and Hetherington, 1992). Natural nitrites can be labeled as "Natural Flavorings" per USDA regulations (USDA, 2005).

Packaging technology has advanced over the past several decades. The advancements in film manufacturing have allowed packing companies to continue to improve and innovate how fresh pork can be packaged. Major packaging advancements include vacuum packaging and modified atmosphere packaging (MAP). A great deal of research has been done over the last two decades to better understand how MAP can be used to improve the appearance and shelf life of fresh pork and other meat products (Luno et al., 2000). Many of the MAP packaging systems use a positive pressure atmosphere so that the gases are forced into the surface of the meat and also for the practical purpose of detecting leaky packages (Stahl, 2007).

Primal cuts are vacuum packaged and transported to stores where they are typically further processed into retail cuts. Vacuum packaging is also used to a lesser extent for retail cuts. Consumer acceptance of vacuum packaged fresh pork or beef has been traditionally low in the United States due to lack of bloomed color (McMillin, 2008).

The objective of this research was to evaluate the influence of low levels of natural nitrites on developing a bright, fresh, pink color in vacuum packaged pork.

Chapter 2 - REVIEW OF LITERATURE

Importance of Meat Color to Consumer

Consumers use color as the primary indicator of quality when purchasing meat (Kropf, 2004). Pork meat color can vary from a bloomed bright pink color (oxymyoglobin), to a deoxymyoglobin pale gray, to a metmyoglobin brown color or to a green color when spoiled. The bright pink color of fresh pork is the result of bloom, which is the oxygenation of the myoglobin protein in the meat (Brewer et al., 2006). Most blooming occurs within the first 30 minutes of exposure to air (Lindahl et al., 2006). Lee et al., (2008) also reported that 30 minutes was an adequate amount of time for pork to bloom from a visual perspective but not from a quantitative instrumental perspective. Optimal fresh pork color is bright pink, which may vary in intensity from cut to cut because of the different amounts of myoglobin in the different muscles of the pork carcass.

Quality of Fresh Pork

Approximately 100 million hogs are harvested every year in the United States (AMI 2010 Meat & Poultry Facts). This equates to a very large fresh pork market. Although much of the pork is further processed, approximately 25% of the harvested meat is sold as fresh pork in retail stores (Wright et al., 2005). Consumers evaluate fresh pork chops based on color, firmness and purge when purchasing fresh pork (Wright et al., 2005).

The quality of fresh and processed pork is affected by the rate of post mortem metabolism, which is in turn influenced by the rate and extent at which the carcass is chilled. The faster the post mortem metabolism, the quicker lactic acid is produced, causing a faster pH decline (Cannon et al., 1995). When muscle is exposed to a lower pH and higher temperatures, proteins within the muscle start to denature. These muscle proteins include actin, myosin and myoglobin. The denaturation of these proteins can result in paler meat (myoglobin) with poor water holding capacity (actin and myosin) (Cannon et al., 1995). Extreme cases of this denaturation are referred to as pale soft and exudative (Briskey, 1964).

There are many factors that can affect the rate of post mortem metabolism and this has been a significant focus of research in the pork industry for many decades (Seideman et al., 1984). Some of this research includes studying the impact of genetics, feed, lairage, pre-slaughter stress and transportation. Wright et al. (2005) found that 12.5% of the pork loins

evaluated in their study were classified as low quality. So as evident by this 2005 study, after all of the research that has been performed to help resolve this issue, poor pork quality is still a big factor in the market today. Cannon et al. (1996) stated that packers reported a 10% incidence of PSE Pork.

When purchasing fresh pork, most consumers can only evaluate the quality of pork by visual appearance, making the color of fresh pork the most important quality characteristics to the consumer. As is suggested by Mancini and Hunt (2005), meat scientists should continue to work on improving muscle color and color stability by focusing on the basic principles of myoglobin chemistry.

Attributes Evaluated by Consumers

Consumers evaluate fresh pork by raw meat evaluation at the point of sale, and cooked meat evaluation at the point of use (Troy and Kerry, 2010). At the point of sale, consumers evaluate fresh meat visually for color, marbling, leanness/fat, and purge in the package. At the point of consumption, key quality characteristics include juiciness, tenderness, marbling, and leanness (Sanders et. al., 2007).

At the point of sale, the color of the fresh meat is the first and most important attribute of the meat from the consumer's perspective (Troy and Kerry, 2010). Fresh pork should have a consistent bright pink color and should retain its color over the period of the products refrigerated shelf life.

Correlations have been reported between meat color and the quality of pork with darker pork chops having a higher pH (Warner et al., 1993, Seideman et al., 1984). Pale, soft and exudative pork has a lower pH and less water holding capacity. Darker chops have a higher pH and more water holding capacity. Darker pork chops will have higher pH and will correlate to a more tender and juicier eating experience (Wright et al., 2005).

Fresh Pork Color

Over the past several decades, optimizing fresh meat color has been a focus of not only the pork industry but also the beef and poultry industry (Seideman et al., 1984; Zhu and Brewer, 1998; Mancini and Hunt, 2005). Research priorities have focused on maintaining meat quality and stabilizing fresh meat color.

Wright et al., (2005) found that consumers categorized darker colored loin chops as higher quality compared to lighter colored pork chops. Blast chilling has been installed at many harvesting facilities to rapidly chill carcasses, which quickly slows post-mortem metabolism and helps minimize protein denaturation. When properly done, this can help maintain pork quality, including a fresh color.

Color and pH are effective quantitative measurements used to sort pork quality (Norman et al., 2004). In their study the three different pork quality groups that were analyzed were developed by the differences in color score using the National Pork Producers Council color standards. Color and pH measurements were made and analyzed on the different boneless loin quality groups. There are many other factors that influence pH of pork such as genetics, animal handling, lairage, feed, stunning methods, chilling and other post-processing procedures and conditions.

Packaging is one of the post-processing procedures that can help stabilize fresh meat color. Modified atmosphere packaging (MAP) has been studied extensively, including looking at different types of gases and mixtures of carbon dioxide, nitrogen, oxygen and carbon monoxide (Sebranek and Houser, 2006).

Carbon monoxide and other myoglobin stabilizing agents, such as nitrites, have also been utilized to develop a bright pink color in fresh pork (Pockat et al., 2006). The color of the myoglobin depends on the oxidative state of the iron and the type of ligand that is bound to it. Nitric oxide myoglobin is a bright-red derivative with a visible spectrum similar to that of oxymyoglobin (Livingston and Brown, 1981). When nitric oxide myoglobin is in anaerobic conditions, such as vacuum packaging, the color of the meat will be bright pink, in the case of pork, or bright red in the case of beef that has greater concentrations of myoglobin in the muscle.

Packaging of Fresh Meat

Proper packaging will significantly impact the shelf life of fresh pork which in turn has allowed for increased transportation distances (Sebranek and Houser, 2006). Changes in packaging has also improved meat quality and color which has led to an increase in consumer expectations and improved color stability.

Pork is sold at the retail level in two basic formats: overwrapped with an oxygen permeable film or modified atmosphere packaging (Troy and Kerry, 2010). Some fresh pork is vacuum packaged for retail sale, but in the U.S. this is a very low percent of the fresh pork

because the vacuum packaged pork is gray in color and considered unacceptable to the consumer. It is estimated that 64% of the U.S. market for fresh meat is now packaged as case-ready (Crews, 2007).

Overwrapped fresh pork has a shelf life of 3-6 days with acceptable color. When oxygen is removed from the packaging, this minimizes aerobic bacterial growth and limits chemical oxidation of the meat or fat. Jeremiah et. al. (1995) reported fresh pork shelf life of up to 9 weeks at -1.5°C when stored under vacuum or carbon dioxide.

Muscle respiration continues to take place after the meat has been packaged and microbial growth will increase for the duration of the storage in the package. Because of this, the atmosphere within the package will remain dynamic in any type of package. Even in vacuum packaged products, there will exist a small amount of air which will be converted to carbon dioxide through either muscle respiration or microbial growth (Sebranek and Houser, 2006).

For consumer acceptance, it is important to stabilize the bloomed color of meat, which will provide a fresh looking pork chop with long shelf life. Meat color has been stabilized by using various packaging systems including overwrapped, modified atmosphere packaging (MAP) and vacuum packaging systems.

Overwrapped Packaging

Overwrapped packaging is the use of polyvinylchloride (PVC) film stretched over the meat which is held in an expanded polystyrene tray and then bonded on the bottom of the tray using heat (Jenkins and Harrington, 1991). The PVC film provides a moisture barrier to the meat while being air permeable. This allows the meat surface to be exposed to environmental oxygen. Meat bloomed in this type of package provides the consumer with a fresh, bright pink pork product. Consumers associate this bright pink color of overwrapped pork cuts with freshness (Jenkins and Harrington, 1991). Film that is used to overwrap meat should have an oxygen permeability of at least 5 liters per square meter per day to allow for the meat to bloom sufficiently (Landrock and Wallace, 1955).

Overwrapped meat is able to bloom, allowing the consumer to view the product in its optimal oxymyoglobin state. The major disadvantage is the limited refrigerated shelf life of the packaged pork, typically 3 to 5 days (Buys et al., 1993).

Modified Atmosphere Packaging

Overwrap packaging has been slowly replaced by case ready products that are produced at a centralized packaging operation. Primals are cut to a specific size or weight that are consumer size specific, allowing for minimal handling at retail stores (McMillin et al., 1994).

Modified atmosphere packaging has evolved over the years, taking advantage of various gases to optimize color and minimize microbial growth (Brody, 2002). Master pack overwrapped meat that was placed in a larger bag containing a high concentration of oxygen helped continue the growth and innovation of the different MAP systems that we have in place today (Brody, 2002). The high oxygen master packs that also contain carbon dioxide help stabilize meat color and inhibit microbial growth.

Current modified atmospheres include high oxygen, no to low oxygen packages and other gases such as nitrogen, carbon dioxide and carbon monoxide. Oxygen and carbon monoxide are used to stabilize fresh color while carbon dioxide is used to inhibit microbial growth. Sebranek and Houser (2006) stated that the most common gases used in MAP systems are oxygen, carbon dioxide and nitrogen. High-oxygen MAP packaged pork is more susceptible to lipid oxidation resulting in quicker rancidity and off-flavors of the products. It has been known for over 100 years that carbon dioxide can help inhibit microbial growth (Roa and Sachindra, 2002). A carbon dioxide concentration of 20-25% is required to have an effect on the microbial growth of packaged meat (Kropf, 2004).

Carbon monoxide is a gas that has been investigated over the last several decades and has been increasingly utilized to stabilize the color of fresh meat (De Santos et al., 2007). Carbon monoxide has a great affinity towards the iron molecule of myoglobin and as a result the stabilized bloomed color of meat, when exposed to carbon monoxide, can last several weeks after exposure (Sebranek and Houser, 2006). Initially there has been some concern for possible health concerns of carbon monoxide packaged meat, but the U.S. Food and Drug Administration (FDA) has approved the use of carbon monoxide at a level of 0.4% or less and has been categorized as a “generally recognized as safe” (GRAS) substance when used at these concentrations (U.S. FDA, 2002). If carbon monoxide is back-flushed into a MAP package containing meat, the color can remain stable for an extended period of time.

