Inhibition of Growth of *Escherichia coli* O157:H7 and *Salmonella* in ground beef using modified atmosphere packaging systems

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Summary
Controversy over the use of modified atmosphere packaging (MAP) containing carbon monoxide (CO) for ground beef has generated petitions to FDA and USDA to re-evaluate its use as an approved packaging component. The use of modified atmosphere packaging systems (MAP) containing CO gas has recently been investigated and has been effective in reducing pathogens such as *Escherichia coli* and *Salmonella* during refrigerated storage. Ground beef products may be subjected to temperature abuse before or after purchase by consumers, therefore, the objectives of this study were to determine if *E .coli* O157:H7 and *Salmonella* growth was inhibited under extreme temperature abuse conditions in various MAP packaging environments compared to traditional PVC overwrap packaging. To evaluate this objective, a cocktail of *Escherichia coli* O157:H7 or *Salmonella* was used to inoculate ground beef patties (80% lean, 20% fat) at a 1 x 10^3 cfu/g inoculation level in four replications. The packaging types were: vacuum bags (VAC), chub (overwrap) and three different modified atmosphere packaging (MAP) trays with a high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide(0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO). Each tray or bag contained two patties. One package from each packaging type was processed after 24 hours of refrigeration in the dark. On day 5 of dark storage at 4ºC, the inoculated ground beef chubs were unpackaged and placed onto plastic trays with overwrap film and were placed in the retail (2.8ºC) for 24 hours. On day 6 the packages were randomly placed in three groups for temperature abuse (2.8ºC walk in cooler in the dark, 8 hours at 21ºC, and 35ºC for 4 hours). After the temperature abuse, the packages were placed back in the dark 2.8ºC walk in cooler. One package from each combination was taken on day 9, 11, 14, and 24 days. *Salmonella* samples were plated onto Rambach agar, *Escherichia coli* O157:H7 samples on MacConkey agar with CT, total mesophilic counts on PCA, and total psychrophiles on APT agar. Our results demonstrate higher numbers of *E. coli* O157:H7 in PVC compared to other packaging types under all abuse condition; and no significant difference between VAC, HO, CO and WOCO. A temperature of storage and a day of sampling effect were observed. Packages stored at 2.8ºC had significantly lower *E. coli* O157:H7 compared to those at 35ºC, but both were statistically the same as packages stored at 21ºC ($P<0.05$ and $P> 0.05$, respectively). Additionally, days 6 and 14 were significantly less than days 9 and 11. *Salmonella* recovery were not significantly different in the packaging type nor the storage temperature ($P>0.05$). However, *Salmonella* concentrations were higher on days 6 and 9, regardless of storage temperature and packaging type. Packaging type by day interaction was highly significant ($P<0.0001$) but all other interactions were not significant ($P>0.05$). When the data is sliced to determine the simple main effects within the study packaging type PVC was significantly different amongst sampling days and on day 11 and 14 of storage there were significant difference amongst packaging types ($P<0.05$). Overall, it was evident that packaging other than overwrap has a positive impact on reducing *E. coli* O157:H7. Samples packaged under these conditions had significantly less *E. coli* O157:H7 growth during temperature abuse and under no abuse conditions, while *Salmonella* counts was significantly different on sampling days 11 and 14 within packaging types and within PVC packaging throughout study. These data suggest packaging types may result in a safer ground beef supply.

Introduction

The use of carbon monoxide (CO) in packaging has been used in the food processing industry for the last forty years in a variety of different perishable food products (Sorheim, 2006). Controversy over this recently approved methodology in ground beef has generated petitions to FDA and USDA to re-evaluate the use of carbon monoxide gas as an approved packaging component. The Food and Drug Administration (FDA) and U.S. Department of Agriculture / Food Safety and Inspection Service (USDA FSIS) requires that approved processes and/or ingredients do not result in the product becoming adulterated or misbranded, which includes making the meat product look better or of greater value than untreated products and the normal spoilage indicators can not be masked (FSIS, 2003). Additionally, the safety of products with regard to pathogen growth has been questioned. Thus, research is needed to provide industry and government officials with scientific data regarding the safety and spoilage characteristics of modified atmosphere packaging systems containing carbon monoxide gas.

The use of modified atmosphere packaging systems (MAP) containing CO gas has recently been investigated and has been effective in reducing pathogens such as *Escherichia coli* O157:H7 *and Salmonella*. A recent study by Brooks et al., 2006 indicated that a reduction in *Escherichia coli* O157:H7 and *Salmonella* 1-2 log cycles compared to pathogen loads in traditional packages packaged in PVC overwrap. This study was the first to use a pathogen processing facility to replicate industry practices to ensure gas conditions were maintained.

Due to the varying abuse that ground beef products may incur before or after purchase by consumers, the importance of subjecting ground beef products to different treatments, such as temperature, time, and packaging methods is extremely important to evaluate. The packaging process is a complex chain of events that affects how well a product maintains a certain temperature from the chill chain to the retail shelf. During this chill chain, a product can easily become abused during transportation, storage/display at retail, and in the hands of the consumer. With this in mind, the objectives of this study were to determine if *E. coli* O157:H7 and *Salmonella* growth was inhibited under extreme temperature abuse conditions in various MAP packaging environments compared to traditional PVC overwrap packaging

Methodology

Culture Preparation

All microbial work was performed at the Texas Tech University BSL2 Food Microbiological lab and in the BSL2 pathogen processing facility in the Animal and Food Science Department under the guidance of Dr. Mindy Brashears. The following cultures were used to create a cocktail for inoculation of the ground beef: *E. coli* O157:H7 strains ATCC 43888 human feces origin, ATCC 43894 human feces origin, ATCC 35150 human feces origin, and A190 origin unknown; and *Salmonella* strains ATCC 9270 creek water origin, ATCC 14028 origin from pools of heart and liver from 4-week old chickens, ATCC 8388 (serovar Meunchen), and ATCC 35640 from pork liver. The *E. coli* O157:H7 and *Salmonella* were grown using a four day procedure to create a cocktail mixture of the multiple strains.

The cocktails were prepared in the following manner. On day one of cocktail preparation, one vial of each strain was removed from a -80 ºC storage freezer. Each vial was scraped for growth with a sterile, disposable 1ul inoculating loop, and mixed in 10ml of sterile BHI broth. This was repeated for each strain

using a new loop and a new tube of 10ml BHI broth. Each inoculated BHI broth tube was incubated overnight, at 37ºC.

On day two, 1.0 ml from each individual overnight BHI culture (from, DAY 1) was placed into 3 new sterile 9.0ml tubes of BHI, for each strain and incubated overnight at 37ºC. Also on day 2, five 1:10 serial dilutions were performed from each overnight BHI culture (from, DAY 1) using 9.0ml BPW tubes. After dilutions were performed 100 ul was plated onto duplicate TSA plates and incubated overnight at 37 ºC.

On day three, the TSA plates (from DAY 2) were counted to determine the CFU/ml, for each strain. Also on day three, the three BHI tubes for each strain was checked for presence of growth (from DAY 2 and from these BHI tubes, 2.0 ml of each strain was placed into 200ml of sterile BHI and incubated overnight at 37ºC.

On day four, the BHI tubes (from DAY 3) were divided into 20-30 ml centrifuge tubes and centrifuged for 10 minutes at 4000 x g. The solvent was decanted and the pellet suspended in minimal volume of BHI/ with 10% glycerol. All suspended pellets (of 1 strain) were pipetted into one 50 ml tube to bring volume of tube to 50ml with BHI/ with 10% glycerol and all 50ml tubes from each strain were combined into a sterile flask. One ml of the cocktail were placed into sterile, cryogenic tubes (2.0ml, size tubes) and stored at -80 ºC.

A sample of the cocktail was taken to verify the concentration of the pathogen in the multi strain cocktail (cfu/ml). The *E. coli* O157:H7 and *Salmonella* cocktails were verified to have a bacterial load of 1.0 x 10^9 cfu/ml.

On the day of processing, 1 tube of frozen culture was thawed by allowing sit on the counter top for 5 minutes. The cocktail was vortexed and serial dilutions were performed to receive a 10^2-10^3cfu/gram inoculum level in the ground beef. An extra tube of culture was sampled on the day of processing and was verified for inoculum load. The tube of culture was covered by ice in a cooler and taken to the pathogen lab.

Ground Beef Processing

Processing occurred at the Texas Tech University Pathogen Laboratory in the Animal and Food Science department under the guidance of Dr. Chance Brooks and Dr. Mindy Brashears. Prior to processing, a three day clean up procedure was conducted in the pathogen lab to ensure that the facility was pathogen free. All equipment was cleaned and then placed in the walk-in cooler to maintain a cold temperature.

Fresh coarse ground beef (80% Lean, 20% Fat) was obtained from two Cargill locations in Texas (Friona and Plainview) three days prior to processing. The ground beef was held in a walk in cooler at 2.8ºC in the dark until processing day. There were four complete replications performed for each of the studies. Replication one and two were performed on the same day with replication one meat coming from Friona and the replication two meat coming from Plainview. Replication three and four were performed on separate days with replication three meat coming from Friona and replication four meat coming from Plainview. On a given processing day, the two replications were performed separately with an intense cleaning procedure between meat types on all equipment and the facility. The meat provided by Cargill processing facilities went through their “test and hold” program to ensure a pathogen free product.

Escherichia coli O157:H7and *Salmonella* samples were prepared on different days to ensure that no cross contamination would occur.

The course ground meat was mixed in a Biro 32 mini mixer in 20 lb batches for 1 minute to break up the meat chucks, then for the additional 2 minutes for the cocktail to be added to the meat and then another 2 minutes ensure uniform distribution of the cocktail in the meat block. Immediately after the mixing, the meat was placed in the walk-in cooler (4 C) in the pathogen lab. Fifteen pounds of inoculated meat was set aside for making chubs. With the remainder made into one-third lb patties using a hand presses with two patties randomly assigned to one of the five packaging types.

The packaging types were: vacuum bags (VAC), chub (which was later overwrapped) and three different modified atmosphere packaging (MAP) trays with a high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO). Each tray or bag contained two patties. The low oxygen carbon monoxide MAP packages and low oxygen MAP both had less than 800ppm of residual oxygen at time of processing. Vacuum packages were made by using 8 X 10 inch Cryovac bags. The MAP trays were processed using a Cryovac #3 tray and tray sealing machine (model CV/VG-S, Semi-Automatic 320 by 500, G. Mondini, Brescia, Italy). The packages were then placed under refrigeration until transport to the Texas Tech Food Microbiology Laboratory. Packages in the Food Microbiology Laboratory were placed in a walk in cooler (4 C) in the dark until processing.

One package from each packaging type was processed after 24 hours of refrigeration in the dark. On day 5 of dark storage in the refrigerator at 4ºC, the inoculated ground beef chubs were unpackaged and placed onto plastic trays with a tight over wrap film (PVC) for the duration of the project. Also on day 5, all of the packaging types were placed in the Food Microbiology Laboratory display coolers (2.8ºC) for 24 hours.

On day 6, the packages were randomly placed in three groups for temperature abuse. One third of the packages were placed directly back in the 2.8ºC walk in cooler in the dark (control, no temperature abuse), another third were left out on the laboratory counter for 8 hours at 21ºC and the last third were placed in incubator at 35ºC for 4 hours. After the temperature abuse occurred, the packages were placed back in the dark 2.8ºC walk in cooler until sampling.

Before abuse and after abuse samples were taken on day 6 from one package from each of the temperature/package combination. One package from each combination was taken on day 9, 11, 14, and 24 days. On day 24, the PVC packages and high oxygen were not tested due to excessive spoilage.

Microbiological Procedure

Packages were opened aseptically using either scissors or a scoopula. Twenty-five grams were weighed into stomacher bags. Buffered peptone water was added and stomached for 1 minute. Serial dilutions were made depending on intended microbial load. *Salmonella* samples were plated onto Rambach agar, *Escherichia coli* O157:H7 samples on MacConkey agar with CT, total mesophilic counts on PCA, and total psychrophiles on APT agar. Plates were inoculated for 24 hours for *Escherichia coli* O157:H7, 48 hours for *Salmonella* and total mesophiles, and 6 days for psychrophiles.

Statistical Analysis

The data was analyzed using a descriptive analysis in SAS program. PROC MIXED program was used to identify the differences between the day, temperature, and packaging type. PROC UNIVARIATE was used to verify normality between days, temperatures, and temperature by packaging type. Significant differences are reported as p-value of less than 0.05.

Results

Escherichia coli O157:H7

Effect of packaging type, effect of day of sampling, and the effect of storage temperature were significant (P-value=0.001, 0.001, and 0.0071, respectively). Additionally there was an interaction between the packaging type and the day of sampling (p-value<0.001).

The effects of packaging type can be made without regard to storage temperature or day of sampling. That is, *Escherichia coli* O157:H7 is higher in PVC than in the other packaging types and *Escherichia coli* O157:H7 was statistically the same in WOCO, CO, VAC, and HO packages (P>0.05).

Additionally, there was a temperature of storage effect found within this study. The packages stored at 2.8ºC were significantly lower than the packages stored at 35ºC, but were statistically the same as packages stored at 21ºC (P<0.05 and P> 0.05, respectively). The packages stored at 21ºC and 35ºC were also statistically the same. All of these similarities and differences were regardless of day of storage or packaging type.

Effect of day of sampling effect can also be made regardless of storage temperature or packaging type. Days 6 and 14 were significantly lower than days 9 and 11 within the study, days 9 and 11 were statistically the same and days 6 and 14 were statistically the same (P<0.05 and P> 0.05, respectively).

Packaging type by day interaction was highly significant (P<0.0001) but all other interactions were not significant (P>0.05). It must be noted that because this is an interaction that is significant in the model it is appropriate to said that "The effect of packaging type on *Escherichia coli* O157:H7 counts is dependent on the day of sampling." When the data is sliced to determine the simple main effects within the study, the following conclusions can be made: When packaging type MAP with high oxygen (HO), PVC, and vacuum packages (VAC) are held constant there are significant differences amongst sampling days (P=0.0012, P<0.001, P<0.001, respectively); and when day of storage 9, 11, and 14 are held constant there are significant difference between packaging type (all P<0.001). All other simple main effects were not significantly different in this trial (P>0.05) as follows: CO (P=0.3076) and WOCO (P=0.4167).

Escherichia coli O157:H7 counts were significantly higher on day 9 than days 6, 11, and 14 within the vacuum packages (VAC) (P <0.05). Additionally, *Escherichia coli* O157:H7 counts were statistically the same on days 6, 11, and 14 within the vacuum packages (VAC) (P >0.05). *Escherichia coli* O157:H7 counts were significantly higher on day 9 and 11 than days 6 and 14 regardless of temperature within the MAP with high oxygen packages (HO) (P <0.05). Additionally, *Escherichia coli* O157:H7 counts were statistically the same on days 6 and 14 within the MAP with high oxygen packages (HO) (P >0.05). *Escherichia coli* O157:H7 counts were significantly higher on day 9, 11, and 14 than day 6 within the

PVC packages. Additionally, *Escherichia coli* O157:H7 counts were statistically the same on days 9, 11, and 14 within PVC packaging (P>0.05).

Escherichia coli O157:H7 counts were statistically the same on days 6 in all the packaging types (P>0.05). *Escherichia coli* O157:H7 counts were statistically the same on days 9 in low oxygen packaging with carbon monoxide (CO), low oxygen packaging without carbon monoxide (WOCO) and MAP with high oxygen packages (HO) (P>0.05). Additionally, *Escherichia coli* O157:H7 counts were statistically the same on days 9 in vacuum packaging (VAC) and overwrap packaging (PVC) (P>0.05). *Escherichia coli* O157:H7 counts was significantly higher on days 9 in the vacuum packaging (VAC) and overwrap packaging (PVC) than the low oxygen packaging with carbon monoxide (CO), low oxygen packaging without carbon monoxide (WOCO) and high oxygen packages (HO) (P<0.05). *Escherichia coli* O157:H7 counts were statistically the same on days 11 in low oxygen packaging with carbon monoxide (CO), low oxygen packaging without carbon monoxide (WOCO) and vacuum packaging (VAC) (P>0.05). Additionally, *Escherichia coli* O157:H7 counts were statistically the same on days 11 in high oxygen packaging (HO) and overwrap packaging (PVC) (P>0.05). *Escherichia coli* O157:H7 counts were significantly higher on days 11 in the high oxygen packaging (HO) and overwrap packaging (PVC) than the vacuum packaging (VAC) , low oxygen packaging with carbon monoxide (CO) and low oxygen packaging without carbon monoxide (WOCO) (P<0.05). *Escherichia coli* O157:H7 counts were significantly higher on day 11 in the overwrap packaging (PVC) than the vacuum packaging (VAC), low oxygen packaging with carbon monoxide (CO) and low oxygen packaging without carbon monoxide(WOCO) (P <0.05). *Escherichia coli* O157:H7 counts were statistically the same on days 14 in the vacuum packaging (VAC) , low oxygen packaging with carbon monoxide (CO), low oxygen packaging without carbon monoxide (WOCO) and vacuum packaging (VAC) (P>0.05). *Escherichia coli* O157:H7 counts were significantly higher on day 14 in the overwrap packaging (PVC) than the high oxygen packaging (HO), vacuum packaging (VAC), the low oxygen packaging with carbon monoxide (CO) and low oxygen packaging without carbon monoxide (WOCO) (P <0.05).

