EFFECT OF STORAGE TEMPERATURE AND TIME ON REDNESS OF 93:7 GROUND BEEF PATTIES IN CARBON MONOXIDE ATMOSPHERIC PACKAGING

By

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Title of Study: EFFECT OF STORAGE TEMPERATURE AND TIME ON REDNESS OF 93:7 GROUND BEEF PATTIES IN CARBON MONOXIDE ATMOSPHERIC PACKAGING

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Abstract: The objective of this study was to investigate storage time and temperature on redness of 93:7 ground beef in CO-MAP (carbon monoxide modified atmosphere) packaging. Three 4.5 kg ground beef chubs with a lean-to-fat ratio (L/F) of 93:7 were collected from Creekstone Farms in Arkansas City, KS. Ground beef chubs were stored for 7 d, then chubs were mixed and finely ground to homogenize sample. Homogenized ground beef sample was then formed into 227 g patties (n = 54*)* and packaged in MAP packaging consisting of 0.4% CO, 30% CO_2, and 69.6% N_2. After packaging, patties were randomly assigned to one of two storage temperatures: 2.5°C or 4.4°C and one of three dark storage times: 1 d, 3 d, or 10 d. On each respective pull day, two patties from each storage temperature were measured for instrumental surface raw color, internal cooked color, and total plate count (TPC). Data were analyzed as a split-split plot using PROC GLIMMIX procedure of SAS. Least square means were calculated and considered significant at $P < 0.05$. There was a significant storage time × storage temperature interaction on raw color measurements. L^* and a^* values increased ($P < 0.05$) at 4.4°C and 2.5°C with an increase in storage time from 1 to 10 d in dark storage. There was no significant effects of storage time or storage temperature on cooked L^* values, a^* values, b^* values, chroma, and hue. At 4.4°C and 2.5°C, TPC increased ($P < 0.05$) with an increase in storage time from 3 to 10 d in dark storage. The current study indicates after 1 and 3 d in dark storage lower storage temperature decreased redness of ground beef patties. Therefore, processors should use favorable temperatures to promote carboxymyoglobin and redness of patties stored in CO-MAP while maintaining other quality and safety attributes.

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CHAPTER I

INTRODUCTION

Meat color is the most important quality attribute consumers look for, associating color with meat freshness and wholesomeness (Mancini and Hunt, 2005). Deviation from this bright cherry-red color results in discarded or discounted products because of a lack of consumer acceptance and possible spoilage (Suman et al., 2014). One of the biggest limiting factors of shelf-life for fresh meat is the loss of color stability throughout storage (Carpenter et al., 2001). It is estimated, discolored meat accounts for 11.07% of discounted retail beef and is responsible for close to $3.73 billion in revenue loss in the U.S. (Ramanathan et al., 2022). Monitoring color to maximize shelf life and consumer acceptability is a priority in meat science research and the meat industry (Schelkopf et al., 2021).

Fresh meat color is determined by the chemical state of myoglobin (Mb) (Mancini and Hunt, 2005). In typical retail packaging, fresh meat is in the aerobic (Oxymyoglobin; OxyMb) state producing a bright cherry-red color, but as meat is displayed longer the discoloration in the form of metmyoglobin (MetMb) increases (Mancini and Hunt, 2005). Over the past several years, technologies have been developed and improved to aid in the

extension of self-life, including the development and use of modified atmospheric packaging (MAP) (Zhao et al., 1994; Skibsted et al., 1994; Tørngren et al., 2018).

Modified atmosphere packaging has become widely used for fresh meat retail packaging (Tørngren et al., 2018); it works by altering the gaseous environment within the vapor barrier film (McMillin et al., 1999). Altering this gaseous environment, aids in protecting fresh meat product from adverse effects associated with extended shelf-life (Skibsted et al., 1994). Carbon monoxide modified atmospheric packaging (CO-MAP) is a type of anaerobic MAP packaging encompassing a gas mixture of carbon dioxide, carbon monoxide (CO), and nitrogen. The use of tri-gas CO-MAP allows for CO to bind with myoglobin to produce carboxymyoglobin (COMb), forming a stable, bright cherry-red color due to Mb increased affinity for binding to CO (Sørheim et al., 1999; Sivertsvik et al., 2002).

Although previous studies reported the use of carbon monoxide (CO) improved color stability, there has been limited research reporting the effect of temperature and/or storage time. Thus, the objective of this study was to investigate the relationship among dark storage time (1 d, 3 d, 10 d) and storage temperature (2.5 °C, 4.4 °C) on redness of 93:7 ground beef patties in carbon monoxide modified atmospheric packaging.

CHAPTER II

REVIEW OF LITERATURE

Meat Color

Meat color is the most important quality factor influencing consumer purchasing decisions at the retail case (Carpenter et al., 2001). For beef consumers, a bright cherry-red product in a package that is esthetically pleasing to the eye are important visual quality attributes the consumer associates with freshness and wholesomeness (Carpenter et al., 2001). Consumer perception of food spoilage and meat discoloration accounts for 15% of retail beef discounts, leading to billions of dollars in revenue loss (Ramanathan et al., 2021). In 2021, revenue loss due to discounted or discarded meat in the U.S. was estimated to be $3.73 billion. With 69% of estimated revenue loss due to discarded discolored meat and 31% of estimated revenue loss due to discounted discolored meat (Ramanathan et al., 2022).The UNEP Food Waste and Index Report 2021, reported global estimates of 931 million tons of food waste from households, food service and retail in 2019, with retail accounting for 16 kg/capita of waste in the U.S. With consumer perception of meat color being the main reason for economic loss, it is important we understand this area of research in meat science.

Meat color is dependent on the sarcoplasmic protein, myoglobin (Mb). Myoglobin is composed of 8 α- helices, containing a centrally located iron atom aligned in the proteins hydrophobic core (Mancini and Hunt, 2005; Aberle, 2012). There are six bonds associated with the iron atom, four bonds connecting the iron atom to the heme ring, the fifth coordination site attaches to the proximal histidine-93, and the 6^{th} coordination site is available to bind reversibly to ligands of diatomic oxygen, carbon monoxide, nitric oxide, and water (Suman and Joseph, 2013). Meat color is determined by the type of ligand binding to the sixth coordinate position. When the sixth ligand is bound, myoglobin will result in one of four chemical forms: deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), carboxymyoglobin (COMb), or metmyoglobin (MetMb) (Mancini and Hunt, 2005; Hunt et al., 2004).

