Extending Shelf Life of Romaine Lettuce Through Modified Atmosphere Packaging and the Effects for Food Safety and Quality

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Abstract

Foodborne related disease outbreaks have been on the rise since 1998. For example, in 2019, approximately 75,233 pounds of salad products were recalled due to contamination with *E. coli* O157:H7. Investigations concluded there was a single grower that supplied romaine lettuce. Outbreaks in 2019 associated with romaine lettuce caused were over 167 people sick with 85 hospitalizations.

Moving forward to prevent future outbreaks, control strategies need to be developed for romaine lettuce. The U.S. FDA has put together a plan, but it will also depend on the work of the farmers to follow guidelines. Additionally, studies by scientists will also be needed to fully understand how certain pathogens, like *E. coli* O157:H7, can remain on the produce. *E. coli* O157:H7 deals with different stresses along the supply chain; for example, *E. coli* O157:H7 can survive on the edible portion of the harvested lettuce for extended periods and adapt to different stresses like temperature changes. Modifying the atmosphere is one control strategy that does help with food quality, but other measures need to be taken to prevent future outbreaks and protect food safety.

Romaine lettuce is a widely consumed product in the US. Consumers are looking for a product that is fresh, convenient, retains its nutrients and, has good sensory qualities. Romaine lettuce is also a highly perishable product resulting in flavor loss, discoloration, decay, softening, shrinkage, and vitamin loss. The use of modified atmosphere packaging (MAP) can extend shelf-life and limit these effects, to provide a fresh product to the consumer. Romaine lettuce is typically packaged with passive MAP. Passive MAP is generated by relying on the natural process of product respiration and the film permeability by relying on the natural respiration rates to attain the desired gas composition over time. Thus, the objective of the study reported here was to conduct a review of MAP and to understand how MAP can help the quality and safety of romaine lettuce.

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Introduction

a. Background and Setting

Modified atmosphere packaging (MAP) is important to extend shelf-life of fresh-cut produce. MAP does not improve the raw material, but instead helps to maintain the existing quality level through the product's life cycle (Toivonen, 2009). Romaine lettuce is a highly perishable product whose product quality can lead to a shorter shelf-life with flavor loss, discoloration, decay, softening, shrinkage, and vitamin loss (Jideani, 2017). Fresh cut produce, like romaine lettuce, can benefit from modified atmosphere packaging to help prevent these factors that shorten the products shelf-life. MAP can retard the respiration rate of the produce, but to have a cost-effective package other factors must be considered such as product weight, temperature, and the packaging material (Caleb, 2013). Romaine lettuce is packaged with passive MAP. This is generated by relying on the natural process of respiration and the film permeability to attain the desired gas composition over time (Caleb, 2013). However, proper post-harvest precooling of the produce is the single most cited issue for maintaining quality in fresh-cut fruits and vegetables (Toivonen, 2009). There is no one fix for giving produce a longer shelf life, but multiple factors can be important for food quality and safety of romaine lettuce.

MAP will alter the gas composition inside a package based on the product's rate of respiration, and help to prevent water loss (Jideani, 2017). There are three different types of packaging that are used for fresh-cut produce: flexible, rigid, and active/intelligent (Toivonen, 2009). Flexible is the most common type of packaging as preformed bags, roll stock, and stand up pouches with the roll stock being the most common for retail applications (Toivonen, 2009). Flexible film MAP serves as a mechanical barrier to help with the movement of water vapor and to help maintain the relative humidity within the package (Caleb, 2013). Rigid packages like clamshell or snap-on lid packages are not true modified atmosphere packages since the gas transmission rates are not engineered or controlled (Toivonen, 2009). This type of rigid packaging is more accurately called a natural aspiration package type.

Respiration and permeation in plastic film packaging take place simultaneously and designing MAP to achieve the desired atmosphere early on and to help keep it maintained as long as possible (Mangaraj, 2009). Films used for MAP are determined based on the oxygen (O_2) permeability that is required to help maintain a concentration of 3% or lower for packaged romaine lettuce (Segall, 1996). Matching the film permeability for the O_2 and CO_2 (carbon dioxide) respiration rates is important for the fresh produce as it will delay tissues decay as well as extend the shelf life. The Danish Technological Institute states that the perforation of the film is the solution to control the atmosphere inside the packaging, steering to a continued transport of O_2 and CO_2 in and out of the package (2008). A decrease in O_2 and an increase in CO_2 inside the packaging is effective for prolonging the shelf life of romaine lettuce (Del Nobile, 2006). Establishing a low O_2 environment helps to control browning (Kim, 2005). Lower O_2 will lead to a slower pace of respiration, the aging processes will be slower and the shelf life extended, but also lower temperatures will give a slower respiration and is essential for MAP (Danish Institute, 2008).

Most plastic films for MAP packaging are constructed of polyvinyl chloride (PVC), polyethylene terephthalate (PETE), polypropylene (PP) or polyethylene (PE) (Mangaraj, 2009). These flexible plastics make up to 90% of the materials used for packaging and providing a large range of permeability to gasses and water vapor (Mangaraj, 2009). PE films are durable, flexible, heat sealable, have a good moisture barrier, good chemical resistance, good low-temperature performance, and are permeable to gasses, making them great for MAP (Toivonen, 2009). Polyolefin is a mixture of PE and PP that is often used as it is light in weight, stable, has good moisture and chemical resistance, and easy processability, making it suited for recycling and reusing (Mangaraj, 2009). Overall, the main goal of MAP is to create an equilibrium of atmosphere that is a low percentage of O_2 and a high enough percentage of CO_2 to be beneficial to the product, but not injurious. This paper will discuss the overall effects of modified packaging and how it can help with maintaining the food quality and safety of romaine lettuce.

b. Statement of the Problem

Fresh produce accounted for 21% of the foodborne illness outbreaks and 34% of the reported illness cases in the US from 1982-2002, with lettuce being the most implicated (Delaquis, 2007). The latest romaine lettuce outbreak 9/20/2019 - 12/20/2019 originated from romaine lettuce that was harvested from the Salinas Valley growing region (California, U.S.). The outbreak resulted in a total of 167 people infected with *E. coli* O157:H7 from a reported 27 states with a total of 85 hospitalizations, including 15 people who developed hemolytic uremic syndrome, a type of kidney failure. The outbreak is now over, and the CDC is no longer advising that people avoid romaine lettuce from this growing region. However, this outbreak and resulting recall affected a lot of people across multiple states. Preventing future outbreaks and recalls like this are important for the safety of all persons. Studying how romaine lettuce is influenced by MAP, as well as understanding the behavior of *E. coli* O157:H7 in leafy vegetables will help to understand potential control strategies to prevent future outbreaks.

c. Significance of the Problem

As stated above from the CDC's webpage, a total of 167 people were infected from eating romaine lettuce in romaine-borne outbreaks in 2019, with people being hospitalized. Recalls also led to no product being on the shelf for consumers to purchase. Since the most recent recall in 2019, romaine lettuce manufacturers are labeling the growing location on the packaging to assist in traceback investigations (to avoid all romaine being pulled from retail shelves and destroyed). Thus, being able to prevent future outbreaks and recalls is important for the safety of all consumers, especially those who consume romaine lettuce.