In a separate SAS analysis for day 24, there were no statistical differences found between the low oxygen with carbon monoxide, the low oxygen without carbon monoxide and the vacuum packaged ground beef (P>0.05). There were no temperature effects found nor any interactions on day 24 found (P >0.05).

<u>*Salmonella*</u>

Effects of day of sampling are highly significant, but the effect of packaging and the effect of storage temperature were not significant (P=0.001, P= 0.1029, P=0.8173, respectively). For the day of sampling effect, an orthogonal polynomial analysis shows a significant curvilinear response: linear and cubic effects. For the *Salmonella* data, there are differences among the day of sampling. In particular, Day 6 and 9 had higher in microbial counts than days 11 and 14 (P<0.05). There were no significant differences in microbial counts between the packaging types (PVC, CO, WOCO, VAC) (P>0.05).

Packaging type by day interaction was highly significant (P<0.0001) but all other interactions were not significant (P>0.05). It must be noted that because this is an interaction that is significant in the model it is appropriate to said that "The effect of packaging type on *Salmonella* counts is dependent on the day of sampling." Therefore no general trends can be made. When the data is sliced to determine the simple main effects within the study, the following conclusions can be made: When packaging type PVC is held constant the days are significantly different; when day of storage 11 is held constant there are significant

difference between packaging type (P<0.001) and when day of storage 14 is held constant there are significant difference between packaging type (P=0.0181). All other simple main effects were not significantly different in this trial (P>0.05) as follows CO (P=0.2187), HO (P=0.1189), VAC (P<0.2349), WOCO (P=0.9207), Day 6 (P=0.6262), and Day 9 (P=0.2421).

When PVC is held constant there are multiple differences found between sampling days. On day 6 the PVC packages have significantly higher *Salmonella* counts than days 11 and day 14 of the study (P<0.0001). Additionally on day 9 the PVC packages have significantly higher *Salmonella* counts than days 11 and day 14 of the study (P<0.0001). PVC packages on days 6 and 9 are statistically the same (P>0.05).

On day 11 of the study, comparisons can be made amongst the packaging types. On day 11, the low oxygen packaging with carbon monoxide (CO) was significantly higher *Salmonella* counts than the PVC packages (P< 0.0001). Also, on day 11 of the trial, the low oxygen packaging without carbon monoxide (WOCO) were significantly higher *Salmonella* counts than the PVC packages (P<0.001). On day 11, the MAP packages with high oxygen (HO) were significantly higher than the PVC packages (P<0.0001). All other packaging type combinations on day 11 of the trial had statistically the same *Salmonella* counts (P>0.05).

On day 14 of the study, comparisons can be made amongst the packaging types. On day 14 of the trial the low oxygen packaging with carbon monoxide (CO) had significantly higher *Salmonella* counts than the PVC packages (p-values 0.0001). Additionally, on day 14 of the trial, the MAP packages without carbon monoxide (WOCO), vacuum (VAC), and MAP packages with high oxygen (HO) were significantly higher *Salmonella* counts than the PVC packages (P<0.001, P<0.0001, P=0.0152, respectively).

The above findings are displayed in Figures 2 and 15 through 22.

Mesophiles & Psychrophiles

Mesophiles and psychrophiles were determined in the *Escherichia coli* O157:H7 study and *Salmonella* study. The only effect that was significant was for the day of sampling with the mesophiles and psychrophiles bacteria. The effect of packaging and the effect of temperature were not significant (P<0.001) for neither mesophiles nor psychrophiles. The day of sampling effect, an orthogonal polynomial analysis shows a curvilinear response: linear, quadratic and cubic effects were significant, which verifies that that SAS model used was correct.

Day 6 was significantly higher than days 9, 11, and 14 for the mesophiles and psychrotroph (P<0.001). No significant differences in microbial counts were shown between the packaging types (PVC, CO, WOCO, VAC) (P>0.001) and for the three storage temperatures (P >0.001). Figures below display the significant differences between days of sampling and display the results of the studies separated by storage temperature, bacteria measured, and study observed.

Package Gas Concentration Readings

Before sampling the modified atmosphere packages on each time point (day 1, 6, 9, 11, and 14), a gas reading was taken of the amount of oxygen, carbon dioxide, and carbon monoxide presence in the package. Table 1 and Table 2 displays these gas combination readings. On the first processing day for *Escherichia coli* O157:H7 replications 1 and 2, reading began to be taken on day 9 instead of 24 hours, therefore there are no readings for replication 1 and 2 for *Escherichia coli* O157:H7 on day 1 or day 6. The MOCON gas reading system was calibrated at the beginning, middle, and end of each replication and when gas composition readings did not to be accurate.

Conclusions

Escherichia coli O157:H7

The effects of packaging type can be made without regard to temperature or day of sampling. That is, *Escherichia coli* O157:H7 is higher in PVC than in the other packaging types and *Escherichia coli* O157:H7 was the same in WOCO, CO, VAC, and HO than the PVC packaging. These differences are regardless of temperature, and regardless of sampling day. Additionally the packages stored at 2.8ºC were significantly lower than the packages stored at 35ºC, but was statistically the same as packages stored at 21ºC($P<0.05$, $P>0.05$ respectively). Days 6 and 14 were significantly lower than days 9 and 11 within the study ($P<0.05$). Packaging type by day interaction was highly significant ($P<0.0001$) but all other interactions were not significant ($P>0.05$). When the data is sliced to determine the simple main effects within the study packaging type PVC, HO, VAC was significantly different amongst sampling days and on day 9, 11 and 14 of storage there were significant difference amongst packaging types ($P<0.05$).

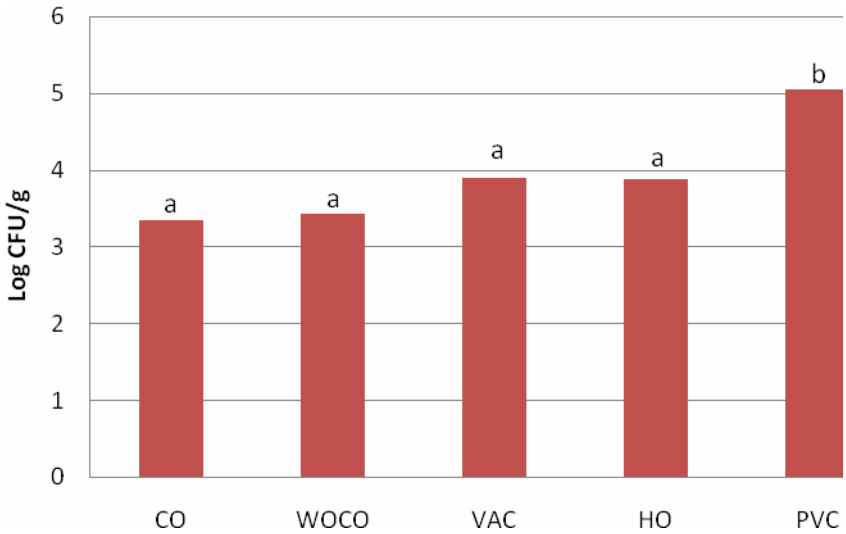
Salmonella

Salmonella recovery were not significantly different in the packaging type nor the storage temperature ($P>0.05$). However, *Salmonella* concentrations were higher on days 6 and 9, regardless of storage temperature and packaging type. Packaging type by day interaction was highly significant ($P<0.0001$) but all other interactions were not significant ($P>0.05$). When the data is sliced to determine the simple main effects within the study packaging type PVC was significantly different amongst sampling days and on day 11 and 14 of storage there were significant difference amongst packaging types ($P<0.05$).

Overall, it was evident that packaging other than overwrap has a positive impact on reducing *E. coli* O157:H7. Samples packaged under these conditions had significantly less *E. coli* O157:H7 growth during temperature abuse and under no abuse conditions, while *Salmonella* counts was significantly different on sampling days 11 and 14 within packaging types and within PVC packaging throughout study. These data suggest packaging types may result in a safer ground beef supply.

Figures

Figure1. Effects of packaging (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO)) on *Escherichia coli* O157:H7 in ground beef hamburger patties temperature abused at 2.8ºC, 21ºC, and 35ºC during a 14 day (PVC and High Oxygen) and 24 day (Vacuum, Without Carbon Monoxide, With Carbon Monoxide) shelf life.



Different subscript letter indicate significant differences at p-value less than 0.05.

Figure2. Effects of storage day on *Salmonella in* ground beef hamburger patties temperature abused at 2.8ºC, 21ºC, and 35ºC during a 24 day shelf life in vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO). Different subscript letter indicate significant differences at p-value less than 0.05

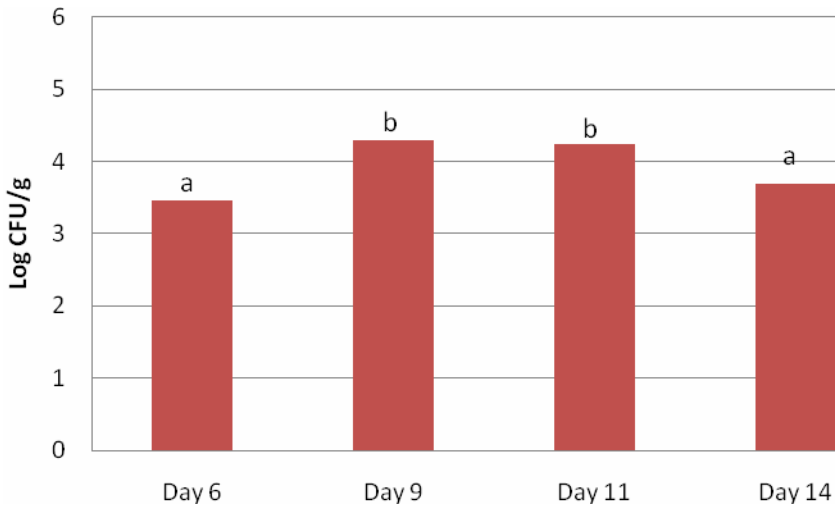


Figure 3. Effects of storage temperature regardless of neither packaging type nor the day of storage on *Escherichia coli* O157:H7 in ground beef hamburger patties over 24 day shelf life. Different subscript letter indicate significant differences at p-value less than 0.05

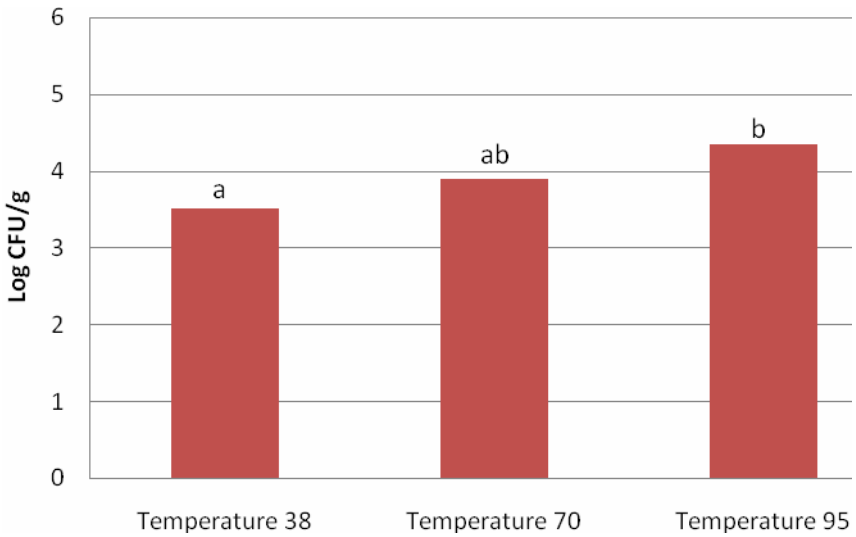


Figure 4. Effects of storage day on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO)) regardless of storage temperature on *Escherichia coli* O157:H7 in ground beef hamburger patties. PVC and HO were not analyzed after day 14.

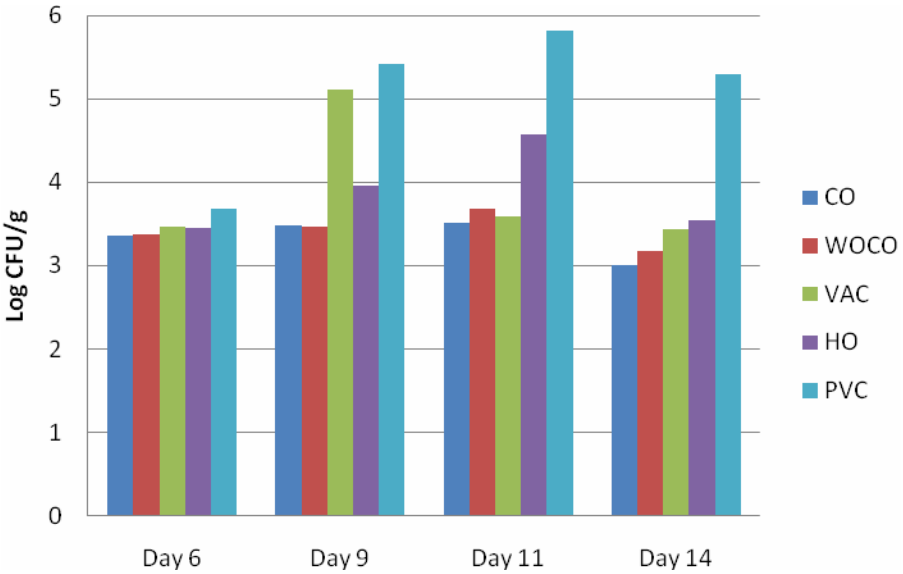


Figure 5. Effect of low oxygen packaging with carbon monoxide MAP (0.4% CO/35% CO_2/64.6% N_2) (CO) on *Escherichia coli* O157:H7 in ground beef hamburger patties temperature abused during a 24 day shelf life. PVC and HO were not analyzed after day 14. Different subscript letter indicate significant differences at p-value less than 0.05.

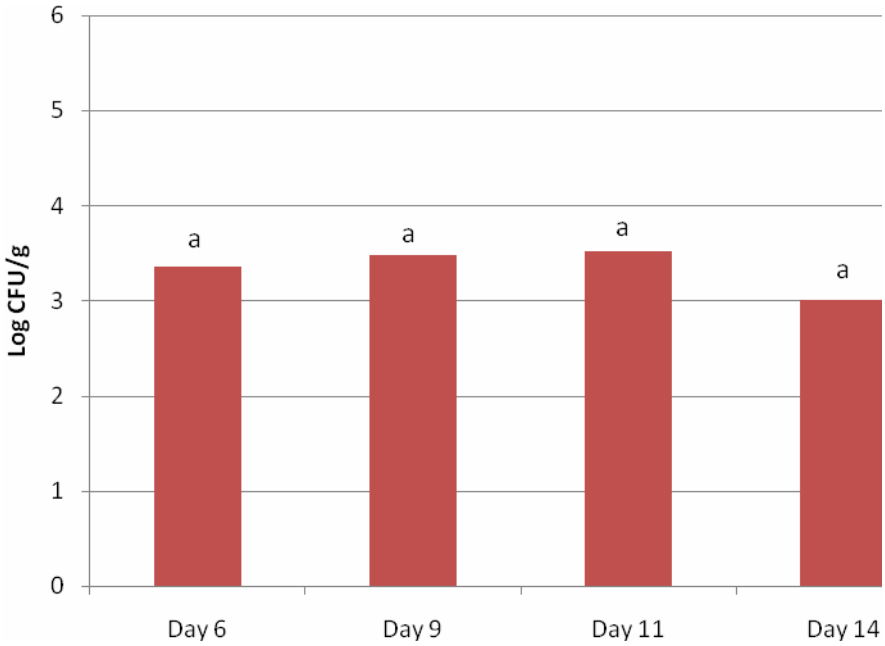


Figure 6. Effect of low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO) on *Escherichia coli* O157:H7 in ground beef hamburger patties temperature abused during a 24 day shelf life. PVC and HO were not analyzed after day 14. Different subscript letter indicate significant differences at p-value less than 0.05.

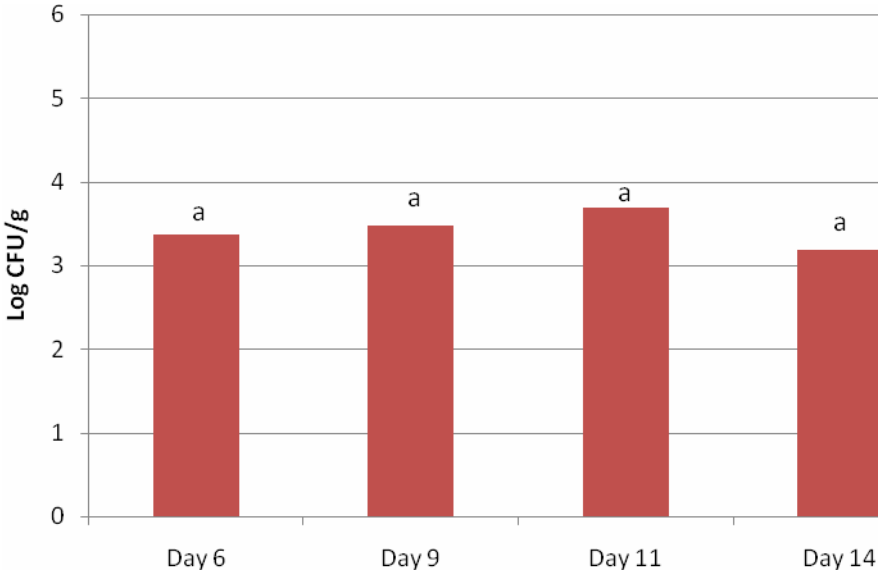


Figure 7. Effect of high oxygen MAP (80%O_2/20% CO_2) (HO) on *Escherichia coli* O157:H7 in ground beef hamburger patties temperature abused a 24 day shelf life. PVC and HO were not analyzed after day 14. Different subscript letter indicate significant differences at p-value less than 0.05.