Deoxymyoglobin has no ligand bound at sixth coordinate site and contains iron in the reduced (ferrous) state (Mancini and Hunt, 2005). Deoxymyoglobin occurs in anaerobic conditions such as vacuum packaged beef and produces a dark purple to dark purplish-red color (Mancini and Hunt, 2005). Oxymyoglobin occurs as oxygen binds at the sixth ligand position and contains iron in the reduced (ferrous) state. As oxygen is introduced to the atmosphere, meat beings the oxygenation process commonly known as "bloom", which produces a bright cherry-red color (Aberle et al., 2012; Carpenter et al., 2012). Carboxymyoglobin is formed when carbon monoxide is bound at sixth ligand position (Mancini and Hunt, 2005), which results in stable bright cherry-red color in the absence of oxygen and contains iron in the reduced state (Mancini and Hunt, 2005).

Metmyoglobin is the oxidized state of myoglobin, formed in low oxygen concentration environments when iron has oxidized, producing a tan to brown color associated with meat discoloration (Ramanathan et al., 2019; Mancini and Hunt, 2005). Due to MetMb high affinity for water, water binds at the sixth ligand binding site instead of oxygen (Suman and Joseph, 2013).

Meat color measurements involve two basic methods: visual appraisal and instrumental analyses. Within instrumental analyses, there are two different approaches which can be used to measure meat color (AMSA, 2012). The first type of instrument used for instrumental analysis is used to determine the physical description of actual perceived color of meat. Use of instruments for physical description include Hunter and CIE-tristimulus. Meat color can be quantitatively measured utilizing the International Commission on Illumination (CIE) *L**, *a**, *b**, corresponding with lightness, redness to greenness, and blueness to yellowness, respectively (Yam and Papadakis, 2004). Holman et al. (2017) reported *a** presented best projection of consumer acceptability of meat color, with an *a** value of 14.5, considered as the minimum value to be acceptable by 95% of consumers. The second type of instrument used is reflectance and transmission spectrophotometry (AMSA, 2012). Transmission spectrophotometry pertains directly to Mb properties (AMSA, 2012). Every Mb derivative has a characteristic absorbance spectrum determining the proportion of each meat surface pigment present (AMSA, 2012). Absorbance wavelengths estimate the relative proportions of Mb redox forms and total Mb concentrations in meat extracts (Tang et al., 2004). Absorbance peaks at 503 nm, 557 nm, and 582 nm are determined to represent wavelengths of maximal absorption for DeoxyMb, OxyMb and MetMb, respectively (Tang et al., 2004).

Carbon Monoxide

Carbon monoxide has become widely used in the U.S. meat industry at low, FDA approved concentrations, due to its ability to produce a very stable bright cherry-red color similar to OxyMb when added into the headspace gas modified atmospheric packaging. Carbon monoxide strongly binds to the iron-porphyrin site on Mb molecule, resulting in a strong stability against autooxidation when compared to OxyMb (Jeong and Claus, 2010). Carbon monoxide has been used by the Norwegian meat industry in fresh meat packaging since 1985 but was not approved in the U.S. until the early 2000's (Sørheim et al., 1999). The use of CO was not approved for beef packaging in the U.S. until 2002. In 2002, the United State Food and Drug Administration (FDA) approved the use of CO in beef packaging systems as a secondary packaging gas and added it to the Generally Recognized as Safe (GRAS) status (USFDA, 2002). Then in 2004 the FDA approved CO for use at a level of 0.4% in Modified atmosphere packaging (MAP) systems for red meat (USFDA, 2004). Since the approval of CO in MAP systems, CO has helped prolong discoloration, extend shelf-life and reduce oxidation of fresh meat products.

Lipid Oxidation

Lipid oxidation is one of the major causes of meat spoilage and quality degradation (Shahidi and Zhong, 2010). Lipid oxidation results in a rancid off-flavor and off odor. For lipid oxidation to occur, an initiator or catalysts must be present (Shahidi and Zhong, 2010). Types of catalysts systems, such as light, enzymes, microorganisms, and heat can lead to the oxidation of lipids present (Shahidi and Zhong, 2010).

During storage of meat and other fat-containing foods, lipid oxidation is the major non-microbial cause of quality deterioration and consequential changes (Gray and Monahan, 1992; Lorenzo and Gómez, 2012). Thiobarbituric acid reactive substance (TBARS) is the process used to determine lipid oxidation production in fresh meat products (Jo and Ahn, 1998). Jakobsen and Bertelsen (2000) stated the amount of lipid oxidation increased with an increase in meat storage time. While high-oxygen MAP (HiOx-MAP) produces an increase in red color stability from 4-7 days, when using PVC the increase in oxygen concentration leads to an increase in lipid oxidation of beef muscles (Jensen et al., 1997; Jakobsen and Bertelsen, 2000). Luño et al. (2000) reported increasing concentrations of CO in MAP packages resulted in increased inhibition of oxidation production. John et al. (2005) reported similar findings stating anaerobic packaging in 0.4% CO-MAP resulted in complete prevention of increased lipid oxidation production. This study also reported HiOx-MAP presented higher variability and a significant increase in TBA over increased storage time on meat product, while no exhibit of significant variation in TBA for CO-MAP steaks over 21 days of storage time (John et al., 2005).

Fat Percentage

While no studies have reported an effect on the formation of carboxymyoglobin in ground beef of increasing fat percentages, some studies have found an effect on the lipid oxidation production and product discoloration in the retail case (Wang et al., 2021; Lavieri and Williams, 2014). Lavieri and Williams (2014) stated fat percentage may have a significant effect on lipid oxidation production in fresh meat, reporting ground beef patties with 30% fat yielded higher TBARS values, than those with 10% fat. Wang et al.

(2021) discovered similar results stating while fat percentage showed no effect on TBARS at the beginning of the study, at the end of the retail display period, patties with a higher fat percentage produced greater lipid oxidation values. In addition to an increase in lipid oxidation. Wang et al. (2021) also found patties from higher fat formulations resulted in a sharper decrease in redness after d 0, underwent greater total color differences throughout retail display, and experienced greater visual discoloration.