d. Purpose of the Project

The purpose of this project was to determine if using MAP will help to extend the shelf life of romaine lettuce so that the quality lasts longer. Additionally, the project also sought to understand the literature on MAP and *E. coli* O157:H7 on romaine lettuce.

e. Project Objectives

Romaine lettuce in two different types of packaging will be evaluated for its O_2 and CO_2 concentrations to determine if the product is modified atmosphere packaged (MAP). There will be a comparison of two different film type packages and two different clamshell (rigid) type packages. Studying how the different gas concentrations change over time will help to determine if the items are MAP, and what effect will it have on the shelf life of the product in terms of food quality.

f. Definition of the Keywords

Romaine Lettuce – a type of lettuce that is grown for human consumption. *Lactuca sativa L.* (Luna, 2013)

Modified Atmosphere Packaging (MAP) – an interaction between the respiration rate of product and the gas transfer through a packaging material (Caleb, 2013). Passive MAP is generated by relying on the natural process of respiration of the product and the film permeability by relying on the natural respiration rates to attain the desired gas composition over time (Caleb, 2013).

Food Safety – when a product is no longer safe for human consumption due to contamination with microorganisms or other toxic substances.

Food Quality – the quality of the product when it is no longer visibly appealing to the consumer and the lettuce leaves show browning or wilting on the surfaces (Manolopoulou, 2010).

Shelf-Life – the length of time of which a product is still considered of good food quality and safety for consumption by a consumer.

Escherichia coli O157:H7 – bacteria strain that is responsible for outbreaks and sporadic diarrhea (Carry, 2009). Exposure to *E. coli* can cause many diseases, ranging from uncomplicated diarrhea to hemorrhagic colitis and hemolytic uremic syndrome which can lead to death (Carry, 2009).

Review of Literature

a. Review of Literature

Studies have been done to review MAP and how effective it can be for prolonging the shelf-life of fresh-cut produce like romaine lettuce. Determining the packaging film for the O_2 permeability is required to maintain an oxygen concentration of 3% in packed romaine (Segall, 1996). The second goal is to determine the extent of MAP to preserve the lettuce quality. The produce in the package is continually respiring and consuming O_2 and producing CO_2 (Segall, 1996). O_2 can diffuse into the bag and the CO_2 can diffuse out because the package has low levels of oxygen and high levels of carbon dioxide, but the atmosphere has the opposite amount with higher levels of O_2, about 21%, with only trace amounts of CO_2 (Segall, 1996). Two factors can diminish the appearance of lettuce: the breakdown of the tissue structure and the development of a red/brown discoloration due to phenolic oxidation (Segall, 1996). Packaging lettuce so that there is 10% CO_2 and 3% O_2 appears to retard the deterioration and extend the shelf-life to an estimated eight days with the proper temperature control (Segall, 1996). Hamza (1996) studied the effects of a controlled atmosphere by flushing a continuous stream of 0.2-2% oxygen and 2-15% carbon dioxide over the lettuce product. Increasing the CO_2 to greater than 10% showed a more reduced enzymatic browning, while 15% CO_2 caused brown lesions. CO_2 can inhibit the polyphenol oxidase activity and the low levels of O_2 can reduce the phenylalanine ammonia-lyases activity being beneficial for quality maintenance (Hamza, 1996). At the end of the study, it was shown that CO_2 levels of about 10% and O_2 levels around a minimum of 1% are beneficial for the lettuce (Hamza, 1996).

MAP has been successful in maintaining the quality of fresh-cut produce, but it can also influence the growth rates of microorganisms present on the produce (Oliveira, 2010). For example, it may enhance the growth of *L. monocytogenes.* The composition of the storage atmosphere generated within the lettuce package during their storage at 5 ^{o}C had no significant effect on the survival and growth of foodborne pathogens on romaine lettuce (Oliveira, 2010). In fact, the results from a study done in Spain (Oliveira, 2010), show that the pathogens have the ability to grow at abuse temperatures no matter if they were stored in air or in modified atmosphere packaging. If the lettuce is already contaminated with the pathogens from processing, they will be able to grow if not kept at the correct temperature conditions.

Extended shelf life, along with the fact that lettuce is often eaten raw, has made this commodity a potentially new challenge for food safety (Chua, 2008). Human pathogens, like *E. coli* can survive passage through acidic environments like the stomach and there is little known about how growth under oxygen partial pressures and how they would impact the pathogens ability to breach the stomach barrier and increase the risk of disease (Chua, 2008). Some believe that using MAP to extend the shelf life could create opportunities for pathogens to grow and survive as the cut plant tissue can release nutrients that would become available for their growth. MAP has a distinct advantage to delay spoilage of the wounded plant tissue so that the product will have a fresh-life appearance for an extended period of time (Chua, 2008). The impending danger of abusive storage temperatures makes it easier for pathogens to grow. In a study with over 1,400 perishable food items (produce and meat) that were examined for temperature abuse at retail locations, over 87% of the samples were kept at the above-recommended temperatures (Chua, 2008).

When distributing lettuce, storage, and display of the produce under refrigerator temperatures is the best for maintaining the product quality. The storage temperature can be the single most important factor affecting the quality of fresh-cut produce (Luo, 2010). Produce can maintain a fresher appearance and last longer with the proper temperature control. Storage temperature plays a huge role in the effectiveness of MAP packaging. The lower the temperature the produce can be stored at can help reduce the respiration rate and retard the growth of spoilage microorganisms (Manolopoulou, 2010). It is ideal to have a temperature between 0-5 ^{o}C. In a study done in Greece, (Manolopoulou, 2010), fresh cut romaine was stored at 0 and 5 ^{o}C and reviewed for quality parameters including appearance, texture, flavor, and nutritional value. The respiration rate at 0 ^{o}C remained unaffected through the ten days of storage but at 5 ^{o}C the lettuce respirated at higher rates with the levels of CO_2 reaching the maximum amount on the seventh day (Manolopoulou, 2010). Pre-harvest conditions as well as the time between harvesting and processing can affect the quality of the raw material and variations in the physiological behavior of the romaine lettuce during the storage (Manolopoulou, 2010). Fresh-cut produce is more susceptible to water loss because more of the product is exposed to the atmospheric conditions. Results from the study, show that storing lettuce at 0 ^{o}C will exhibit the lowest amount of quality deterioration and that a PE or PVC film is the best to protect fresh-cut romaine lettuce from water loss (Manolopoulou, 2010).

Produce may get processed and packaged promptly after harvest, but delays of up to 12 hours or more do frequently occur (Kim, 2005). A study done in the USDA Beltsville Agricultural Research Center examined the issue of delayed MAP packaging effects on quality maintenance and shelf-life of romaine lettuce (Kim, 2005). A delay in packaging can affect the gas, off-odor, color, CO_2, injury, and volatile production, but it could also be an alternative method to help optimize and balance O_2 (Kim, 2005). Seasonal variations and post-harvest storage can affect the respiration rate and the quality of lettuce. Having a large variation in the respiration rate, with the oxygen concentrations too high or even too low, can result in tissue discoloration of the lettuce

(Kim, 2005). Delaying the packaging of lettuce affected the rate of the CO_2 accumulation that occurred in the packages (Kim, 2005). Overall, the results indicated that the impact of delayed packaging on the product quality depends on the atmosphere within the package. Lower O_2 and higher CO_2 and with a delay of 12 hours before packaging was best and beneficial in reducing ethanol in the package, delaying off-odors, and alleviating injury to the lettuce (Kim, 2005).