There are many considerations and decisions that need to be made when using a MAP system. These considerations include muscle cuts, injected and enhanced products, phosphate

type and meat color during transit and display (Smith, 2001). Where the muscle comes from on the pig will make a difference as to how much myoglobin it contains. What is added to the meat in the form of injected/enhancement solutions will change the chemistry within the meat. These factors can affect how much or what type of gas or gases should be used to inhibit microbial growth or modify and stabilize meat color.

Shelf life of a meat product using MAP is a science that requires proper gas mixes, matching of product and packaging materials, online analysis of packaged products, and leak detection of packages (Stahl, 2007). Optimal head space to prevent package collapse is 2 to 3 times the volume of the meat (Gill and Gill, 2005).

Some of the advantages of MAP packaging include the color of fresh pork and the fact that there is less purge in MAP packaged meat than there is in vacuum packaged meat (Sebranek and Houser, 2006). Some of the disadvantages of MAP packaging include the capital investment required, the cost of equipment, the specific gas mixtures required and the cost of these gases, along with possible worker safety concerns of these gases if leaked into the centralized production facility (Sebranek and Houser, 2006).

Vacuum Packaging

Before MAP packaging was developed, primals were vacuum packaged to extend shelf life. Vacuum packaging is mainly used to package primals that are shipped to retailers where they are fabricated into retail cuts and overwrapped or placed in a self-serve display case (McMillin, 2008, Seideman et al., 1984).

Jenkins and Harrington (1991) stated that consumers associated a bright red bloomed color with the freshness of meat in the self-serve display case and therefore began to expect this bloomed color in air permeable packaged meats. While vacuum packaged pork has several advantages over MAP or overwrap packaged pork, color of the product is not one of them.

Daraba (2005) reported changes in the surface of pork *longissimus dorsi* myoglobin states over a period of days in both aerobic and anaerobic conditions. There were very large changes in the percent of oxymyoglobin and metmyoglobin in anaerobic conditions. The oxymyoglobin and metmyoglobin decreased to less than 10% and the deoxymyoglobin increased to over 50% by day 6 of refrigerated display. Chops that were cut and exposed to oxygen, and then vacuum packaged changed in color, going from bright pink (oxymyoglobin state) to dull gray (deoxymyoglobin state) within a day or two. When a chop was cut and exposed to the

atmosphere, about 80% of the myoglobin was in the oxymyoglobin state but that quickly changed. The percent of oxymyoglobin went from about 80% to less than 40% within 2 days when vacuum packaged. Daraba (2005) reported that after the initial drop in oxymyoglobin, it only slowly decreased over time as did the metmyoglobin.

Daraba (2005) stated that the consumer associates the bright red color of pork with freshness and good quality eating; efforts to market fresh meat with color characteristics other than this have been mostly unsuccessful. The volume of fresh pork vacuum packaged retail cuts is small in the U.S. This is more of a niche market where the retailer educates the consumer on the advantages of this type of packaging. One major advantage of vacuum packaged pork is the lower cost in packaging compared to case ready MAP packaged products. Vacuum packaged products have a smaller footprint per packaged pound so shipping costs can be reduced by maximizing the units per box and boxes per refrigerated trailer. A retailer can also take advantage of the reduced unit size, allowing more units per display case.

Vacuum packaged meat has an increased shelf life compared to overwrap because it serves as an anaerobic environment. Jeremiah et al., (1997) have shown that vacuum packaged pork can have a storage life of up to 9 weeks if stored at -1.5°C . Many of the injected pork products had a shelf life of over 25-30 days when they were vacuum packaged and stored at 4°C .

Jeremiah et al., (1995) evaluated carbon dioxide controlled atmosphere packaging (CAP) and vacuum packaged loin portions for quality attributes such as drip loss, surface discoloration, shelf life, odor, and flavor. Samples were evaluated in this study at 3 week intervals for up to 15 weeks. They found that packaging treatment only slightly affected the purge and had little influence on the other quality attributes measured. Vacuum packaged pork had a small improvement in drip loss compared to CAP.

The main advantage of vacuum packaged pork compared to overwrapped pork is shelf life of the product. Overwrapped pork has a shelf life of approximately 6 days whereas vacuum packaged pork can have a shelf life of approximately 30 days or longer. The three major advantages that vacuum packaging has over MAP are the cost of package material and gases, shipping cost, and freezer shelf life. Modified atmosphere packaged pork will form ice crystals on the meat when frozen, leading to freezer burn. Vacuum packaged pork will store extremely well in the freezer. The main disadvantage of vacuum packaged pork is the gray color of the meat (Pockat et al., 2006).

Myoglobin

Myoglobin is the principle protein that is responsible for meat color (Mancini and Hunt, 2005). Myoglobin is a member of the heme proteins that also includes hemoglobin and cytochrome C. This section of the literature review discusses myoglobin chemical and biochemical activities and reactions.

Myoglobin in muscle and hemoglobin in blood transport oxygen and carbon dioxide throughout a hog's body. Hemoglobin is the larger molecule with a molecular weight of 67,000, whereas myoglobin has a molecular weight of approximately 16,700 (Seideman et al., 1984). Myoglobin accounts for 10% of the iron in an animal while it is living, but 95% of the iron after it has been bled and eviscerated (Clydesdale and Francis, 1971). The concentration of myoglobin depends on the species, muscle type, age of the animal, and how the meat is handled after slaughter (Livingston and Brown, 1981). The concentration of myoglobin in pork has been measured at 2.5-7.0 mg/g (Topel et al., 1966)

There is one iron atom per myoglobin molecule. Iron is a transition metal that has eight electrons in its valance shell. Because of this it has a lower electronegativity and will give up two or three of these electrons to form ferrous (Fe^{2+}) or ferric (Fe^{3+}) iron cations. Iron will form bonds with ligands. A ligand is an ion or molecule that will bind ionically or covalently with transition metals. With iron, it will typically form bonds with 5 to 6 ligands. In the case of the iron atom in the myoglobin molecule, four of the six ligands are heme pyrrole nitrogens, which are very stable, and the fifth is a histidine, which is also stable. This leaves the position of the sixth ligand open for binding with either oxygen, carbon monoxide, nitric oxide or some other molecule (Livingston and Brown, 1981). The iron atom within the myoglobin molecule is fairly sterically hindered and therefore, the molecule or atom that can potentially form the sixth ligand would need to be an atom or molecule that is small in size and yet have a strong electronegativity to bind with the iron (Takano, 1977).

Commercially, nitric oxide in cured meats and carboxymyoglobin in fresh meats are used to stabilize the myoglobin in the ferrous state (Livingston and Brown, 1981). Nitric oxide and carbon monoxide bind to the myoglobin to form bright red derivatives. Nitric oxide and carbon monoxide will dissociate from the myoglobin at a very slow rate. The difference between these two ligands is that when nitric oxide disassociates, it will react with the oxygen present to form nitrate (NO_3). When carbon monoxide disassociates, it will not react with oxygen because it is a

stable molecule by itself so it can re-associate with myoglobin to reform carboxymyoglobin (Livingston and Brown, 1981). Nitric oxide is not stable with the heme iron but when heated it is converted to di-nitric heme complex which is quite stable but somewhat sensitive to photo oxidation (Livingston and Brown, 1981). Fresh meats that are packaged with a very small amount of carbon monoxide will remain in the carboxymyoglobin state for a long period of time even when exposed to oxygen.

States of Myoglobin

There are basically four forms of myoglobin when looking at fresh meat color. These forms are deoxymyoglobin, which is purple in color, oxymyoglobin and carboxymyoglobin which are red and metmyoglobin which is brown (Mancini and Hunt, 2005).

In a pork chop that is exposed to air, there are typically three states of myoglobin present. Oxygen in the air will bind with the myoglobin to form oxymyoglobin, which is pink in color. The majority of this covalent bonding usually takes 30 minutes or less for the predominant color of the surface of the pork chop to turn pink, commonly referred to as blooming (Brewer et al. 2001). Muscle respiration will continue and the myoglobin just under the exposed surface will continue to be oxidized to the metmyoglobin state, which will be brown in color. The deep tissue of the meat which has not been exposed to oxygen will be in the purple deoxymyoglobin state (Seideman et al., 1984).

Oxidation of myoglobin to the metmyoglobin state can be caused by several factors that reduce the oxygen tension of the meat surface causing dissociation of the oxygen from the oxymyoglobin. These factors can include high temperatures, low pH, ultraviolet light, high salt concentrations, low oxygen atmospheres and aerobic bacteria (Seideman et al., 1984).

An equilibrium exists on the surface of fresh meat packaged in a high oxygen permeable film (Pirko and Ayres, 1957). Oxymyoglobin is deoxygenated to the reduced myoglobin state which is immediately oxidized to metmyoglobin. During the first 3-4 days of retail display, oxymyoglobin is the predominate product. After 3-4 days, the equilibrium shifts to where metmyoglobin predominates in higher concentrations on the meat surface and the meat will slowly start to turn brown.

When pork is vacuum packaged, the color will turn from bright pink, or oxymyoglobin, to gray or brown which is the metmyoglobin state, within a few hours. This is caused by low oxygen tension, which favors metmyoglobin formation. When residual oxygen is converted to

carbon dioxide through muscle respiration, metmyoglobin will be reduced to the gray/purple deoxymyoglobin state. This takes a longer time because this is an enzymatic biochemical reaction of respiration (Seideman et al., 1984).

Luno et al. (2000) suggested that the carboxymyoglobin chemical state be included as a relevant state of myoglobin because of the increased interest in the use of carbon monoxide in fresh meat. Under anaerobic conditions such as vacuum packaging, nitric oxide myoglobin can be generated by reacting nitrite with deoxymyoglobin (Koizumi and Brown, 1971). Pockat et al. (2006) showed that using low levels of nitrite impregnated in film, nitric oxide myoglobin could also be included as a relevant state of myoglobin in fresh meat.

Nitrites

Nitrite is used in the meat industry to cure meat (Honikel, 2008). The nitrite used for this is most often in the sodium or potassium salt form. The upper limit of sodium nitrite in massaged or pumped pork is 200 ppm, for comminuted pork it is 156, and for bacon it is 120 ppm (USDA, 1995). High levels of nitrite can be poisonous and therefore nitrite used to cure meats is tightly regulated by the USDA.

Nitrite binds with iron in myoglobin and when heated, forms a stable nitrosyl hemochrome pink color in cured meat. Nitrosylhemochrome is quite stable as long as it is protected from oxygen and light. The use of nitrite helps stabilize cured color and also provides microbial inhibition (Honikel, 2008).

The pH of fresh pork is typically between 5.5 and 6.2. The pKa of nitrous acid is 3.37, which means that at a pH of 3.37, 50% of the nitrous acid is dissociated. At a pH of 5.5 or above, 99% or more of the sodium nitrite will be dissociated, which means that the nitrite will be free to react with water and then eventually with iron in myoglobin (Honikel, 2008).