Packaging

Packaging has grown into a crucial element in the meat manufacturing process, due to its aid in food safety of products and added convenience to food handling (Skandamis and Nychas, 2002). The objective of packaging is to maintain the optimal quality properties of meat during storage, transportation, and display (McMillin, 2008). Packing technologies have been a pivotal part in advances in quality improvements and the reduction and elimination of pathogens (Belcher, 2006). There are three occurrences that have played a part in the advancement of packaging year after year including: the need to reduce labor cost, push for convenient meat items and ready to eat meals that are fresh and high quality, and delivering a safe and consistent food item to costumer every time (Belcher, 2006). As case-ready products have increased in popularity, the criteria for successful packaging have become crucial for the meat industry. These advancements allow packaging to obtain the longest shelf life of the product, be visually appealing, clearly identify product, and have product properly labeled upon arrival to retail stores (Belcher, 2006).

Shelf-Life

Packaging is one of the most important breakthroughs for the extension of fresh meat shelf-life (McMillin, 2008). Over the last several decades, advancements in new technologies have been developed for the continuation of active and intelligent packaging in projection to increase shelf-life of fresh meat products, while maintaining meat wholesomeness and quality (McMillin, 2008). Shelf-life is defined as the period of time between packaging and product properties (appearance, texture, flavor, color and aroma) remaining acceptable by the consumer (Singh and Singh, 2005). Several variables influence the shelf-life of packaged fresh meat including packaging type and head space, gas flush, additives, and storage temperatures (Hotchkiss, 1989). Jeremiah and Gibson (2001) reported meat storage at -1.5 °C provided the greatest color stability without freezing product and produced a longer shelf-life, but with increased temperatures above -1.5 °C the color becomes progressively less stable. Packaging advancements over the years including the development of modified atmosphere packaging (MAP), aid in the progression of additional shelf-life of fresh meat product.

Modified Atmosphere Packing

Fresh beef is merchandized in a variety of ways within the retail sector of the beef industry. The most common type of case-ready packaging at retail is modified atmosphere packaging (MAP) (NAMI, 2016). Modified atmosphere packaging has become widely used for fresh meat retail packaging (Tørngren et al., 2018). MAP is economically competitive and practical for the meat industry due to its cost economics and efficiencies associated with the development of packaging materials and equipment, and processing leading to MAP incorporation into several case-ready systems (Zhao et

al., 1994). MAP aids in protecting fresh meat products against declining effects associated with increased shelf-life including off-flavor and aromas, discoloration, texture, nutritional value, and microbial growth (Skibsted et al., 1994).

Modified atmosphere packaging is a type of packaging that involves a vacuum sealed plastic tray containing an absorbent pad (McMillin, 2008). During the MAP packaging process atmospheric air is flushed out of the package and a purified, pre-formulated gas mixture is flushed into the package, and then sealed in a vapor barrier material (McMillin et al., 1999). Gases approved for the use in MAP packaging consist of oxygen, carbon dioxide, nitrogen, and carbon monoxide (Arvanitoyannis, 2012). There are two common types of modified atmosphere gas flushes that can be used in MAP packaging including: High Oxygen packaging (HiOx-MAP) consisting of 80% oxygen / 20% carbon and Carbon Monoxide packaging (CO-MAP) consisting of 0.4% carbon monoxide / 30-40% carbon dioxide / 60-70% nitrogen (Sivertsvik et al., 2002). Of these gases, oxygen is used to produce a bright cherry-red colored meat product, carbon dioxide is incorporated to decrease and/or inhibit spoilage and aerobic bacteria growth, carbon monoxide is used as a replacement for oxygen, producing a more stable bright cherry-red product that has a longer shelf-life and less susceptible to autooxidation, and nitrogen is a bulk filler to help prevent deflation of package as CO_2 dissolves in meat (Arvanitoyannis, 2012). However, it is important to note nitrogen has no effect on meat color or microbial growth (McMillin, 2017).

Carbon Monoxide MAP Packaging (CO-MAP)

Using carbon monoxide in MAP packaging allows for a unique combination of allowing for a long microbiological shelf-life and a stable, bright cherry-red colored fresh

meat product (Sivertsvik et al., 2002). Carbon monoxide can extend shelf-life while maintaining a high quality, fresh meat product due to its ability to reduce MetMb formation and oxidation by combining with myoglobin to form bright cherry-red color carboxy (Wolfe, 1980). Furthermore, Rogers et al. (2014) stated CO in CO-MAP stabilized red meat color throughout retail display regardless of storage temperatures.

A type of CO-MAP packaging called "master bags" has become popular in the fresh meat case-ready industry. Master bags were developed to aid in providing fresh meat to be packaged in traditional Styrofoam trays overwrapped with polyvinyl chloride film (PVC) with long and sufficient storage life and subsequent retail display life in case-ready packaging (Kennedy et al., 2005). When using master bags, case ready meat product will be placed on a polystyrene foam tray and overwrapped with high O_2 permeable PVC film (Kennedy et al., 2005). Once the primary package is sealed 2 to 6 trays will then be placed inside a larger master bag (secondary package) (Kennedy et al., 2005). Next, all residual air is pulled out of the master bag and flushed with a purified gas mixture then sealed (Kennedy et al., 2005), and placed into a box that limits the amount of light allowed. Once at retail store level, master packs can be opened and primary packages are directly placed into display cases for consumer purchasing (Kennedy et al., 2005). The use of low oxygen MAP within the master bag allows for the necessary migration and binding of gases within secondary package to migrate through PVC film into primary package and produces the necessary bacteriostatic effects to ensure a reasonable shelf-life, while O_2 permeable PVC overwrap allows optimal bloom of meat once master bag is opened (Kennedy et al., 2005).

There are several advantages of using CO-MAP packaging in the meat industry. One of the most significant advantages of using CO-MAP packaging is its ability to produce a more stable, desirable, bright cherry-red color, along with higher *a** values, for a longer period of time in comparison to HiOx-MAP packaging and polyvinyl chloride (PVC) packaging (Hunt et al., 2004). John et al. (2005) reported beef top sirloin steaks packaged in HiOx-MAP packaging retained a bright cherry-red color for 7 d, had some brown evident after 14 d and were completely brown by d 21, while steaks packaged in 0.4% CO-MAP retained a bright cheery-red color throughout the 21 d period. Tørngren (2003) also reported beef packaged in CO-MAP helped hinder premature browning. Premature browning results in the interior of ground beef to look fulling cooked below necessary cooking temperatures (Hague et al., 1994). Hunt et al. (1995) reported chemical state of internal Mb prior to cooking has effects on premature browning, noting patties with OxyMb or MetMb internal color were more susceptible to premature browning. Furthermore, John et al. (2004) stated raw ground beef patties exposed to 0.4% CO avoided premature browning and high TBA values.