Other than having a longer shelf-life for the produce, having safe lettuce to eat is important as well to prevent future recalls, illnesses, and hospitalizations, and even deaths. There is not just one thing that needs to be done to prevent illness outbreaks but guidelines that also should be followed. The commodity-specific food safety guidelines for the lettuce and leafy greens supply chain, is a guide that was prepared by different members of the lettuce and leafy greens industry and published by the FDA in 2006. Members include the International Fresh-Cut Produce Association, Produce Marketing Association, United Fresh Fruit and Vegetable Association, and Western Growers. Lettuce is harvested either mechanically or by hand, and then consumed uncooked/raw. Many touchpoints can cause cross-contamination either when the produce is handled by workers or when it comes into contact with equipment (FDA, 2006). Another factor in keeping the lettuce safe is that water used needs to be of appropriate microbial quality (FDA, 2006). All water needs to be tested regularly to meet standards set by the Environmental Protection Agency (EPA) and the World Health Organization (WHO) (FDA, 2006). Fresh cut produce should go through one or more vigorous wash processes, with the wash water containing disinfectants that are effective in eliminating free-floating or exposed microorganisms (FDA, 2006). There are scientific studies that demonstrate that washing produce in cold, chlorinated water will reduce the microbial population by 90-99% (FDA, 2006).

Plants depend more on the availability of water than on any other single environmental factor because water influences cell expansion and leaf water status. Therefore, it might be expected that irrigation affects the post-harvest quality of produce (Luna, 2013). A study done in Spain (Luna, 2013) reviewed the influence of both deficit and excess irrigation water on the quality of fresh-cut romaine lettuce. The influence of different irrigation regimes on the respiration rate, browning and microbial of the lettuce was reviewed. Water can influence not only the microbial quality but the overall quality and shelf-life of vegetables (Luna, 2013). Texture was significantly reduced, and dry matter content decreased significantly when lettuce was cultivated under the highest excess of irrigation water (+75%). Fresh cut lettuce cultivated with the most severe deficit and the extreme excess of water also showed the highest respiration rate compared to the standard (Luna, 2013). The results show that the microbial population in romaine lettuce increased when the irrigation regimes were increased, however, the microbial load after the ten days of storage was similar despite the differences in irrigation regimes (Luna, 2013). Water management can be improved by the use of capacitance probes that can measure the humidity of the soil and adjust for the correct water dose. Using the optimum water amount for each lettuce type, the environmental sustainability will improve without compromising the quality of the produce and give improvement to the quality of raw materials and extend the shelf life of the fresh-cut product (Luna, 2013).

There was a study done in Israel about the effects of MAP on the survival of *Salmonella enterica* on washed romaine lettuce leaves (Horev, 2012). Not many lettuce recalls have been attributed to *Salmonella,* but it can be beneficial to know this pathogens survival on lettuce to help reduce it and prevent any outbreaks. Romaine lettuce leaves were inoculated and then packaged either in passive MAP, active MAP, and a control. The concern is that under MAP conditions, *Salmonella* can be relatively tolerant to high CO_2 and low O_2 levels. In the end, there was an advantage with active MAP over passive to help maintain the CO_2 levels, and with the enriched atmosphere can result in a more efficient control of microflora, but not for *Salmonella* (Luna, 2013); in fact, active MAP does not protect against *Salmonella* growth but could favor its survival. This means that producers need to use the appropriate pre and postharvest sanitation to prevent contamination and the microbial safety of ready-to-eat lettuce products.

Pathogens can contaminate the fresh produce at any time in the field or along the supply chain from farm to table. Many washing and sanitizing methods can help to reduce the microbial load on the lettuce but cannot be relied upon to eliminate the foodborne pathogens (Luo, 2010). A study by Lou et al. (2010) determined if packaged lettuce can support the growth of *E. coli.* Three different trials were done. For trial 1 and 2, the lettuce was initially inoculated with 10^3 CFU/g of *E. coli* and for trial 3, the cells of the pathogen were first stored at 5 ^oC for 48 hours before using as an inoculum to stimulate the cold and nutrient stress that would likely be found in the produce field. The lettuce was stored at either 5 ^oC or 12 ^oC with a best if used by date of 10 days later. For both trials 1 and 2 the *E. coli* grew significantly post-inoculation on the lettuce when at 12 ^{o}C, but at 5 ^{o}C there was no significant growth, and even a slight decline in *E. coli* growth for trial 2. For trial 3, *E. coli* grew significantly overtime for the lettuce at 12 ^{o}C but decreased overtime for the lettuce stored at 5 ^{o}C; initially at 2.7 CFU/g decreasing to 1.9 CFU/g by day ten. This shows that temperature abuse can accelerate the growth of spoilage microorganisms like *E. coli*. It is important to control the temperature during processing, distribution, and storage and understand that any temperature abuse will facilitate the rapid proliferation of *E. coli* before any signs of produce deterioration (Luo, 2010).

Understanding *E. coli* in leafy green vegetables, like romaine lettuce, during production, after harvest, storage, processing, and in packaging is essential for establishing control measures (Delaquis, 2007). There are critical gaps in knowledge identified with uncertainty about the location of bacterial cells on or in the plant tissues, behavior in products stored at low temperatures, and the influence of environmental stresses (Delaquis, 2007). Studies indicate that *E. coli* has the ability to survive on the edible portion of the harvested lettuce for extended periods of time (Carey, 2009). Humans can become infected with *E. coli* either by direct or indirect contact with infected animals, individuals, contaminated surfaces, or inadequately treated water. The fate of *E. coli* is not fully understood outside the animal host, as it can persist for long times as it has been recovered from manure, soil, contaminated irrigation water, and crops (Delaquis, 2007). This also indicates that *E. coli* can survive multiple types of stresses in a production environment. Most outbreaks involving *E. coli* suggest that the leafy greens are contaminated in the field. There is data from a United States Department of Agriculture (USDA) survey that shows an analysis of 4,000 leafy green samples in 2002, that lettuce is more likely to be contaminated with generic *E. coli* than any other type of produce (Delaquis, 2007). In different studies done, the cells of the pathogen can be introduced to the plant in different ways and can be recovered from the roots or leafy parts, but not always both, and sometimes is not recovered at all (Delaquis, 2007). This suggests that *E. coli* can be transferred from the soil and the water to the plant, but the true nature of the interaction is not known.

