FINAL REPORT

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ELIMINATION OF *ESCHERICHIA COLI* O157:H7, GENERIC *ESCHERICHIA COLI*, AND *SALMONELLA* SPP. ON BEEF TRIMMINGS PRIOR TO GRINDING USING A CONTROLLED PHASE CARBON DIOXIDE ($_{CP}CO_2$) SYSTEM.

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PREFACE

The beef industry is continuously facing costly product recalls and court cases resulting from ground beef contaminated with *Salmonella* spp. and *Escherichia coli* O157:H7. Additionally, current test and hold programs enforced by the government and implanted by manufacturers leave an enormous logistical and economic burden on the industry. A wealth of antimicrobial technologies have been researched, developed, and implemented at the pre-chilled carcass level to control these and other pathogens, however, subsequent handling during chilling and fabrication, and occasional issues such as improper carcass spacing during chilling, can increase the risk of recontamination and overgrowth on raw meat products.

Effective decontamination of beef trimmings is utmost important because this product carries an elevated risk for *Salmonella* spp. and *E. coli* O157:H7 contamination, this is a step in the integrated beef manufacturing process which would add an important antimicrobial hurdle prior to grinding. Currently, there are no validated decontamination technologies available that effectively control meatborne pathogens on trimmings while providing acceptable quality in finished ground beef.

SafeFresh Technologies and Kansas State University have designed a novel antimicrobial technology for beef trimmings, by manipulating and controlling the various phases of carbon dioxide (CO_2) inside an enclosed system.

Controlled Phase Carbon Dioxide ($_{CP}CO_2$) in gaseous state dissolves in water to form carbonic acid, thus lowering pH anywhere between 3 and 4. The pH of the meat can be continuously maintained at <3.5 when pressures around 1000 psi (pounds per square inch) are exerted with a constant flow of $_{CP}CO_2$, overcoming the buffering capacity of the meat surface.

As pressures are raised slightly to exceed 1100 psi at 36°C, $_{CP}CO_2$ enters a supercritical phase, acquiring additional antimicrobial activity by becoming a dense gas, with the highly effective solvent of its liquid phase. The solvent property of the supercritical gas substantially affects lipids, which are an integral component of microbial cell membranes. The cell membrane, loses its ability to regulate the influx of hydrogen ions into the cellular cytoplasm, thus carbonic acid will acidify the cytoplasm, becoming lethal to the organism.

A final antimicrobial component of $_{CP}CO_2$ in the SafeFresh system design is the rapid compression and decompression of the system. The pressure differential during depressurization will cause $_{CP}CO_2$ to pass through its solid phase, forming ice crystals inside the meat, thus causing physical damage to bacterial cell membranes.

With all of these chemical and physical stresses being alternately applied in a controlled manner, the SafeFresh decontamination system may provide a synergistic series of bactericidal effects within the chamber.

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RESEARCH OBJECTIVE

The main objective of this research was to confirm the effectiveness of the SafeFresh (SafeFresh Technologies, Mercer Island, WA) controlled phase carbon dioxide ($_{CP}CO_2$) system in reducing pathogen levels in beef trim prior to grinding.

This investigation was initiated by performing a series of preliminary studies. The first study, was conducted to evaluate the effect of $_{CP}CO_2$ as an antimicrobial on sterile filter papers challenged with multiple strains of generic *Escherichia coli, E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. Different pressures were tested in order to determine the effects on bacterial populations.

Subsequently, a second study, subdivided in four parts, was conducted to evaluate the effects of $_{CP}CO_2$ on non-challenged and challenged beef trimmings. In the first part of the study, the effect of subcritical and supercritical carbon dioxide on the normal microflora of beef trimmings was analyzed. In the second part, residual *E. coli* O157:H7 populations were analyzed on beef trimmings treated with $_{CP}CO_2$ below the supercritical region with subsequent modified atmosphere packaging. The third and fourth parts of the study focused on the effects of $_{CP}CO_2$ on beef trimmings challenged with generic *E. coli* and *E. coli* O157:H7, respectively; both parts also included subsequent modified atmosphere packaging of the beef trimmings after $_{CP}CO_2$ application in the supercritical region.

Findings of the previous study directed the investigation into exploring the effects of $_{CP}CO_2$ in the supercritical region and additional modified atmosphere packaging on the organoleptic attributes and the microbiological safety of ground beef.

A third study was conducted to confront these concerns by analyzing the quality of ground beef manufactured with beef trimmings treated with $_{CP}CO_2$, and challenged with generic *E. coli, E. coli* O157:H7, and *Salmonella* spp, based on the information gathered during the first two preliminary studies.

Finally, the last study designed to evaluate the effects of $_{CP}CO_2$ as an antimicrobial intervention process for beef trimmings destined for grinding is discussed.

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Abstract

This experiment was designed to evaluate antimicrobial, quality, and shelf life effects of controlled phase carbon dioxide ($_{CP}CO_2$) on beef trimmings destined for ground beef.

Studies showed 1500 psi $_{CP}CO_2$ for 15 min achieved the highest lethality ($P<0.05$) in challenged beef trimmings (TR) and ground beef (GR). Total Plate Count (TPC), Generic *E. coli* (GEC), *E. coli* O157:H7 (O157), and *Salmonella* spp. (SS) reached 0.83, 0.96, 1.00, and 1.06 log reductions, respectively. Bacterial reductions in ground beef (GR) were similar to beef trimmings (TR) ($P\geq0.05$).

In raw patties, CIE L^* values showed significant pressure by packaging interaction (L^*, $P<0.05$). CIE a^* values showed significant pressure by packaging by days of display interaction (a^*, $P<0.05$). CIE b^* scores showed significant packaging by days of display interaction (b^*, $P<0.05$). However, after 5 days of simulated retail display, CIE L^* and a^*, reflectance (630/580nm) ratios were similar for all treatments ($P\geq0.05$). CIE b^* scores after 5 days of display were most acceptable at 1500 $_{CP}CO_2$ (b^*, $P\geq0.05$) , regardless of the packaging conditions.

In cooked patties, CIE L^* values were similar ($P\geq0.05$) when comparing packaging conditions within the treatments. After 5 days of refrigerated display, CIE L^* values were slightly higher for patties packaged under 100% flushed CO_2 (CO2) when compared to aerobic trays (AT). CIE a^* and b^* values and reflectance ratios exhibited very similar trends, both scores were similar (a^*, b^*, 630/580, $P\geq0.05$) after simulated retail display for all the treatments. No differences were observed for crude protein (%CP) and crude fat (%CF) after 5 d of simulated display (%CP, %CF, $P\geq0.05$). The extent of lipid oxidation, after 5 days of simulated retail display, scored higher in AT than CO2, with the highest values reached at 1500 psi $_{CP}CO_2$ ($P<0.05$). TBAR values in CO2 patties were able to maintain similar values to the control all throughout refrigerated storage ($P\geq0.05$).

Ground beef patties manufactured from treated beef trimmings scored higher values for tenderness ($P< 0.05$) when compared to non-treated. 750 psi $_{CP}CO_2$ appeared to have worse scores for juiciness, beef flavor intensity or off flavor intensity ($P<0.05$) than the 1500 psi $_{CP}CO_2$ treatment or the control.

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Effects of $_{CP}CO_2$ on Filter Paper Disks Challenged with Food Pathogens

Objective

This experiment was designed to evaluate the feasibility of using controlled phase carbon dioxide ($_{CP}CO_2$) as an antimicrobial control against multiple strains of generic *Escherichia coli, E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. inoculated on sterile filter paper disks, and to analyze the microbial effectiveness of $_{CP}CO_2$ as a function of the pressure in the system.