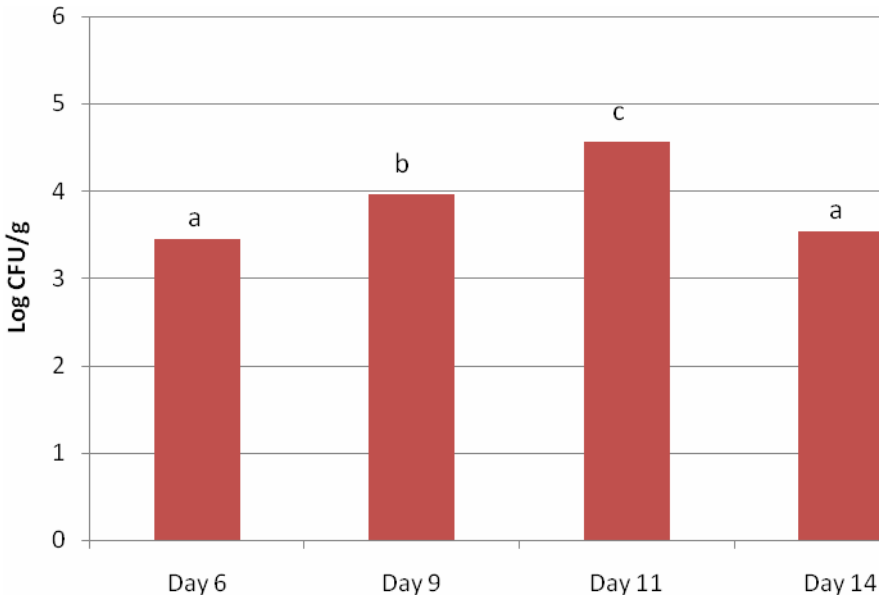


Figure 8. Effect of vacuum packaging (VAC) on *Escherichia coli* O157:H7 in ground beef hamburger patties temperature abused during a 24 day shelf life. PVC and HO were not analyzed after day 14. Different subscript letter indicate significant differences at p-value less than 0.05. Different subscript letter indicate significant differences at p-value less than 0.05.

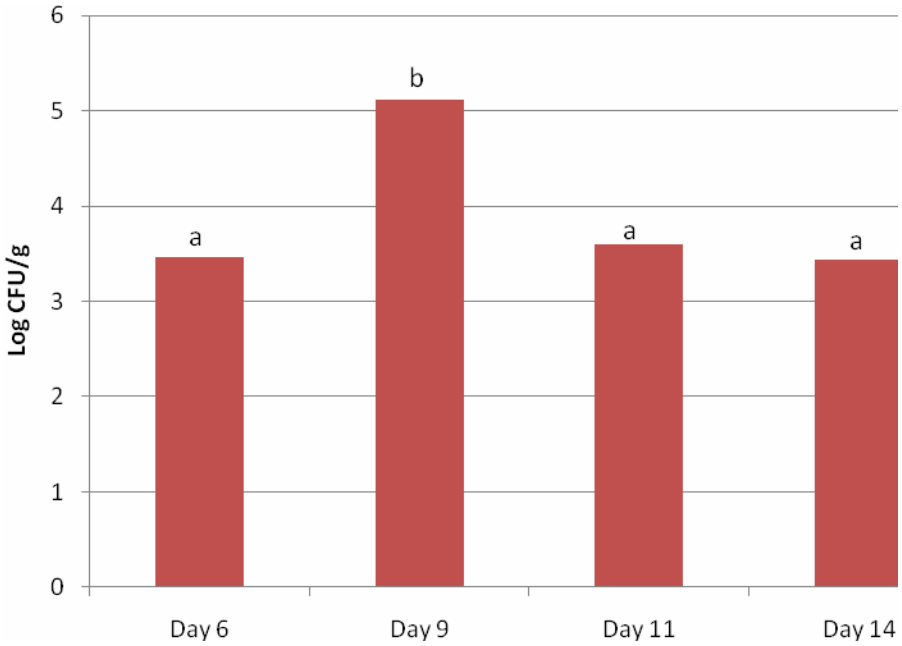


Figure 9. Effect of overwrap packaging (PVC) on *Escherichia coli* O157:H7 in ground beef hamburger patties temperature abused during a 24 day shelf life. PVC and HO were not analyzed after day 14. Different subscript letter indicate significant differences at p-value less than 0.05.

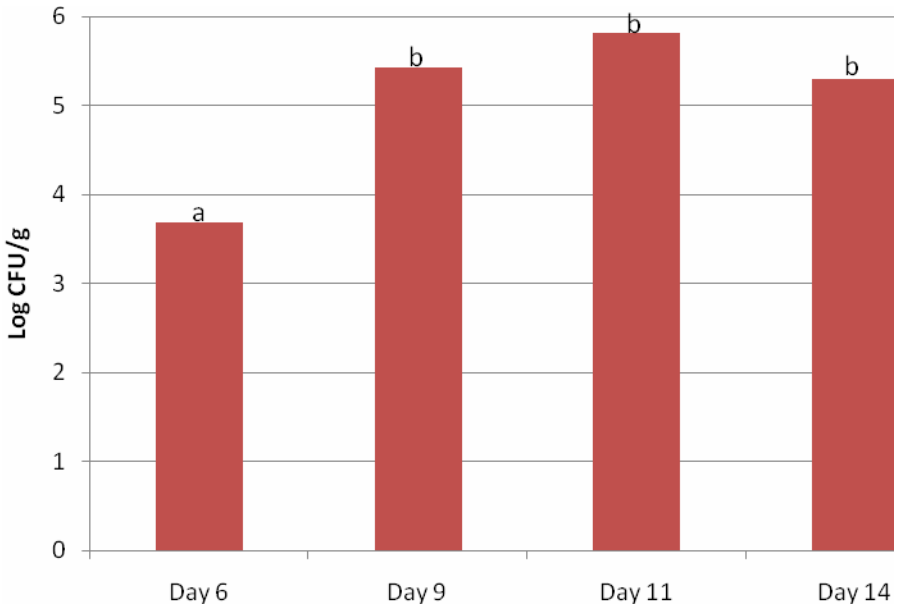


Figure 10. Effects of storage day 6 on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO)) regardless of storage temperature on *Escherichia coli* O157:H7 in ground beef hamburger patties. Different subscript letter indicate significant differences at p-value less than 0.05.

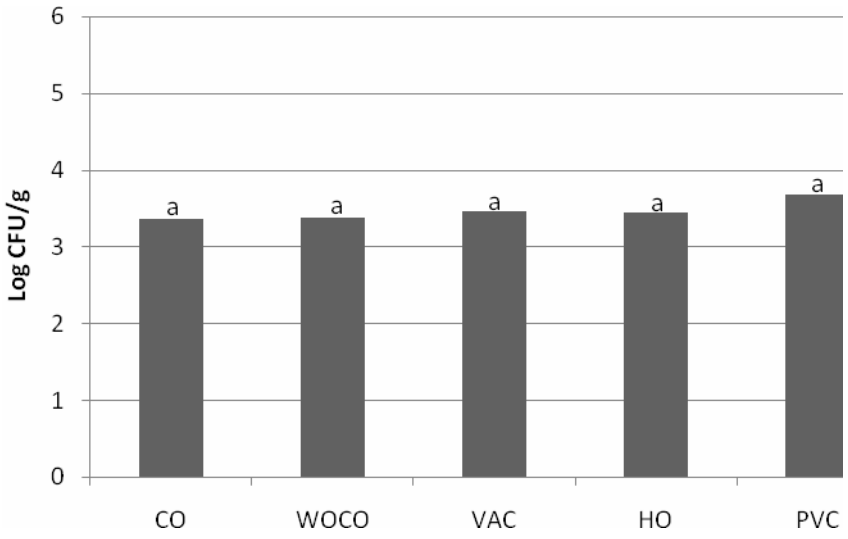


Figure 11. Effects of storage day 9 on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO)) regardless of storage temperature on *Escherichia coli* O157:H7 in ground beef hamburger patties. Different subscript letter indicate significant differences at p-value less than 0.05.

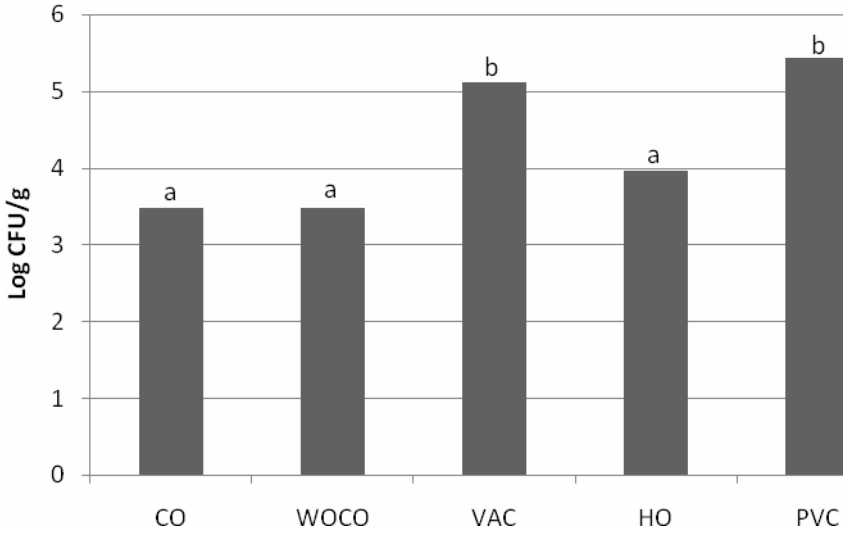


Figure 12. Effects of storage day 11 on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO)) regardless of storage temperature on *Escherichia coli* O157:H7 in ground beef hamburger patties. Different subscript letter indicate significant differences at p-value less than 0.05.

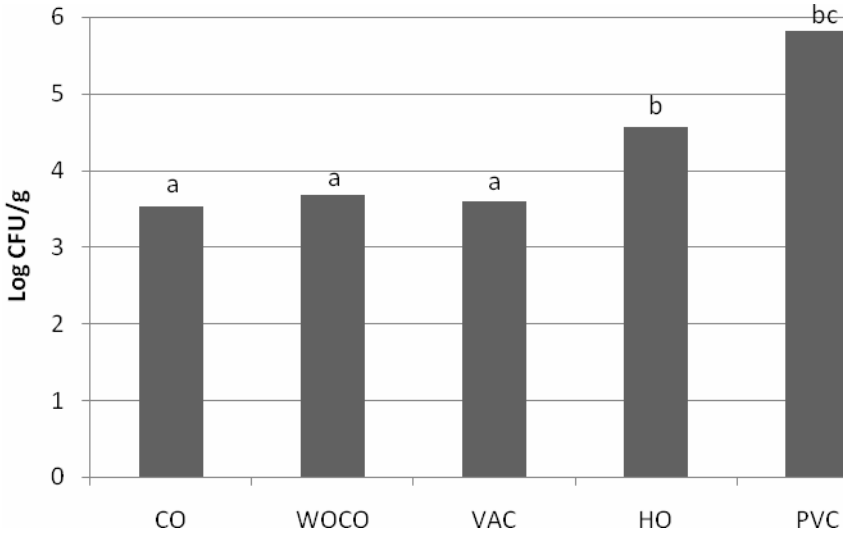


Figure 13. Effects of storage day 14 on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO)) regardless of storage temperature on *Escherichia coli* O157:H7 in ground beef hamburger patties. Different subscript letter indicate significant differences at p-value less than 0.05.

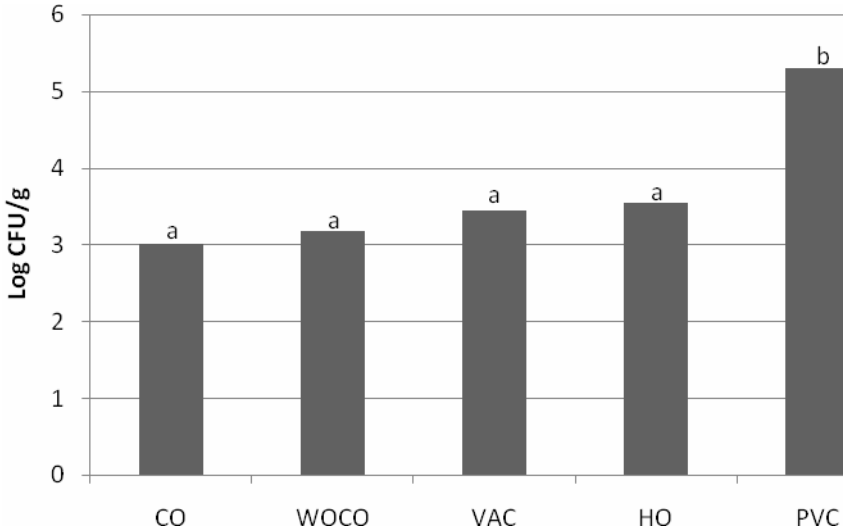


Figure 14. Effects of day on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO)) on *Salmonella in* ground beef hamburger patties temperature abused at 2.8ºC during a 24 day shelf life. PVC and HO were not analyzed after day 14.

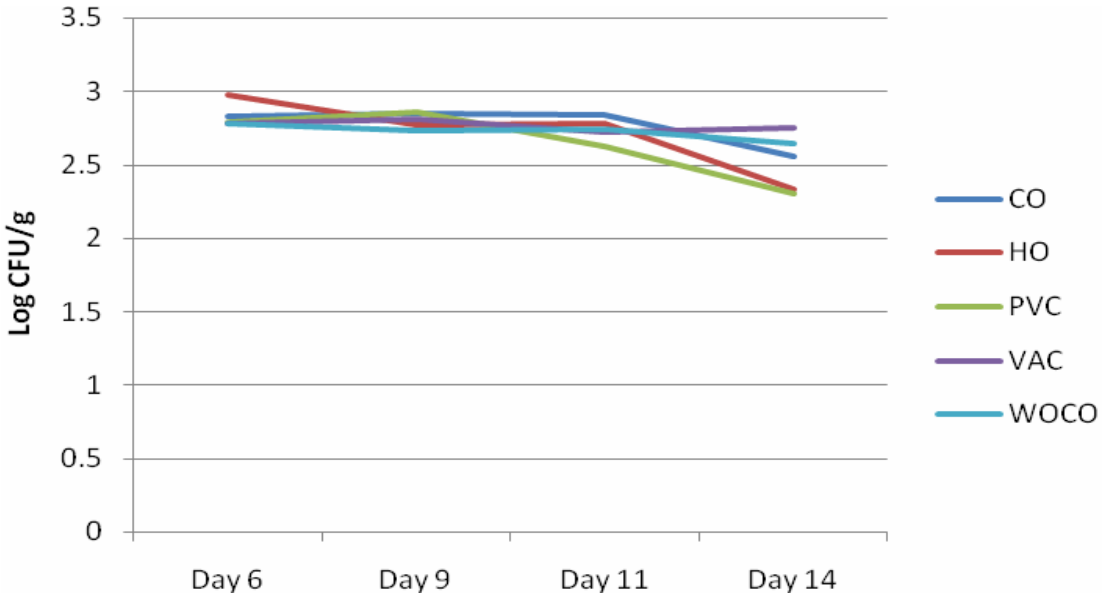


Figure 15. Effects of day on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2) (WOCO)) on *Salmonella in* ground beef hamburger patties temperature abused at 21ºC during a 24 day shelf life. PVC and HO were not analyzed after day 14.

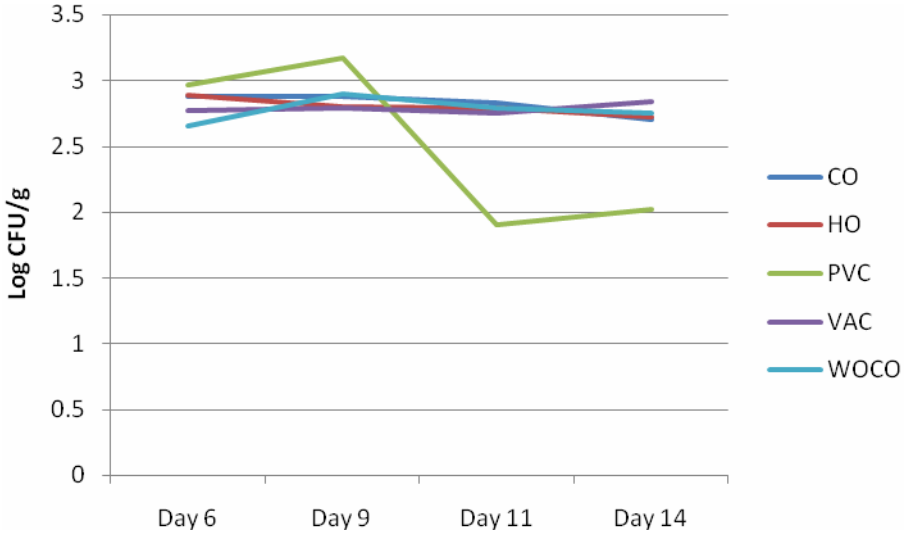


Figure 16. Effects of day on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) on *Salmonella in* ground beef hamburger patties temperature abused at 35ºC during a 24 day shelf life. PVC and HO were not analyzed after day 14.