Plants, like romaine lettuce, possess a wide array of chemical defenses that can provide some protection against a microbial invasion (Delaquis, 2007). It is known that pathogens can survive in or on leafy greens for a long time, but the growth in or on plants under field conditions is not demonstrated. From farm to table of lettuce, there are many different points that the lettuce could become contaminated. Harvesting can lead to the mechanical injury of the plant. Cross-contamination with different contact surfaces can occur. Slicing and shredding before packaging alters the biochemical processes and provides ample opportunity for the microbial invasion of the tissues (Delaquis, 2007). Environmental conditions associated with food production, processing, and storage have influenced the competition between indigenous microorganisms and pathogens (Carey, 2009). While various environmental conditions can affect *E. coli's* growth and survival, the indigenous microflora can also influence pathogen populations and gene expression (Carey, 2009). *E. coli* can be transferred between cut lettuce by dry or wet conditions and washing in solutions with sanitizers, like chlorine, can help to restrict the water transfer of pathogens (Delaquis, 2007). When bacterial cells are subject to low temperatures, they can suffer injury, with *E. coli* populations declining the most at 5^{o}C or less, but much remains to be learned about the nature of stress responses of this pathogen (Delaquis, 2007).

b. Summary

It is very important to emphasize the importance of strict hygiene during production, processing, and packaging to avoid the contamination of lettuce before and after washing or sanitizing and to maintain the correct storage chain temperatures until consumption, as recommended by producers (Oliveira, 2010). Modified atmosphere packaging will help to increase the shelf life of produce. Packaging lettuce so that there is 10% CO_2 and 3% O_2 appears to retard the deterioration and extend the shelf-life to an estimated eight days with the proper temperature control (Segall, 1996). There needs to be more understanding of foodborne pathogens, like *E. coli*, and their stress responses and how they can infect the produce leaves and their survival in the plants. Most of the time, romaine lettuce gets infected at the farm level or even with processing and then the temperature abuse will just encourage the pathogens to grow. It is very important to stress the importance of temperature control during processing, distribution, and storage and that any temperature abuse will facilitate the rapid proliferation of *E. coli* prior to any signs of produce deterioration (Luo, 2010). By identifying the conditions which affect the relationship between the indigenous microflora and pathogenic bacteria, strategies to advance produce safety can be improved upon (Carey, 2009). Improved knowledge of the plant-microbe interactions, and the influence of the indigenous microflora on pathogen virulence, will improve our understanding of the threat of *E. coli* on fresh produce like romaine lettuce (Carey, 2009).

Project Methodology and Design

a. Design

Fresh-cut produce, like romaine lettuce, is packaged into three different packaging types: flexible, rigid, and active/intelligent packaging. Flexible packaging is the most common type of packaging being either preformed bags, roll stock, or stand-up pouches (Toivonen, 2009). Rigid packaging has a rigid tray where the product is contained and a removable lid. An example of rigid packaging would be clamshell or snap-on lid. Both flexible packaging and rigid packaging were studied for the modified atmosphere packaging of the containers. For each type of packaging, there were two different brands analyzed for comparison; so, four different items being tested in total. All the packages were studied from the day of purchase to beyond the shelf life listed on the product packaging to determine all changes in the atmosphere over time.

Ten packages of each type (40 in total) were purchased for this study. One bag was left as the control for the color analysis and appearance observations each day and not tested for the atmosphere. Seven packages of each type and brand were used for a testing period of 14 days. The packages were tested for the oxygen (O_2) and carbon dioxide (CO_2) concentrations inside. Packages were tested every other day (except weekends) starting with day 0 being the day the items were purchased from the grocery stores and ending with day 14. Days of testing were 0, 2, 4, 7, 9, 11, 14. Starting day 0, one product was tested from each brand and type of packaging (four in total). Then each day from there, the product that had been tested the previous day was also tested again for changes in the atmosphere as well as a new package of the same type tested for comparison. Refer to table 1 in the appendices for the flow of the experiment design.

b. Methodology

Lettuce Material. Romaine lettuce was purchased from local grocery stores in the area. Two different brands of flexible packaging type and two different type of rigid tray-type packaging were purchased. Kroger and Dole romaine lettuce in 10oz flexible film bags and Kroger and Food Lion romaine lettuce in 5oz rigid clamshell boxes. The lettuce was purchased fresh from the grocery stores so that the products had the maximum shelf life. All lettuce was transferred to a walk-in refrigerator where the temperature was 3 ^{o}C and stored for the remainder of the study. The temperature of the refrigerator was checked each day and recorded.

Headspace analysis. The headspace gases of each container were analyzed using a Dansensor Checkpoint 3 in the Human and Agricultural Biosciences Building 1, located on Virginia Tech campus. This portable gas analyzer would analyze the O_2 and CO_2 concentrations in the headspace of each container. Atmospheric analysis was also taken each day as a calibration for the machine.

Color analysis/Appearance. Each day pictures were taken of all four of the products. Notes were taken on the color of the lettuce inside the packaging as well as if any moisture accumulation was noticed. Refer to the picture section in the appendices for day 0 and day 14 pictures of the products to see the quality decrease.

c. Data Collection

Starting each day, temperature was taken in degrees Celsius of the refrigerator and recorded. There was a manual recording chart for the temperature and an alarm that would go off if the temperature exceeded the set limit. The Dansensor Checkpoint 3 was used to take the atmospheric O_2 and CO_2 concentrations daily before any product was tested as a way to calibrate the machine. The atmospheric results were recorded. All four packages of the control lettuce were removed from the refrigerator and reviewed for the appearance and color of the lettuce as well as pictures were taken of each product. Every day of testing the control lettuce was reviewed for color and appearance and pictures were taken. Next, the lettuce to be tested, starting with bag/box one of each of the four different types on day 0 were removed from the refrigerator. A small, round foam sticker was placed on the packaging before testing with the Dansensor Checkpoint 3 to help maintain the seal on the packaging. The needle from the Dansensor Checkpoint 3 was inserted through the foam sticker seal, and into the headspace of the packaging carefully avoiding puncturing any actual lettuce product. Results for O_2 and CO_2 concentrations were recorded for each product that had been tested. Each day more products of the same type were tested as discussed in the design above so that the last day all seven packages of each brand and both packaging types were tested for analysis and O_2 and CO_2 concentrations.

Summary of Outcomes, Discussions, and Recommendations

a. Project Outcomes and Results

Results show that both Kroger and Dole romaine lettuce in the 10oz film bags are truly MAP packaged. The Kroger and Food Lion romaine lettuce in the 5oz clamshell boxes are not MAP packaged as the O_2 and CO_2 concentrations inside the packaging was about the same as the atmosphere O_2 and CO_2 concentrations. The average temperature inside the refrigerator each day was 3^oC. The atmosphere conditions for O_2 averaged 20.9% over the 14 days, and 0.043% for CO_2. Graphs that represent the change in O_2 and CO_2 concentrations overtime per each product type that was tested are listed in the appendices.

A results summary table is listed in the appendices. It shows how from the first day to the last day of testing that the O_2 concentrations did decrease, or remain the same for both bag film packages and the CO_2 did increase over the 14 days just as it would be expected for a MAP packaged item. For the clamshell boxes, the O_2 and CO_2 both remained relatively the same for the 14-day testing and that the results measured are close to the atmospheric conditions. The table also shows the manufacturer use by date that was printed on the package and what was determined in the study as the spoilage date due to daily observations of the romaine lettuce quality.

b. Observations

Daily observations were made of each of the four products in the packaging for their color, appearance in the bag, and any moisture accumulation. It was determined that if the product showed a significant amount of browning inside the bag and any wilting or excessive moisture that the product was no longer of good quality and fit for consumption by the consumer. Refer to the picture section in the appendices for day 0 and day 14 pictures of the products to see the quality decrease.