Packaging in CO also aids in flavor acceptability, as it decreases the microbial growth that is commonly associated with an off flavor including those from lipid oxidation and the production of off-odors (Jayasingh et al., 2002). Brooks et al. (2008) stated CO-MAP meat resulted in reduced growth of spoilage organisms and pathogenic bacteria. Though only permitted for use at levels ≤ 0.4%, Gee and Brown (1980) found at CO levels of < 30%, P. aeruginosa, E.coli, achromobactin, and P. fluorescens growth are inhibited. However, CO at low levels (< 1%) has relatively little to no effect on bacterial growth on meat (Gee and Brown, 1980). In conjunction with other gases, like CO_2,

microbial growth can be controlled as a result of carbon dioxides bacteriostatic and fungistatic properties (Thippareddi and Phebus, 2002).

Though there are several advantages to CO-MAP packaging, there are also some disadvantages. The biggest disadvantage of using CO in MAP packaging is the negative image perceived by consumers, due to CO being a potentially hazardous gas (Cornforth and Hunt, 2008). Another disadvantage is misconception concerning CO treated products looking fresh, but long stability of color masks high bacterial levels and product spoilage (Cornforth and Hunt, 2008). However, due to these disadvantages extensive research has continued to ensure the freshness and wholesomeness of CO-MAP packaged products (Clark et al., 1976; Watts et al., 1978; Gee and Brown, 1978; and Sørheim et al., 2001).

Conclusion

With fresh meat discoloration producing the largest portion of product loss, packaging improvements are imperative for increasing shelf-life. Though vacuum packaging is a sustainable option for increasing shelf-life of fresh meat, many consumers are unwilling to purchase a product in dark-purple, purple DeoxyMb state associated with anaerobic packaging. The use of CO in MAP packaging has become extremely common for case-ready products, due to its ability to keep products a stable bright cherry-red color for an extended period of time with no adverse effects on microbial growth and increased lipid oxidation. With an increase in CO-MAP for case-ready products, it is important to understand the effect of storage temperature, fat percentage and/or storage time can have on carboxymyoglobin formation, microbial growth, and lipid oxidation.

CHAPTER III

EFFECT OF STORAGE TEMPERATURE AND TIME ON REDNESS OF 93:7 GROUND BEEF PATTIES IN CARBON MONOXIDE ATMOSPHERIC PACKAGING

ABSTRACT

The objective of this study was to investigate storage time and temperature on redness of 93:7 ground beef in CO-MAP (carbon monoxide modified atmosphere) packaging. Three 4.5 kg ground beef chubs with a lean-to-fat ratio (L/F) of 93:7 were collected from Creekstone Farms in Arkansas City, KS. Ground beef chubs were stored for 7 d, then chubs were mixed and finely ground to homogenize sample. Homogenized ground beef sample was then formed into 227 g patties (n = 54*)* and packaged in MAP packaging consisting of 0.4% CO, 30% CO_2, and 69.6% N_2. After packaging, patties were randomly assigned to one of two storage temperatures: 2.5°C, or 4.4°C and one of three dark storage times: 1 d, 3 d, or 10 d. On each respective pull day, two patties from each storage temperature were measured for instrumental surface raw color, internal cooked color, and total plate count (TPC). Data were analyzed as a split-split plot using PROC GLIMMIX procedure of SAS. Least square means were calculated and considered significant at $P < 0.05$. There was a significant storage time × storage temperature interaction on raw color measurements. L^* and a^* values increased ($P < 0.05$) at 4.4°C

and 2.5°C with an increase in storage time from 1 to 10 d in dark storage. There was no significant effects of storage time or storage temperature on cooked *L** values, *a** values, *b** values, chroma, and hue. At 4.4°C and 2.5°C, TPC increased ($P < 0.05$) with an increase in storage time from 3 to 10 d in dark storage. The current study indicates after 1 and 3 d in dark storage lower storage temperature decreased redness of ground beef patties. Therefore, processors should use favorable temperatures to promote carboxymyoglobin and redness of patties stored in CO-MAP while maintaining other quality and safety attributes.

INTRODUCTION

Meat color is the most important quality attribute consumers evaluate, as they associate color with meat freshness and wholesomeness (Mancini and Hunt, 2005). Deviation from a bright cherry-red color results in discarded or discounted products because of a lack of consumer acceptance and possible spoilage (Suman et al., 2014). One of the biggest limiting factors of shelf-life for fresh meat is the loss of color stability throughout storage (Carpenter et al., 2001). It is estimated, discolored meat accounts for 11.07% of discounted retail beef and is responsible for close to $3.73 billion in revenue loss in the U.S. (Ramanathan et al., 2022). Monitoring color to maximize shelf life and consumer acceptability is a priority in meat science research and the meat industry (Schelkopf et al., 2021).

Fresh meat color is determined by the relative chemical state of myoglobin (Mb) (Mancini and Hunt, 2005). In retail, fresh meat is in the aerobic (Oxymyoglobin; OxyMb) state producing a bright cherry-red color, but as meat is displayed longer the discoloration in the form of metmyoglobin (MetMb) increases (Mancini and Hunt, 2005). Over the past

several years, technologies have been developed and improved to aid in the extension of self-life, including the development and use of modified atmospheric packaging (MAP) (Zhao et al., 1994; Skibsted et al., 1994; Tørngren et al., 2018).

Modified atmosphere packaging has become widely used for fresh meat retail packaging (Tørngren et al., 2018); it works by altering the gaseous environment within the vapor barrier film (McMillin et al., 1999). Altering this gaseous environment, aids in protecting fresh meat product from adverse effects associated with extended shelf-life (Skibsted et al., 1994). Carbon monoxide modified atmospheric packaging (CO-MAP) is a type of anaerobic MAP packaging encompassing a gas mixture of carbon dioxide, carbon monoxide (CO), and nitrogen. The use of tri-gas CO-MAP allows for CO to bind with myoglobin to produce carboxymyoglobin (COMb), forming a stable, bright cherry-red color due to Mb increased affinity for binding to CO (Sørheim et al., 1999; Sivertsvik et al., 2002).