Materials and Methods

Preparation of Bacterial Cultures

Bacterial cultures were obtained from the Food Microbiology Laboratory stock culture collection at Kansas State University (KSU). The following cultures were utilized: *Listeria monocytogenes* 101M, 109, and 108M (Larry Beuchat, UGA); Generic *E. coli* ATCC 14763 (Jackie Staats, KSU), ATCC 23740 (Microb. Genet. Res. Unit, London); *E. coli* O157:H7 ATCC 43890 (Jackie Staats, KSU), ATCC 43895, and KSU01 (CDC); *S.* Enteritidis USDA-FSIS 15060; *S.* Montevideo (Larry Beuchat, UGA); and *S.* Seftenburg subsp. Cholerasuis ATCC 43485. Stock cultures were cultivated by placing one impregnated bead into a 5 ml solution of Difco® Tryptic Soy Broth (TSB) and incubating for 24 h at 35°C. Next, a 0.05 ml loop of the respective culture was inoculated into a 5 ml solution of TSB and incubated for 24 h at 35°C. After incubation, 1 ml of the respective culture was inoculated into 49 ml TSB and incubated for 24 h at 35°C. After incubation, bottles of respective cultures were mixed together in equal parts to create a 50 ml cocktail containing 10^9 to 10^{10} CFU/ml of *Listeria monocytogenes, Salmonella* spp., and generic *E. coli* or *E. coli* O157:H7. Cultures were confirmed by cultivation on selective and differential media, and biochemical analysis of presumptive colonies using API 20E kits.

Twelve sterile filter paper #1 disks (Whatman International Ltd. Maidstone, England) with 5.5 cm diameter and a total surface area of 47.5 cm^2 were sterilized for 24 hours. Sterilization was achieved by placing them individually inside a Petri dish under UV light exposure inside a Sterile Guard II Class II Type A/B3 Laminar Flow Hood Model SG600 (The Baker Company, Sanford, Maine, US). Four filter paper disks were each inoculated on both surfaces with a three-strain cocktail of *Listeria monocytogenes*, *Salmonella* spp., generic *E. coli*, or *E. coli* O157:H7. Inoculation was performed by aseptically dipping the disks into the respective inoculum until saturation. The four filter paper disks were hung and the inoculum was allowed to drip for 10 minutes in order to remove liquid excess and to allow for proper bacterial cells attachment to disk surfaces. This procedure was repeated for every pressure tested.

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After inoculation, four filter papers disks were placed randomly inside the experimental laboratory model of a vessel (Atlas/Parker, Des Plaines, IL) described in Appendix A. Each filter paper disk was aseptically attached to the shaft inside the chamber with a sterile paper clip. Filter papers were treated with the following treatments:

- 1200 psi for 3 min at 36°C
- 1700 psi for 3 min at 36°C
- 2100 psi for 3 min at 36°C

After safely closing the vessel, $_{CP}CO_2$ was applied according to the protocol entitled: "Protocol for Application of $_{CP}CO_2$" found in Appendix B.

Pressures and temperatures during the study were measured in psi and °C respectively, recorded by an OM-CP-Quadprocess 4 channel 4-20 mA Data Logger (Omega Engineering, Inc.; Stamford, CT), and electronically stored with the Omega® Data Logging Software Ver. 2.00.43c for Windows®. The study was conducted at the KSU Food Safety Processing Laboratory. Four replications were performed. Statistical Analysis was conducted in recovered bacterial populations in a Randomized Complete Block Design, using the General Linear Model from SAS (SAS, 2003).

Microbiological Analysis

After $_{CP}CO_2$ application, each filter paper disk was aseptically extracted from the vessel, placed in 30 ml of 0.1% sterile peptone water (PW) and homogenized in a stomacher for one minute. Microbiological samples were serially diluted in sterile PW and spiral plated using a Whitley automatic spiral plater (Don Whitley Scientific ltd., Shipley, West Yorkshire, England). Samples were cultured onto Modified Oxford Agar (MOX; Oxoid ltd., Basingstoke, Hampshire, England), MacConkey Sorbitol Agar (MSA; Difco, Detroit, MI), and Xylose Lysine Desoxycholate Agar (XLD; Difco, Detroit, MI) to enumerate *Listeria monocytogenes*; generic *E. coli* and *E. coli* O157:H7, and *Salmonella* spp. respectively. Plates were incubated at 37°C for 24 and colony forming units (CFU) were enumerated manually using a spiral plate dark field Quebec colony counter (Model 330; American Optical Company, Buffalo, NY). Average recoveries were converted into log and average reductions were calculated as a difference between the respective inoculated controls and their treated samples.

Results and Discussion

The effects of antimicrobial effectiveness of $_{CP}CO_2$ as a function of pressure or microorganism tested could not be clearly established, as there were no statistical differences in bacterial recoveries (Table 1) among any of the pressures tested, challenged pathogens or the interactions between the effects ($P \geq 0.05$).

No bacterial colonies forming units were recovered after application of $_{CP}CO_2$ at 1200 psi for generic *E. coli*, providing the highest numerical reductions for this pathogen (5.7 log);

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however, this treatment provided the lowest numerical reductions for *Listeria monocytogenes* (4.81 log) and *Salmonella* spp. (5.69 log).

Only *L. monocytogenes* was recovered after 3 min exposure to the 1700 psi $_{CP}CO_2$ treatment, which provided levels of reductions of 5.7, 6.33, and 5.81 log for generic *E. coli*, *E. coli* O157:H7, and *Salmonella* spp., respectively.

Application of 2100 psi $_{CP}CO_2$ for 3 min provided the same level of reductions for *Salmonella* spp. (5.81 log) as the 1700 psi $_{CP}CO_2$ for 3 min treatment, rendering the highest reduction for *Listeria monocytogenes* (5.60 log). However, this treatment was the least effective for generic *E. coli* and *E. coli* O157:H7 ($P<0.05$) with reductions of only 4.04 and 5.16 log, respectively (Figure 1). Nevertheless, reduction levels obtained from this study (Table 2) demonstrated $_{CP}CO_2$ as an extremely efficient antimicrobial treatment, as all the pressures tested consistently achieved an average of at least 4.04 log reductions over the inoculated controls across the challenging microorganisms, similar to those reported in a study by Kamihira et al. (1997).

Table 1. Least Square Means of Generic *E. coli*, *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. Recoveries (Log CFU/cm^2) on Filter Paper Circles Treated with $_{CP}CO_2$.

TREATMENT	*Generic Escherichia coli*	*Listeria monocytogenes*	*Escherichia coli O157:H7*	*Salmonella spp.*
CTRL	*6.50*	*6.86*	*7.13*	*6.61*
1200	*0.80*	*2.05*	*1.12*	*0.92*
1700	*0.80*	*1.82*	*0.80*	*0.80*
2100	*2.46*	*1.25*	*1.97*	*0.80*

Averages of 4 replications.

Table 2. Least Square Means of Generic *E. coli*, *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. Reductions (Log CFU/cm^2) on Filter Paper Circles Treated with $_{CP}CO_2$.

TREATMENT	*Generic Escherichia coli*	*Listeria monocytogenes*	*Escherichia coli O157:H7*	*Salmonella spp.*
1200 psi	*5.70^{ax}*	*4.81^{ax}*	*6.02^{ax}*	*5.69^{ax}*
1700 psi	*5.70^{ax}*	*5.04^{ax}*	*6.33^{ax}*	*5.81^{ax}*
2100 psi	*4.04^{ax}*	*5.60^{ax}*	*5.16^{ax}*	*5.81^{ax}*

Averages of 4 replications.