Nitrates and nitrites can be found naturally in plants. It is important to know the concentration of natural nitrates and nitrites when used to cure meat products. The concentration of nitrate or nitrite within different plant materials can depend on the amount of light, type of soil, temperature, humidity, the plant maturity, harvesting time, storage time and source of nitrogen (Tamme et al., 2006). Some of the different types of vegetables that accumulate nitrate and typically a lower level of nitrite include but are not limited to: radish, mustard, spinach, beetroot, lettuce, celery, and parsley. There are many other types of vegetables that contain nitrate and lower levels of nitrite (Santamaria, 2006). Nitrate can be converted to nitrite using a

fermentation process. This fermentation can be done on vegetables containing nitrates to keep the source of nitrite used to cure meat natural (USDA, 2005).

Measuring the Color of Meat

Color of fresh pork is very important to consumers and serves as the main attribute consumers use to evaluate their purchase (Troy and Kerry, 2010). A fresh pink pork color can be equated by the consumer with long shelf-life and good quality eating (Honikel, 2008).

There are several ways to evaluate fresh pork color. Two visual fresh pork color scales have been developed in an attempt to score chops by color level. These are the Japanese Pork Color Standards (JPCS) and the National Pork Producers Council Pork Quality Standards (NPPC) (NPPC, 1999). They use similar scoring methods that employ a 6 point scale with 1 being extremely pale and 6 being extremely dark. These scales can be used to help quantify the color of different cuts.

There are also instrumental measurements of color that include colorimeters and spectrophotometers. Colorimeters use a light source that will hit the fresh meat with a known amount of energy, the reflected light is then passed through filters and the amount of light reflected back is measured with a detector. Spectrophotometers use refracted light that is transmitted to the surface of the meat product and is then reflected back to a detector. The amount of energy reflected back in both of these types of measurements are quantified within specific wavelengths and are expressed as either Hunter L*, a*, b* and CIE color spaces L*, a* and b* values (Brewer et al., 2006). L* is a measurement of white to black, a* is a measurement of red to green and b* is a measurement of yellow to blue. The L* values range from 0 to 100, with 0 being perfect black and 100 being perfect white. A positive a* value is red and a negative value is green. A positive b* value indicates yellow while a negative value is blue. These two different methods may derive different color values and the results cannot be compared or interchanged (Brewer et al., 2001). From these measurements Hue and Chroma can be calculated. Hue is a measure of the color angle and is calculated as the inverse tangent of b^*/a^* . Chroma is a measure of color intensity and is calculated as the square root of a^* squared plus b^* squared (RMC, 1991).

De Santos, et al. (2007) stated in their research that they evaluated meat color in high oxygen MAP, carbon monoxide MAP, and PVC overwrap packaging. They found that raw pork packaged in carbon monoxide had the lowest hue angle values which was consistent with a more

true red color. Chroma values of cooked pork fluctuated as endpoint temperatures were varied in their research and it was suggested that this may be a result of the beginning heat-induced protein denaturation.

Summary

Fresh meat is a very important food source in our society today and will continue to be so in the future. Most consumers evaluate the freshness of the meat by its appearance including color of lean and fat, amount of purge in the package, and consistency of the meat itself. Color is a key quality characteristic from a consumer's perspective. Equipment has been developed over the last several decades to better quantify and evaluate the color of the meat.

The pork industry has spent a great deal of time and money to improve the quality of pork. Meat scientists may define the quality of pork as pH, drip loss, shear force, and color. Color and purge are the characteristics that the consumer may use to evaluate fresh pork at the point of purchase. Tenderness and juiciness are quality characteristics the consumer may use to evaluate cooked pork at the point of consumption.

Packaging technology of fresh pork has dramatically changed over the last several decades. Modified atmosphere packaging is used to optimize the color of fresh pork and/or to help inhibit microbial growth, thus prolonging shelf life. Vacuum packaging has many advantages because of the anaerobic environment. Other advantages include low material cost, shipping weight optimization, and aerobic microbial inhibition. The major disadvantage of vacuum packaged fresh pork is the color of the meat which is dull and gray.

The dull gray color of vacuum packaged fresh pork is a result of the myoglobin transitioning to the deoxymyoglobin state because of the anaerobic conditions. Myoglobin that is bound with oxygen, carbon monoxide or nitric oxide is bright pink in color. If nitrite is present in fresh pork, in the absence of oxygen, the color will be bright pink.

Natural nitrite are found in "flavorings" such as celery, mustard and parsley. These flavorings and others can be used in fresh pork to develop a bright pink color when it is vacuum packaged.

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Chapter 3 - THE EFFECT OF NATURAL NITRITES ON COLOR OF VACUUM PACKAGE FRESH PORK AND COOKED PORK

Abstract

The objective of this research was to evaluate the effect of natural nitrites on objective color of vacuum packaged fresh pork. Sections of *longissimus dorsi* muscle (approximately 18 cm) were injected with solutions containing 0, 3, 6, 9 or 12 ppm of natural nitrite. Sections were sliced into chops (2.54 cm) and individually vacuum packaged. Raw chop surface L*, a* and b* values were measured at 1, 5, 15 and 30 days post packaging. At 1, 15 and 30 days post packaging chops were cooked and surface L*, a* and b* values were measured. Hue and Chroma values were calculated for all color measurements. Linear and quadratic contrasts were evaluated on treatments for all measured and calculated color values. A linear ($P < 0.05$) increase was detected on the L* values for days of vacuum storage treatment, all other raw color measurements and calculations for levels of natural nitrite and days of vacuum package storage were found to be quadratic ($P < 0.05$). Cooked L* and Hue values for days of vacuum storage were found to decrease linearly ($P < 0.05$), all other days of storage and levels of nitrite treatments were found to be quadratic ($P < 0.05$) in relationship to the measured and calculated cooked color values. All raw chops containing nitrite had higher a* and Chroma values at all evaluation days than those containing no added nitrite. Raw chops containing nitrite had lower L*, higher b* and Hue values than the 0 ppm chops ($P < 0.05$). Raw chops containing natural nitrite were darker, redder, more yellow and more intense in color than those without nitrite. The longer the chops were vacuum packaged and then cooked, the lower the L* values were ($P < 0.05$). Cooked chops containing nitrite were redder, less yellow and lower in Hue and Chroma values than cooked chops with no added nitrite ($P < 0.05$). These results indicate that low levels of nitrite can alter fresh and cooked pork color during vacuum storage. To balance the increased redness and darkness of the raw chops with the increased redness of the cooked chops, 3 ppm of natural nitrite was found to be the optimal treatment.

Keywords: color, natural nitrite, pork, vacuum package

Introduction

The preferred storage environment for fresh pork is under vacuum. Vacuum packaging significantly increases fresh pork shelf-life (Jeremiah et al., 1995) and has become one of the major forms of packaging primal cuts, in part because of its superior maintenance of meat color (Seideman et al., 1984). Consumers associate a bright pink color with fresh pork (Zhu and Brewer, 1981, 1998). Color is the most important attribute that the consumer uses to evaluate fresh pork (Lee et al., 2008). Myoglobin bound with oxygen is referred to as a bloomed color or oxymyoglobin.

When fresh pork is vacuum packaged, oxymyoglobin is converted to the natural state of deoxymyoglobin and the color changes from bright pink to gray (Bekhit and Faustman, 2005). Seideman et. al (1984) stated that when meat is vacuum packaged, the formation of metmyoglobin is initially favored because of low oxygen tension. Over time metmyoglobin pigments will be reduced to the deoxymyoglobin state. Pork in the deoxymyoglobin state is a dull gray color. Because of the lack of oxygen to support bacterial growth and protein and lipid oxidation, vacuum packaged meat will have a longer shelf life than meat packaged in high oxygen packaging (Jeremiah, 2001).

Myoglobin without anything bound to the 6th ligand is called deoxymyoglobin and it is gray in color (Livingston and Brown, 1981). This is the state that myoglobin is in when it is vacuum packaged. The color change from oxymyoglobin to deoxymyoglobin will take several hours after the pork has been vacuum packaged (Seideman et. al, 1984).

When oxygen binds to the heme portion of the myoglobin, it forms oxymyoglobin giving pork a bright pink color. The heme can form ligands with different small molecules such as oxygen, carbon dioxide, carbon monoxide and nitric oxide. The color of the pork will depend on what compound and how much of it binds with the myoglobin (Pockat et al., Patent Application 2006).

Because nitrites are not typically used in fresh meats, limited research has been conducted on the use of nitrites to stabilize fresh meat color under anaerobic conditions. When nitrite is present in the meat it provides a nitric oxide molecule that binds with the myoglobin to form nitrosylmyoglobin (Hood, 2005).

Nitrosylmyoglobin (NOMb) is similar to oxymyoglobin in the visible spectrum as a bright red derivative (Livingston and Brown, 1981). In the presence of oxygen, the

nitrosylmyoglobin will be converted to metmyoglobin which is brown in color. In an anerobic environment such as vacuum packaging, the nitrosylmyoglobin is stable and will remain in this bright red state. The nitric oxide dissociates from the myoglobin about 1 million times slower than oxygen but it is still considered an unstable complex (Livingston and Brown, 1981).

Natural nitrates and nitrites are found in vegetables such as celery, lettuce, and radishes and these nitrates can be naturally fermented to nitrites. The USDA labeling requirement for these natural nitrites is currently “Natural Flavorings” (USDA, 2005).

Color of vacuum packaged pork can be potentially modified by use of very low levels of natural nitrite, 12 ppm or less, injected into the meat. The objective of this research was to evaluate the effect of low levels of natural nitrite, fermented from vegetable powder, on the color of vacuum packaged fresh pork.

Materials and Methods

Pork *longissimus* muscle, North American Meat Processors Association # 413C, samples (n=30) were randomly chosen from a commercial plant. Refrigerated loins had been vacuum packaged and utilized for this experiment within 1 week post mortem. The loins were randomly selected with an average weight of 3.76 kg. A randomized complete block design was used to assign loins to 3 replications (10 loins/replication). Each loin was fabricated into 5 equal sections (~18 cm). Sections were randomly assigned to one of 5 nitrite treatments: 0, 3, 6, 9 and 12 ppm. On the same day but prior to treatment, the pH of each loin section was measured using a pH meter with a glass tip probe (Meat Probes, Inc., Topeka KS).

All sections were injected with a solution of salt (Morton Salt Inc., Chicago, IL), phosphate (ICL Performance Products LP, St. Louis, MO), 60% solution of sodium lactate (MAK Wood Inc, Graffton, WI), and sodium diacetate (Jungbunzlauer Suisse AG, Landenburg, Germany) that incorporated a pre-converted natural nitrite (VegStable™ 504) at 0, 3, 6, 9 or 12 ppm. Target injection levels of this solution in the loin sections was 12%. Table 1 illustrates the composition of each nitrite injection solution. A Schroder IMAX 350 injector (Wolf-tec, Inc., Kingston, NY) was used to inject the loins. Each section was weighed before and after injection to determine injection yield (Table 2). Nitrite concentration of the VegStable™ 504 (Florida Food Product’s Inc., Eustis, FL) powder was measured using a Skalar SAN++ Continuous Flow Analyzer (Skalar, Inc., Buford, GA)(Method 4500-NO²-B, Standard Methods for The

Examination of Water and Wastewater). Final nitrite was calculated by assuming an 11.5% retention (Table 2) of the solution by the loin section.