Although previous studies reported the use of carbon monoxide (CO) improved color stability, there has been limited research reporting the effect of fat percentage, temperature and/or storage time. Thus, the objective of this study was to investigate the relationship among dark storage time (1 d, 3 d, 10 d) and storage temperature (2.5 °C, 4.4 °C) on redness of 93:7 ground beef patties in carbon monoxide modified atmospheric packaging.

MATERIALS AND METHODS

Raw materials, processing, and proximate composition analysis

Three, 4.5 kg ground beef chubs, with a lean to fat ratio (L/F) of 93% lean:7% fat were collected from Creekstone Farms in Arkansas City, KS (packaging date established

as d 0) and transported to the Robert M. Kerr Food and Agricultural Products Center at Oklahoma State University. Upon arrival ground beef chubs were stored for 7 d at an average of 3 ± 0.5°C. Each chub was opened and finely ground with a 3 mm plate utilizing a Biro mixer grinder (Model AFMG-24, Biro Manufacturing Company Marblehead, OH) to homogenize the sample. Ground beef batch was placed into a lug prior to packaging. Proximate analysis was measured to calculate the percentage of protein, fat, and moisture of composite meat sample (Table 1). Protein, fat, and moisture content for 93:7 ground beef was 20.89, 8.75, and 71.54%, respectively. Ground beef samples were measured using NIR with AOAC (2007.04) approved near infrared spectrophotometer (FoodScan Lab Analyzer, Serial No. 91753206, Foss, NIR systems Inc., Slangerupgade, Denmark, 2014). Homogenized ground beef sample was then formed into 227 g patties (n = 54) using a Weston Double Burger Press (07-0701 Double Burger Express, Weston Brands, Southern Pines, NC)

Patties were packaged into modified atmospheric packaging (MAP) with a certified gas blend consisting of 0.4% CO, 30% CO_2, and 69.6% N_2. Patties were placed in white MAP trays (Rock-Tenn DuraFresh^{TM} rigid trays), obtained from Cryovac Sealed Air (Duncan, SC) and sealed with a Mondini semi-automatic tray-sealing machine (Model CV/VG-5, G Mondini S.P.A., Cologne, Italy) utilizing a multi-layer barrier film (LID 1050, Cryovac Sealed Air, Duncan, SC). Immediately prior to being sealed, the certified gas blend was flushed into the package. Using a head space analyzer (Bridge 900131 $O_2/CO_2/N_2$, Illinois Instruments, Ingleside, IL), the percentage of O_2, CO_2, and CO was verified to ensure the proper gas flush was achieved. Once sealed, patties were

randomly assigned to one of two temperature storage treatments: 2.5°C or 4.4°C and one of three storage times: 1 d, 3 d, or 10 d.

Fresh meat color

After d 1, 3 and 10 of storage, two patties from each storage temperature were measured for instrumental surface color, using a HunterLab 4500L MiniScan EZ Spectrophotometer (2.5-cm aperture, illuminant A, and 10° standard observer angle; HunterLab Associates; Reston, VA). Readings were taken in triplicate across the patty surface and averaged. Instrumental color provided CIE *L**, *a**, b*, determining surface color. CIE *L** was utilized to measure lightness (white to black), the higher the value, the lighter (whiter) the product. CIE *a** was utilized to measure redness (red to green), with higher, positive values representing red color and negative values representing green color. CIE *b** was utilized to measure yellowness to blueness, with a positive value representing yellow and negative value representing blue. Chroma $\left[\sqrt{(a^{*2}+b^{*2})}\right]$ was determined using CIE *a** and *b** values, representing the red intensity of the color (AMSA, 2012). Hue angle$\left(\tan^{-1}\left(\frac{b*}{a*}\right)\right)$, was determined using CIE *a** and *b** values, representing color present.

Cooked meat color

After d 1, 3 and 10 of storage, two patties from each storage temperature were cooked utilizing an XLT Impingement Oven (model 3240-TX, BOFI Inc., Wichita, KS). The oven temperature was set at 177°C, and patties were cooked to 71°C, following USDA Food Safety and Inspection Service guidelines. Internal temperature was monitored by inserting a handheld probe thermometer (AccuTuff 340, Atkins, Gainesville, FL) into the geometric center of each patty. Each patty was then placed back

into respectively labeled MAP tray and allowed to cool for 5 min at room temperature. Next, patties were bisected parallel to the cooked surface to measure instrumental internal cooked color. Cooked color was read in triplicate across interior cooked surface across both sides of each patty, using a HunterLab 4500L MiniScan EZ Spectrophotometer. Instrumental color provided CIE *L**, *a**, *b**, determining cooked internal color. Chroma and hue were determined using CIE *a** and *b**.

Microbiology

Total plate count (TPC) was obtained from a composite sample from each patty and storage temperature combination on pull d 1, 3, and 10. Ten grams of sample from each patty was homogenized in a sterile stomacher bag, containing 90 mL of sterile 0.1% peptone water. Each stomacher bag was pummeled for 30 s at 230 rpm using a Stomacher-400. For TPC analysis, one mL of homogenate was plated on 3M™ Petrifilm™ Aerobic Count Plate (St. Paul, MN, USA), with respective decimal dilutions. Plates were incubated at 37°C for 48 h in a VWR Forced Air General Incubator (5.4 ft^3; VWR, Radnor, PA). After 48 h, plates were removed and counted according to the 3M™ Petrifilm™ Aerobic Count Plate Interpretation Guide using an Interscience Scan 100 pressure sensitive pad (Interscience; Woburn, MA), to determine TPC per cm^2.

Statistical analysis

The experimental design was a split-split plot. Within the whole plot, ground beef chubs were the experimental unit and were formed into patties: 93% lean: 7% fat (n = 54). Within the sub-plot (split-factor), each patty was considered the experimental unit randomly assigned to 1 of 2 storage temperature of 2.5°C or 4.4°C. Within the sub-sub plot, patties within each storage temperature served as experimental units randomly

assigned to 1 of 3 storage times (1 d, 3 d, or 10 d) in dark storage. The fixed effects were storage temperature and days in dark storage and random effect was the ground beef chub.

Simple means were calculated for pH, proximate analysis, and headspace analysis. All other data were analyzed using PROC GLIMMIX of SAS 9.4 (SAS Institute Inc., Cary, North Carolina), where main effects were pull day, temperature, and their interactions. Non-significant interactions were removed from the model. Least square means were calculated and considered significant at $P < 0.05$, using ANOVA testing to indicate significance. Using the PDIFF option, means were separated and deemed significant at $P < 0.05$.