[a] *Least square means within a row bearing different letters are different ($P<0.05$).*

[x] *Least square means within a column bearing different letters are different ($P<0.05$).*

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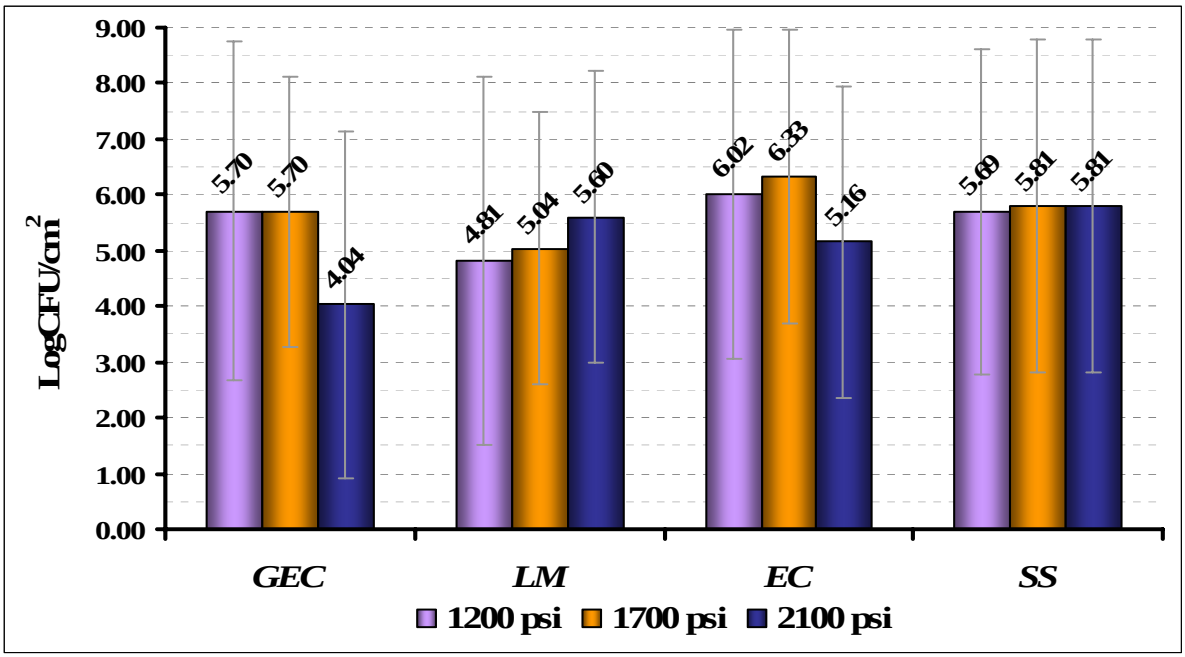
Figure 1. Effect of Controlled Phase Carbon Dioxide ($_{CP}CO_2$) Application as a Function of Pressure (1200 psi, 1700 psi, 2100 psi) on Average Reductions (Log CFU/cm^2) in Filter Paper Disks Challenged with Food Pathogens as Compared to Non Treated Controls (CTRL).



[a] CFU = Colony Forming Units.
[b] Generic *E. coli* (GEC), *Listeria monocytogenes* (LM), *E. coli* O157:H7 (EC) and *Salmonella* spp. (SS)

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Effects of $_{CP}CO_2$ on Non-Challenged and Challenged Beef Trimmings and Ground Beef

Objective

This exploratory study, subdivided in four parts, was conducted to assess the feasibility of using controlled phase carbon dioxide ($_{CP}CO_2$) as an antimicrobial control against the normal microflora of ground beef and against pathogenic bacteria in beef trimmings prior to grinding. The objective of these series of experiments was to determine the most important factors to be included as the main independent variables of one final study.

In the first part of the study, non-challenged ground beef samples were exposed to $_{CP}CO_2$ under subcritical conditions (1470 and 3000 psi for 5 min at 29°C), and supercritical conditions (2000 and 5000 psi for 7.5 min and 2000 psi for 15 min at 36°C).

In the second part of the study, fresh meat cubes samples challenged with *E. coli* O157:H7 were exposed to $_{CP}CO_2$ at 1000 psi for 5 min, 10 min, and 240 min. The 240 min samples were also analyzed for additional lethality after storage under modified atmospheric packaging conditions (bag flushed with CO_2, and stored in a display case refrigerator at 4°C for 4 days).

The third part of this experiment tested fresh meat cubes samples challenged with generic *E. coli* exposed to the effect of supercritical $_{CP}CO_2$ at 2400 psi for 3 min, 1800 psi for 7 min, and 1300 psi for 5 min. These treatments were applied at 36°C. All meat samples were additionally tested for increased lethality effects after storage under modified atmospheric packaging conditions (bag flushed with 100% CO_2 stored in a display case refrigerator at 4°C for 10 days).

Finally, during the fourth part of this study, fresh meat samples challenged with *E. coli* O157:H7 were exposed to supercritical $_{CP}CO_2$ at 1100 psi for 5 min, 1600 psi for 7 min, and 2100 psi for 3 min. All treatments were applied at 36°C and additionally tested for increased lethality effects after storage under modified atmospheric packaging conditions (bag flushed with 100% CO_2 stored in a display case refrigerator at 4°C for 10 days).

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Part 1. Residual Populations of Normal Microflora in Non-challenged Ground Beef Samples Treated with $_{CP}CO_2$

Materials and Methods

Preparation of Samples

Fresh ground beef meat stored at 4°C was obtained from a retail store. Ground meat was weighted in 10g batches and each batch was aseptically mixed with 20g of Wetsupport™ desiccant (ISCO, Inc.; Lincoln, NE) in order to maintain a 2:1 w/w ratio. Two extraction vessels were sterilized and 2g of mixture were aseptically placed inside the extraction vessel.

Treatment of Samples

Duplicate ground beef samples were treated under different pressures and exposure times with $_{CP}CO_2$ under subcritical (at 29°C) and supercritical (at 36°C) conditions inside a Pepmaster GA CO_2 extractor (Suprex Corporation, now ISCO, Lincoln, NE) calibrated at a flow rate of 3 ml/min of CO_2. The study was conducted at the KSU Food Chemistry Laboratory.

Meat samples were treated inside the extractor chamber, according to the manufacturer operating instructions, with the following treatments:

- 1470 psi for 5 min at 29°C
- 3000 psi for 5 min at 29°C
- 2000 psi for 7.5 min at 36°C
- 2000 psi for 15 min at 36°C
- 3000 psi for 5 min at 36°C
- 5000 psi for 7.5 min at 36°C

Individual controls for the experiments consisted of non-treated meat samples. One control was used for every set of treated samples. Statistical Analysis was conducted in two replications in a Randomized Complete Block Design using the General Linear Model from SAS (SAS, 2003).

Microbiological Analysis

After treatment, samples were weighted, diluted with 20 ml of 0.1% sterile peptone water (PW), and homogenized in a stomacher for one minute. After homogenization, each sample was serially diluted in sterile PW, spiral plated onto Tryptic Soy Agar (TSA; Difco, Detroit, MI) and incubated at 37°C for 24h to enumerate the Total Plate Count (TPC).

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Colony forming units of endogenous microflora were enumerated manually using a spiral plate dark field Quebec colony counter (Model 330; American optical company, Buffalo, NY). Colony forming units were converted into log and reductions were calculated as the difference between respective non-treated controls and the average of their treated duplicates.

Results and Discussion

Results of this experiment (Table 3) demonstrated that $_{CP}CO_2$ pressurized under supercritical conditions (36°C) provided a higher lethality effect on the endogenous microflora (TPC), when compared to subcritical conditions (29°C), regardless the pressure and time of exposure combination utilized.

Samples treated with $_{CP}CO_2$ under supercritical conditions (5000 psi for 7.5 min, 2000 psi for 7.5 min, 2000 psi for 15 min, and 3000 psi for 5 min) showed similar recovery levels of 5.42, 5.39, 5.25 and 5.53 log CFU/g, respectively (P≥0.05). These same treatments achieved reduction levels of 2.23, 1.97, 1.88, and 1.83 log, in that order, over their correspondent non-treated controls.

In the other hand, samples treated with $_{CP}CO_2$ under subcritical conditions at 1470 psi for 5 min and 3000 psi for 5 min, also yielded similar recoveries (P≥0.05) of 6.79 and 6.87 log CFU/g, respectively. These particular treatments only provided reductions of 0.74 and 0.67 log, respectively.

Table 3. Least Square Means of Total Plate Count Recoveries and Reductions in Ground Beef Exposed to 1470, 2000, 3000, and 5000 psi $_{CP}CO_2$ in a Supercritical Fluid Extractor.

Treatments	*Temp.*	*Time*	*Control (Log CFU[z]/g)*	*Recoveries (Log CFU/g)*	*Log Reductions*
1470 psi	*29°C*	*5.0 min.*	*7.53*	*6.79[b]*	*0.74[b]*
3000 psi	*29°C*	*5.0 min.*	*7.54*	*6.87[b]*	*0.67[b]*
2000 psi	*36°C*	*7.5 min.*	*7.36*	*5.39[a]*	*1.97[a]*
2000 psi	*36°C*	*15.0 min.*	*7.13*	*5.25[a]*	*1.88[a]*
3000 psi	*36°C*	*5.0 min.*	*7.36*	*5.53[a]*	*1.83[a]*
5000 psi	*36°C*	*7.5 min.*	*7.65*	*5.42[a]*	*2.23[a]*

Average of 1 replication with two duplicates.
[ab] Least square means within a column bearing different letters are different (P<0.05).
[z] CFU=Colony Forming Units

No statistical differences in microbial recoveries were attributable to the combination of pressure and exposure time applied (P≥0.05). However, it is evident that higher pressures provided slightly larger numerical values in APC recoveries within both of the temperatures tested.