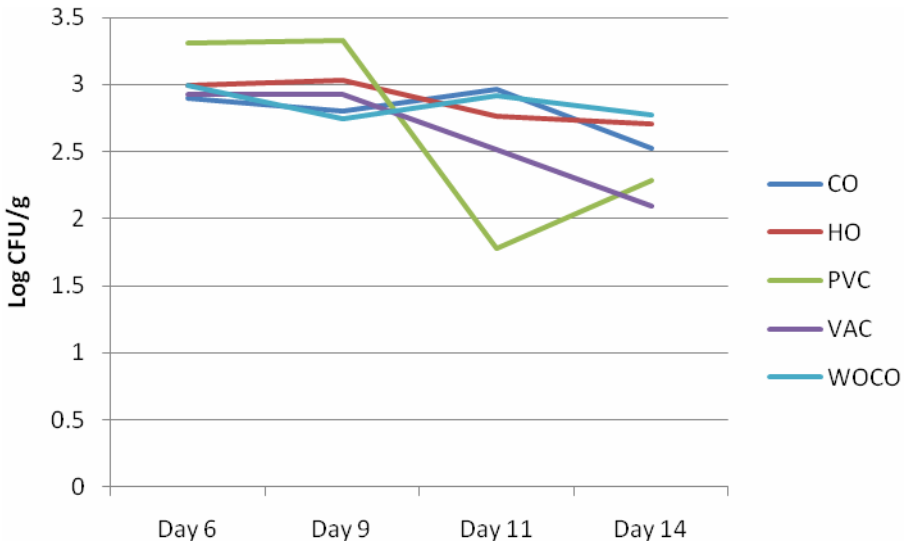


Figure 17. Effects of packaging type on sampling day (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) on *Salmonella in* ground beef hamburger patties temperature abused during a 24 day shelf life. PVC and HO were not analyzed after day 14.

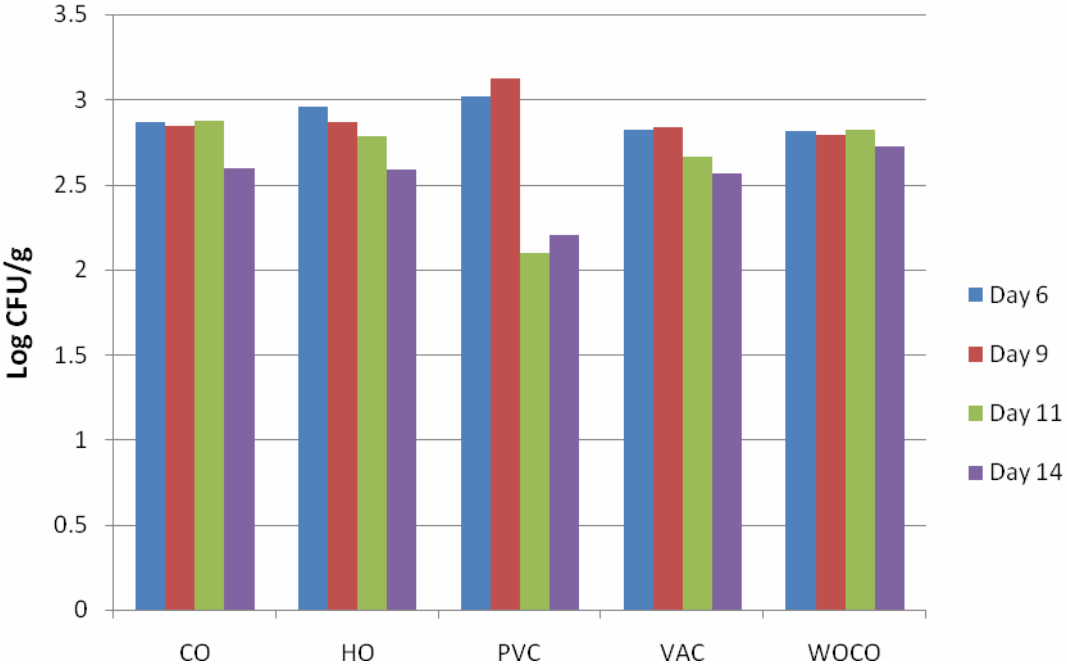


Figure 18. Effects of day on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) on *Salmonella in* ground beef hamburger patties temperature abused during a 24 day shelf life. PVC and HO were not analyzed after day 14.

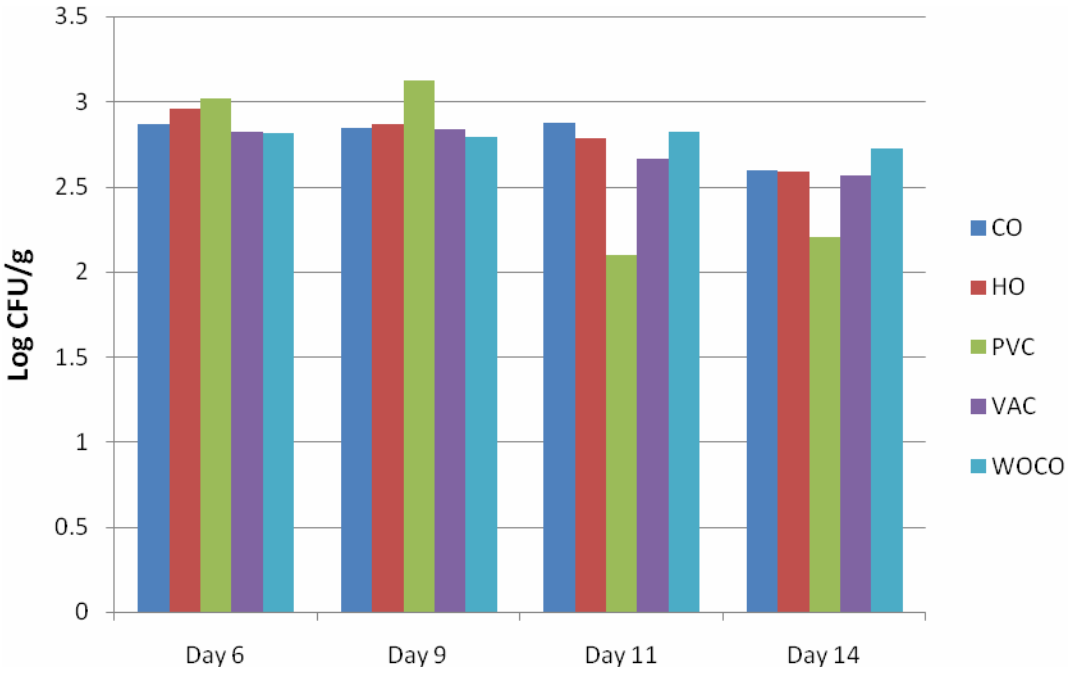


Figure 19. Effects of Day 11 of storage on *Salmonella* is dependent on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) in ground beef hamburger patties temperature abused during a 24 day shelf life. Different subscript letter indicate significant differences at p-value less than 0.05.

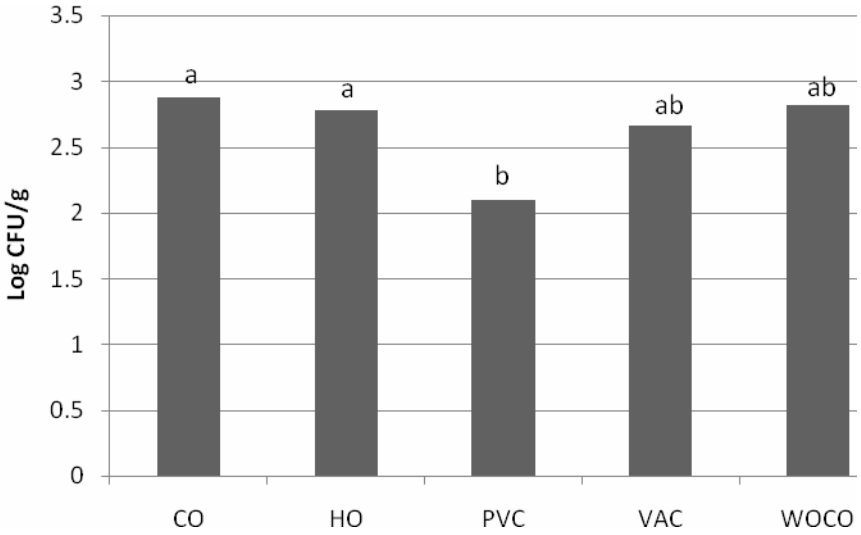


Figure 20. Effects of Day 14 of storage on *Salmonella* is dependent on packaging type (Vacuum package (VAC), Overwrap (PVC), and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) in ground beef hamburger patties temperature abused during a 24 day shelf life. Different subscript letter indicate significant differences at p-value less than 0.05.

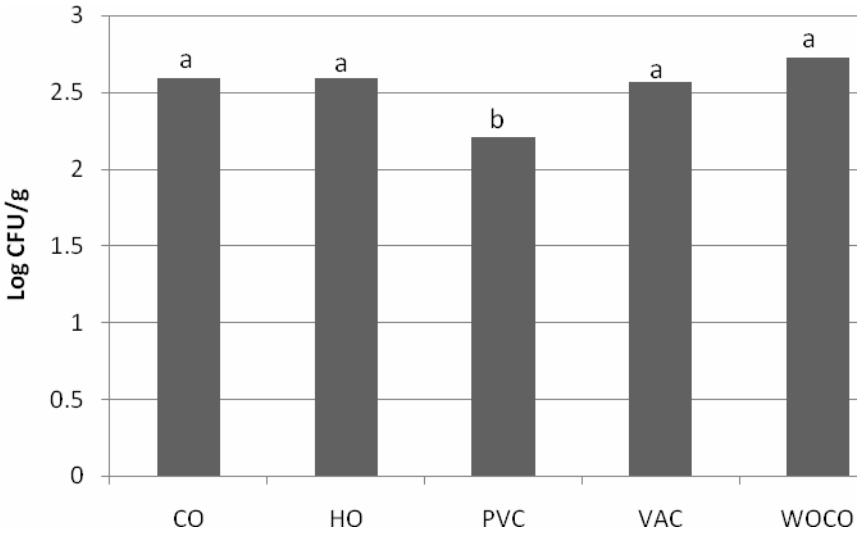


Figure 21. Effects of PVC packaging on *Salmonella* is dependent on sampling in ground beef hamburger patties temperature abused during a 14 day shelf life. Different subscript letter indicate significant differences at p-value less than 0.05.

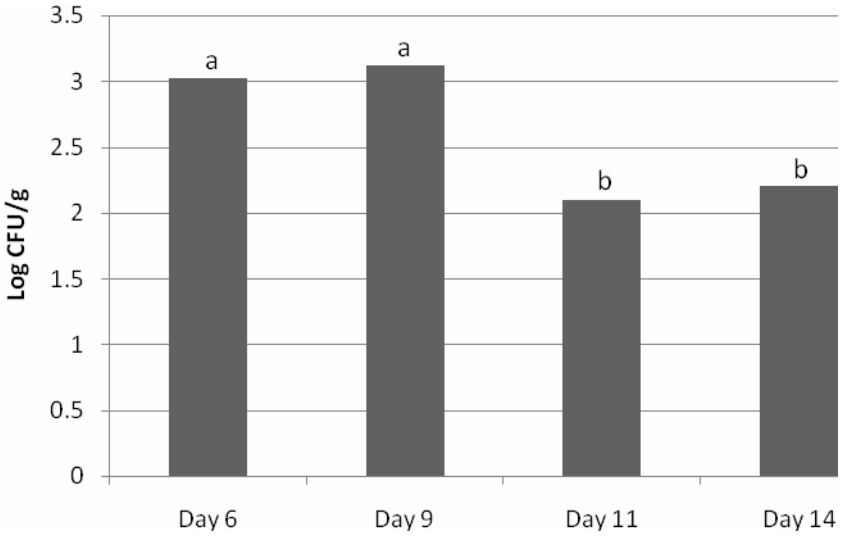
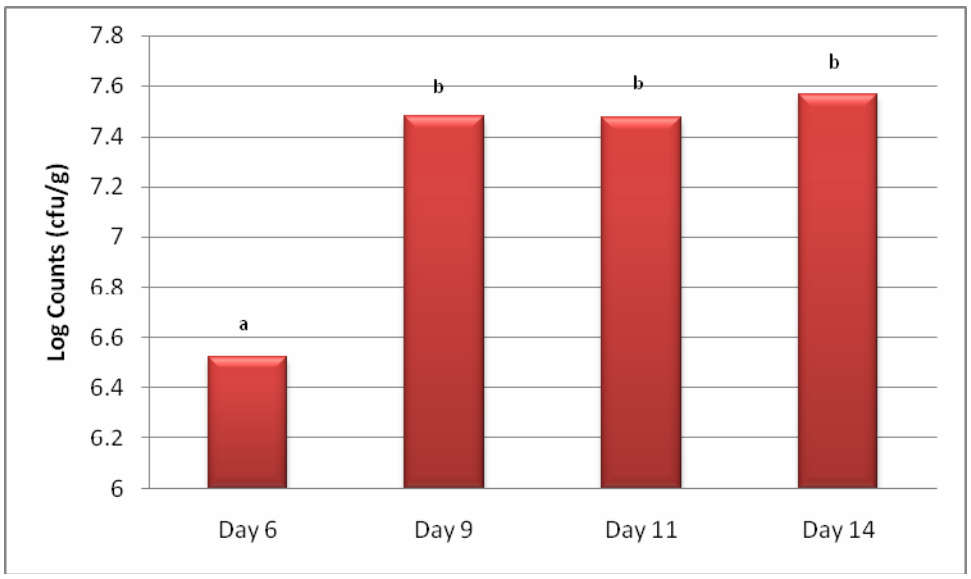
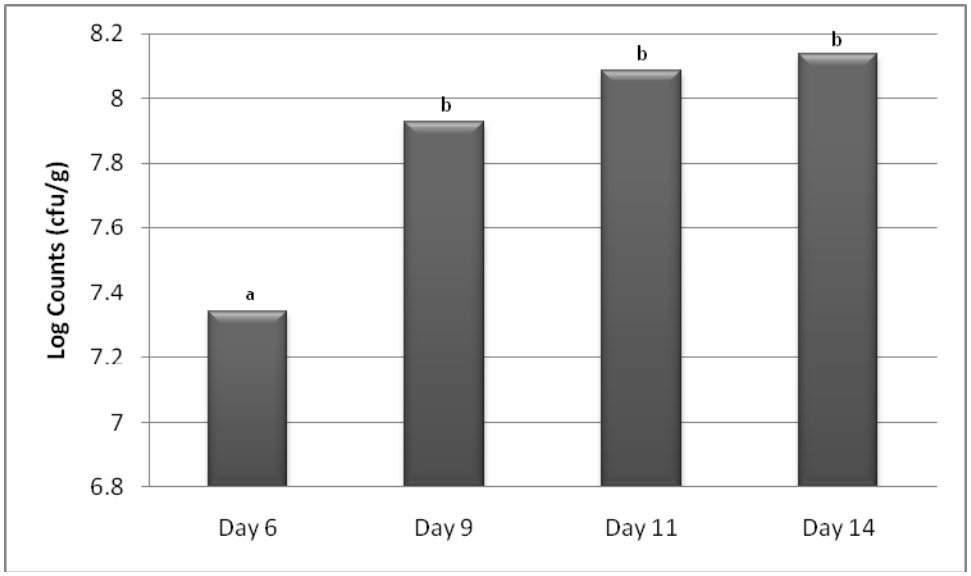


Figure 22. Effects of storage day on Mesophiles in ground beef hamburger patties temperature abused at 2.8ºC, 21ºC, and 35ºC during a 24 day shelf life in vacuum packages, PVC, and three MAP packages with a high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO).



Different subscript letter indicate significant differences at p-value less than 0.001

Figure 23. Effects of storage day on Psychrophiles in ground beef hamburger patties temperature abused at 2.8ºC, 21ºC, and 35ºC during a 24 day shelf life in vacuum packages, PVC, and three MAP packages with a high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO).



Different subscript letter indicate significant differences at p-value less than 0.001

Figure 24. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide(0.4% CO/35% CO_2/64.6% N_2)(CO), and low oxygen packaging without carbon monoxide MAP (35%CO_2/65% N_2)(WOCO)) on Mesophiles in ground beef hamburger patties temperature abused at 2.8ºC during a 24 day shelf life with *Escherichia coli* O157:H7.