RESULTS AND DISCUSSION

Raw color

L values*

There was a dark storage time × storage temperature effect on *L** values (Table 2). At 4.4°C and 2.5°C, lightness of patties increased ($P < 0.05$) with an increase in storage time from 1 to 10 d in dark storage. These results are paralleled with John et al. (2005) and Sakowaska et al. (2017) reporting *L** values significantly increased with extended CO exposure. Santos et al. (2007) also found *L** values increased with increased storage time in pork chops. Additionally at all storage times, *L** values were similar ($P > 0.05$) for patties at both storage temperatures.

*a *and chroma values*

There was a dark storage time × storage temperature effect on *a** values and chroma (Table 3). At 4.4°C and 2.5°C, redness (*a**) of patties significantly increased ($P <$

0.05) with an increase in storage time from 1 to 10 d in dark storage. Paralleled with the current study, others have found *a** values increased with increased storage time when beef is exposed to CO (Jayasingh et al., 2001; Sakowska et al., 2017; Sørheim et al., 1999). Sørheim et al. (1999) also found 0.4% CO-MAP resulted in elevated *a** values and a bright cherry-red color throughout a 21-d period. After 1 and 3 d in dark storage, redness of patties decreased ($P < 0.05$) with a decrease in storage temperature from 4.4°C to 2.5°C; however, patties held in dark storage for 10 d increased ($P < 0.05$) in redness with a decrease in storage temperature from 4.4°C to 2.5°C. Jayasingh et al. (2001) observed ground beef packaged in modified atmosphere packaging with CO maintained a red color for a full 8 week study with $a^* \geq 14$.

Chroma indicates the relative saturation of color; a greater number indicates color with greater red intensity and an increase in redness. At 2.5°C, red intensity of patties increased ($P < 0.05$) with an increase in storage time from 1 to 10 d in dark storage while at 4.4°C red intensity only increased ($P < 0.05$) from 1 to 3 d in dark storage but was similar ($P > 0.05$) after 3 and 10 d in dark storage. Paralleled with raw *a** values, after 1 and 3 d in dark storage red intensity of patties decreased ($P < 0.05$) with a decrease in storage temperature from 4.4°C to 2.5°C; however, after 10 d in dark storage chroma values increased ($P < 0.05$) with a decrease in storage temperature from 4.4°C to 2.5°C. Jeong and Claus (2011) found similar results, reporting CO exposure increased chroma values along with *a** values.

b* and hue values

There was a dark storage time × storage temperature effect on *b** values and hue angle (Table 4). At 4.4°C, yellowness of patties increased ($P < 0.05$) with an increase in

storage time from 1 to 3 d in dark storage, but did not change ($P > 0.05$) in b^* value from 3 to 10 d in dark storage. At 2.5°C, b^* value of patties increased ($P < 0.05$) as dark storage time increased. Yellowness of patties was similar ($P > 0.05$) at both storage temperatures (4.4°C to 2.5°C) across all three storage times. Other researchers found yellowness increased in patties and steaks packaged in 0.4% CO-MAP compared to other packaging types (Grobbel et al., 2008; Sakowska et al., 2016). Grobbel et al. (2008) also reported even though CO-MAP increased b^* values, yellowness did not change during storage from d 7-21.

Hue angle indicates the true red axis; a greater number indicates color further from true red color and an increase in discoloration. At 2.5°C, hue angle of patties decreased ($P < 0.05$) with an increase in storage time from 1 to 10 d in dark storage, while at 4.4°C hue angle did not change ($P > 0.05$) from 1 to 3 d in dark storage but decreased ($P < 0.05$) from 3 to 10 d in dark storage. Additionally after 1 d in dark storage, hue angle of patties was greater ($P < 0.05$) in patties stored at 2.5°C compared to patties stored at 4.4°C. However after 3 and 10 d in storage, hue angle of patties was similar ($P > 0.05$) at both storage temperatures.

Cooked color

L* values

Cooked colors lightness of patties were similar ($P > 0.05$) at both storage temperatures and all three storage times (Table 5). Grobbel et al. (2008) reported there was no significant effect of packaging type, including 0.4% CO-MAP, or storage time on cooked L^* values of beef steaks.

*a** values and chroma

Cooked *a** values were collected to evaluate if exposure to CO resulted in the formation of persistent pinking or premature browning in ground beef patties. Observed results were similar to perceived notions, showing little to no variation in cooked *a** values (Table 6). These results are inconsistent with De Santos et al. (2007) reporting *a** values of cooked ground beef patties increased with increased storage time in CO-MAP.

Changes in cooked chroma values were paralleled to cooked *a** values, showing little to no variation in red intensity of patties (Table 6). Other researchers have reported CO treated ground beef patties resulted in a pinker internal color after cooking compared to other packaging systems (Sørheim et al., 2001; John et al., 2004). John et al. (2004) also reported cooked ground beef patties exposed to CO resulted in a slight pink appearance but faded quickly after slicing. However, in the current study there was little to no variation in *a** and chroma values and patties showed no indication of a pink internal cooked color.

b values and hue angle*

Parallel with cooked *a** values and chroma, there was little to no variation in *b** values and hue angle of cooked patties at different storage times or storage temperatures (Table 7). John et al. (2004) reported the interior of CO-treated patties had lower hue angles (hue < 38), indicating a more red color than patties in other packaging systems. This is inconsistent with the current study, having high cooked hue angle values (hue > 55) and patties showing no indication of a pink internal cooked color.

Total Plate Count

There was a dark storage time × storage temperature effect on total plate count (Table 8). At 4.4°C and 2.5°C, TPC of patties were similar ($P > 0.05$) with an increase in storage time from 1 to 3 d in dark storage, but significantly increased ($P < 0.05$) from 3 to 10 d in dark storage. Sakowska et al. (2017) found similar results, reporting microbial growth increased with increased storage time. Additionally, TPC of patties at both storage temperatures were similar at 1, 3, and 10 d of dark storage. Cornforth and Hunt (2006) found CO-MAP packaging helped to inhibit increased growth of spoilage and pathogenic bacteria during refrigerated storage and resulted in longer microbial shelf-life; however this is inconsistent with the current study as TPC significantly increased ($P < 0.05$) after 10 d in dark storage.