Table 1. Composition of Injection Solutions of Different Natural Nitrite Levels

Ingredients ^b	Nitrite Level				
	0 ppm	3 ppm	6 ppm	9 ppm	12 ppm
Water	83.50%	83.34%	83.17%	83.00%	82.83%
Salt	1.85%	1.85%	1.85%	1.85%	1.85%
Sodium Phosphate	4.17%	4.17%	4.17%	4.17%	4.17%
Postassium Lactate	9.27%	9.27%	9.27%	9.27%	9.27%
Sodium Diacetate	1.21%	1.21%	1.21%	1.21%	1.21%
VegStable™ 504 ^a	0.00%	0.16%	0.33%	0.50%	0.67%
Total	100.00%	100.00%	100.00%	100.00%	100.00%

^a VegStable™ 504 is a fermented celery powder with an average nitrite level of 1.49%

^b Target injection level of 12.00%

Table 2. Actual Average Ingredient Pump Levels of Loin Sections

Rep.	Nitrite Level	Green Weight (kg)	Target Pump Weight (kg) ^a	Actual Pump Weight (kg)	% Actual Pump
1	0	7.26	8.13	8.21	13.1%
1	3	7.48	8.38	8.53	13.9%
1	6	7.26	8.13	8.23	13.4%
1	9	7.76	8.69	8.60	10.8%
1	12	7.67	8.59	8.75	14.2%
2	0	7.85	8.79	8.94	13.9%
2	3	7.35	8.23	8.28	12.7%
2	6	7.60	8.51	8.60	13.1%
2	9	7.71	8.64	8.85	14.7%
2	12	7.10	7.95	7.92	11.5%
3	0	6.96	7.80	7.80	12.1%
3	3	7.23	8.10	8.19	13.2%
3	6	6.99	7.82	7.89	13.0%
3	9	6.83	7.65	7.76	13.6%
3	12	6.96	7.80	7.78	11.7%

^a Target Pump was 12% over green weight

Three chops were cut from each section and evaluated for color as is shown in Appendix A. Loin sections were cut into 2.5-cm thick chops and then individually vacuum packaged (<25 mm Hg) using a MULTIVAC™ R140 (Multivac, Inc., Kansas City, MO) with a coextruded linear low density polyethylene sealant and an ethyl vinyl alcohol barrier film. The bottom film (side of color measurements) was a 6.0 mil thick film with an oxygen transmission rate of 2 cc/m²/day at 23°C and 0% RH and water transmission rate of 5 g/m²/day at 38°C and 100% RH. The top film was a 4.0 mil thick film with an oxygen transmission rate of 3 cc/m²/day at 23°C and 0% R.H and water transmission rate of 7 g/m²/day at 38°C and 100% RH.

Objective color measurements of L*, a* and b* were taken with a Minolta colorimeter (Model CR-400 colorimeter, Konica Minolta, Ramsey NJ) at the D65 illuminant, 10° standard observer, 6 mm orifice and was calibrated using the white calibration tile from the manufacture. The film used to vacuum package the chops was placed over the tile when calibrating the colorimeter. Hue (inverse tangent of b*/a*) and Chroma (square root of (a* squared plus b* squared)) were calculated from the a* and b* values.

Raw chop color measurements were taken at 1, 5, 15, and 30 days of refrigerated display. Color was taken on each chop at three randomly selected locations of the *longissimus dorsi* lean portion. All color measurements were made on the same side of the chop each time for both the fresh and the cooked chops. Chops were stored in the display case, face up which was the same side that color measurements were taken.

Chops were stored in a refrigerated display case that was maintained at a temperature of approximately 2°C (Table 3), set at a 10 min defrost every 5 hr. The HUSSMAN™ display case (Husmann, Bridgeton, MO) was set up with four shelves and contained fluorescent light bulbs at 3000°K under each shelf. Chops were kept in columns by loin in the display case from front to back according to nitrite level. These samples were rotated once every 5 days to ensure that the temperature and lighting within the display case had little to no effect on the outcome of the results.

Table 3. Average Temperature of Display Case for Pork Chop Storage

	Average Temperature °C	SEM ^a
Rep #1	2.61	0.02
Rep #2	2.17	0.02
Rep #3	2.50	0.02

^a Standard Error of Means (SEM)

At days 1, 15 and 30 days of storage, one chop from each loin section was cooked in the vacuum package in a steam jacketed hot water bath at $82^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 15 minutes. Internal temperature of the chop was not measured in order to keep them in the vacuum package. The chops were cooled for approximately 45 minutes in a 3°C cooler. The packages were wiped dry and surface color measurements were made on the same side of the chops as the raw measurements were made.

Statistical analysis

Results were analyzed using the Mixed Procedure of Statistical Analysis System (SAS 9.2, 2008 Cary, NC). The experimental design was a randomized complete block design and was blocked by loin. The statistical model included the main effects of natural nitrite level, days of storage, and replication as well as the interaction between natural nitrite level and days of storage. Section pH within each loin was included in the model as a covariate. When mean differences were observed ($P < 0.05$), means were separated using the least significant difference test. Linear and quadratic relationships were evaluated for levels of nitrite and days of storage.

Results and Discussion

P-values for the main effects of nitrite level and days of storage and nitrite x days of storage interactions are presented in Table 4. A nitrite level x days of storage interaction was found for raw a^* and chroma and cooked a^* , b^* , and hue values.

Table 4. Effect of natural nitrite levels in pork chops vacuum packaged.

P-values for nitrite levels (ppm), days of storage and their interactions for instrumental color of vacuum packaged pork chops.

Trait	Main Effects		Interactions			Nitrite Level		Days of Storage	
	Nitrite Level (ppm)	Days of Storage	Nitrite Level ppm x Days of Storage	Rep.	Raw pH	Linear	Quadratic	Linear	Quadratic
Raw									
L* ¹	<0.001	<0.001	0.9050	0.0025	<0.001	<0.001	0.0051	<0.001	0.2576
a* ²	<0.001	<0.001	<0.001	0.1586	0.3251	<0.001	<0.001	<0.001	0.0368
b* ³	<0.001	<0.001	0.2410	<0.001	0.4915	<0.001	<0.001	<0.001	<0.001
Hue ⁴	<0.001	<0.001	0.8643	<0.001	0.8000	<0.001	<0.001	<0.001	<0.001
Chroma ⁵	<0.001	<0.001	0.0069	0.0253	0.1874	<0.001	<0.001	<0.001	<0.001
Cooked									
L* ¹	0.1153	<0.001	0.9197	0.4345	0.0154	0.5179	0.0239	<0.001	0.8690
a* ²	<0.001	0.0014	<0.001	0.0878	<0.001	<0.001	<0.001	0.9613	0.0003
b* ³	<0.001	<0.001	<0.001	0.2996	0.0332	<0.001	<0.001	<0.001	0.0118
Hue ⁴	<0.001	<0.001	<0.001	0.3074	0.0665	<0.001	<0.001	<0.001	0.1307
Chroma ⁵	<0.001	<0.001	0.3691	0.0801	<0.001	0.3864	<0.001	0.0005	<0.001

¹ CIE L* as a measure of black to white (0 to 100) respectively.

² CIE a* as a measure of red (positive value) and green (negative value).

³ CIE b* as a measure of yellow (positive value) and blue (negative value).

⁴ Hue = inverse tangent of (b*/a*).

⁵ Chroma = square root of ((a*)² + (b*)²).

Raw Color

Nitrite levels influenced instrumental color measurements of pork chops (Table 5).

Nitrite level of 0 ppm was lighter (P<0.05) with a higher L* value, than all of the chops with added nitrite. No differences in L* values (P>0.05) were observed among nitrite levels.

Wright et al. (2005) studied the characterization of U.S. pork in the retail market place; and found that 12.5% of the pork chops at retail stores were classified as “low quality”. High quality pork had darker L* values compared to average or low quality pork. Therefore, chops with natural nitrite would be perceived as higher quality chops.

Days of storage L* means for raw chops are presented in Table 6. As storage time increased, chops became lighter (higher L* values). Pork *longissimus dorsi* chops at 1 day of

display had ($P < 0.05$) the lowest (darkest) L^* values and chops at 5 days of display had ($P < 0.05$) lower L^* values than chops at 30 days.

Brewer et al., (2001) demonstrated that bloom time had no significant effect on Hunter L^* values but Hunter a^* values increased from 8.89 on freshly cut pork to 11.00 within 10 min. They studied several muscle groups including *gluteus medius*, *longissimus lumborum et thoracis*, *semimembranosus*, *biceps femoris* and *triceps brachii*. Brewer et al., (2001) found no muscle x bloom time interactions. Their Hunter a^* value results were more similar to the differences we obtained when comparing deoxymyoglobin to nitrosylmyoglobin in *longissimus dorsi*. There is an increase in redness on day 5 compared to day 1 of storage in chops with added nitrite, showing that it takes days to fully convert to the nitrosylmyoglobin state.

Table 5. Effect of natural nitrite levels on instrumental color of vacuum packaged raw pork chops

Trait	Nitrite Level (ppm)					SEM ¹	P-value	
	0	3	6	9	12		Linear	Quadratic
L^* ²	54.3 ^b	53.1 ^a	53.4 ^a	53.3 ^a	53.2 ^a	0.25	<0.001	0.0051
b^* ³	0.77 ^a	2.31 ^b	2.83 ^c	3.33 ^d	3.38 ^d	0.12	<0.001	<0.001
Hue ⁴	0.11 ^a	0.28 ^b	0.34 ^c	0.37 ^d	0.39 ^d	0.13	<0.001	<0.001

¹ Standard Error of Means (SEM)

^{a, b, c} and ^d means with different superscripts differ.

² CIE L^* as a measure of black and white (0-100) respectively.

³ CIE b^* as a measure of yellow (positive value) and blue (negative value).

⁴ Hue = inverse tangent of (b^*/a^*).

Table 6. Effect of days of vacuum storage on instrumental color of raw pork chops

Trait	Day of Vacuum Storage				SEM ¹	P-value	
	1	5	15	30		Linear	Quadratic
L* ²	52.2 ^a	53.3 ^b	53.5 ^{bc}	54.9 ^c	0.24	<0.001	0.2576
b* ³	2.15 ^b	3.59 ^d	2.84 ^c	1.53 ^a	0.11	<0.001	<0.001
Hue ⁴	0.26 ^b	0.42 ^d	0.34 ^c	0.16 ^a	0.01	<0.001	<0.001

¹ Standard Error of Means (SEM)

a, b, c and d means with different superscripts differ.