Kroger 10oz film bags had an expiration date of 02/17/2020, day 7 of the testing. The romaine lettuce was grown in Florida and Yuma, Arizona. The lettuce weight without packaging was 10.01oz. Day 0 the lettuce was all green without any visible signs of browning or moisture in the bag. Day 2 moisture accumulation was noticed in the bag, but all the lettuce was still green. Day 4 showed moisture in the bag, and some of the bags still had a few pieces, about four or five, that displayed a few brown spots and wilting. Day 7, the same as the expiry date of the lettuce, all bags of lettuce showed that there was some browning on the stems and moisture in the bags as well. The majority of the bag showed that the lettuce was still green and would have still been fit for consumption. Day 9 showed that the browning increased and much of the lettuce was wilting and moisture was still observed. On this day 9 the lettuce would have not been of good enough quality for the consumption by a consumer. All observations beyond this day, the lettuce looked browner, more wilting and some bags had a brown like slime noticed in the packaging.

Dole 10oz film bags had an expiration date of 02/16/2020, day 6 of testing. The romaine lettuce was grown in Florida and Yuma, Arizona. The lettuce weight without packaging was 9.04oz. Day 0 the lettuce was bright green and there were no visible signs of browning or moisture. Day 2 and day 4 of observations there was a little moisture accumulation noticed in the bags and all the lettuce was still bright green in color. Day 7 some of the stems of some of the lettuce pieces had started to show some red/browning. The lettuce had expired according to the manufacturer the day before this but based on observations the quality of the lettuce was still fit for consumption. Day 9 more of the lettuce was browning and wilting, and it was determined to not be of good quality for consumption. All observations beyond this day, the lettuce looked browner, more wilting and some bags had a brown like slime noticed in the packaging.

Kroger 5oz clamshell boxes had an expiration date of 02/18/2020, day 8 of testing. The baby red and green romaine lettuce was grown in Phoenix and Yuma, Arizona. The lettuce weight without packaging was 5.12oz. Day 0 the lettuce was still all red and green, without any visible signs of browning or moisture. Day 2 and day 4 there was moisture that was noticed on some of the sides of the packaging box, but the lettuce was still green and red. Day 7 the lettuce was still green and red but just on the end of the stems of some of the pieces there was some browning that was occurring as well as a few pieces that were wilting. Enough of the container was of good quality for consumption. Day 9, the expiration day being the day before, the stems were still showing browning and there was a decrease in the amount of moisture noticed in the packaging from the previous observation. Day 11 all boxes had observations of browning and wilting of the lettuce. It was determined that the lettuce was no longer good quality and not fit for consumption.

Food Lion 5oz clamshell boxes had an expiration date of 02/20/2020, day 10 of testing. The baby red and green romaine lettuce was grown in Phoenix and Yuma, Arizona. The lettuce weight without the packaging was 4.22oz. Day 0 the lettuce was green and red with no visible signs of moisture or browning. Day 2 and day 4 the lettuce was still green and red with no visible brown spots. Moisture was noticed on the top and the bottom of the packaging container. Day 7 the lettuce was still green and red with a slight browning just on the ends of the stems of some of the pieces as well as moisture still noticed in the packaging. Day 9 more browning was noticed at the end of the stems as well as some wilting. Moisture was still noticed in the packaging, but it was less than the day before. On day 11, the expiration date is the day before, all boxes showed signs of the lettuce wilting and browning, and no moisture shown in the boxes. It would be determined today that the lettuce would not be of good quality for consumption by the consumer.

c. Project Outcomes and Results Analysis

Initially all data was used from each film bag of lettuce and graphed per bag over 14 days. However, the measured data observed was not the same as other studies from the literature. The foam seal that was used for the testing of each bag did not keep the bags sealed with true MAP inside. This means that once the bag was tested the MAP seal was broken and any further testing was unusable for the study. Over the 14 days the O_2 levels were increasing and CO_2 levels were decreasing, to becoming more like the atmospheric conditions where O_2 levels are higher than CO_2. When looking at only the initial testing day of each of the seven bags and graphing that over time, it is shown in both Graph 1 and Graph 2, there the O_2 concentration remains the same or decreases, while the CO_2 increases. Since lettuce is a living produce item it is respiring and giving off some CO_2, seen by the increase, and the permeability of the film bags is allowing the O_2 to escape and remain low, so that there is a longer shelf life for the product. For the Dole bag on day 14, the bag was tested twice however the results for the headspace analysis of both O_2 and CO_2 were significantly different it was determined that the seal on that product was not good from being processed at the manufacturing facility and thus the data was not used.

For the clamshell type packaging, Graph 3 and 4 show the data for what is inside one package over the 14-day testing period graphed with the atmospheric O_2 and CO_2 conditions. All packages over time showed about the same results. As seen in the graphs, the atmosphere and what is inside the packaging are about the same, with just a slightly higher amount of CO_2 in the packaging. That is due to the lettuce being a living product item and is respiring and giving off some gas. But because the packaging is not sealed 100% some of the gas can escape leaving with just very low numbers for the CO_2 percentages and the O_2 being at about equilibrium with the air. Due to the concentrations of the gases being about the same, the clamshell type package is not a modified atmosphere package.

It is not known the exact plastic material that was used for the film bags. For the clamshell it is polyethylene terephthalate (PETE) material as it was listed on the packaging itself. Polyolefin is most widely used for plastics in the food industry. Polyolefin is a polyethylene/polypropylene mixture. It has the properties of good flexibility, strength, lightness, stability, moisture, chemical resistance, and easy processability (Mangaraj, 2009). So, one could make a guess that is what the film packaging is made from seeing as it is most widely used and the properties are what you would want for packaged lettuce, but there is no way to be sure without being in direct contact with a manufacturer or by further testing. MAP relies on the relationship between the product respiration and the package transmission rate but is only effective if a consistent temperature is maintained throughout the produces life cycle (Toivonen, 2009).

In the study, lettuce packaged in films with modified atmosphere, where O_2 levels kept low and CO_2 levels increased slowly, were able to last longer than the expiration date listed on the package by the manufacturers. This means that the MAP was successful with the consistent temperature that products were kept at in the refrigerator. Based on the literature review from above, having levels of CO_2 around 10% and O_2 levels around 1% is what is successful for the MAP to prolong the shelf-life of the product which is about what was measured in the study. The clamshell package is not a true MAP product since the package gas transmission rates are not engineered or controlled but more of a natural aspiration package (Toivonen, 2009). The atmosphere in the package was very similar to the measured atmosphere not in the package. However, the lettuce did last beyond the listed expiration date by the manufacturers.

d. Implications, Impacts and Recommendations

From the study it seems that both MAP packaged lettuce as well as not MAP packaged lettuce, can last beyond the date of expiration listed by the manufacturer. It may be helpful in future projects to work more closely with a manufacturer of the lettuce to know the exact packaging material that is used to better understand the exact rate of gas transmission through the packaging material as well as to understand any pre-harvesting techniques used that could also help for the extended shelf life of the product. Regardless it does seem that both types of packaging will help to extend the shelf life of the lettuce, but that it does also depend on the consumer to purchase the lettuce and make sure to keep it at correct temperatures and avoid any temperature abuse that could potentially lead to lesser quality and any safety issues.

e. Dissemination Plan

This information would be useful for romaine lettuce farmers and manufacturers. To let them know about extending the shelf life of their products for good quality as well as safety for the food. It would be good for consumers, and even retailers that sell lettuce to know the importance of temperature control for lettuce products to help with the safety and the quality of the products as well.