CONCLUSION

In previous research, the use of carbon monoxide in MAP packaging has been shown to improve the color stability and shelf-life of ground beef in dark storage and retail display. The current study indicates that lower storage temperature decreased redness of ground beef patties with 1 and 3 days of dark storage but increased redness after 10 d dark storage. Lower temperatures decrease oxygen consumption; hence the conversion of oxymyoglobin to carboxymyoglobin may be limited early in the storage period. Additionally increased storage time resulted in elevated L^* and a^* values, but increased overall microbial growth. In conclusion, varying storage temperatures and storage times in CO-MAP could influence instrument color analysis and microbial growth. Therefore, processors should use favorable temperatures to promote carboxymyoglobin and redness of patties stored in CO-MAP mother bags while

maintaining other quality and safety attributes. Further research should further investigate the effect of varying fat percentages, dark storage temperatures, and length of CO exposure on the extension of retail stability and quality.

Table 1. pH and mean proximate composition (%) of 93:7 ground beef

Component	93:7
pH	5.7
Protein	20.9
Fat	8.8
Moisture	71.5

Table 2. Least squares means for L^*[1] (dark storage[2] × storage temperature) of raw patties (n = 12) in dark storage for 1, 3, or 10 d from both storage temperatures (2.5°C, 4.4°C)

			Temperature (ºC)	
Parameter	Fat	Day	4.4 ºC	2.5 ºC
L* values	7%	1	46.9[c]	47.3[c]
		3	49.4[b]	49.0[b]
		10	50.9[a]	50.1[a]
		SEM[3] = 0.15		

[a-c] Least squares means with different subscripts are significantly different ($P < 0.05$)
[1] L^* values: higher values indicate lighter color
[2] Total time the patties spent in dark storage prior to reading
[3] SEM = Standard error of the mean

Table 3. Least squares means for a^*[1] and chroma[2] (dark storage[3] × storage temperature) of raw patties (n = 12) in dark storage for 1, 3, or 10 d from both storage temperatures (2.5°C, 4.4°C)

			Temperature (ºC)	
Parameter	Fat	Day	4.4 ºC	2.5 ºC
a* values	7%	1	26.8[e]	24.7[f]
		3	32.7[c]	30.9[d]
SEM = 0.33		10	33.7[b]	34.9[a]
Chroma	7%	1	32.2[d]	30.2[e]
		3	39.1[b]	37.3[c]
SEM[4] = 0.34		10	40.0[b]	41.5[a]

[a-f] Least squares means within different parameter with different subscripts are significantly different ($P < 0.05$)

[1] a^* values: higher values indicate redder color

[2] Chroma values: higher values indicate greater red intensity

[3] Total time the patties spent in dark storage prior to reading

[4] SEM = Standard error of the mean

Table 4. Least squares means for b^*[1] and hue[2] (dark storage[3] × storage temperature) of raw patties (n = 12) in dark storage for 1, 3, or 10 d from both storage temperatures (2.5°C, 4.4°C)

			Temperature (ºC)	
Parameter	Fat	Day	4.4 ºC	2.5 ºC
b* values	7%	1	17.9^{c}	17.3^{c}
		3	21.4^{b}	20.8^{b}
SEM = 0.30		10	21.6^{ab}	22.6^{a}
Hue	7%	1	33.7^{bc}	35.1^{a}
		3	33.2^{bc}	33.9^{b}
SEM[4] = 0.26		10	32.7^{d}	32.9^{cd}

[a-d] Least squares means within different parameter with different subscripts are significantly different ($P < 0.05$)
[1] b^* values: higher values indicate greater yellowness
[2] Hue values: higher values indicate true red axis
[3] Total time the patties spent in dark storage prior to reading
[4] SEM = Standard error of the mean

Table 5. Effects of days in dark storage[1] and storage temperature on *L**[2] values of patties (n = 12) cooked to 71°C

Parameter		L*
Day	1	52.2
	3	52.9
	10	50.0
	SEM = 2.12	
Temperature	4.4ºC	51.9
	2.5ºC	51.5
	SEM[3] = 1.73	

[1] Total time the patties spent in dark storage prior to reading
[2] *L** values: higher values indicate lighter color
[3] SEM = Standard error of the mean

Table 6. Effects of days in dark storage[1] and storage temperature on *a**[2] values and chroma[3] of patties (n = 12) cooked to 71°C

Parameter		a*	Chroma
Day	1	11.8	21.1
	3	12.1	21.9
	10	11.7	21.3
		SEM = 0.49	SEM = 0.75
Temperature	4.4ºC	11.8	21.6
	2.5ºC	11.9	21.3
		SEM[4] = 0.40	SEM = 0.61

[1] Total time the patties spent in dark storage prior to reading
[2] *a** values: higher values indicate redder color
[3] Chroma values: higher values indicate greater red intensity
[4] SEM = Standard error of the mean

Table 7. Effects of days in dark storage[1] and storage temperature on *b**[2] values and hue[3] of patties (n = 12) cooked to 71°C

Parameter		b*	Hue
Day	1	17.5	55.9
	3	18.3	56.6
	10	17.9	56.8
		SEM = 0.63	SEM = 0.76
Temperature	4.4ºC	18.1	56.9
	2.5ºC	17.7	56.0
		SEM[4] = 0.52	SEM = 0.62

[1] Total time the patties spent in dark storage prior to reading
[2] *b** values: higher values indicate greater yellowness
[3] Hue values: higher values indicate true red axis
[4] SEM = Standard error of the mean

Table 8. Least squares means for Total Plate Count (dark storage[1] × storage temperature) of raw patties (n = 12) in dark storage for 1, 3, or 10 d from both temperatures (2.5°C, 4.4°C)

		Temperature (ºC)	
Fat	Day	4.4ºC	2.5ºC
7%	1	4.66[b]	4.80[b]
	3	4.50[b]	4.69[b]
	10	6.99[a]	6.89[a]
	SEM[2] = 0.20		

[a-b] Least squares means with different subscripts are significantly different ($P < 0.05$)
[1] Total time the patties spent in dark storage prior to reading
[2] SEM = Standard error of the mean

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APPENDICES

Figure 1. Temperature curve from room set at 3.0 °C over a 7 d period, read every 30 min