Results from this experiment also demonstrated that $_{CP}CO_2$ under subcritical conditions may not be suitable as an antimicrobial treatment (Figure 2), as reductions

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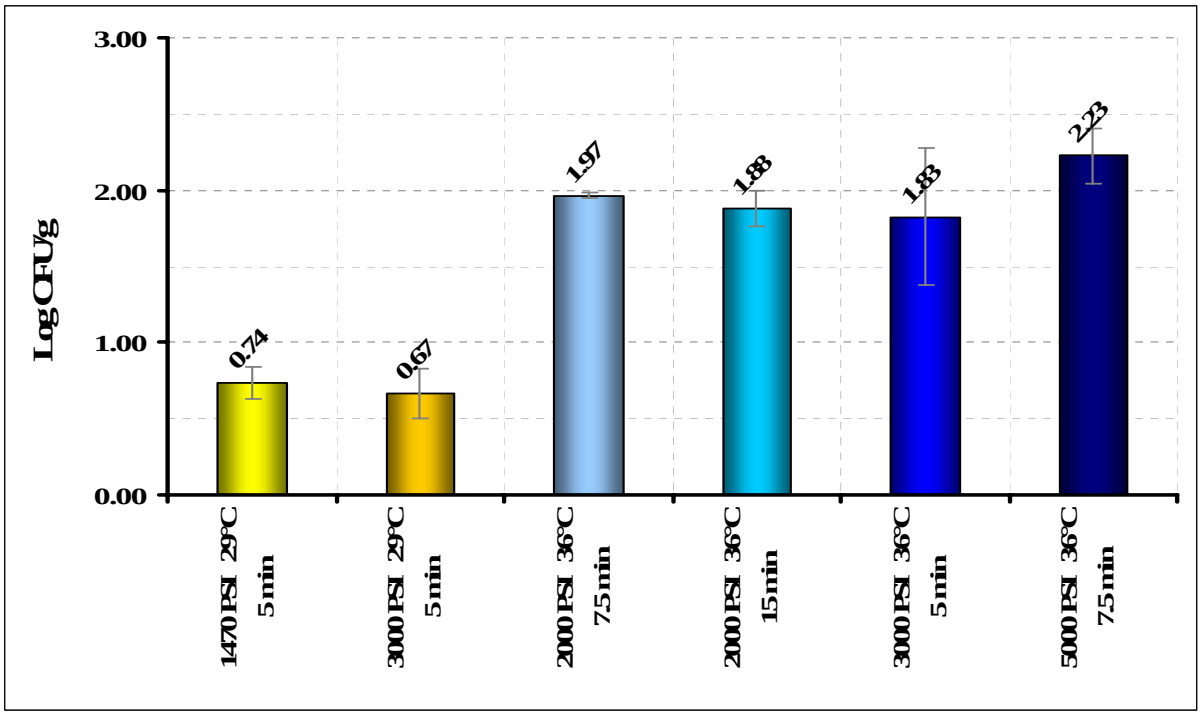
obtained under subcritical conditions (29°C) were substantially lower than those obtained under supercritical conditions (36°C), regardless of the pressure and time of exposure combination applied ($P<0.05$).

Figure 2. Effect of Controlled Phase Carbon Dioxide ($_{CP}CO_2$) Application as a Function of Pressure (1470 psi, 2000 psi, 3000 psi, 5000 psi) and time (5, 7.5, and 15 min) under Subcritical (29°C) and Supercritical (36°C) Conditions on Average Reductions (Log CFU/g) of Total Plate Counts in Ground Beef.



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Part 2. Residual Populations of *Escherichia coli* O157:H7 in Challenged Fresh Beef Trimmings Treated with $_{CP}CO_2$

Materials and Methods

Preparation of Samples

Bacterial cultures in this study included five different strains of *Escherichia coli O157:H7* obtained from the Food Microbiology Laboratory stock culture collection at Kansas State University (KSU). The following cultures were utilized: ATCC 43890 and ATCC 43889, obtained from Jackie Staats at KSU Veterinary School; ATCC 43895, obtained from a raw ground meat sample implicated in hemorrhagic colitis outbreak; and USDA-FSIS 011-82 and USDA-FSIS 380-94.

Stock cultures were cultivated by placing one impregnated bead into a 5 ml solution of Difco® Tryptic Soy Broth (TSB) and incubating for 24 h at 35°C. Next, a 0.05 ml loop of the respective culture was inoculated into a 5 ml solution of TSB and incubated for 24 h at 35°C. Following incubation, samples were centrifuged (13,300 x g at 4°C), and the supernatant decanted and the pellet was re-suspended with 50 ml of 0.1% peptone and centrifuged (13,300 x g at 4°C) a final time. The peptone was decanted and the remaining pellet was re-suspended with 10 ml of 0.85% saline solution. The five 10 ml bottles of respective culture were mixed together to create a 50 ml cocktail containing 10^9 to 10^{10}CFU/ml of *E. coli* O157:H7. The cell density of this suspension was determined by plating appropriate dilutions on MSA (MacConkey Sorbitol Agar) plates incubated for 48 hours at 35°C. Cultures were confirmed by cultivation on selective and differential media, and biochemical analysis of presumptive colonies using API 20E kits.

Inoculation and Treatment

Fresh beef meat was obtained from the meat lab at KSU. A select top round roast stored at 4°C was aseptically cut into ca. 1 in. cubes. Individual pieces were aseptically placed in a previously sterilized hanging device. Samples were inoculated inside a “bio-containment” chamber by “misting” the surface of the meat with approximately 10 ml of the inoculum. This was done ensuring that all 6 sides of each piece of meat received the same exposure to the inoculum. Samples were held for 30 min at room temperature in a sterile laminar flow cabinet (SterilGARD II ®, The Baker Company, Sanford, ME) to allow proper bacterial attachment to the surface of the meat.

Meat samples were treated with $_{CP}CO_2$ inside our experimental vessel (Appendix A), according to the general protocol (Appendix B), with the following parameters:

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- 1000 psi for 5 min at 36°C
- 1000 psi for 10 min at 36°C
- 1000 psi for 4 hours at 36°C

A non-treated inoculated meat sample was microbiologically analyzed as the control. The 240 min samples were also analyzed for additional lethality on residual bacterial populations after storage under modified atmospheric packaging conditions by placing a sub-sample from each treatment in a 3 ml standard barrier 10 x 15 in Nylon/PE bag, flushing with 100% CO_2 for 10 seconds, and heat-sealing the bag. Sub-samples were immediately stored in a display case refrigerator at 4°C for 4 days. Pressure and temperatures during the study were measured in psi and °C, respectively, recorded by an OM-CP-Quadprocess 2 channel Data Logger (Omega Engineering, Inc.; Stamford, CT) and electronically stored with the Omega® Data Logging Software Ver. 2.00.03c for Windows®. The study was conducted at the KSU Food Safety Processing Laboratory. Statistical Analysis was conducted in three replications with a Randomized Complete Block Design using the General Linear Model from SAS (SAS, 2003).

Microbiological Analysis

After treatment, samples were weighted, diluted with 50 ml of 0.1% sterile peptone water (PW) and homogenized in a stomacher for one minute. After homogenization, samples were serially diluted in sterile PW and spiral plated to enumerate *E. coli* O157:H7 onto MacConkey Sorbitol Agar (MSA; Difco, Detroit, MI). The plates were incubated at 37°C for 24 hrs. The colony forming units were enumerated manually using a spiral plate dark field Quebec colony counter (Model 330; American optical company, Buffalo, NY). Colony forming units were converted into log and reductions were calculated as the difference between respective non-treated controls and the average of their treated duplicates.