² CIE L* as a measure of black to white (0 to 100) respectively.

³ CIE b* as a measure of yellow (positive value) and blue (negative value).

⁴ Hue = inverse tangent of (b*/a*).

A nitrite level x days of storage interaction occurred for the raw a* measurements (Figure 1). The control (0 ppm) samples were (P<0.05) less red than any of the chops with natural nitrite at all days of storage. At day 1, chops with 9 and 12 ppm nitrite were (P<0.05) redder than chops with 0 and 3 ppm nitrite.

At day 5, chops with natural nitrite did not differ (P>0.05) in a* values. At day 15 of storage, chops with 9 ppm nitrite had (P<0.05) higher (redder) a* values than those with 6 ppm and less. In addition, chops with 12 ppm had (P<0.05) higher a* values than those at 0 and 3 ppm. At day 30 of storage, chops with 9 ppm nitrite also had (P<0.05) higher a* values than chops with 6 ppm and less.

For chops with no added nitrite, chops displayed at days 5 and 30 had (P<0.05) higher a* values than those at 1 and 15 days of storage. For chops with 3 ppm nitrite, chops stored at 1 day had (P<0.05) the lowest a* values and those stored for 5 days were (P<0.05) redder than those displayed for 30 days. For chops with 6 ppm nitrite, chops stored for 1 day had (P<0.05) the lowest a* value and those stored for 15 days had (P<0.05) lower a* values than those displayed for 5 and 30 days. For chops with 9 and 12 ppm, chops displayed for 1 day had (P<0.05) the lowest a* values and those displayed for 30 days were (P<0.05) redder than those displayed for 5 and 15 days.

Instrumental color results indicate that a* was 1.0 or more units lower for no added nitrite compared with nitrite-treated chops. Differences in a* value among nitrite-treated chops were minimal during early storage and became more pronounced during longer storage. As a result, chops with 3 ppm nitrite were intermediate to no-nitrite chops and chops with higher levels of

nitrite (6-12 ppm). However, small levels of nitrite were very effective in increasing the red color of vacuum packaged chops.

Nitrosylmyoglobin (NOMb) is similar to oxymyoglobin in the visible spectrum; it is a bright red derivative. In the presence of oxygen, the nitrosylmyoglobin will be converted to metmyoglobin which is brown in color. In an anaerobic environment such as vacuum packaging, the nitrosylmyoglobin is stable and will remain in this bright red state (Livingston and Brown, 1981).

Little research has been done on the state of myoglobin in anaerobic conditions with nitrite, nitrosylmyoglobin, specifically the color and color stability of this molecule. Because oxymyoglobin is similar in the visible spectrum to nitrosylmyoglobin, similarities and differences can be looked at between these two different myoglobin states. A great deal of research has been performed on oxymyoglobin such as bloom time and color stability. The bloom time for pork is less than 1 hour going from the deoxymyoglobin to oxymyoglobin state. The change in raw pork color due to nitrite, under anaerobic conditions, takes longer, going from oxymyoglobin to metmyoglobin, to nitric oxide metmyoglobin transient intermediate to the red nitrosylmyoglobin (Aberle, et. al, 2001).

The results from this research were different compared to the results from Brewer et al. (2006). This would be expected as Brewer was measuring the changes between deoxymyoglobin and oxymyoglobin. Our research compared the deoxymyoglobin state to the nitrosylmyoglobin state. The results from Brewer et al. (2006) did not show a significant ($P=0.23$) change in Minolta a^* values between the unbloomed verses bloomed pork chops but the Hunter a values did have a significant a value increase with bloom. In fact, the Minolta a^* bloomed values decreased compared to the unbloomed Minolta a^* values, just the opposite of the Hunter a values.

Nitric oxide can bind with myoglobin in the vacuum package chops to form the brown nitric oxide metmyoglobin transient intermediate. Under anaerobic conditions the intermediate can slowly be converted to the bright pink nitrosylmyoglobin (Parthasarathy and Bryan, 2012). Further, the main benefits of nitrite used in curing meat include stabilized pink cured color, inhibition of rancidity and clostridial growth.

When exposed to nitric oxide, metmyoglobin will convert to nitric oxide metmyoglobin transient intermediate which will then slowly convert to nitrosylmyoglobin which in pork is a bright pink color. The nitrosylmyoglobin is stable in anaerobic conditions. This conversion

process takes several days as is shown in Figure 1, a^* values are ($P < 0.05$) higher at day 5 than at day 1 for vacuum packaged raw chops with added nitrite.

Shown in Rosenvold and Anderson (2003), there was about a 1 unit increase in a^* value comparing a chop over a 1 hour bloom time, time 0 color taken immediately after cutting. These same chops have approximately a 2 unit increase in b^* from an unbloomed to a bloomed state. There was little change in the L^* values during the first hour of bloom. The results of Rosenvold and Anderson (2003) were more similar in a^* and b^* changes to the results obtained in this research.

One percent of nitrite present in meat of pH from 5.5 to 6.0 is N_2O_3 (dinitrogen trioxide). Dinitrogen trioxide is reduced to nitric oxide by endogenous reductants such as cysteine, cytochromes, quinones and NADH (Alberle et. al, 2001). These reductants decrease in effectiveness over time. When nitrite is added to meat, the most significant reductant is the myoglobin itself going to metmyoglobin, therefore the surface of the meat turns brown after nitrite has been added. To ensure there is an adequate supply of reductants available when curing meats, often small amounts of exogenous reducing agents such as sodium ascorbate or sodium erythorbate are added (Alberle et. al, 2001).

Livingston and Brown (1981) found that in anaerobic environments such as vacuum packaging, nitrosylmyoglobin is stable and will remain bright red. Nitrosylmyoglobin and oxymyoglobin are similar in the visible spectrum.

The main effect of nitrite level on b^* value is reported in Table 5. Chops with 0 ppm nitrite had ($P < 0.05$) the lowest (less yellow) b^* values and chops with 9 and 12 ppm had ($P < 0.05$) the highest. In addition, chops with 6 ppm nitrite had ($P < 0.05$) lower b^* values than 9 and 12 but had ($P < 0.05$) higher b^* values than 3 ppm.

Rosenvold and Anderson (2003) found an increase in b^* values from the *longissimus dorsi* muscle going from approximately 5 to approximately 7 from an unbloomed (deoxymyoglobin) to a bloomed (oxymyoglobin) state.

The b^* value changed over time as the chops were stored in vacuum packaging resulting in a quadratic relationship (Table 6). Chops had ($P < 0.05$) the lowest b^* value on day 30 of storage and had ($P < 0.05$) the highest on day 5 of storage. At day 1 of storage the b^* values were ($P < 0.05$) lower than days 5 and 15.

All chops with natural nitrite had ($P < 0.05$) higher hue values than chops with no added nitrite (Table 5). Chops with 9 and 12 ppm nitrite were ($P < 0.05$) higher in hue value than chops

with 3 and 6 ppm nitrite and chops with 6 ppm nitrite had ($P < 0.05$) higher hue values than chops with 3 ppm nitrite. Zhu and Brewer (1998) suggested that a lower hue value would be indicative of pork that would be less red and more gray.

A quadratic response was observed for days of storage and hue angle. Hue value was lowest ($P < 0.05$) on day 30 of storage, followed by day 1 and then day 15. The highest ($P < 0.05$) hue value was found on day 5 of storage. The color of the chops were changing over time as is indicated by the hue values.

Chroma is a measure of color intensity. Chroma values increase as the intensity of the color increases (AMSA, 1991). In a nitrite x days of storage interaction, chops with no added nitrite had ($P < 0.05$) lower chroma values than all of the chops with added nitrite at all days of storage. At day 1, 5, and 15, chops with 9 and 12 ppm were ($P < 0.05$) higher in chroma values than chops with 3 ppm. At day 30, chops with 9 and 12 ppm nitrite had ($P < 0.05$) higher chroma values than chops with 3 and 6 ppm nitrite. Furthermore, chops with 6 ppm nitrite had ($P < 0.05$) higher chroma values than chops with 3 ppm nitrite. Chops with nitrite were more intense in color compared to chops without natural nitrite.

Chops with 0 ppm nitrite had a ($P < 0.05$) higher chroma value at day 5 than at day 15. For chops with 3 ppm added nitrite, chops stored at day 5 had the ($P < 0.05$) highest chroma value. Furthermore, chops at day 15 of storage had ($P < 0.05$) higher chroma values than those at day 1. For chops with 6 ppm nitrite, chops stored at day 1 were ($P < 0.05$) lowest in chroma values and chops stored at day 5 were higher in chroma values than day 15. For chops with 9 ppm nitrite, chops stored at day 1 were ($P < 0.05$) lower in chroma values than all other chops stored at days 5, 15, and 30 days. Chops with 12 ppm nitrite, as is shown in Figure 2, had ($P < 0.05$) the lowest chroma values at day 1. Also, chops stored at day 15 were ($P < 0.05$) higher in chroma values than day 1 but ($P < 0.05$) lower in chroma value than day 5. Chroma (color intensity) of the chops changed over time as described above, the higher the chroma values, the more intense the color.

Linear and quadratic contrasts were analyzed for treatments on the main effects. For raw pork data, all effects were found to have quadratic relationships to treatments with the exception of L^* values on days of storage which was found to have a linear contrast. The a^* , b^* , hue, and chroma values increased to a greater extent going from 0 ppm to 3 ppm natural nitrite compared to the remaining 3 ppm incremental increases, thus a quadratic effect was detected. A quadratic

effect was also detected in L* values and natural nitrite levels, but a decrease in L* values was observed when nitrite was added to the pork chops.

A linear effect was detected for L* values and days of display, the longer the chops were stored the lighter the chops became. A quadratic effect was detected for a*, b*, hue, and chroma values and days of display. The b*, hue, and chroma values were at their highest values at day 5, a* was at its highest value at day 30 of storage.

Cooked L , a* , b* color*

There was not a significant (p=0.12) effect of nitrite level on cooked L* values, shown in Table 4. The effect of days of vacuum packaging on cooked chop L* value is shown in Table 8. At day 1 the average L* value of the cooked chops was 75.0. The longer the chops were held in vacuum packaging in the raw state and then cooked in the vacuum packaging, the darker the chops became. The chops at day 15 were (P<0.05) lighter than those at day 1. The chops at day 30 were (P<0.05) lighter (greater L*) than those at days 1 and 15.

Table 7. Effect of natural nitrite levels on instrumental color of vacuum packaged cooked pork chops

Trait	Nitrite Levels (ppm)					SEM ¹	Linear	Quadratic
	0	3	6	9	12			
L* ²	74.0	74.6	74.8	74.4	74.4	0.29	0.5179	0.0239
Chroma ³	10.33 ^c	9.22 ^a	9.37 ^a	9.97 ^b	9.82 ^b	0.14	0.3864	<0.001

¹ Standard Error of Means (SEM)

^{a, b, and c} means with different superscripts differ.

² CIE L* as a measure of black to white (0 to 100) respectively.

³ Chroma = square root of ((a*)² + (b*)²).