Conclusion

Between 2009 and 2018 the FDA and CDC identified over 40 different foodborne outbreaks of Shiga toxin-producing *E. coli* (STEC) infections within the United States with a confirmed or suspected link to leafy greens (FDA, 2020). Leafy greens are grown outside so they can be exposed to several of sources of potential pathogen contamination like soil, animals, and water. These leafy greens are also consumed raw without a heating or cooking step to help eliminate any microbial hazards. Due to the reoccurring nature of these outbreaks associated with the leafy greens, the FDA has developed a commodity-specific action plan, 2020 Leafy Greens STEC Action Plan (FDA, 2020). The FDA plans to act and address work in areas of prevention, response, and addressing knowledge gaps to help improve the safety of leafy greens.

As part of prevention, the FDA would like to focus on areas of advancing the agricultural water safety, reinforcing the importance of ensuring standards and emphasizing to the growers the importance of using good agricultural practices (GAPs) for the water used. Enhancing the inspection, auditing, and certification programs to help out farms in identifying their deficiencies in their approach to food safety (FDA, 2020). The FDA is conducting test samples of the romaine lettuce grown where the recent outbreaks occurred as well as trying to engage the sampling protocols like what to sample, when and where and how often to sample to help detect *E. coli* potentially at the time of harvest before it would be processed and shipped out for human consumption. It is important for the farmers to have an increased awareness and to address concerns about the adjacent and nearby lady use, especially the land that involves livestock production as it has the potential to be a source of pathogens (FDA, 2020).

Response is to ensure that the outbreak activities are conducted as quickly as possible and thoroughly as possible as this is essential for preventing illnesses (FDA, 2020). The FDA will publish an official outbreak investigation report including the traceback and all the sampling activities that were conducted for the most recent outbreak in California. In addition to the outbreak report, the FDA plans to conduct follow-up surveillance during the Fall 2020 growing and harvesting season to possibly identify factors that may have contributed to the outbreaks as well as identifying farms that could have supplied the contaminated product (FDA, 2020). The public health agencies have gotten better at detecting foodborne illnesses, but the ability to be able to trace it back to the source is not effective due to the lack of modernized food traceability capabilities (FDA, 2020). Being able to have a farm to table traceability throughout all of the leafy green supply chain would help make it easier to shorten outbreaks, narrow product warnings and prevent any illnesses.

While the FDA and stakeholders have greatly expanded what is known about leafy green food safety, there are still knowledge games and these can be explored in new ways through the use of new and emerging technologies (FDA, 2020). The FDA is working with the Arizona Department of Agriculture and other members of the leafy green industry to better understand the ecology of human pathogens and how they can survive in various reservoirs and how they can move throughout the environment. Using data from previous outbreaks like rainfall levels, temperature, economic indicators, genetic information would also hold additional clues for understating the factors that contribute to outbreaks with *E. coli* (FDA, 2020).

Since there are multiple ways that the pathogens may enter the leafy greens either in the field, during harvesting, production, irrigation water, in the home/restaurant, there is not going to be just one fix that needs to be done to help prevent future outbreaks. Hurdles, combining several mitigating approaches, should happen as to decrease the risk due to pathogenic microbes as well as to improve the microbial stability, shelf-life, nutritional properties, and sensory quality of leafy greens (Mogren, 2018). The main points of contamination for leafy greens are production, harvest, washing and processing, packaging and handling, distribution, display and retail (Mogren, 2018). At any of these points the lettuce can be contaminated but if there is a prevention step at each of these steps too, there could be a better approach to ensuring the safety of leafy greens.

The produce industry must rely on preventive measures like GAPs and HACCP and with this approach, it requires decisions based on evidence and framework for describing the decision process within the whole supply chain (Mogren, 2018). One of the most important factors of plant and microbial activities and growth is temperature. Harvested leafy vegetables should be cooled as soon as possible to help prevent water loss and respiration (Mogren, 2018). High outside temperatures during harvesting can promo respiration, so the post-harvest temperature management is the most critical hurdle for leafy greens and should not exceed 5 ^{o}C (Mogren, 2018). Low temperatures are also not often maintained during transport, retail storage, display, and even at the home of consumers in their refrigerators. Next it is important to monitor pathogen and indicator species in water sources, as well as the available physical and chemical water treatment methods to remove human pathogens from the irrigation water source (Mogren, 2018). Methods like subsurface drip irrigation, used to avoid direct contact with the edible parts of the crop, could help prevent contamination, but more knowledge is required on the risks posed by root contamination and internalization of the bacteria (Mogren, 2018).

Once harvested, lettuce leaves with continue to respire and if placed in sealed packages they will modify their atmosphere due to the consumption of O_2 and releasing $CO_{2.}$ This is where the packaging material comes into play that MAP is used as the extension of the shelf-life. However, extending the shelf-life of the lettuce could also allow for the growth of pathogenic bacteria, while also inhibiting the growth of organisms that usually make the consumer aware of spoilage by off-odors (Mogren, 2018). A pH value below 4.6 will help inhibit the growth and toxin production of any different pathogens but many microorganisms, like *E. coli* are capable of growth or survival at pH values lower than this limit (Mogren, 2018). Co-existence in bacterial communities is controlled by the access to space, nutrient use and availability, production of antimicrobial compounds and other strategies to acquire resources (Mogren, 2018). An invading microorganism like a human pathogen must successfully co-exist and compete with the already adapted and established bacterial community in order to exist (Mogren, 2018). It has also been concluded that bacteriophages could reduce the number of pathogens by at least one log of 90% so they could be useful as one amongst several hurdles to ensure food safety (Mogren, 2018).

In the end, it is seen that not just one prevention step is going to prevent all future outbreaks regarding human foodborne pathogens. Leafy greens like romaine lettuce would benefit from combining multiple hurdles to help improve its food safety. Lettuce being packaged MAP is more for keeping the quality of the lettuce to last longer since it is a fresh-picked commodity. Other factors like water used for processing, GAPs, and temperature control from harvest, processing, to the retail and the consumer level, also has to be properly controlled. Farmers need to work with the FDA and other officials to better understand issues that can cause the outbreaks to help prevent them. Also, more studies need to be done on pathogenic bacteria like *E. coli* to help understand its response in the environment can help to take the necessary steps to prevent human illnesses.