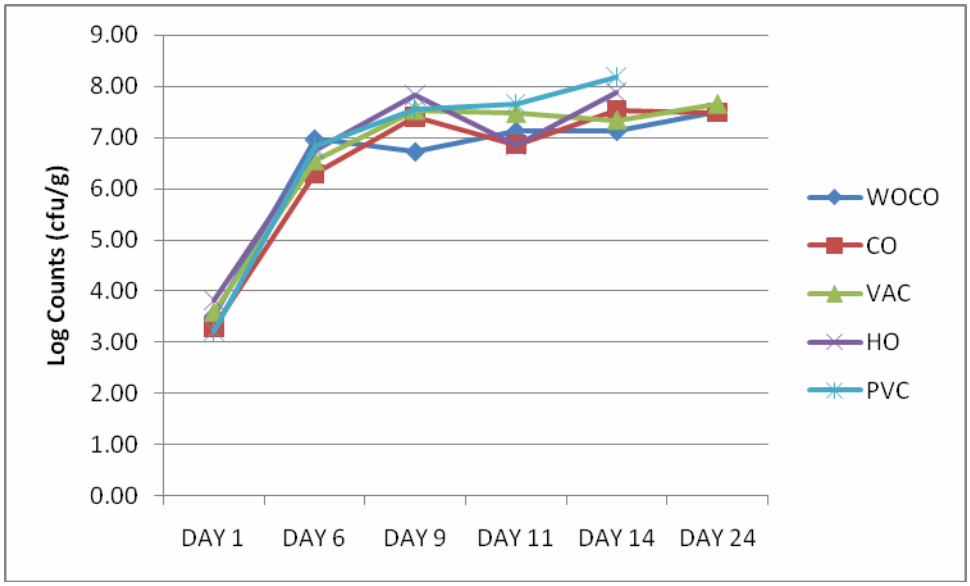


Figure 25. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2)(CO), and low oxygen packaging without carbon monoxide (35%CO_2/65% N_2)(WOCO)) on Mesophiles in ground beef hamburger patties temperature abused at 21ºC during a 24 day shelf life with *Escherichia coli* O157:H7.

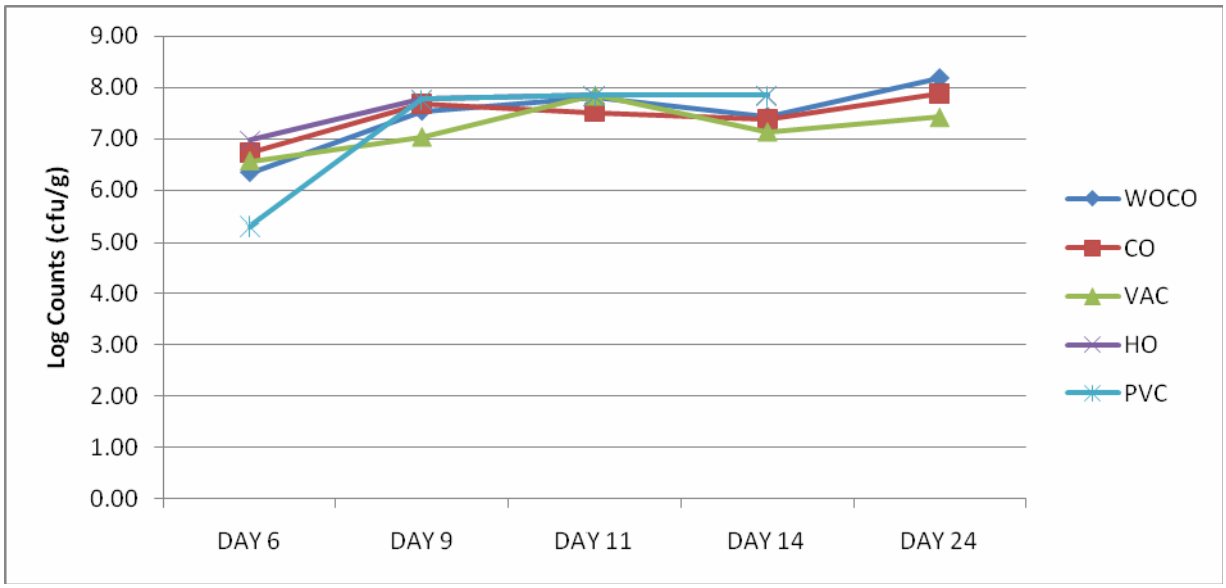


Figure 26. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2)(CO), and low oxygen packaging without carbon monoxide(35%CO_2/65% N_2)(WOCO)) on Mesophiles in ground beef hamburger patties temperature abused at 35ºC during a 24 day shelf life with *Escherichia coli* O157:H7.

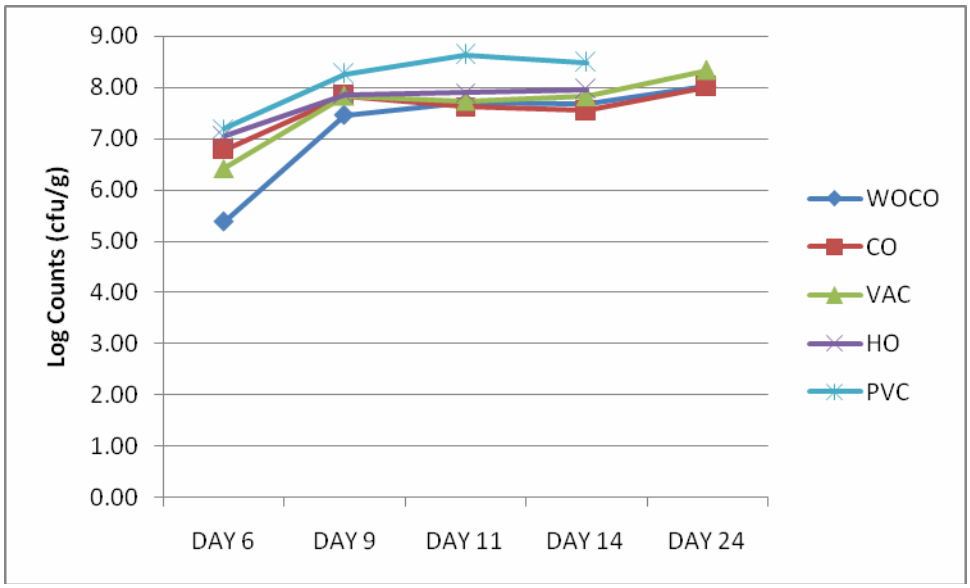


Figure 27. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) on Mesophiles in ground beef hamburger patties temperature abused at 2.8ºC during a 24 day shelf life with *Salmonella spp.*

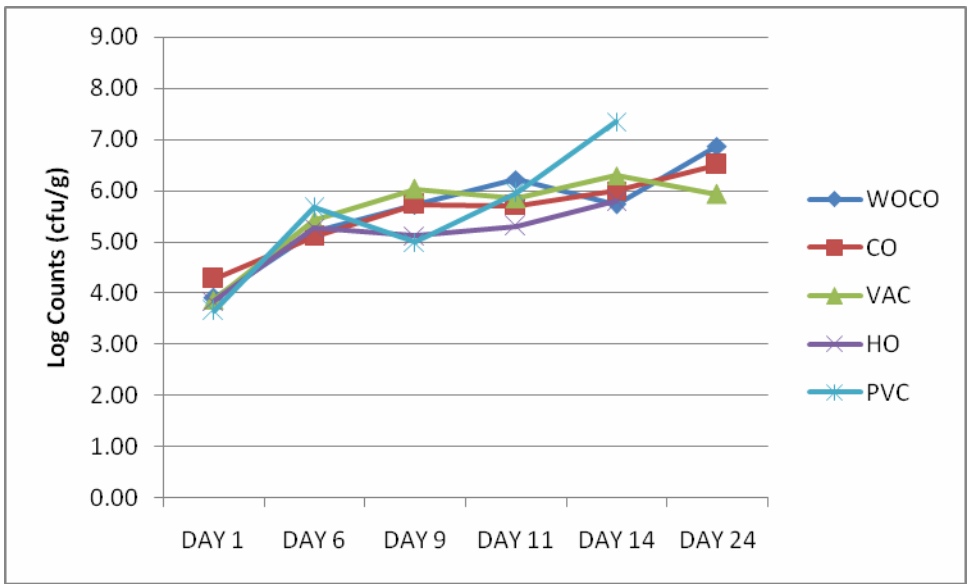


Figure 28. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) on Mesophiles in ground beef hamburger patties temperature abused at 21ºC during a 24 day shelf life with *Salmonella spp.*

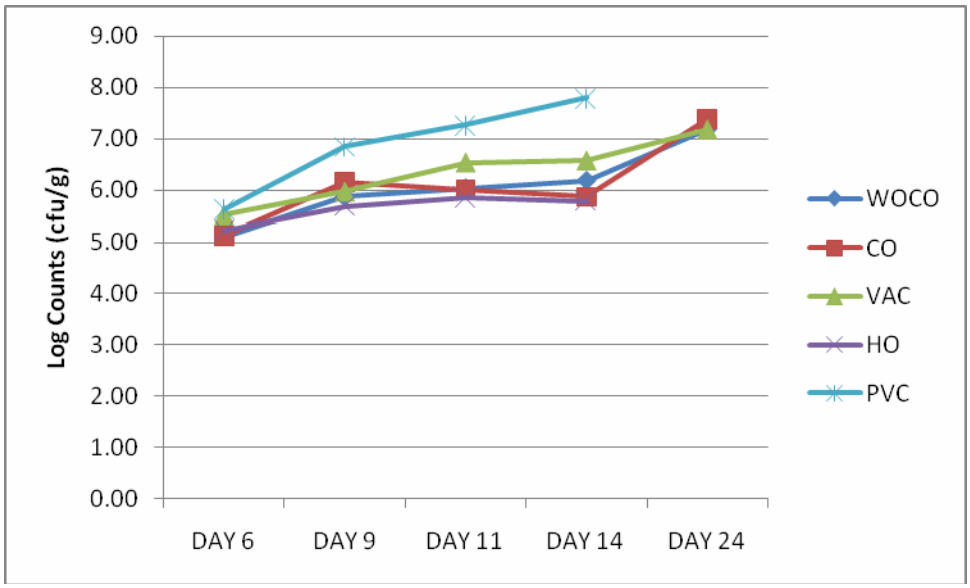


Figure 29. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) on Mesophiles in ground beef hamburger patties temperature abused at 35ºC during a 24 day shelf life with *Salmonella spp.*

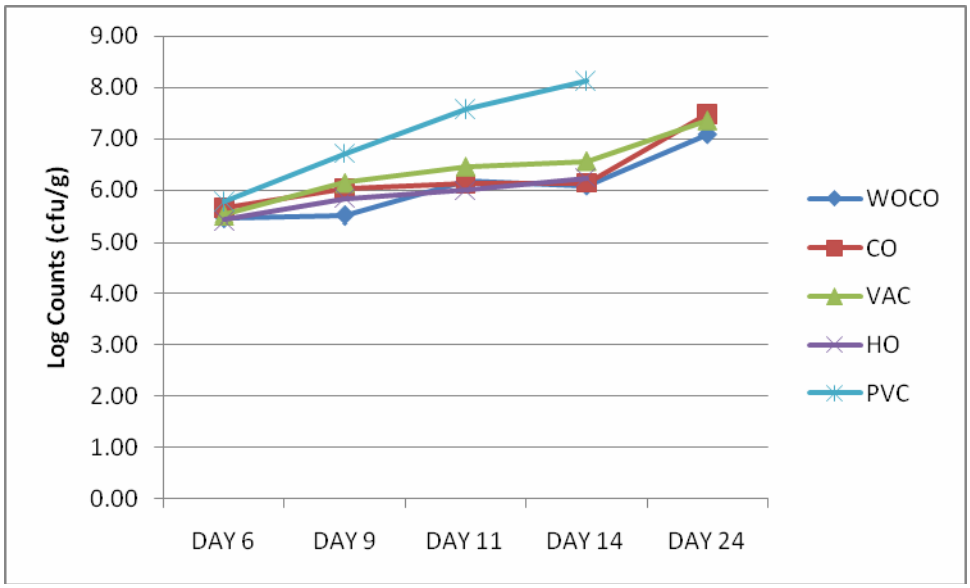


Figure 30. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2)(CO), and low oxygen packaging without (35%CO_2/65% N_2)(WOCO)) on Psychrotrophs in ground beef hamburger patties temperature abused at 2.8ºC during a 24 day shelf life with *Escherichia coli* O157:H7.

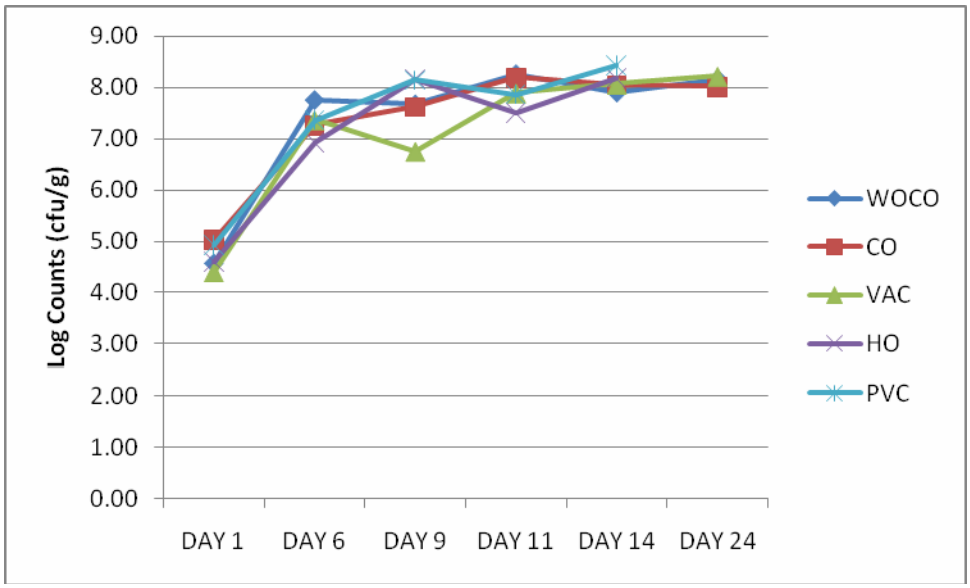


Figure 31. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2)(CO), and low oxygen packaging without carbon monoxide(35%CO_2/65% N_2)(WOCO)) on Psychrotrophs in ground beef hamburger patties temperature abused at 21ºC during a 24 day shelf life with *Escherichia coli* O157:H7.

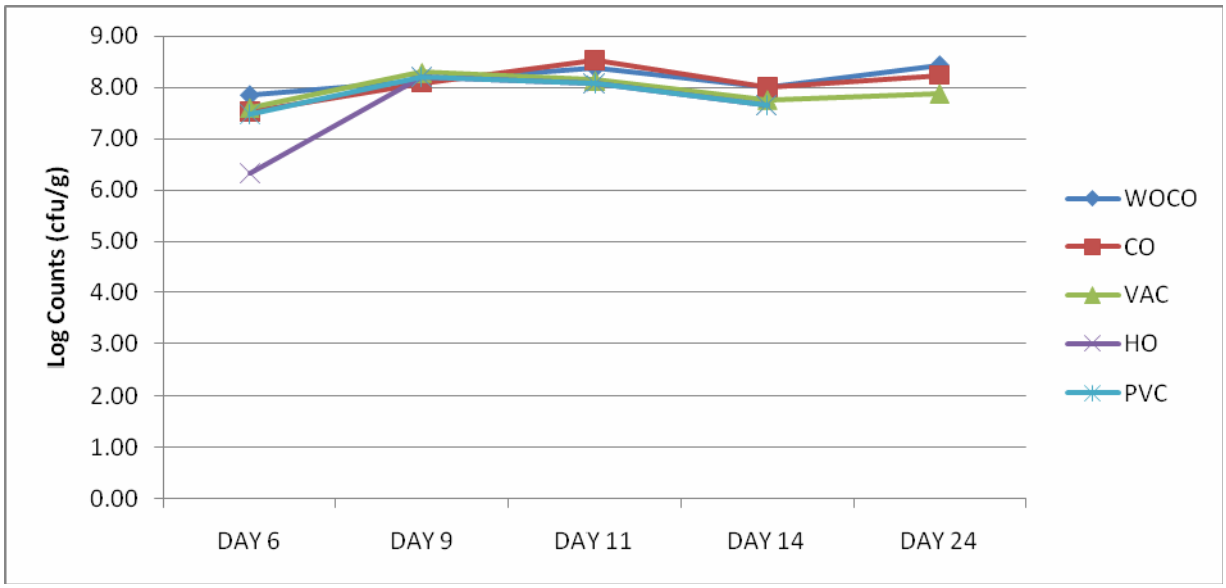


Figure 32. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO_2/64.6% N_2)(CO), and low oxygen packaging without (35%CO_2/65% N_2)(WOCO)) on Psychrotrophs in ground beef hamburger patties temperature abused at 35ºC during a 24 day shelf life with *Escherichia coli* O157:H7.