Table 8. Effect of days of vacuum storage on instrumental color of vacuum packaged cooked pork chops

Trait	Days of Vacuum Storage			SEM ¹	Linear	Quadratic
	1	15	30			
L* ²	75.0 ^c	74.5 ^b	73.9 ^a	0.26	<0.001	0.869
Chroma ³	9.80 ^b	10.03 ^b	9.40 ^a	0.12	0.0005	<0.001

¹ Standard Error of Means (SEM)

^{a, b, and c} means with different superscripts differ.

² CIE L* as a measure of black to white (0 to 100) respectively.

³ Chroma = square root of $((a^*)^2 + (b^*)^2)$.

There was a significant ($P < 0.05$) nitrite level x days of storage interaction on the a^* values of the cooked chops (Figure 3). The a^* value of cooked chops with no natural nitrite increased over time, day 15 chops were ($P < 0.05$) redder than chops at day 0 and chops at day 30 were ($P < 0.05$) redder than chops at day 0 and 15. Cooked chops with 3 ppm and 6 ppm nitrite were ($P < 0.05$) less red at day 30 than at days 0 and 15. For those chops with 9 and 12 ppm nitrite, a^* values were ($P < 0.05$) not different at days 1, 15 and 30.

At day 1, cooked chops with 0 ppm were ($P < 0.05$) less red than all chops with nitrite and chops with 6, 9 and 12 ppm were ($P < 0.05$) redder than chops with 3 ppm. At day 15, cooked chops with 0 ppm were the ($P < 0.05$) least red. At day 15, cooked chops with 6 ppm nitrite were redder than those with 3 ppm and chops with 9 and 12 ppm were ($P < 0.05$) redder than chops with 6 ppm nitrite. For chops stored for 30 days and then cooked, chops with 6 ppm were ($P < 0.05$) redder than chops with 0 and 3 ppm nitrite. In addition, chops with 9 and 12 ppm nitrite were ($P < 0.05$) redder (higher a^* value) than those with 0, 3, and 6 ppm nitrite. Chops with 9 and 12 ppm natural nitrite were not ($P < 0.05$) different in a^* cooked color on all days. At day 30, cooked chops with 0 and 3 ppm nitrite were not ($P < 0.05$) different in a^* value.

One interesting aspect of cooked a^* values were that the chops without nitrite ($P < 0.05$) increased in cooked a^* values the longer they were stored but the chops with lower levels of natural nitrite, 3 and 6 ppm, decreased ($P < 0.05$) in cooked a^* values from day 15 to day 30. This convergence of 0 ppm and 3 and 6 ppm continues to the point where at day 30, there was no ($P = 0.97$) difference in a^* values between 0 and 3 ppm chops. Additional research needs to be done to better understand what was happening with cooked chops without nitrite. Could this be a persistent pinking concern that the pork industry should evaluate? More research could also be

performed on evaluating cooked chops with nitrite to better understand the decrease in cooked a^* values from day 15 to 30 on chops with 6 ppm nitrite or less.

A nitrite level x days stored interaction for b^* values of the cooked chops ($P < 0.05$) was observed, shown in Figure 4.

Chops with no nitrite, when cooked were ($P < 0.05$) more yellow than any of the cooked chops with nitrite, at all days (1, 15 and 30) of evaluation. At day 1 and 15, cooked chops with 3 and 9 ppm nitrite were ($P < 0.05$) more yellow than cooked chops with 6 and 12 ppm nitrite. At day 30, cooked chops with 3 ppm nitrite were ($P < 0.05$) more yellow than chops with 6, 9 and 12 ppm nitrite.

For chops with 0 ppm nitrite, chops stored and then cooked at day 30 were less yellow than chops cooked at days 1 and 15. Cooked chops with 3 ppm nitrite were ($P < 0.05$) not different in b^* value on all days. This was also the case for chops with 6 ppm nitrite. Cooked chops with 9 ppm nitrite were less yellow at day 30 than day 1; this was also the case for chops with 12 ppm nitrite.

The b^* values for the cooked chops containing nitrite were fairly narrow in range. The average b^* value of cooked chops containing nitrite range from 4.50 (12 ppm at day 30) to 5.49 (3 ppm at day 1). Chops with no added nitrite (0 ppm) ranged in b^* values from 7.38 at day 30 to 8.71 at day 15.

A nitrite level x days stored interaction for hue values of the cooked chops ($P < 0.05$) was observed, shown in Figure 5. As can be seen in Figure 5, the hue value for chops with 0 ppm nitrite were ($P < 0.05$) higher than all chops with nitrite on all days of storage. This is the opposite of what is seen in the raw chops where the 0 ppm chops were lower in hue value. This inversion of the slope of the hue going from a positive slope when raw to a negative slope when cooked is mainly due to the change in b^* values. Chops with nitrite were redder and more yellow in color when raw but when cooked the chops with nitrite were much less yellow than the chops without nitrite. Chops with 0 ppm nitrite were ($P < 0.05$) lowest in hue values on day 30 of storage and at day 1 of storage chops were ($P < 0.05$) highest in hue value. Also, chops stored at day 15 were ($P < 0.05$) lower than day 1 but ($P < 0.05$) higher than day 30 in hue value. For chops with 3 ppm nitrite, day of storage 1 and 15 were ($P < 0.05$) lower than day 30 in hue values. Chops containing 6 ppm nitrite were not ($P < 0.05$) different in hue values on any days of storage, this was also the case for chops with 9 ppm nitrite. Hue values were ($P < 0.05$) highest at day 1 of storage for chops with 12 ppm nitrite.

At day 1 of storage, chops with 6, 9, and 12 ppm nitrite were ($P < 0.05$) lowest in hue value and chops with 3 ppm were ($P < 0.05$) lower than chops with 0 ppm but ($P < 0.05$) higher than all other chops in hue values. This was also the case at day 15 of storage. At day 30 of storage, chops with 6 ppm nitrite were ($P < 0.05$) higher in hue value than chops with 12 ppm nitrite and chops with 3 ppm nitrite were ($P < 0.05$) higher than all other chops with nitrite.

As is shown in Table 7, cooked chops with 0 ppm nitrite had ($P < 0.05$) higher chroma values than all chops with nitrite. Furthermore, chops with 3 and 6 ppm nitrite were ($P < 0.05$) higher in chroma (intensity) than chops with 9 and 12 ppm nitrite. Shown in Table 8, the chroma values were ($P < 0.05$) lowest at 30 days of storage and then cooked. Cooked chops with less nitrite were generally more intense in color. This was a result mainly due to the large increase in b^* values when cooked, as chroma is the product of the square root of a^* squared plus b^* squared.

Linear and quadratic contrasts were analyzed for treatments on the main effects. For cooked pork data, all effects were found to have a quadratic relationship to treatments with the exception of L^* and hue values on days of storage which were found to have linear relationships. The L^* and a^* values increased to a greater extent going from 0 ppm to 3 ppm natural nitrite compared to the remaining 3 ppm incremental increases, thus a quadratic effect was detected. A quadratic effect was also detected in b^* , hue, and chroma values and natural nitrite levels, large decreases in these values were observed when nitrite was added to the pork chops. Continued decreases were observed with additional nitrite addition but these decreases weren't as large.

A linear effect was detected for L^* and hue values and days of display, the longer the chops were stored the lighter and less yellow the chops became respectively. A quadratic effect was detected for a^* , b^* , and chroma values and days of display. The b^* and hue values decreased the longer they were stored and then cooked, the chroma values were lowest at day 30 of storage which means the color was least intense at day 30 of storage, post cooked.

Conclusion

A bright pink bloomed pork chop is what is acceptable and expected of fresh pork. Pork exposed to oxygen develops this bright pink color in less than 1 hour and oxymyoglobin is the predominate state of myoglobin on the surface of the chop.

Nitrosylmyoglobin can be formed in fresh pork when nitrite is added in anaerobic conditions such as vacuum packaging. Color of nitrosylmyoglobin in fresh pork is bright pink.

Vacuum packaged pork without nitrite will be turn grey in color and the predominate state of myoglobin on the surface of the meat is deoxymyoglobin.

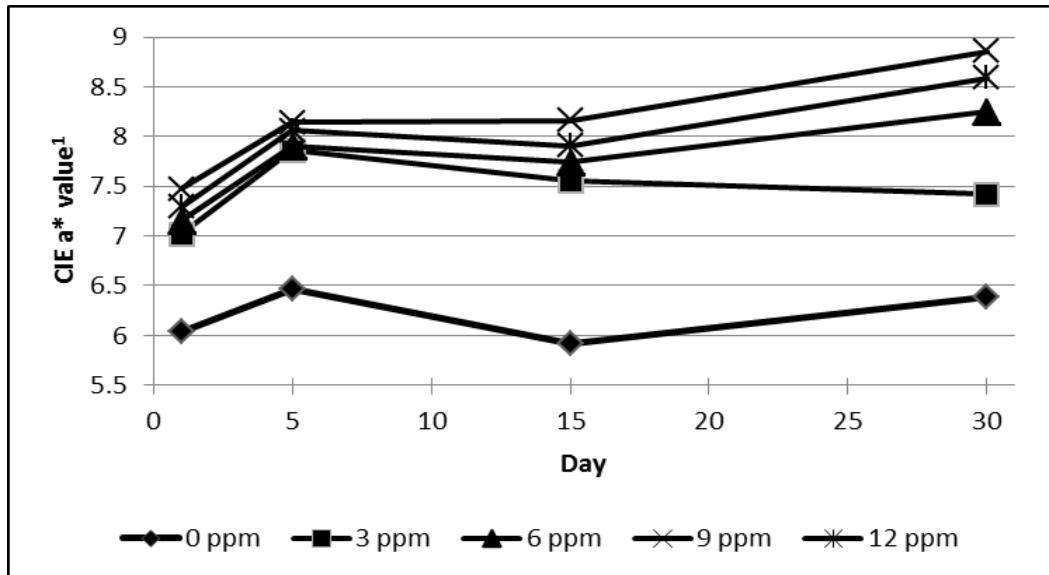
Reflectance color measurements closely parallel that of what the eyes and brain see. The results from this research indicate that the color differences between deoxymyoglobin and nitrosylmyoglobin are similar to the color differences between deoxymyoglobin and oxymyoglobin.

Vacuum packaged raw chops with added nitrite were redder, darker, more yellow, and more intense in color than without added nitrite. When these same chops were cooked, the chops with nitrite were redder, less yellow and lower in hue and chroma values compared to chops with no nitrite. The ideal levels of natural nitrite were at concentrations below 9 ppm, optimally at 3 ppm, in fresh pork to minimize any issue with persitent pinking in the cooked product. At 3 ppm nitrite in fresh pork, the raw vacuum packaged color is still redder and darker and more intense in color than chops without nitrite.

Much of the fresh pork color research over the last several decades has looked at Modified Atmosphere Packaging (MAP) to optimize fresh pork color. MAP packaged pork exist in the market today and provides the consumer a good option for fresh pork, typically optimizing the color and appearance of fresh pork. The MAP commercial systems in packers and product consumables can be expensive compared to overwrap or vacuum packaging consumables and required packing equipment.