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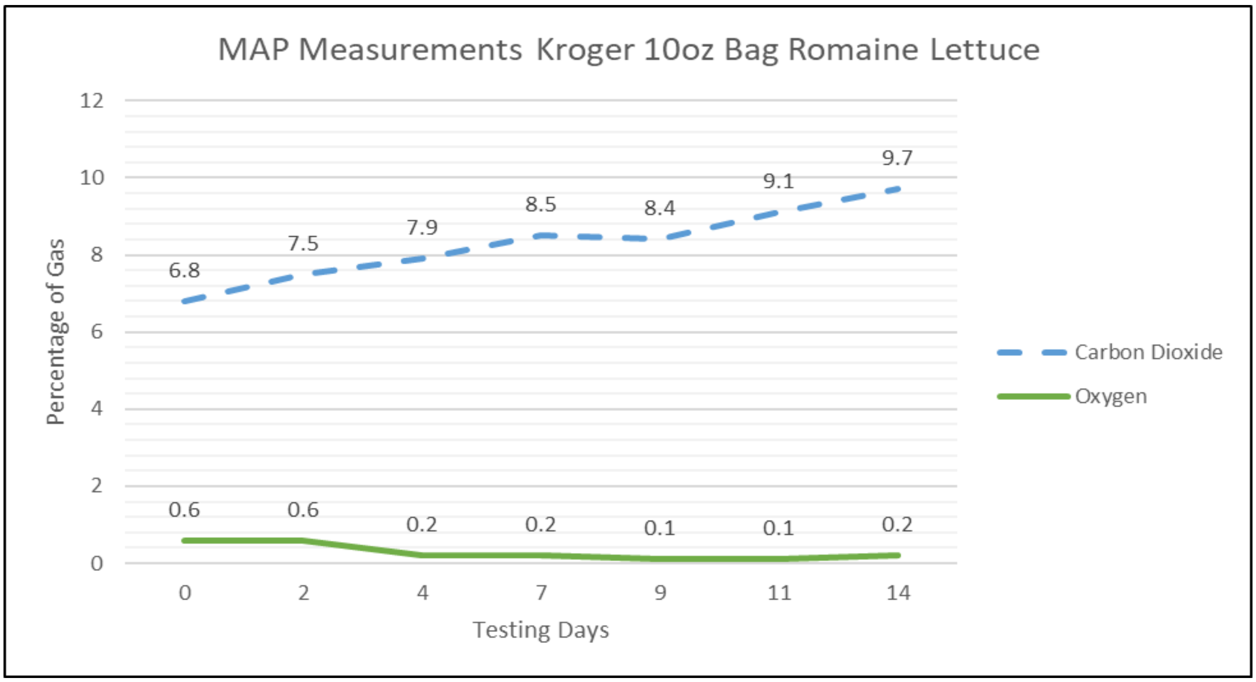
Appendices

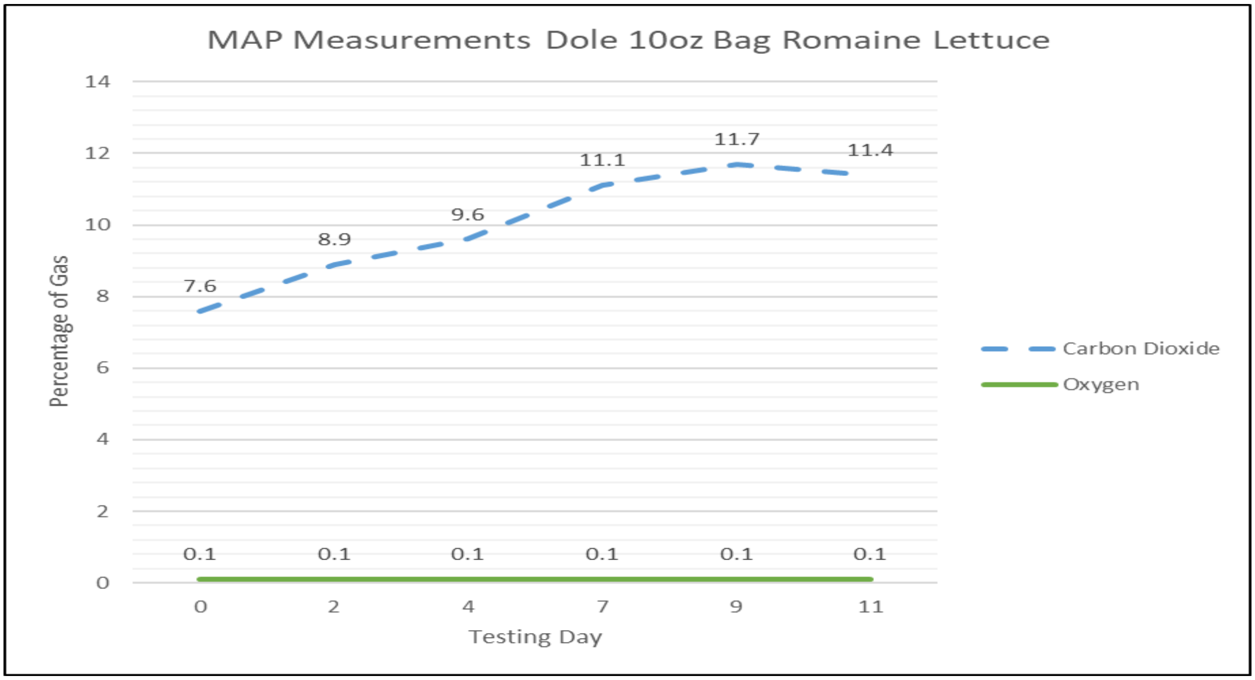
a. Acknowledgements

The author would like to thank Brett Driver for his help and assistance with setting up the study as well as providing the space and the equipment needed as well as the processing and evaluation of the data. Also, would like to thank Dr. Eifert for his guidance and support during the study. Funding for the project was provided by Virginia Tech.