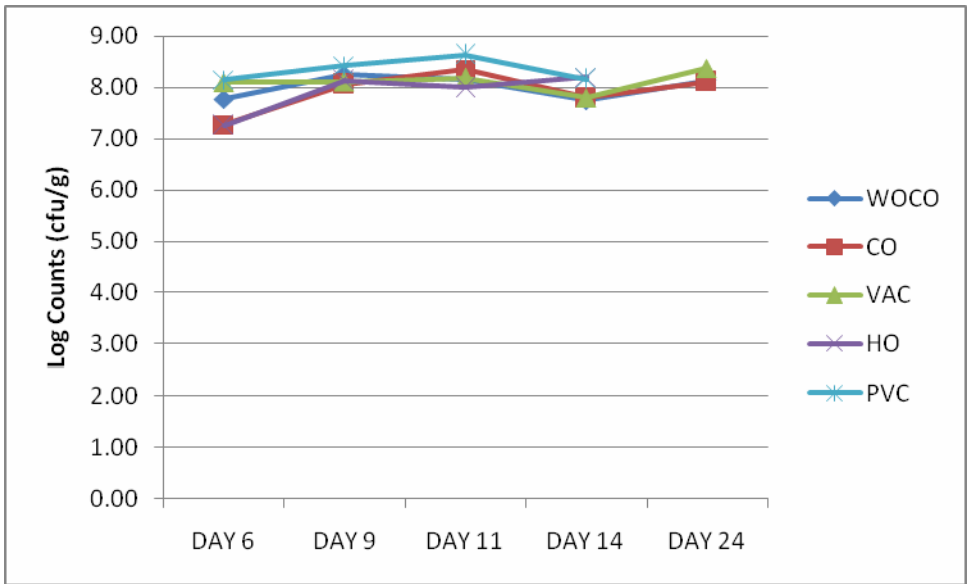


Figure 33. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) on Psychrotrophs in ground beef hamburger patties temperature abused at 2.8ºC during a 24 day shelf life with *Salmonella spp.*

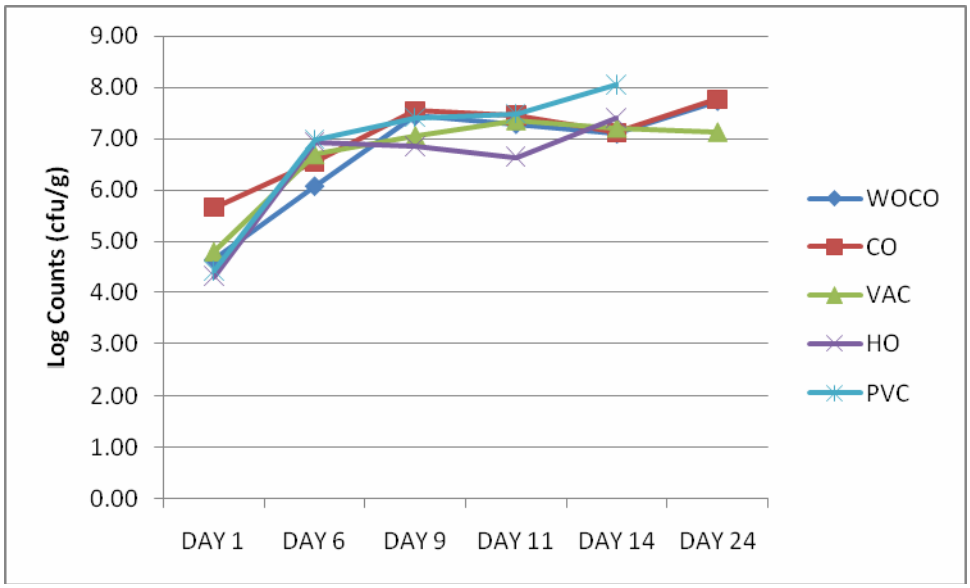


Figure 34. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) on Psychrotrophs in ground beef hamburger patties temperature abused at 21ºC during a 24 day shelf life with *Salmonella spp.*

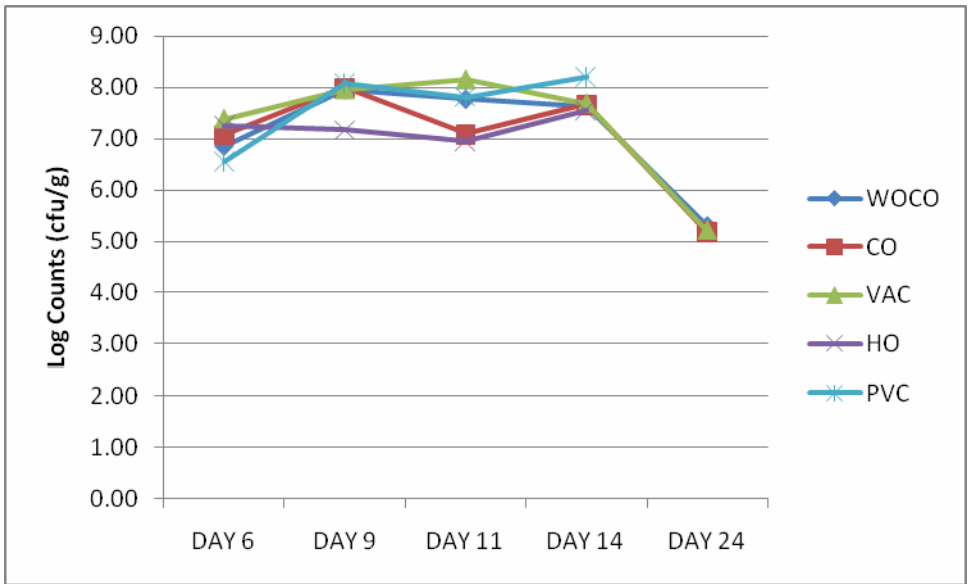


Figure 35. Effects of day on packaging type (Vacuum package, PVC, and three different MAP packages with high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO)) on Psychrotrophs in ground beef hamburger patties temperature abused at 35ºC during a 24 day shelf life with *Salmonella spp.*

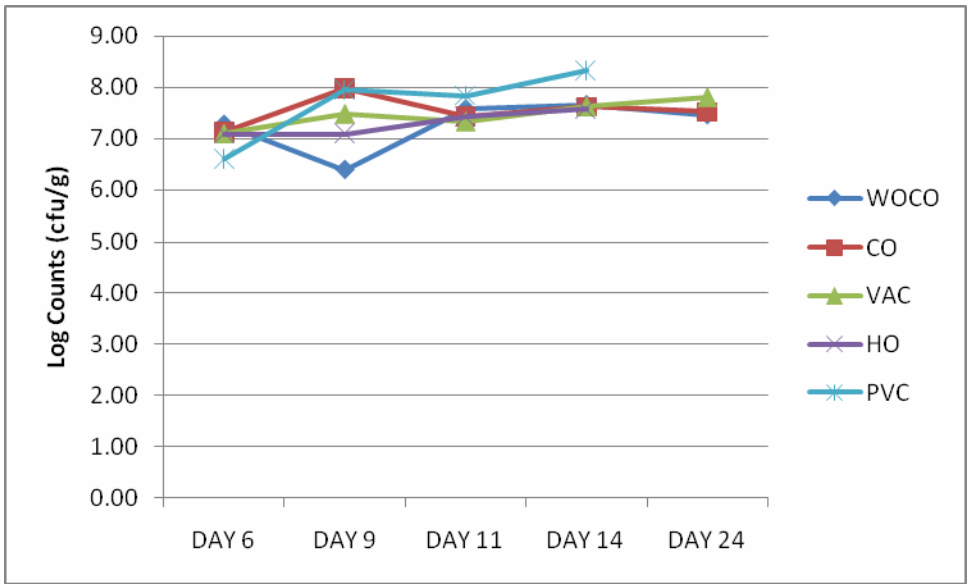


Table 1: Gas Concentration of each package of hamburger patties containing *Escherichia coli* O157:H7, temperature abused at 2.8ºC, 21ºC, and 35ºC during a 24 day shelf life in three MAP packages with a high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO).

			24 hr			Day 6			Day 9		
Packaging	REP	Temp	O2	CO2	CO	O2	CO2	CO	O2	CO2	CO
WOCO	1	38	0	23.45	0.03	0.07	23.97	0	0	23.37	0.37
WOCO	2	38	0	22.99	0	0.08	23.98	0.02	0	25.86	0.19
WOCO	3	38	0	28.23	0.44	0	23.44	0.39	0	24.3	0.48
WOCO	4	38	0	28.6	0.09	0	30.9	1.57	0	33.18	2.53
CO	1	38	0	30.08	0.35	0	25.98	1.57	0	26.25	0.7
CO	2	38	0	28.23	0.44	0	35.04	1.44	0	34.3	2.67
CO	3	38	0.01	26.77	0.31	0	26.38	0.31	0.47	23.68	0.31
CO	4	38	0	23.45	0.32	0	24.51	0.34	0	24.72	0.33
HO	1	38	75.41	14.79	0	75.25	15.77	0	75.01	18.21	0.09
HO	2	38	74.63	14.37	0	73.41	15.73	0	70.74	20.34	0.01
HO	3	38	78.12	14.42	0.04	76.77	13.3	0.16	71.62	21.73	0.7
HO	4	38	77.75	14.34	0.06	75	13.2	0.11	57.88	30.62	0.1
WOCO	1	70	NA	NA	NA	NA	NA	NA	0	26.45	0.25
WOCO	2	70	NA	NA	NA	NA	NA	NA	0	24.75	0.32
WOCO	3	70	NA	NA	NA	0	22.35	0.65	0	23.96	0.54
WOCO	4	70	NA	NA	NA	0	30.27	2.35	0	35.78	1.44
CO	1	70	NA	NA	NA	NA	NA	NA	0	26.98	0.34
CO	2	70	NA	NA	NA	NA	NA	NA	0	26.42	0.44
CO	3	70	NA	NA	NA	0	23.97	2.58	0	27.27	1
CO	4	70	NA	NA	NA	0	27.8	1.91	0	36.18	2.66
HO	1	70	NA	NA	NA	NA	NA	NA	71.78	16.11	0.07
HO	2	70	NA	NA	NA	NA	NA	NA	66.23	29.06	0.08
HO	3	70	NA	NA	NA	72.07	14.34	0.45	66.42	24.35	0.4
HO	4	70	NA	NA	NA	61.1	25.03	0.92	59.54	29.82	0.21
WOCO	1	95	NA	NA	NA	NA	NA	NA	0	21.42	0.22
WOCO	2	95	NA	NA	NA	NA	NA	NA	0	27	0.31
WOCO	3	95	NA	NA	NA	0	27.84	0.93	0	25.05	1.24
WOCO	4	95	NA	NA	NA	0	31.76	2.05	0	32.24	2.37
CO	1	95	NA	NA	NA	NA	NA	NA	0	24.31	0.35
CO	2	95	NA	NA	NA	NA	NA	NA	0.56	26.42	0.36
CO	3	95	NA	NA	NA	0	31.76	2.05	0	32.24	2.37
CO	4	95	NA	NA	NA	0	25.56	0.51	0	26.76	0.74
HO	1	95	NA	NA	NA	NA	NA	NA	68.02	22.67	0.14
HO	2	95	NA	NA	NA	NA	NA	NA	72.65	21.85	0.05
HO	3	95	NA	NA	NA	73.13	18.26	1.03	69.32	22.18	0.7
HO	4	95	NA	NA	NA	63.05	30.37	0.54	59.39	30.07	0.35

			Day 11			Day 14			Day 24		
Packaging	REP	Temp	O2	CO2	CO	O2	CO2	CO	O2	CO2	CO
WOCO	1	38	0.11	22.69	0.09	0	27.46	0.12	0	27.82	3.13
WOCO	2	38	0.02	27.23	0.09	0	32.45	0.41	0	34.41	3.03
WOCO	3	38	0	25	0.86	0.09	22.77	0.42	0	31.67	0.27
WOCO	4	38	0	29.73	1.59	0	29.06	2.19	0	38.27	1.61
CO	1	38	0	26.94	1.41	0	27.6	1.6	0	34.35	1.21
CO	2	38	0	32.46	1.83	0	30.19	2.47	0	33.22	1.41
CO	3	38	0	25.64	0.41	0	28.17	0.66	0	26.76	0.87
CO	4	38	0	29.52	0.39	0	28.13	0.79	0	35.58	3.2
HO	1	38	70.03	22.65	0.07	64.15	29.73	0.12	NA	NA	NA
HO	2	38	66.42	26.16	0.09	64.33	29.71	0.1	NA	NA	NA
HO	3	38	68.97	22.51	0.07	64.93	24.29	0.64	NA	NA	NA
HO	4	38	56.82	33.12	0.72	50.27	37.85	1.13	NA	NA	NA
WOCO	1	70	0	24.94	0.4	0	26.12	0.58	0	30.77	3.04
WOCO	2	70	0	29.53	0.53	0	28.01	0.42	0	25.97	3.03
WOCO	3	70	0	24.96	0.5	0	22.78	1.23	0	31.98	0.63
WOCO	4	70	0	30.29	2.16	0	31.75	1.62	0	34.95	1.64
CO	1	70	0	25.24	0.37	0	24.82	0.42	0	27.44	0.87
CO	2	70	0	28.38	0.54	0	33.5	0.05	0	34.4	1.3
CO	3	70	0	26.47	0.97	0	29.01	1.63	0	31.22	0.96
CO	4	70	0	31.03	2.37	0	35.39	2.48	0	36.25	2.07
HO	1	70	69.96	20.89	0.28	66.1	27.74	0.32	NA	NA	NA
HO	2	70	64.03	26	0.37	63.48	28.22	0.18	NA	NA	NA
HO	3	70	65.92	25.64	0.09	61.46	28.6	0.64	NA	NA	NA
HO	4	70	53.21	36.36	1.27	50.61	37.03	1.19	NA	NA	NA
WOCO	1	95	0.03	22.99	0.21	0	24.65	0.02	0	29.81	3.09
WOCO	2	95	0	25.41	0.33	0	28.01	0.81	0	25.72	1.62
WOCO	3	95	0	25.28	0.51	0	27.35	0.3	0	33.58	0.96
WOCO	4	95	0	32.17	2.31	0	32.12	1.79	0	34.45	1.47
CO	1	95	0	26.06	0.6	0	26.06	0.05	0	30.28	3.19
CO	2	95	0.01	29.41	0.64	0	24.53	0.26	0	35.39	3.16
CO	3	95	0	32.17	2.31	0	32.12	1.79	0	34.45	1.47
CO	4	95	0	25.28	0.85	0	30.38	1.73	0	31.24	1.22
HO	1	95	66.27	24.27	0.26	61.4	31.29	0.31	NA	NA	NA
HO	2	95	67.97	24.78	0.27	63.41	28.84	0.41	NA	NA	NA
HO	3	95	64.81	26.1	0.02	58.78	32.01	0.13	NA	NA	NA
HO	4	95	57.13	35.35	1.43	55.02	34.35	0.83	NA	NA	NA

Table 2: Gas Concentration of each package of hamburger patties containing *Salmonella* spp., temperature abused at 2.8ºC, 21ºC, and 35ºC during a 24 day shelf life in three MAP packages with a high oxygen blend (80%O_2/20% CO_2) (HO), a low oxygen packaging with carbon monoxide (0.4% CO/35% CO2/64.6% N2) (CO), and low oxygen packaging without carbon monoxide (35%CO2/65% N2) (WOCO).