The use of natural nitrite in vacuum packaged pork provides a cost effective solution for fresh pork. The color of fresh pork vacuum packaged with added nitrite is similar to that of bloomed pork that is typically found in overwrapped product. Overwrapped fresh pork will have a typical shelf life of 6-7 days depending on the display atmosphere and temperature conditions. Properly chilled vacuum packaged pork can have a shelf life of greater than 4 weeks.

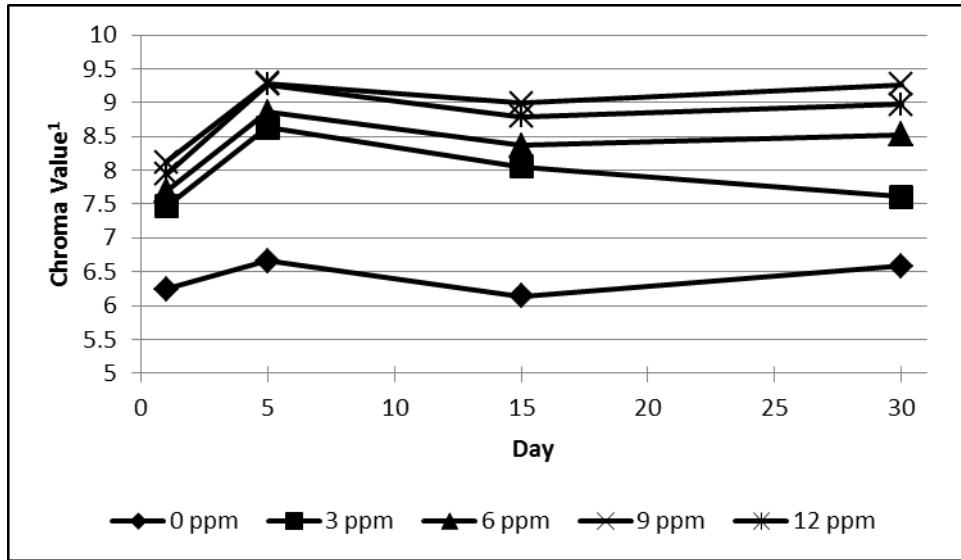
Figure 1. Minolta a* values (redness) interaction means for natural nitrite levels (ppm) x days stored of vacuum packaged raw pork chops



Standard Error of Means (SEM) = 0.18

¹ CIE a* value as a measure of red (positive value) and green (negative value).

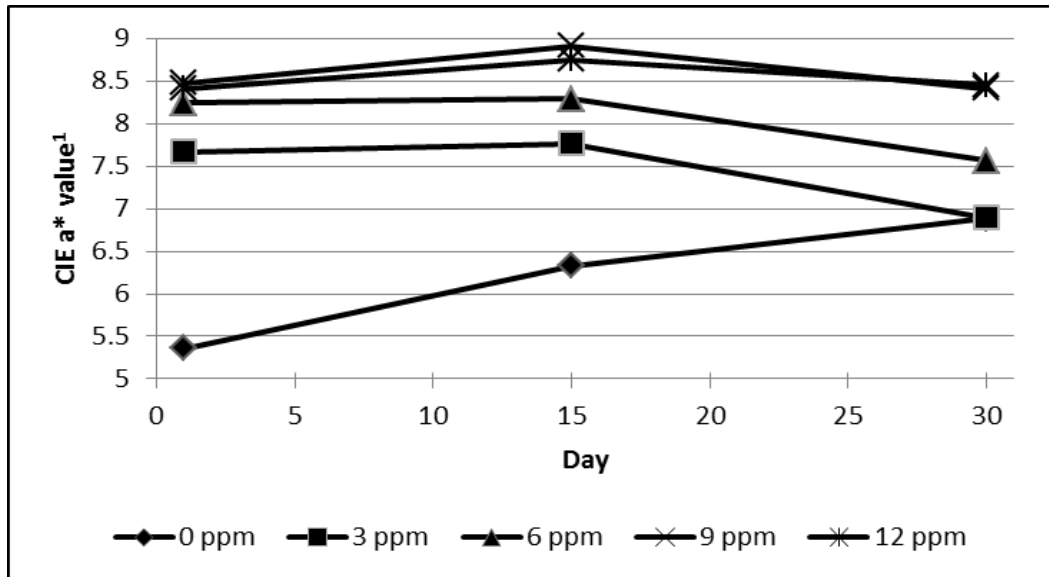
Figure 2. Minolta chroma values (intensity) interaction means for natural nitrite levels (ppm) x days stored of vacuum packaged raw pork chops



Standard Error of Means (SEM) = 0.21

¹ Chroma value as calculated by square root of $((a^*)^2 + (b^*)^2)$.

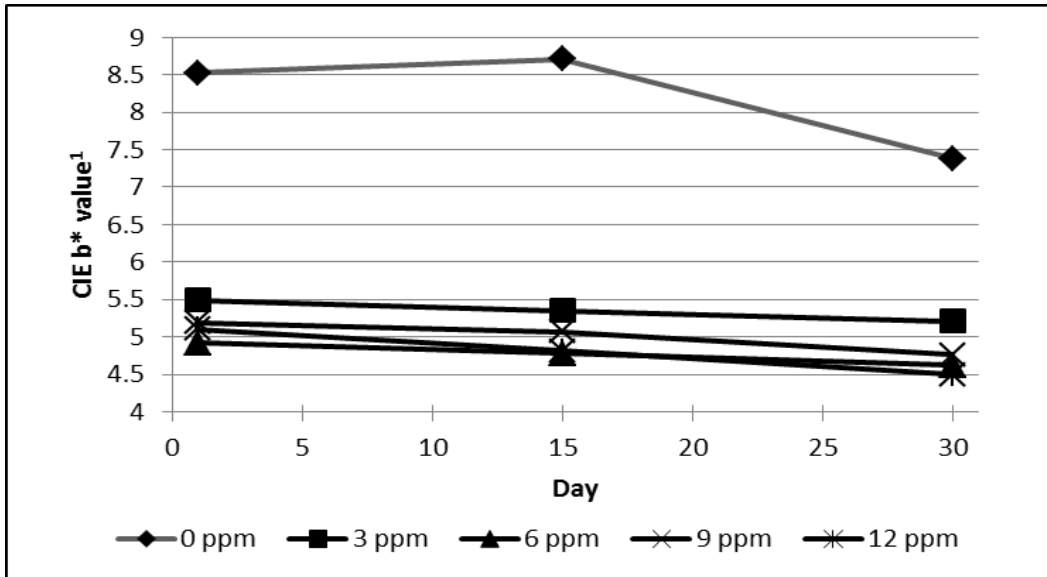
Figure 3. Minolta a* values (redness) interaction means for natural nitrite levels (ppm) x days stored of vacuum packaged cooked pork chops



Standard Error of Means (SEM) = 0.21

¹ CIE a* value as a measure of red (positive value) and green (negative value).

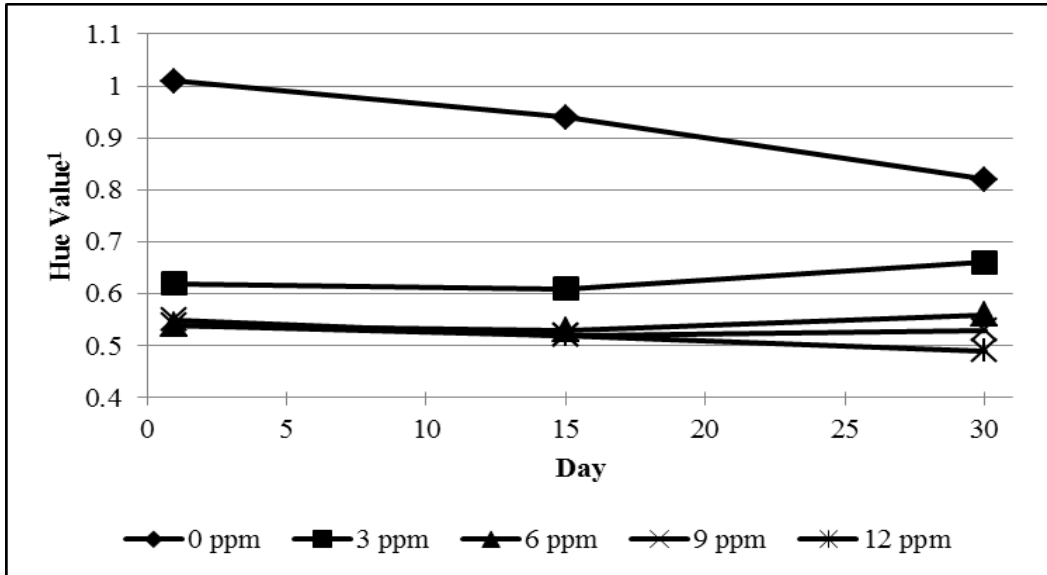
Figure 4. Minolta b* values (yellowness) interaction means for natural nitrite levels (ppm) x days stored of vacuum packaged cooked pork chops



Standard Error of Means (SEM) = 0.13

¹ CIE b* value as a measure of yellow (positive value) and blue (negative value).

Figure 5. Hue values interaction means for natural nitrite levels (ppm) x days vacuum stored of cooked pork chops



Standard Error of Means (SEM) = 0.21

¹ Hue as a calculated value of inverse tangent of (b*/a*).