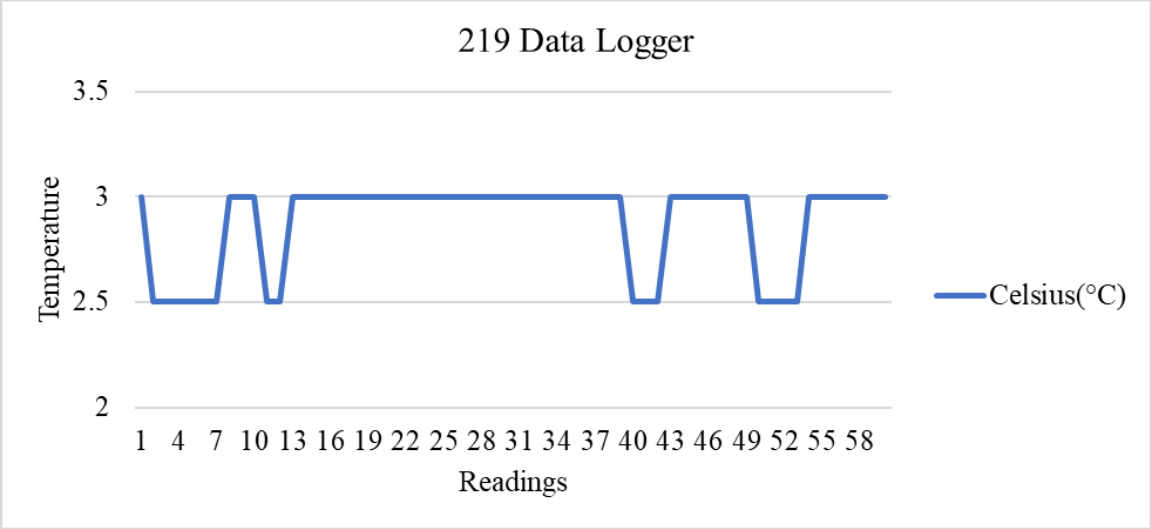


Figure 2. Temperature curve from room set at 2.5 °C over a 10 d period, read every 30 min

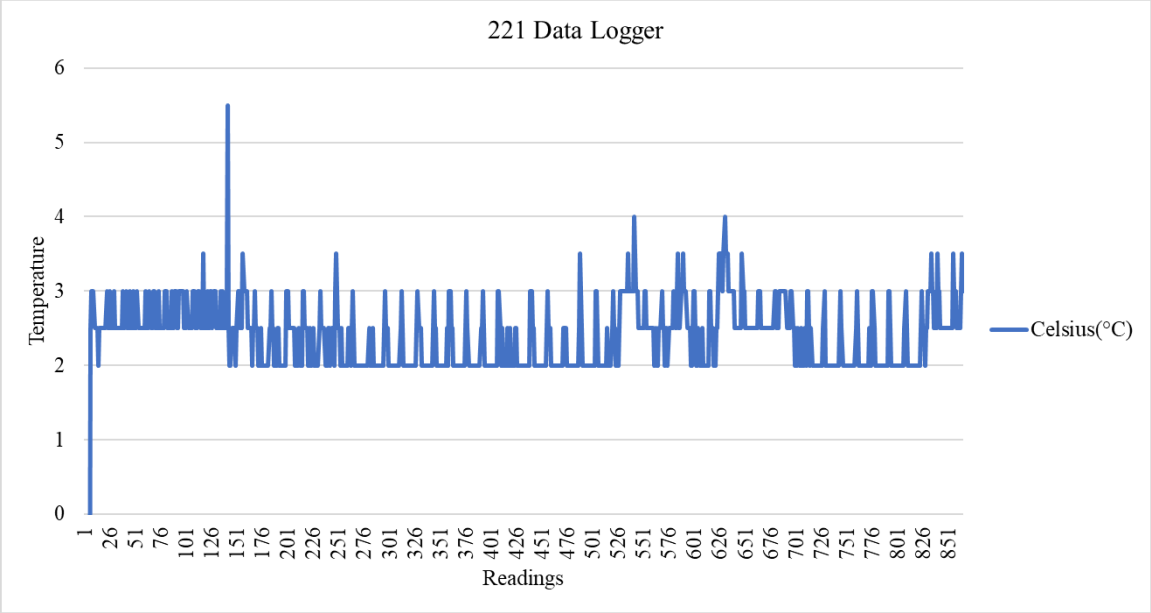
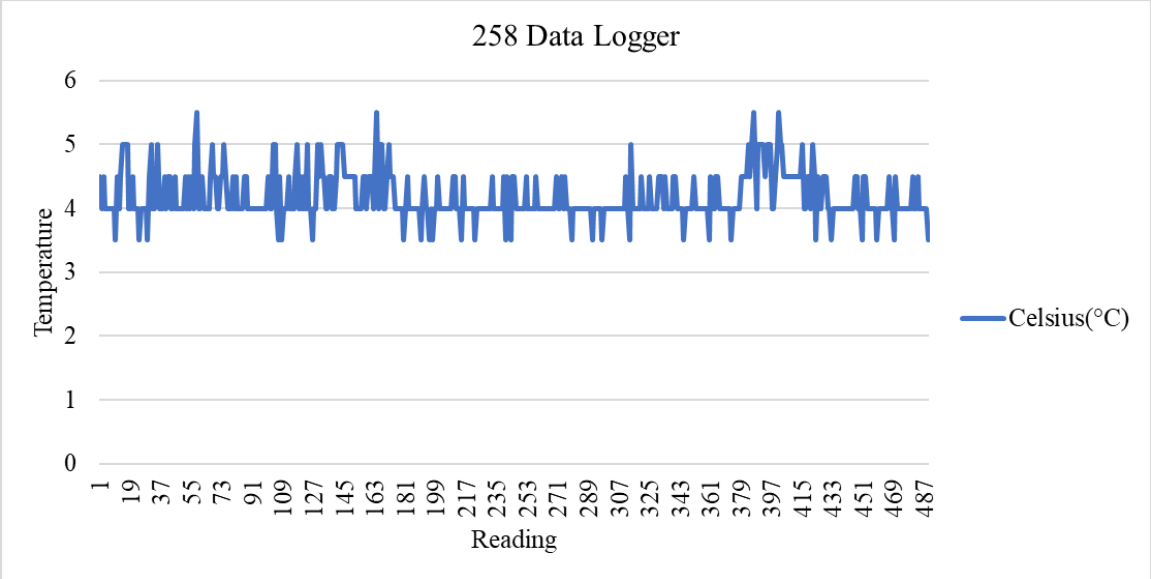


Figure 3. Temperature curve from room set at 4.4 °C over a 10 d period, read every 30 min



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