Results and Discussion

E. coli O157:H7 recoveries in treated samples were in the order of 6.71, 6.92, and 7.11 log CFU/g when exposed to 1000 psi at 36°C during 5, 10, and 240 min, respectively (Table 6, Figure 10). Statistical analysis confirmed that bacterial recoveries as a function of the exposure time were not statistically different (P≥0.05). Nevertheless, numerical results also confirmed that pressurized $_{CP}CO_2$ was able to reduce *E. coli* O157:H7 populations by at least 0.66 log when pressurized up to 1000 psi at 36°C (Table 4).

Time of exposure inside the chamber, as previously mentioned, had no apparent effect on the level of bacterial recoveries, perhaps because supercritical conditions were not completely established inside the vessel, as the pressure used was very close to the critical pressure of 1066 psi (1000 psi).

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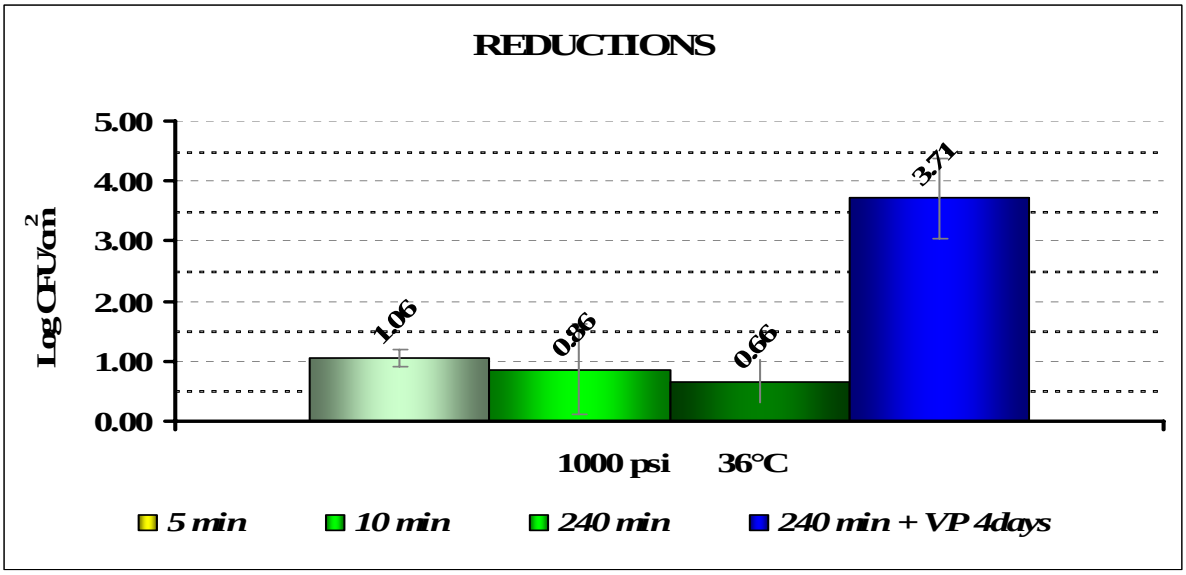
Table 4. Least Square Means of *E. coli* O157:H7 Recoveries and Reductions in Fresh Meat Cubes Exposed to 1000 psi $_{CP}CO_2$ for 5, 10, and 240 min at 36°C.

Treatments	*Temp.*	*Time*	*Recoveries (Log CFU/g)*	*Log Reductions*
Control	*36°C*	*Non treated*	*7.78*	
1000 psi	*36°C*	*5 min*	*6.71*	*1.06[b]*
1000 psi	*36°C*	*10 min*	*6.92*	*0.86[b]*
1000 psi	*36°C*	*240 min*	*7.11*	*0.66[b]*
1000 psi + 100% CO_2 day 4	*36°C*	*4 days*	*4.06*	*3.71[a]*

Average of 3 replications.
[ab] *Least square means within a column bearing different letters are different (P<0.05).*

It was also observed that modified atmosphere packaging with 100% flushed CO_2 and immediate storing for 4 days at 4°C further reduced bacterial populations to 3.71 log CFU/g, providing and additional lethality effect of 2.65 log. This data suggested the opportunity of further investigation of modified atmosphere packaging with 100% flushed CO_2 in order to compare its efficiency versus traditional vacuum packaging and to determine its organoleptic effects on beef trim, because of its great features not only as a bacteriostatic control measure, but as a very efficient bactericide as well (Figure 3).

Figure 3. Effect of Controlled Phase Carbon Dioxide ($_{CP}CO_2$) Application as a Function of Pressure (1000 psi), Time (5, 10, and 240 min) and Vacuum Package(VP) on Average Reductions (Log CFU/g) of *E. coli* O157:H7 in Ground Beef.



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Part 3. Residual Populations of Generic *Escherichia coli* in Challenged Fresh Beef Trimmings Treated with $_{CP}CO_2$

Materials and Methods

Preparation of Samples

Bacterial cultures in this study included two different strains of generic *Escherichia coli* obtained from the Food Microbiology Laboratory stock culture collection at Kansas State University (KSU). Cultures utilized included Generic *E. coli* ATCC 14763 (Jackie Staats, KSU) and ATCC 23740 (Microb. Genet. Res. Unit, London) and were cultivated by placing one impregnated bead into a 5 ml solution of Difco® Tryptic Soy Broth (TSB) and incubating for 24 h at 35°C. Next, a 0.05 ml loop of the respective culture was inoculated into a 5 ml solution of TSB and incubated for 24 h at 35°C. Following incubation, samples were centrifuged (13,300 x g at 4°C), and the supernatant decanted and the pellet was re-suspended with 50 ml of 0.1% peptone and centrifuged (13,300 x g at 4°C) a final time. The peptone was decanted and the remaining pellet was re-suspended with 50 ml of 0.85% saline solution. 25 ml of respective cultures were mixed together to create a 50 ml cocktail containing 10^9 to 10^{10} CFU/ml of generic *E. coli.* The cell density of this suspension was determined by plating appropriate dilutions on MSA (MacConkey Sorbitol Agar) plates incubated at 37°C for 48 hours. Cultures were confirmed by cultivation on selective and differential media, and biochemical analysis of presumptive colonies using API 20E kits.

Inoculation and Treatment

Fresh beef meat was obtained from the Meats Laboratory at Kansas State University (KSU). A select top round roast stored at 4°C was aseptically cut into ca. 1 in. cubes with a total surface area of 38.5 cm^2. Individual pieces were aseptically placed in a previously sterilized hanging device. Samples were inoculated inside a “bio-containment” chamber by “misting” the surface of the meat with approximately 10 ml of the inoculum. This was done ensuring that all 6 sides of each piece of meat received the same exposure to the inoculum. Samples were held for 30 min at room temperature in a sterile laminar flow cabinet (SterilGARD II ®, The Baker Company, Sanford, ME) to allow proper bacterial attachment to the surface of the meat. Meat samples were treated with $_{CP}CO_2$ inside an experimental laboratory model of a vessel (Appendix A). After safely closing the vessel, meat samples were treated with the general procedure (Appendix B) with the following specifications:

- 1300 psi for 5 min at 36°C
- 1800 psi for 7 min at 36°C
- 2400 psi for 3 min at 36°C

All of these treatments were additionally tested for increased lethality effects after storage under modified atmospheric packaging conditions. Immediately after CO_2 treatment,

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for the 2400 psi treatment only, five samples were placed individually in 3 ml standard barrier 10 x 15 in Nylon/PE bags, vacuum packaged and stored in a display case refrigerator at 4°C). From every treatment, an additional sample was flushed with 100% CO_2 for 10 seconds placed in a 3 ml standard barrier 10 x 15 in Nylon/PE bag, heat-sealed and placed under the same storage conditions. Samples were analyzed for bacterial populations after 1, 2, 4, 6 and 10 days of storage. A non-treated inoculated meat sample was microbiologically analyzed as the control. Statistical Analysis was conducted in two replications with a Split Plot Design using the General Linear Model from SAS (SAS, 2003).

Pressure and temperatures during the study were measured in psi and °C, respectively. These parameters were recorded by an OM-CP-Quadprocess 4 channel 4-20 mA Data Logger (Omega Engineering, Inc.; Stamford, CT) and electronically stored with the Omega® Data Logging Software Ver. 2.00.43c for Windows®. The study was conducted at the KSU Food Safety Processing Laboratory. Colony forming units were converted into Log and reductions were calculated as the difference between respective non-treated controls and the average of their treated duplicates.