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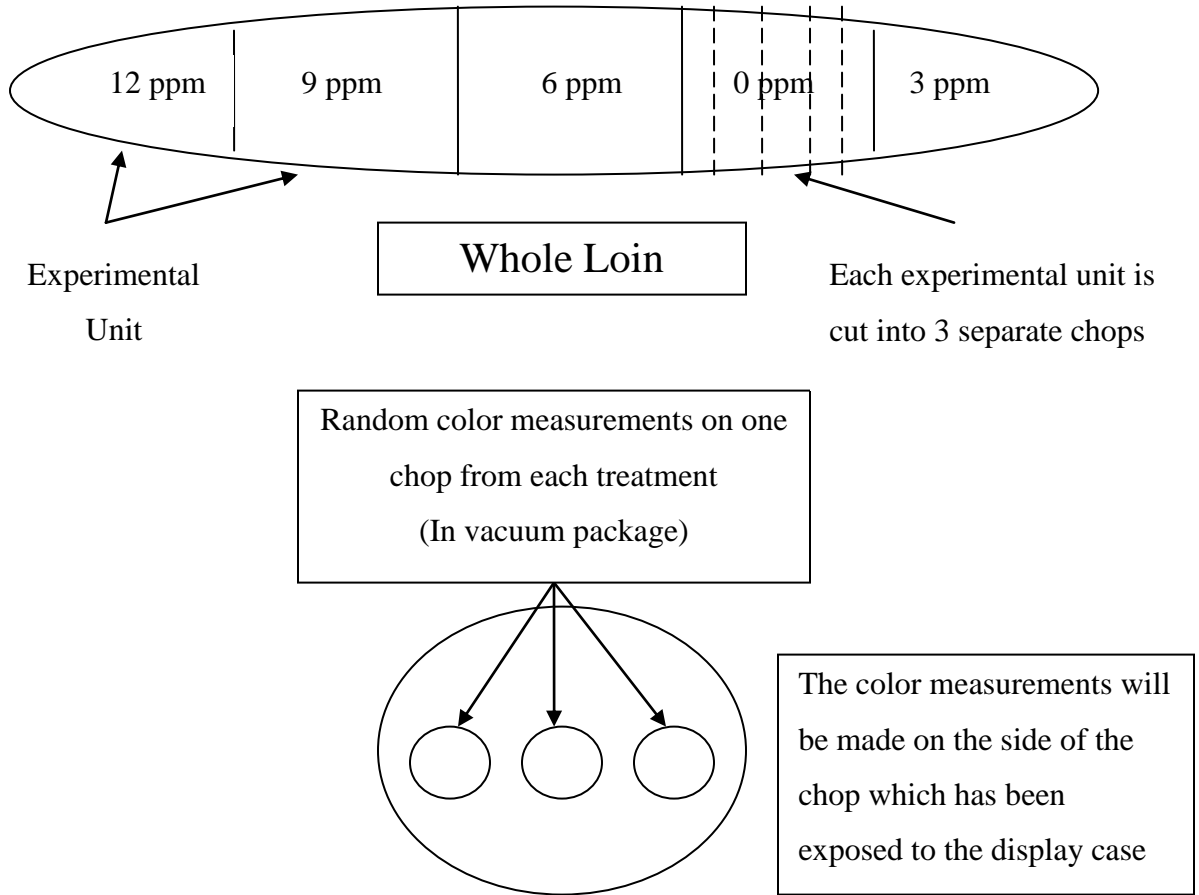
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Appendix A - Loin Treatment Diagram

Figure A.1 – Schematic of Loin Section and Chop Treatments and Analysis



Appendix B - % Nitrite in VegStable™ 504

Table B.1 – Nitrite Analysis of VegStable™ 504

	% of sodium nitrite in VegStable™ 504 (Lab Results)	% nitrite in VegStable™ 504 (Calculated)
	2.23%	1.49%
	2.29%	1.53%
	2.36%	1.57%
	2.23%	1.49%
	2.20%	1.47%
	2.25%	1.50%
	2.22%	1.48%
	2.15%	1.43%
Average =	2.24%	1.50%

Appendix C - Raw L* value, nitrite level (ppm) x days stored

Table C.1 – Minolta L* values (darkness) interaction means for natural nitrite levels (ppm) x days stored of raw pork chops

Nitrite Level (ppm)	Days of Vacuum Storage			
	1	5	15	30
0 ppm	53.1	53.6	54.6	55.9
3 ppm	51.8	53.1	53.2	54.5
6 ppm	52.1	53.3	53.5	54.8
9 ppm	52.2	53.4	53.2	54.5
12 ppm	52.1	53.0	53.1	54.6

Standard Error of Means (SEM) = 0.37

L* as a measure of black and white (0-100) respectively.

Appendix D - Raw a* value, nitrite level (ppm) x days stored

Table D.1 – Minolta a* values (redness) interaction means for natural nitrite levels (ppm) x days stored of raw pork chops

Nitrite Level (ppm)	Days of Vacuum Storage			
	1	5	15	30
0 ppm	6.04 ^{a1}	6.46 ^{b1}	5.92 ^{a1}	6.39 ^{b1}
3 ppm	7.02 ^{a2}	7.86 ^{c2}	7.55 ^{bc2}	7.42 ^{b2}
6 ppm	7.15 ^{a23}	7.90 ^{c2}	7.74 ^{b23}	8.25 ^{c3}
9 ppm	7.47 ^{a3}	8.14 ^{b2}	8.16 ^{b4}	8.86 ^{c4}
12 ppm	7.29 ^{a3}	8.06 ^{b2}	7.91 ^{b34}	8.59 ^{c34}

Standard Error of Means (SEM) = 0.18

a* as a measure of red (positive value) and green (negative value).

Day is alpha superscripted within row. ^{a, b,} and ^c with different superscripts differ.

Nitrite level is numerically superscripted within column. ^{1, 2, 3} and ⁴ with different superscripts differ.

Appendix E - Raw b* value, nitrite level (ppm) x days stored

Table E.1 - Minolta b* values (yellowness) interaction means for natural nitrite levels (ppm) x days stored of raw pork chops

Nitrite Level (ppm)	Days of Vacuum Storage			
	1	5	15	30
0 ppm	0.46	1.40	1.02	0.21
3 ppm	2.09	3.53	2.62	1.01
6 ppm	2.49	4.00	3.09	1.74
9 ppm	2.84	4.46	3.71	2.33
12 ppm	2.87	4.55	3.75	2.36

Standard Error of Means (SEM) = 0.21

b* as a measure of yellow (positive value) and blue (negative value).

Appendix F - Raw Hue value, nitrite level (ppm) x days stored

Table F.1 - Hue values, interaction means for natural nitrite levels (ppm) x days stored of raw pork chops

Nitrite Level (ppm)	Days of Vacuum Storage			
	1	5	15	30
0 ppm	0.05	0.21	0.15	0.02
3 ppm	0.26	0.42	0.32	0.13
6 ppm	0.31	0.47	0.37	0.20
9 ppm	0.34	0.50	0.42	0.24
12 ppm	0.35	0.51	0.44	0.26

Standard Error of Means (SEM) = 0.21

Hue = inverse tangent of (b*/a*).

Appendix G - Raw Chroma value, nitrite level (ppm) x days stored

Table G.1 - Chroma values interaction means for natural nitrite levels (ppm) x days stored of raw pork chops

Nitrite Level (ppm)	Days of Vacuum Storage			
	1	5	15	30
0 ppm	6.24 ^{ab1}	6.66 ^{b1}	6.14 ^{a1}	6.58 ^{ab1}
3 ppm	7.47 ^{a2}	8.63 ^{c2}	8.05 ^{b2}	7.61 ^{ab2}
6 ppm	7.69 ^{a23}	8.86 ^{c23}	8.37 ^{b23}	8.53 ^{bc3}
9 ppm	8.12 ^{a3}	9.29 ^{b3}	8.99 ^{b4}	9.27 ^{b4}
12 ppm	7.94 ^{a3}	9.27 ^{c3}	8.79 ^{b34}	8.97 ^{bc34}

Standard Error of Means (SEM) = 0.21

Chroma = square root of $((a^*)^2 + (b^*)^2)$.

Day is alpha superscripted within row. ^a, ^b, and ^c with different superscripts differ.

Nitrite level is numerically superscripted within column. ¹, ², ³ and ⁴ with different superscripts differ.

Appendix H - Cooked L* value, Day by Nitrite Table

Table H.1 - Minolta L* values (darkness) interaction means for natural nitrite levels (ppm) x days stored of cooked pork chops

Nitrite Level (ppm)	Days of Vacuum Storage		
	1	15	30
0 ppm	73.3	74.2	73.6
3 ppm	75.3	74.5	74.0
6 ppm	75.1	74.9	74.4
9 ppm	75.0	74.2	73.9
12 ppm	75.1	74.5	73.5

Standard Error of Mean (SEM) = 0.40

L* as a measure of black and white (0-100) respectively.

Appendix I - Cooked a* value, Day by Nitrite Table

Table I.1 - Minolta a* values (redness) interaction means for natural nitrite levels (ppm) x days stored of cooked pork chops

Nitrite Level (ppm)	Days of Vacuum Storage		
	1	15	30
0 ppm	5.36 ^{a1}	6.33 ^{b1}	6.88 ^{c1}
3 ppm	7.67 ^{b2}	7.76 ^{b2}	6.89 ^{a1}
6 ppm	8.24 ^{b3}	8.29 ^{b3}	7.56 ^{a2}
9 ppm	8.47 ^{a3}	8.91 ^{a4}	8.41 ^{a3}
12 ppm	8.41 ^{a3}	8.75 ^{a34}	8.45 ^{a3}

Standard Error of Means (SEM) = 0.21

a* as a measure of red (positive value) and green (negative value).

Day is alpha superscripted within row. ^{a, b} and ^c with different superscripts differ.

Nitrite level is numerically superscripted within column. ^{1, 2, 3} and ⁴ with different superscripts differ.

Appendix J - Cooked b* value, Day by Nitrite Table

Table J.1- Minolta b* values (yellowness) interaction means for natural nitrite levels (ppm) x days stored of cooked pork chops

Nitrite Level (ppm)	Days of Vacuum Storage		
	1	15	30
0 ppm	8.53 ^{b3}	8.71 ^{b3}	7.38 ^{a3}
3 ppm	5.49 ^{a2}	5.35 ^{a2}	5.21 ^{a2}
6 ppm	4.92 ^{a1}	4.78 ^{a1}	4.62 ^{a1}
9 ppm	5.19 ^{b12}	5.07 ^{ab12}	4.76 ^{a1}
12 ppm	5.10 ^{b1}	4.81 ^{ab1}	4.50 ^{a1}

Standard Error of Means (SEM) = 0.13

b* as a measure of yellow (positive value) and blue (negative value).

Day is alpha superscripted within row. ^a and ^b with different superscripts differ.

Nitrite level is numerically superscripted within column. ^{1,2} and ³ with different superscripts differ.

Appendix K - Cooked Hue value, Day by Nitrite Table

Table K.1 - Hue values, interaction means for natural nitrite levels (ppm) x days stored of cooked pork chops

Nitrite Level (ppm)	Days of Vacuum Storage		
	1	15	30
0 ppm	1.01 ^{c3}	0.94 ^{b3}	0.82 ^{a4}
3 ppm	0.62 ^{a2}	0.61 ^{a2}	0.66 ^{b3}
6 ppm	0.54 ^{a1}	0.53 ^{a1}	0.56 ^{a2}
9 ppm	0.55 ^{a1}	0.52 ^{a1}	0.53 ^{a12}
12 ppm	0.54 ^{b1}	0.52 ^{a1}	0.49 ^{a1}

Standard Error of Means (SEM) = 0.21

Hue = inverse tangent of (b*/a*).

Day is alpha superscripted within row. ^{a, b} and ^c with different superscripts differ.

Nitrite level is numerically superscripted within column. ^{1,2,3} and ⁴ with different superscripts differ.

Appendix L - Cooked Chroma value, Day by Nitrite Table

Table L.1 - Chroma values interaction means for natural nitrite levels (ppm) x days stored of raw pork chops

Nitrite Level (ppm)	Days of Vacuum Storage		
	1	15	30
0 ppm	10.10	10.79	10.11
3 ppm	9.48	9.48	8.71
6 ppm	9.62	9.59	8.90
9 ppm	9.95	10.26	9.70
12 ppm	9.86	10.00	9.59

Standard Error of Means (SEM) = 0.20

Chroma = square root of $((a^*)^2 + (b^*)^2)$.