			24 hr			Day 6			Day 9		
Packaging	REP	Temp	O2	CO2	CO	O2	CO2	CO	O2	CO2	CO
WOCO	1	38	0	26.53	0	0	25.67	0	0	29.15	2.44
WOCO	2	38	0	26.9	0	0	27.4	0	0	34.57	2.18
WOCO	3	38	0	25.66	0	0	26.4	0	0	32.08	2.19
WOCO	4	38	0	26.32	0	0	29.53	0	0	32.36	1.24
CO	1	38	0	26.66	0.33	0	29.35	0.22	0	39.96	0.03
CO	2	38	0	30.17	0.37	0	31.42	0.43	0	30.87	2.02
CO	3	38	0	28.64	0.37	0	29.5	0.4	0	34.3	2.48
CO	4	38	0	34.56	0.38	0	32.67	0.34	0	31.85	2.65
HO	1	38	78.47	14.8	0	66.57	24.32	1.46	65.06	21.66	1.32
HO	2	38	78.11	13.56	0	64.02	24.44	1.39	65.92	21.29	0.86
HO	3	38	77.19	14	0.04	78.92	13.41	0	64.22	28.23	1.33
HO	4	38	78.3	13.62	0	76.74	12.53	0	66.9	24.47	1.15
WOCO	1	70	NA	NA	NA	0	29.08	3.04	0	31.88	2.43
WOCO	2	70	NA	NA	NA	0	33.55	3.05	0	29.63	2.43
WOCO	3	70	NA	NA	NA	NA	NA	NA	0	31.63	1.92
WOCO	4	70	NA	NA	NA	NA	NA	NA	0	31.11	2.07
CO	1	70	NA	NA	NA	0	34.01	3.01	0	27.94	2.31
CO	2	70	NA	NA	NA	0	34.25	3.09	0	31.57	2.13
CO	3	70	NA	NA	NA	NA	NA	NA	0	31.46	2.06
CO	4	70	NA	NA	NA	NA	NA	NA	0	32.81	1.7
HO	1	70	NA	NA	NA	NA	NA	NA	64.87	26.36	1.15
HO	2	70	NA	NA	NA	NA	NA	NA	65.19	24.65	1.28
HO	3	70	NA	NA	NA	NA	NA	NA	64.87	26.36	1.15
HO	4	70	NA	NA	NA	NA	NA	NA	65.19	24.65	1.28
WOCO	1	95	NA	NA	NA	0	34.59	1.82	0	29.22	2.33
WOCO	2	95	NA	NA	NA	0	32.86	1.32	0	28.26	2.42
WOCO	3	95	NA	NA	NA	NA	NA	NA	0	29.27	1.24
WOCO	4	95	NA	NA	NA	NA	NA	NA	0	32.06	0.8
CO	1	95	NA	NA	NA	0	33.06	2.37	0	30.93	2.31
CO	2	95	NA	NA	NA	0	35.53	2.44	0	32.96	2.63
CO	3	95	NA	NA	NA	NA	NA	NA	0	32.44	2.35
CO	4	95	NA	NA	NA	NA	NA	NA	0	29.03	1.33
HO	1	95	NA	NA	NA	67.21	21.84	1.43	63.15	23.03	1.41
HO	2	95	NA	NA	NA	63.51	24.63	1.63	58.27	31.02	1.79
HO	3	95	NA	NA	NA	NA	NA	NA	64.87	24.26	1.54
HO	4	95	NA	NA	NA	NA	NA	NA	66.11	23.32	1.1

			Day 11			Day 14			Day 24		
Packaging	REP	Temp	O2	CO2	CO	O2	CO2	CO	O2	CO2	CO
WOCO	1	38	0	33.25	1.23	0	34.67	2.1	0	33.55	3.31
WOCO	2	38	0	35.94	1.35	0	34.82	2.24	0	36.38	3.02
WOCO	3	38	0	31.7	1.87	0	29.87	1.43	0	29.65	1.55
WOCO	4	38	0	30.79	1.88	0	34.62	1.42	0	35.07	1.76
CO	1	38	0	33.64	1.83	0	32.95	1.69	0	33.53	1.6
CO	2	38	0	36.63	1.51	0	36.95	2.04	0	31.45	1.81
CO	3	38	0	32.45	1.37	0	35.9	1.11	0	36.62	2.13
CO	4	38	0	33.54	1.45	0	33.9	1.13	0	34.93	2.16
HO	1	38	68.5	30.82	1.52	59.36	32.51	1.48	N/A	N/A	N/A
HO	2	38	65.8	35.55	0.9	53.56	38.99	1.36	N/A	N/A	N/A
HO	3	38	60.3	30.87	1.29	57.64	35.54	1.55	N/A	N/A	N/A
HO	4	38	63.1	27.77	0.81	61.73	28.97	1.37	N/A	N/A	N/A
WOCO	1	70	0	30.47	1.4	0	30.96	1.02	0	32.2	3.24
WOCO	2	70	0	30.47	1.4	0	32.34	1.1	0	33.68	2.82
WOCO	3	70	0	30.79	1.51	0	35.85	1.63	0	29.01	1.5
WOCO	4	70	0	32.51	1.54	0	33.42	1.72	0	28.78	1.47
CO	1	70	0	33.17	1.95	0	34.09	1.03	0	35.83	2.83
CO	2	70	0	35.33	2.51	0	36.8	1.94	0	38.2	3.13
CO	3	70	0	30.16	1.95	0	35.5	1.22	0	32.02	1.66
CO	4	70	0	33.53	1.49	0	32.89	2.04	0	33.26	1.75
HO	1	70	63.5	26.65	1.16	69.63	34.56	1.45	N/A	N/A	N/A
HO	2	70	64.2	13.5	1.38	62.56	29.12	1.2	N/A	N/A	N/A
HO	3	70	63.5	26.65	1.16	69.63	34.56	1.45	N/A	N/A	N/A
HO	4	70	64.2	13.5	1.38	62.56	29.12	1.2	N/A	N/A	N/A
WOCO	1	95	0	32.87	1.49	0	34.5	0.96	0	30.73	2.9
WOCO	2	95	0	32.87	1.49	0	38.49	1.99	0	35.17	3.08
WOCO	3	95	0.03	22.99	0.21	0	24.65	0.02	0	29.81	3.09
WOCO	4	95	0	25.41	0.33	0	28.01	0.81	0	25.72	1.62
CO	1	95	0	37.21	1.58	0	35.92	1.95	0	32.55	3.27
CO	2	95	0	34.94	1.46	0	37.45	2.11	0	37.04	3.04
CO	3	95	0	26.06	0.6	0	26.06	0.05	0	30.28	3.19
CO	4	95	0.01	29.41	0.64	0	24.53	0.26	0	35.39	3.16
HO	1	95	63	26.92	1.69	57.11	32.34	1.32	N/A	N/A	N/A
HO	2	95	62.4	27.92	1.69	54.3	38.95	1.49	N/A	N/A	N/A
HO	3	95	68	24.78	0.27	63.41	28.84	0.41	N/A	N/A	N/A
HO	4	95	66.3	24.27	0.26	61.4	31.29	0.31	N/A	N/A	N/A

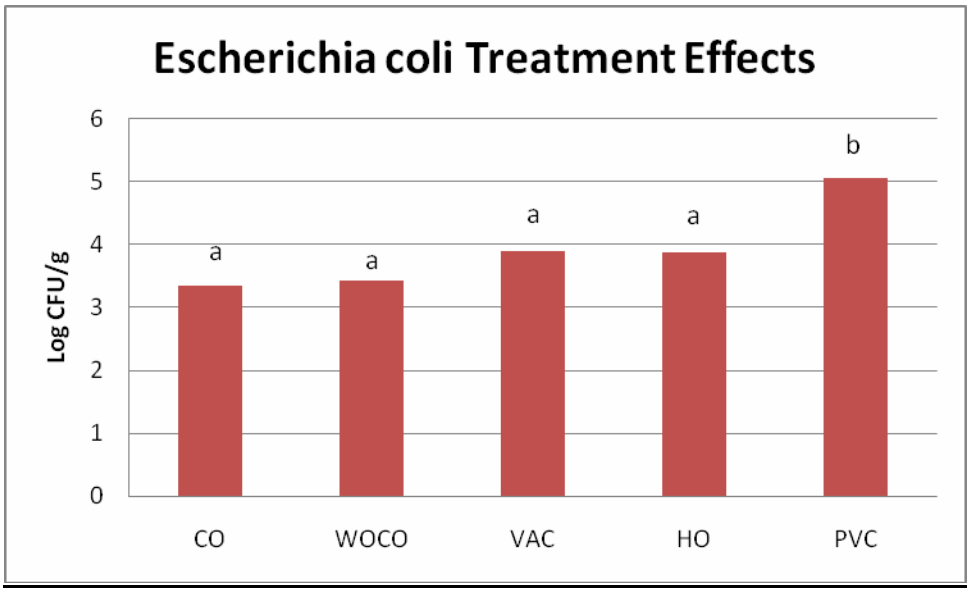
***Escherichia coli* O157:H7 SAS Summary**

Effect	**P-Value (Significance is at alpha less than 0.05)**
PACK or Package Type	<0.001
TEMP or Storage Temperature	0.0071
Day	<0.001
PACK * TEMP	0.9923
PACK * DAY	<0.001
DAY * TEMP	0.9963
PACK * TEMP * DAY	0.9883

Effect	**Estimate**
CO or Low oxygen with carbon monoxide	3.3406
WOCO or Low oxygen without carbon monoxide	3.4261
VAC or vacuum	3.9006
HO or High oxygen	3.8783
PVC or Overwrap	5.0548
Temperature 2.8ºC	3.5175
Temperature 21ºC	3.8999
Temperature 35ºC	4.3429
Day 6	3.4656
Day 9	4.2874
Day 11	4.2351
Day 14	3.6920

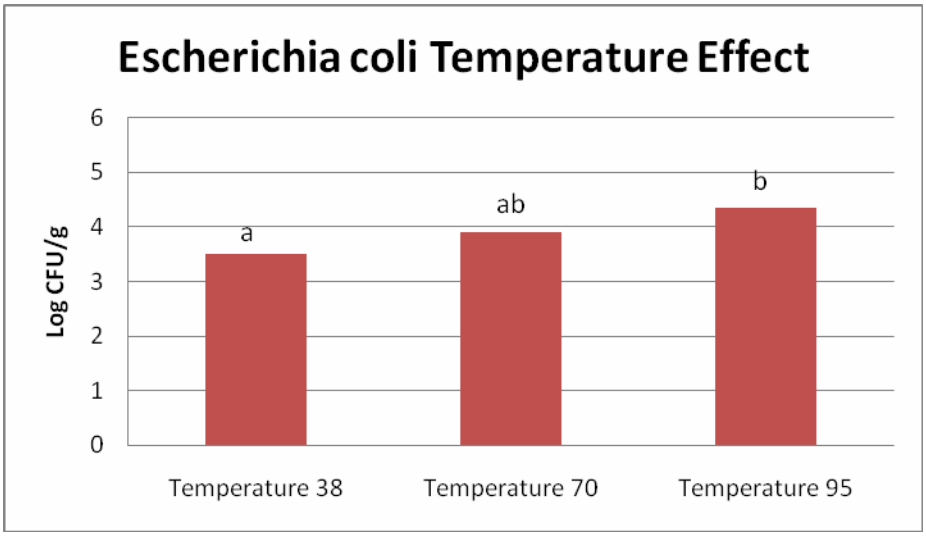
Packaging Effects regardless of sampling day and temperature

- Low oxygen with carbon monoxide is the same as High Oxygen package (P-Value=0.0997).
- Low oxygen with carbon monoxide is different then the Overwrap package (P-Value<0.001).
- Low oxygen with carbon monoxide is the same as Vacuum package (P-Value=0.0868).
- Low oxygen with carbon monoxide is the same as Low oxygen without carbon monoxide (P-Value=0.7903).
- High oxygen is different than the Overwrap package (P-value=0.0007).
- High oxygen is the same as Vacuum package (P-value=0.9448).
- High oxygen is the same as Low oxygen without carbon monoxide (P-value=0.1641).
- Overwrap is different than Vacuum package (P-value=0.0008).
- Overwrap is different than the Low oxygen without carbon monoxide (P-value<0.001).
- Vacuum is same as the Low oxygen without carbon monoxide (P-vale=0.1448).



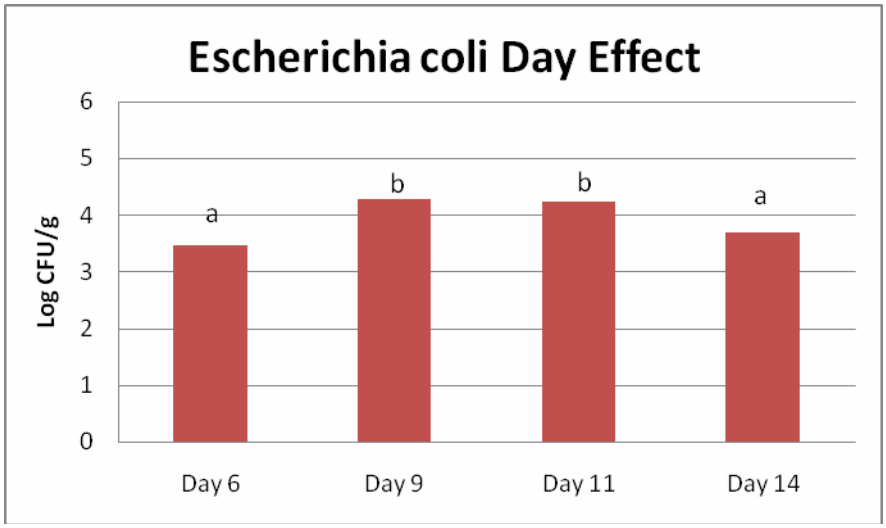
Temperature Effects regardless of packaging type and sampling day

- Temperature 2.8 is the same as Temperature 21 (P-value=0.1297).
- Temperature 2.8 is different than Temperature 35 (P-value=0.0018).
- Temperature 21 is the same as Temperature 35 (P-value=0.0805).



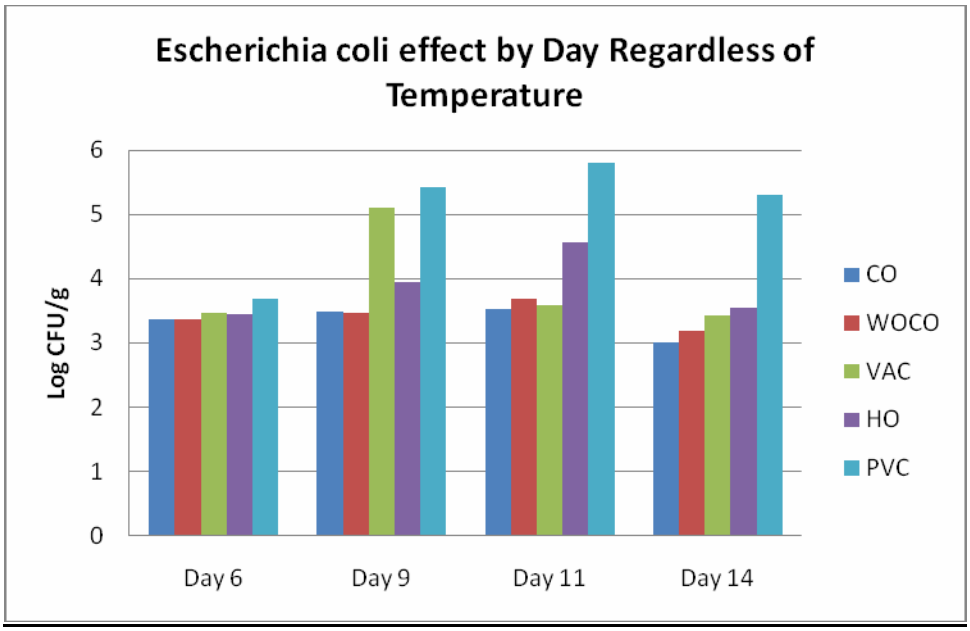
Sampling Day Effects regardless of packaging type and storage temperature

- Day 6 is different than day 9 (P-value<0.001).
- Day 6 is different than day 11 (P-value<0.001).
- Day 6 is the same as day 14 (P-value=0.0981).
- Day 9 is the same as day 11 (P-value=0.7002).
- Day 9 is different than day 14 (P-value<0.001).
- Day 11 is different than day 14 (P-value=0.0001).



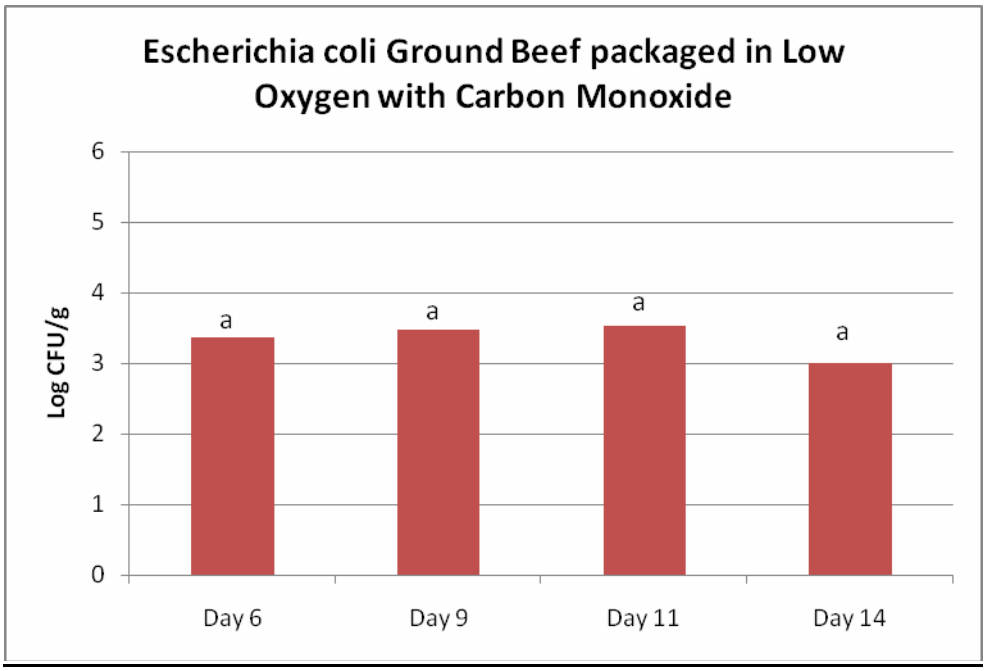
Sampling Day Effects regardless of storage temperature

- General Overview of all the data.