Microbiological Analysis

After exposure and storage times, samples were weighted, diluted with 50 ml of 0.1% sterile peptone water (PW) and homogenized in a stomacher for one minute. After homogenization, samples were serially diluted in sterile PW and spiral plated onto MacConkey Sorbitol Agar (MSA; Difco, Detroit, MI) to enumerate residual populations of generic *E. coli*. Plates were incubated at 37°C for 48 hours. The colony forming units were enumerated manually using a spiral plate dark field Quebec colony counter (Model 330; American optical company, Buffalo, NY).

Results and Discussion

$_{CP}CO_2$ application at 36°C yielded similar recoveries (P≥0.05) for generic *E. coli* populations in non-packaged (NP) beef cube samples exposed to 1300 psi for 5 min and 2400 psi $_{CP}CO_2$ for 3 min, with 6.90 and 6.92 log CFU/g, respectively. 1800 psi $_{CP}CO_2$ for 7 min showed the lowest level of recoveries (P<0.05) with 6.69 log CFU/g. However, when compared to their inoculated controls (7.75, 8.13, and 7.76 log CFU/g, respectively), 2400 psi $_{CP}CO_2$ for 3 min achieved the highest level of lethality among non-packaged samples, reducing generic *E. coli* populations by 1.22 log cycles (P<0.05). These results shown in Table 5 suggest that greater pressures have a larger bactericidal effect when compared with time of exposure.

After flushing with 100% CO_2, 2400 psi $_{CP}CO_2$ for 3 min consistently achieved the highest reductions among treatments (P<0.05), reaching the optimum after 6 days of storage, with an additional lethality of 1.72 log cycles reduction for a total reduction of 2.94 log cycles. In all cases, flushing with 100% CO_2 prior to storage at 4°C contributed to further reduce counts after 1, 2, 4, 6, and 10 days of storage. However, there were no statistical

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differences in reduction levels attributable to refrigerated storage time within any of the treatments for the 100% CO_2 flushed packages (P≥0.05).

Table 5. Least Square Means of Generic *E. coli* Recoveries and Reductions in Fresh Meat Cubes Exposed to $_{CP}CO_2$ at 36°C.

Treatments	*Temp.*	*Time*	*Control (Log CFU/g)*	*Recoveries (Log CFU/g)*	*Log Reductions*
2400 psi	*36°C*	*3 min*	*8.13*	*6.92*	*1.21*
2400 psi + VP day 1	*36°C*	*3 min*	*8.13*	*6.72*	*1.41*
2400 psi + 100 % CO_2 day 1	*36°C*	*3 min*	*8.13*	*6.26*	*1.87*
2400 psi + VP day 2	*36°C*	*3 min*	*8.13*	*Lost **	
2400 psi + 100 % CO_2 day 2	*36°C*	*3 min*	*8.13*	*5.45*	*2.68*
2400 psi + VP day 4	*36°C*	*3 min*	*8.13*	*5.92*	*2.21*
2400 psi + 100 % CO_2 day 4	*36°C*	*3 min*	*8.13*	*5.33*	*2.80*
2400 psi + VP day 6	*36°C*	*3 min*	*8.13*	*5.82*	*2.31*
2400 psi + 100 % CO_2 day 6	*36°C*	*3 min*	*8.13*	*5.19*	*2.94*
2400 psi + VP day 10	*36°C*	*3 min*	*8.13*	*5.23*	*2.90*
2400 psi + 100 % CO_2 day 10	*36°C*	*3 min*	*8.13*	*5.28*	*2.85*
1,800 psi	*36°C*	*7 min*	*7.76*	*6.69*	*1.07*
1800 psi + 100 CO_2 day 1	*36°C*	*7 min*	*8.13*	*6.45*	*1.31*
1800 psi + 100 CO_2 day 2	*36°C*	*7 min*	*8.13*	*6.09*	*1.67*
1800 psi + 100 CO_2 day 4	*36°C*	*7 min*	*8.13*	*5.84*	*1.92*
1800 psi + 100 CO_2 day 6	*36°C*	*7 min*	*8.13*	*5.75*	*2.01*
1800 psi + 100 CO_2 day 10	*36°C*	*7 min*	*8.13*	*5.76*	*2.00*
1,300 psi	*36°C*	*5 min*	*7.75*	*6.90*	*0.85*
1300 psi + 100 CO_2 day 1	*36°C*	*5 min*	*7.75*	*6.32*	*1.43*
1300 psi + 100 CO_2 day 2	*36°C*	*5 min*	*7.75*	*5.82*	*1.93*
1300 psi + 100 CO_2 day 4	*36°C*	*5 min*	*7.75*	*5.73*	*2.02*
1300 psi + 100 CO_2 day 6	*36°C*	*5 min*	*7.75*	*5.59*	*2.16*
1300 psi + 100 CO_2 day 10	*36°C*	*5 min*	*7.75*	*5.47*	*2.28*

Average of 1 replication with two duplicates.
**During depressurization of the vessel, sample was expelled through the exhaust outlet.*

Vacuum packaging (VP) was only tested after the 2400 psi $_{CP}CO_2$ for 3 min treatment. VP yielded additional lethality effects, with a marginal reduction of 0.22 log cycles after 1 day of refrigerated storage, achieving the highest additional lethality after 10 days of refrigerated storage with an additional reduction of 1.68 log cycles (P<0.05), achieving a total reduction of 2.9 log.

Flushing with 100% CO_2 was more effective when compared to vacuum packaging, (P<0.05). Additional 1.73, 1.06, and 1.43 log reductions were observed after 6 days of display storage for the 2400 psi, 1800 psi, and 1300 psi, respectively (Figure 4). After application of 2400 psi $_{CP}CO_2$ for 3 min, packages flushed with 100% CO_2 achieved 2.94 log

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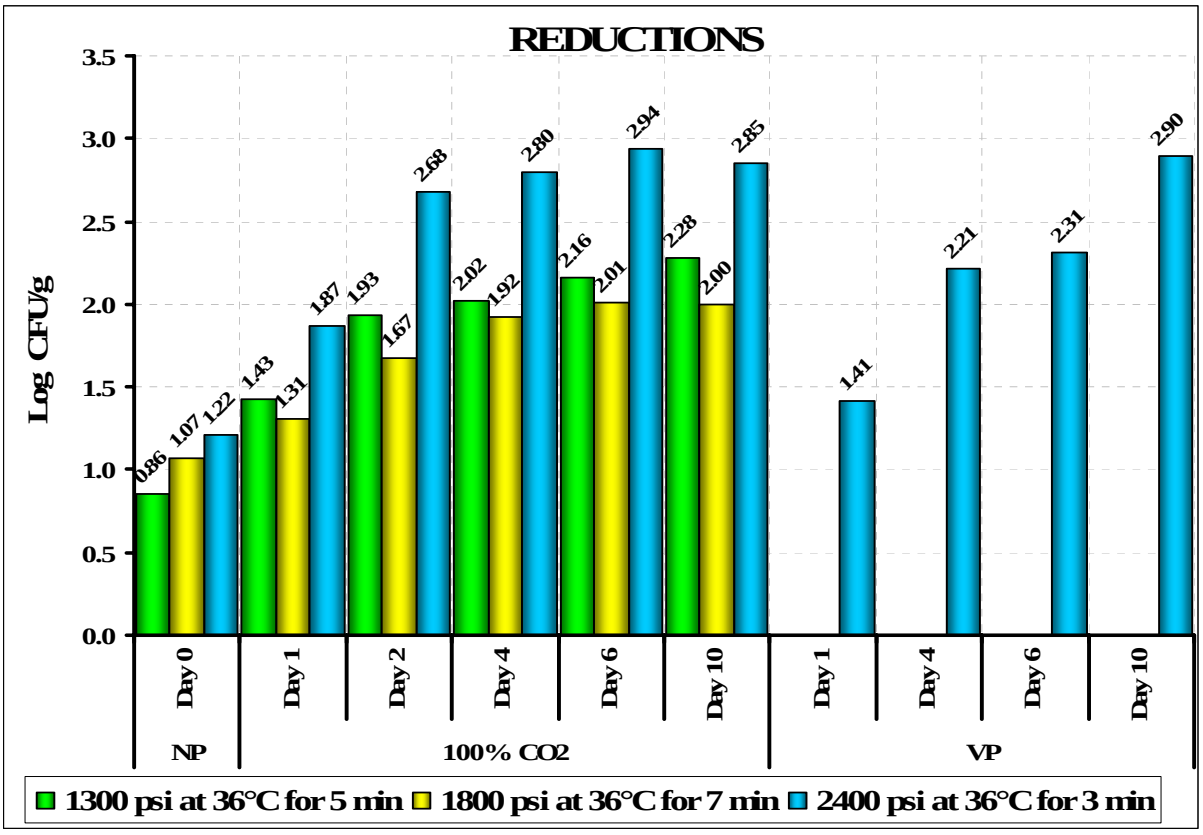
reductions after a 6 day storage period. Vacuum packaging (VP) reached approximately the same level of reductions (2.90 log) only after 10 days of storage (P≥0.05).