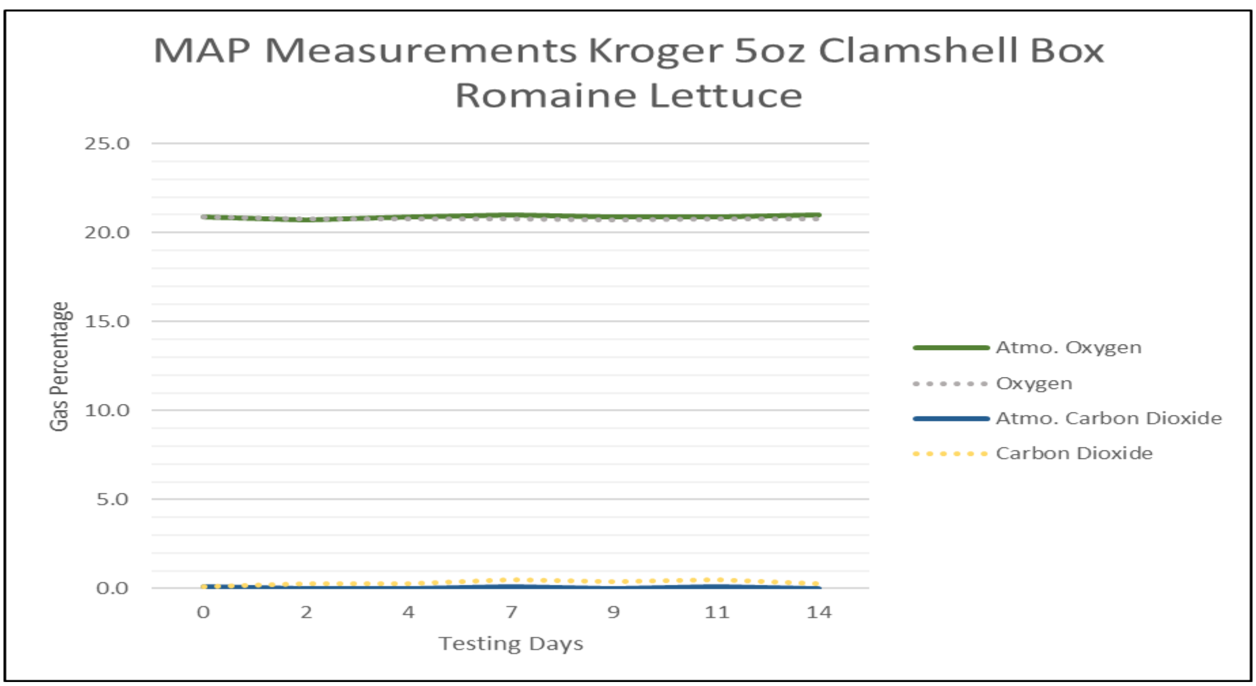
b. Graphs



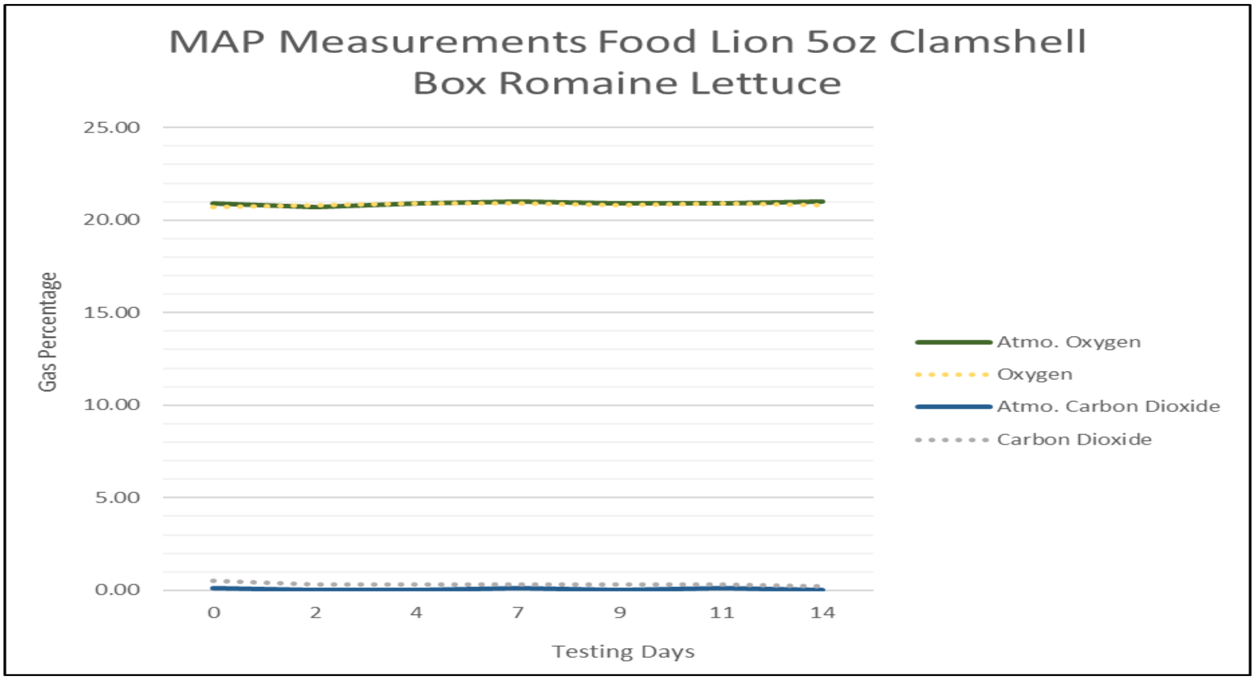
(Graph 1 – the measurement of change of O_2 and CO_2 over the course of 14 days in plastic film 10oz bag of Kroger romaine lettuce)



(Graph 2 – the measurement of change of O_2 and CO_2 over the course of 11 days in plastic film 10oz bag of Dole romaine lettuce)



(Graph 3 – the measurement of change of O_2 and CO_2 and atmospheric O_2 and CO_2 over the course of 14 days in plastic clamshell 5oz box of Kroger romaine lettuce)



(Graph 4 – the measurement of change of O_2 and CO_2 and atmospheric O_2 and CO_2 over the course of 14 days in plastic clamshell 5oz box of Food Lion romaine lettuce)

c. Tables

Product Type	Testing Day 0	Testing Day 2	Testing Day 4	Testing Day 7	Testing Day 9	Testing Day 11	Testing Day 14
Kroger 10oz film bag	Bag 1	Bag 1, 2	Bag 1, 2, 3	Bag 1, 2, 3, 4	Bag 1, 2, 3, 4, 5	Bag 1, 2, 3, 4, 5, 6	Bag 1, 2, 3, 4, 5, 6, 7
Dole 10oz film bag	Bag 1	Bag 1, 2	Bag 1, 2, 3	Bag 1, 2, 3, 4	Bag 1, 2, 3, 4, 5	Bag 1, 2, 3, 4, 5, 6	Bag 1, 2, 3, 4, 5, 6, 7
Kroger 5oz clamshell box	Box 1	Box 1, 2	Box 1, 2, 3	Box 1, 2, 3, 4	Box 1, 2, 3, 4, 5	Box 1, 2, 3, 4, 5, 6	Box 1, 2, 3, 4, 5, 6, 7
Food Lion 5oz clamshell box	Box 1	Box 1, 2	Box 1, 2, 3	Box 1, 2, 3, 4	Box 1, 2, 3, 4, 5	Box 1, 2, 3, 4, 5, 6	Box 1, 2, 3, 4, 5, 6, 7

(Table 1 – items and the corresponding days tested)

Product Type	% O_2 First Day	% O_2 Last Day	% CO_2 First Day	% CO_2 Last Day	Package Use by Date	Spoilage/Not Fit for Consumption
Kroger 10oz film bag	0.6 %	0.2 %	6.8 %	9.7 %	Day 7	Day 9
Dole 10oz film bag	0.1 %	0.1 %	7.6 %	11.4 %	Day 6	Day 9
Kroger 5oz clamshell box	20.9 %	20.8 %	0.1 %	0.3 %	Day 8	Day 11
Food Lion 5oz clamshell box	20.7 %	20.8 %	0.5 %	0.2 %	Day 10	Day 11

(Table 2 – results summary from all products tested)

d. Pictures

<table>
<tr><th>Product Type</th><th>Day 0 Of Observations</th><th>Day 14 of Observations</th></tr>
<tr><td>Kroger 10oz film bag</td><td>
</td><td>
</td></tr>
<tr><td>Dole 10oz film bag</td><td>
</td><td>
</td></tr>
<tr><td>Kroger 5oz clamshell box</td><td>
</td><td>
</td></tr>
<tr><td>Food Lion 5oz clamshell box</td><td>
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