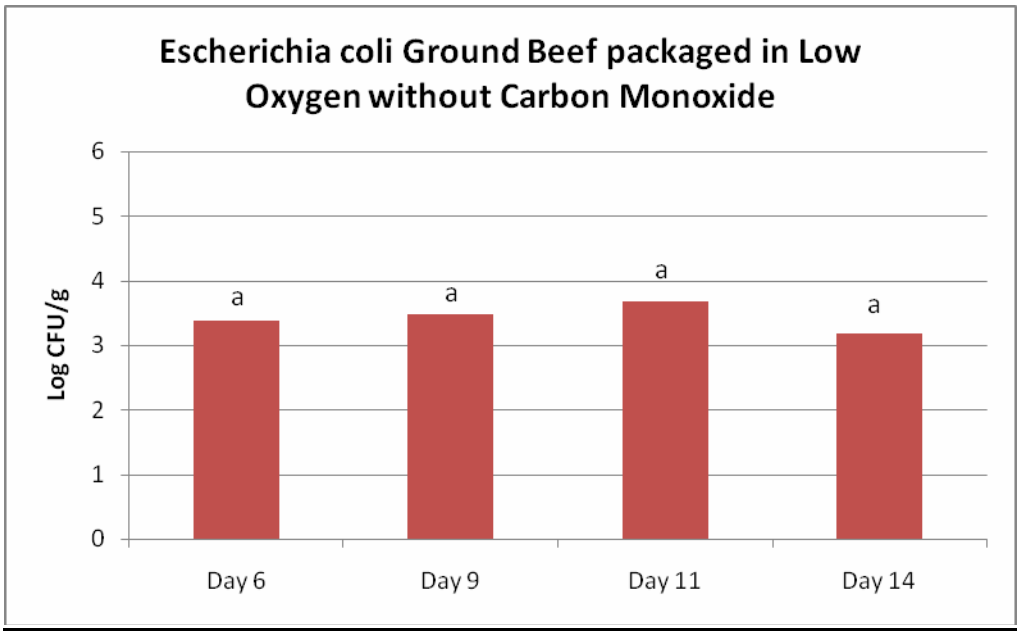
Effect of *Escherichia coli* inoculated ground beef in low oxygen packaging with carbon monoxide over time

- *Escherichia coli* O157:H7 was not affected by low oxygen packaging with carbon monoxide over the 24 day shelf life regardless of temperature (p-value >0.05).



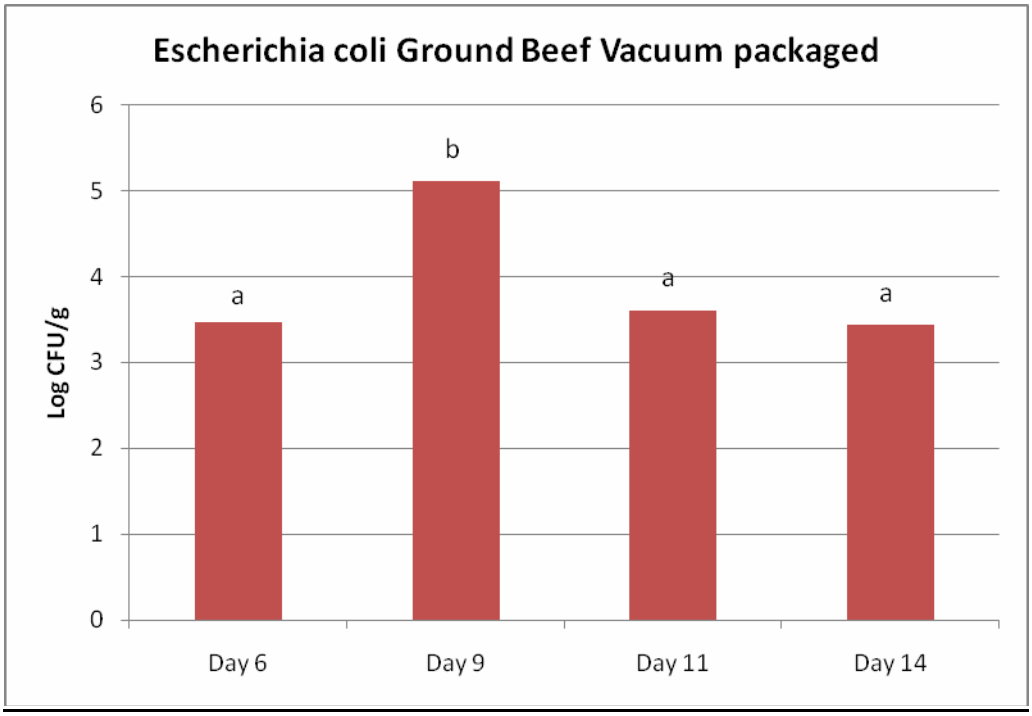
Effect of *Escherichia coli* inoculated ground beef in low oxygen packaging without carbon monoxide over time

- *Escherichia coli* O157:H7 were not affected by low oxygen packaging without carbon monoxide over the 24 day shelf life regardless of temperature (p-value >0.05).



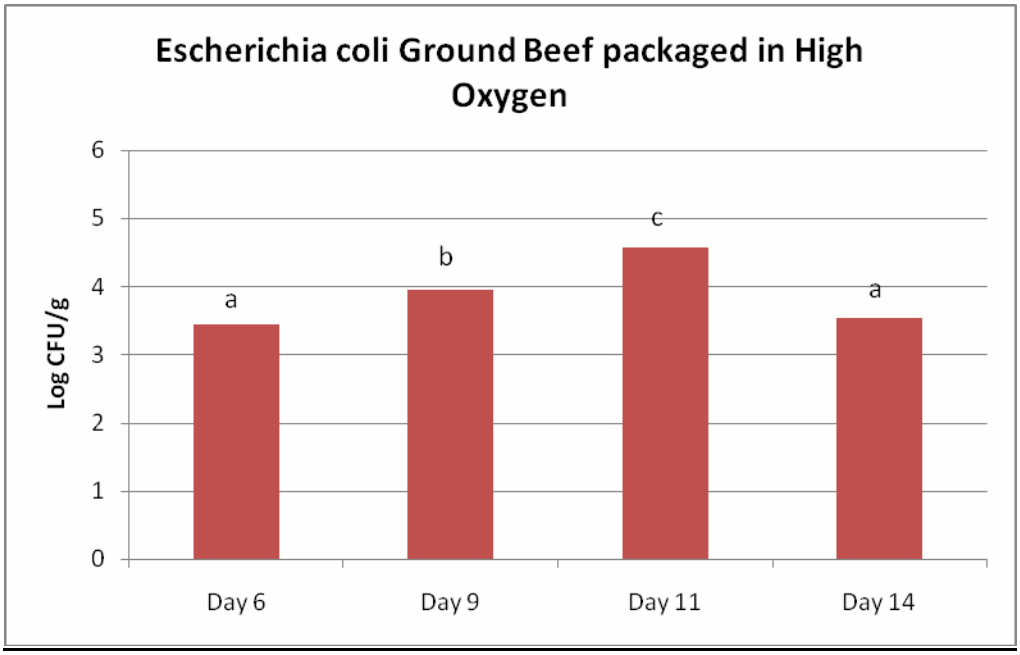
Effect of *Escherichia coli* inoculated ground beef in vacuum packaging over time

- *Escherichia coli* O157:H7 were significantly higher than day 9 than days 6, 11, and 14 regardless of temperature (p-value <0.05).
- *Escherichia coli* O157:H7 were statistically the same on days 6, 11, and 14 regardless of temperature (p-value >0.05).



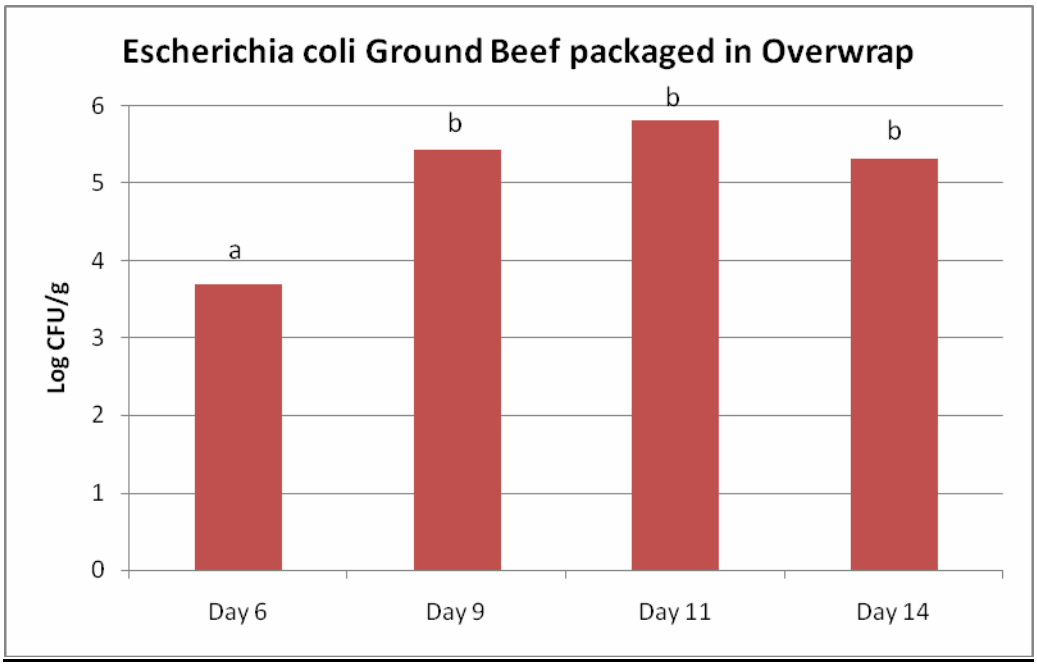
Effect of *Escherichia coli* inoculated ground beef in high oxygen packaging over time

- *Escherichia coli* O157:H7 were significantly higher on day 9 and 11 than days 6 and 14 regardless of temperature (p-value <0.05).
- *Escherichia coli* O157:H7 were significantly higher on day 9 than day 11 regardless of temperature (p-value <0.05).
- *Escherichia coli* O157:H7 were statistically the same on days 6 and 14 regardless of temperature (p-value >0.05).



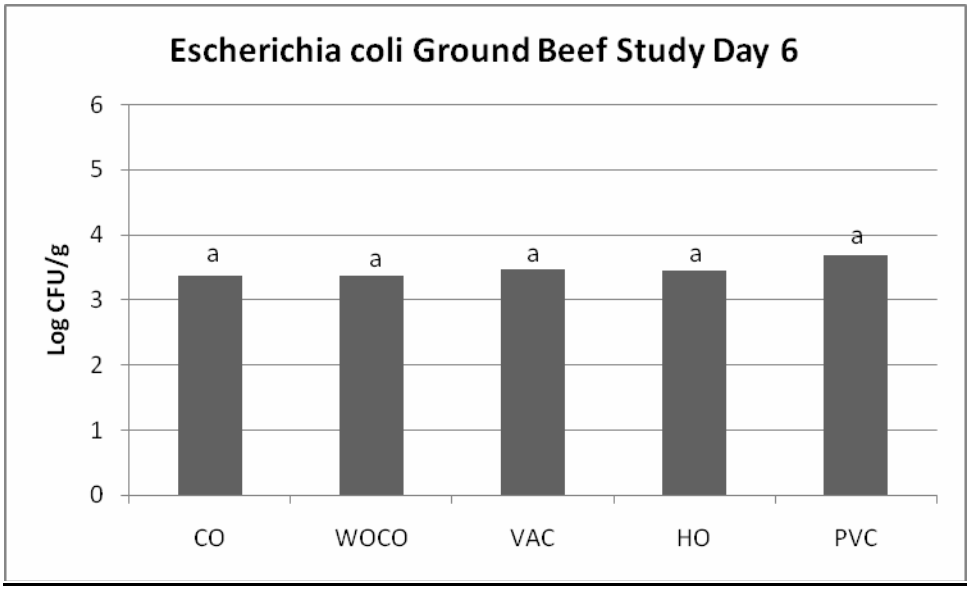
Effect of *Escherichia coli* inoculated ground beef in overwrapped packages over time

- *Escherichia coli* O157:H7 were significantly higher on day 9, 11, and 14 than day 6 regardless of temperature (p-value <0.05).
- *Escherichia coli* O157:H7 were statistically the same on days 6, 11 and 14 regardless of temperature (p-value >0.05).



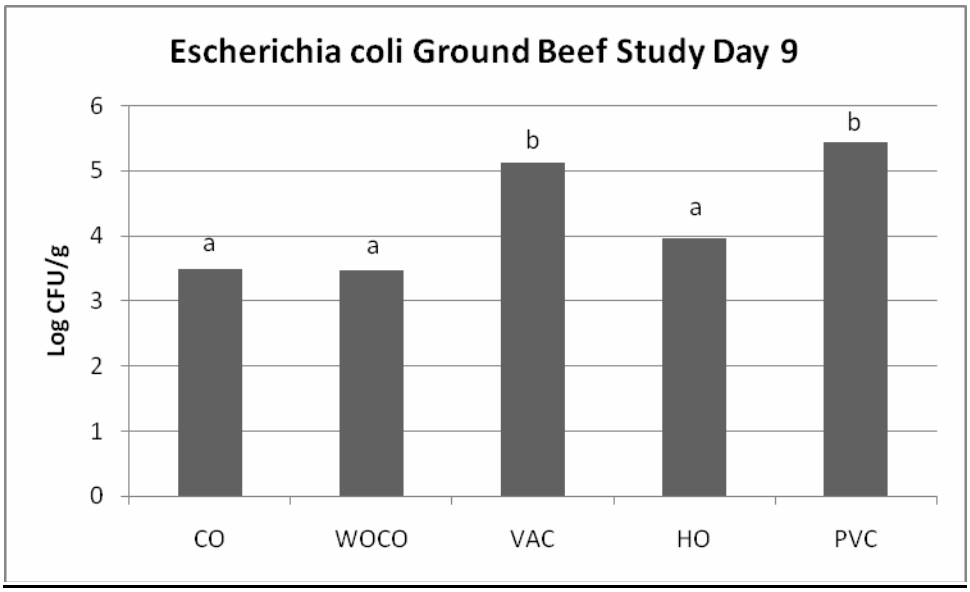
Effect of *Escherichia coli* inoculated ground beef in various packages on Day 6

- *Escherichia coli* O157:H7 were statistically the same on days 6 in all the packaging types regardless of temperature (p-value >0.05).



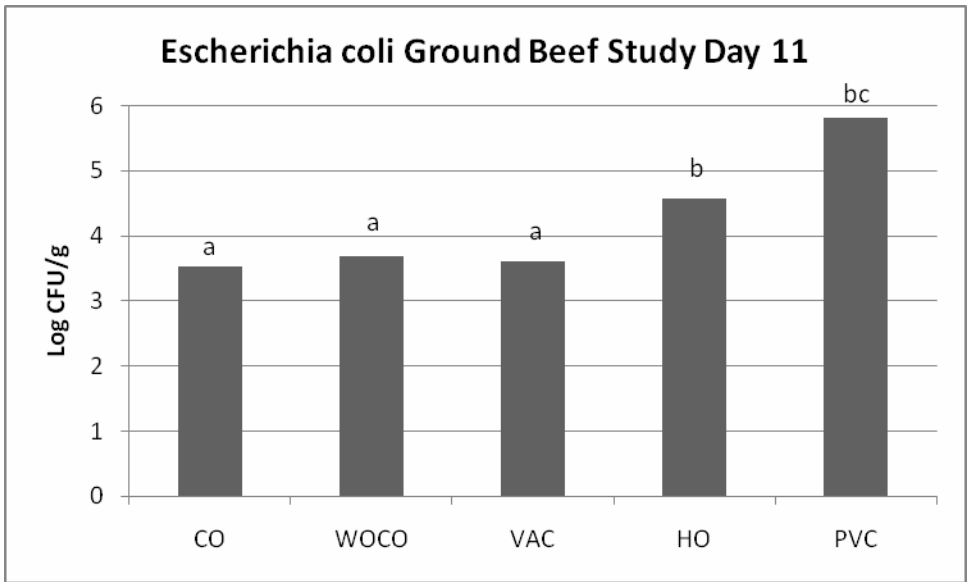
Effect of *Escherichia coli* inoculated ground beef in various packages on Day 9

- *Escherichia coli* O157:H7 were statistically the same on days 9 in low oxygen packaging with carbon monoxide, low oxygen packaging without carbon monoxide and high oxygen packages regardless of temperature (p-value >0.05).
- *Escherichia coli* O157:H7 were statistically the same on days 9 in vacuum packaging and overwrap packaging regardless of temperature (p-value >0.05).
- *Escherichia coli* O157:H7 was significantly higher on days 9 in the vacuum packaging and overwrap packaging than the low oxygen packaging with carbon monoxide, low oxygen packaging without carbon monoxide and high oxygen packages regardless of temperature (p-value <0.05).



Effect of *Escherichia coli* inoculated ground beef in various packages on Day 11

- *Escherichia coli* O157:H7 were statistically the same on days 11 in low oxygen packaging with carbon monoxide, low oxygen packaging without carbon monoxide and vacuum packaging regardless of temperature (p-value >0.05).
- *Escherichia coli* O157:H7 were statistically the same on days 11 in high oxygen packaging and overwrap packaging regardless of temperature (p-value >0.05).
- *Escherichia coli* O157:H7 was significantly higher on days 11 in the high oxygen packaging and overwrap packaging than the vacuum packaging, the low oxygen packaging with carbon monoxide and low oxygen packaging without carbon monoxide regardless of temperature (p-value <0.05).
- *Escherichia coli* O157:H7 was significantly higher on day 11 in the overwrap packaging than the vacuum packaging, the low oxygen packaging with carbon monoxide and low oxygen packaging without carbon monoxide regardless of temperature (p-value <0.05).



Effect of *Escherichia coli* inoculated ground beef in various packages on Day 14

- *Escherichia coli* O157:H7 were statistically the same on days 14 in the vacuum packaging, low oxygen packaging with carbon monoxide, low oxygen packaging without carbon monoxide and vacuum packaging regardless of temperature (p-value >0.05).
- *Escherichia coli* O157:H7 was significantly higher on day 14 in the overwrap packaging than the high oxygen packaging, vacuum packaging, the low oxygen packaging with carbon monoxide and low oxygen packaging without carbon monoxide regardless of temperature (p-value <0.05).