Figure 4.Interaction of $_{CP}CO_2$ exposure (1300 psi for 5 min, 1800 psi for 7 min, 2400 psi for 3 min) at 36°C with refrigerated storage (Day 0, 1, 2, 4, 6, and 10) by Type of Packaging (100% Flushed CO_2 and Vacuum Package) on Average Generic *E. coli* Reductions (Log CFU[a]/g) in Fresh Meat Cubes.



Based on informal observations, it was perceived that regardless of the pressure used, after $_{CP}CO_2$ application, meat samples appeared to have a slightly brown discoloration on the exterior surface.

The interior of several samples was exposed by cutting the cubes in half, the cross section from treated trimmings revealed a normal purplish color with a brown layer next to the surface of the meat (Figure 4); in uncut meat, the deoxymyoglobin form predominates in a normal muscle. When fresh meat is cut, myoglobin is in its reduced form (purple); on exposure to air, oxygen binds rapidly to the free sixth co-ordination binding site induced by the ferrous state, forming oxymyoglobin (Bright cherry red). Upon diffusion of oxygen deeper into the muscle tissue, a layer of oxymyoglobin is formed on the surface of the meat. At low oxygen partial pressures, where oxygen concentrations range between 0.5% and 2%, myoglobin is rapidly reversed to metmyoglobin resulting in a brown layer on the surface of

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the meat. Meat cubes, however, bloomed to a red cherry color after being exposed to air for few minutes (Figure 6).

Figure 5. Meat Cubes after $_{CP}CO_2$ Application (A). Cross section revealed a normal purplish color with a brown layer next to the surface of the meat (B).

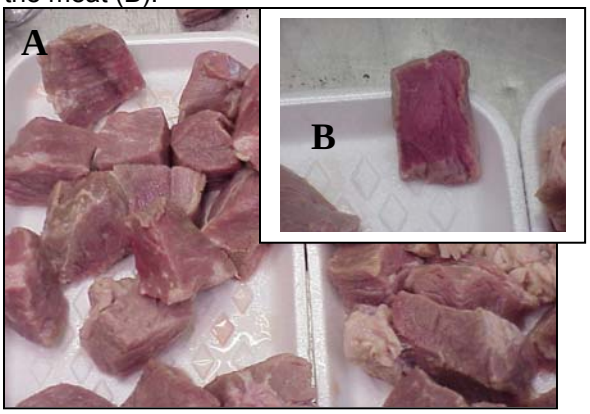

Figure 6. Bloomed Meat Cubes. After $_{CP}CO_2$ application, meat cubes bloomed to a red cherry color after being exposed to air for few minutes.

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Part 4. Residual Populations of *E. coli* O157:H7 in Challenged Fresh Beef Trimmings Treated with $_{CP}CO_2$

Materials and Methods

Preparation of Samples

Bacterial cultures in this study included three strains of *Escherichia coli* O157:H7 obtained from the Food Microbiology Laboratory stock culture collection at Kansas State University (KSU). Cultures used included *E. coli* O157:H7 ATCC 43890 (Jackie Staats, KSU), ATCC 43895, and KSU01 (CDC).

Stock cultures were cultivated by placing one impregnated bead into a 5 ml solution of Difco® Tryptic Soy Broth (TSB) and incubating for 24 h at 35°C. Next, a 0.05 ml loop of the respective culture was inoculated into a 5 ml solution of TSB and incubated for 24 h at 35°C. Following incubation, samples were centrifuged (13,300 x g at 4°C), and the supernatant decanted and the pellet was re-suspended with 50 ml of 0.1% peptone and centrifuged (13,300 x g at 4°C) a final time. The peptone was decanted and the remaining pellet was re-suspended with 20 ml of 0.85% saline solution. 20 ml of respective cultures were mixed together to create a 60 ml cocktail containing 10^9 to 10^{10}CFU/ml of *E. coli* O157:H7. The cell density of this suspension was determined by plating appropriate dilutions on MSA (MacConkey Sorbitol Agar) plates incubated for 48 hours at 35°C. Cultures were confirmed by cultivation on selective and differential media, and biochemical analysis of presumptive colonies using API 20E kits.

Inoculation and Treatment

Fresh beef meat was obtained from the Meats Laboratory at Kansas State University (KSU). A select top round roast stored at 4 °C was aseptically cut into excised cores (3.5 cm diameter x c.a. 3.5 cm thick). Individual pieces were aseptically placed in a previously sterilized hanging device. Samples were inoculated inside a “bio-containment” chamber by “misting” the surface of the meat with approximately 10 ml of the inoculum. This was done ensuring that all sides of the sample received the same exposure to the inoculum. Samples were held for 30 min at room temperature in a sterile laminar flow cabinet (SterilGARD II ®, The Baker Company, Sanford, ME) to allow proper bacterial attachment to the surface of the meat.

Meat samples were treated with $_{CP}CO_2$ inside the experimental laboratory model of a vessel (Appendix A). After safely closing the vessel, meat samples were treated as follows:

- 1100 psi for 5 min at 36°C
- 1500 psi for 1 min at 36°C
- 1600 psi for 7 min at 36°C
- 2100 psi for 3 min at 36°C

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All of these treatments were additionally tested for increased lethality effects after storage under modified atmospheric packaging conditions. Immediately after every treatment, a sample was placed individually in a 3 ml standard barrier 10 x 15 in Nylon/PE bag, flushed with 100% CO_2 for 10 seconds, heat-sealed, and stored in a display case refrigerator at 4°C for 10 days). Samples were analyzed for bacterial populations after 1, 2, 4, and 6 days of storage. A non-treated inoculated meat sample was microbiologically analyzed as the control.

Pressure and temperatures during the study were measured in psi and °C, respectively. These parameters were recorded by an OM-CP-Quadprocess 4 channel 4-20 mA Data Logger (Omega Engineering, Inc.; Stamford, CT) and electronically stored with the Omega® Data Logging Software Ver. 2.00.43c for Windows®. The study was conducted at the KSU Food Safety Processing Laboratory. Statistical Analysis was conducted in a Split Plot Design with two replications using the General Linear Model from SAS (SAS, 2003).

Microbiological Analysis

After treatment, samples were diluted with 50 ml of 0.1% sterile peptone water (PW) and homogenized in a stomacher for one minute. After homogenization, samples were serially diluted in sterile PW and individually spiral plated onto MacConkey Sorbitol Agar (MSA; Difco, Detroit, MI) to enumerate *E. coli* O157:H7. Plates were incubated at 37°C for 48 hrs. The colony forming units were manually enumerated using a spiral plate dark field Quebec colony counter (Model 330; American optical company, Buffalo, NY). Colony forming units were converted into log and reductions were calculated as the difference between the averages of respective non-treated controls and treated samples.

Results and Discussion

Results from this experiment indicate that $_{CP}CO_2$ application was able to achieve a immediate reduction on *E. coli* O157:H7 populations of at least 0.60 log cycles on fresh beef core samples, no differences among the treatments utilized was statistically different ($P<0.05$).

As illustrated in Table 5, the highest lethality immediately after $_{CP}CO_2$ application was achieved by pressurizing at 1100 psi for 5 min, reaching 0.81 log. After pressurizing at 1100 psi for 5 min, packaging with 100% CO_2 also demonstrated to be effective to further reducing bacterial counts of *E. coli* O157:H7 by additional 1.28 log, obtained after four days of refrigerated storage, for a total reduction of 2.14 log. However, reductions achieved after two days of storage were statistically similar ($P\geq0.05$) for those treatments with the best numerical reductions immediately after $_{CP}CO_2$ application (0.81 for 1100 psi for 5 min and 0.81 for 1500 psi for 1 min). Reductions achieved across all treatments in this experiment (Figure 7) were slightly lower when compared to those achieved in the previous experiment, possibly due to differences in the physiological responses of *E. coli* O157:H7 and generic *E. coli*.

Meat core samples in this experiment were approximately the same surface area as the 1 inch square cubes tested in the prior experiments (38.5 and 38.7 cm^2, respectively) and should have not contributed to the differences seen in bacterial kill between the experiments. In

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this case, time or pressure did not reveal significant differences on *E. coli* O157:H7 reductions, with all time/pressure treatment combinations, providing <1 Log CFU/cm^2.

Table 5. Least Square Means of Generic *E. coli* Recoveries and Reductions in Fresh Meat Cubes Exposed to 1300 psi CO_2 at 36°C.

Treatments	Temp.	Time	*Recoveries (Log CFU/cm^2)*	*Log Reductions*
1100 psi	*36°C*	*5 min*	*6.80*	*0.81*
1100 psi + 100% CO2 day 1	*36°C*	*5 min*	*6.23*	*1.37*
1100 psi + CO2 day 2	*36°C*	*5 min*	*5.50*	*2.10*
1100 psi + CO2 day 4	*36°C*	*5 min*	*5.46*	*2.14*
1100 psi + CO2 day 6	*36°C*	*5 min*	*5.47*	*2.13*
1500 psi-1 min	*36°C*	*1 min*	*6.53*	*0.81*
1500 psi + CO_2 day 1	*36°C*	*1 min*	*6.29*	*1.05*
1500 psi + CO_2 day 2	*36°C*	*1 min*	*5.50*	*1.84*
1500 psi + CO_2 day 4	*36°C*	*1 min*	*5.39*	*1.95*
1500 psi + CO_2 day 6	*36°C*	*1 min*	*5.96*	*1.38*
1600 psi	*36°C*	*7 min*	*6.19*	*0.74*
1600 psi + CO2 day 1	*36°C*	*7 min*	*5.72*	*1.21*
1600 psi + CO2 day 2	*36°C*	*7 min*	*5.90*	*1.03*
1600 psi + CO2 day 4	*36°C*	*7 min*	*5.37*	*1.56*
1600 psi + CO2 day 6	*36°C*	*7 min*	*5.54*	*1.39*
2100 psi	*36°C*	*3 min*	*6.33*	*0.60*
2100 psi + CO_2 day 1	*36°C*	*3 min*	*6.67*	*0.26*
2100 psi + CO_2 day 2	*36°C*	*3 min*	*6.84*	*0.09*
2100 psi + CO_2 day 4	*36°C*	*3 min*	*5.89*	*1.04*
2100 psi + CO_2 day 6	*36°C*	*3 min*	*5.59*	*1.34*

Average of 1 replication with two duplicates.

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Figure 7. Interaction of $_{CP}CO_2$ exposure (1100 psi for 5 min, 1500 psi for 1 min, 1600 psi for 3 min, 2100 psi for 3 min) at 36°C with refrigerated storage (Day 0, 1, 2, 4, 6) by Type of Packaging (Not Packaged [NP], 100% Flushed CO_2 [100% CO2], and Vacuum Packaged [VP]) on Average *E. coli* O157:H7 Reductions in Fresh Meat Cubes.

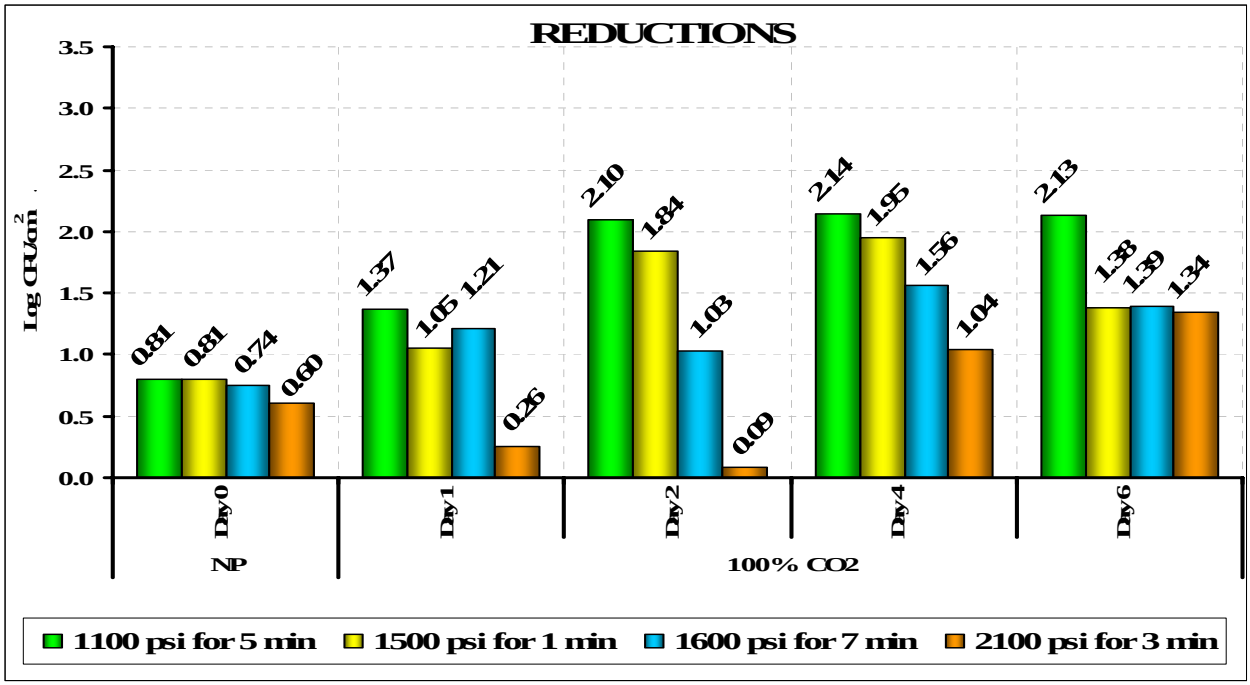

Table 6 illustrates a summary for the lethality obtained from all previous studies. Figure 18 illustrates the results of the studies performed in filter paper disks (A) and in beef trimmings and ground beef (B).

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Table 6. Summary Least Square Means of Bacterial Reductions from Preliminary Studies Sorted by Pressure and Time of Exposure.

Studies	*Code*	*Pressure (psi)*	*Time of Exposure (min)*	*Temp. (°C)*	*Microorganisms*	*RED. (log)*
Study 2 Part 2	*A1*	*1000*	*5*	*29*	*E. coli O157:H7*	*1.06*
Study 2 Part 2	*A2*	*1000*	*10*	*29*	*E. coli O157:H7*	*0.86*
Study 2 Part 2	*A3*	*1000*	*240*	*29*	*E. coli O157:H7*	*0.66*
Study 2 Part 4	*K*	*1100*	*5*	*36*	*E. coli O157:H7*	*0.81*
Study 1	*L2*	*1200*	*3*	*36*	*E. coli O157:H7*	*6.02*
Study 1	*L1*	*1200*	*3*	*36*	*Generic E. coli*	*5.70*
Study 1	*L4*	*1200*	*3*	*36*	*L. monocytogenes*	*4.81*
Study 1	*L3*	*1200*	*3*	*36*	*Salmonella spp.*	*5.80*
Study 2 Part 3	*G*	*1300*	*5*	*36*	*Generic E. coli*	*0.86*
Study 2 Part 1	*A1*	*1470*	*5*	*29*	*Aerobic Plate Count*	*0.74*
Study 2 Part 4	*J*	*1500*	*1*	*36*	*E. coli O157:H7*	*0.81*
Study 2 Part 4	*I*	*1600*	*7*	*36*	*E. coli O157:H7*	*0.74*
Study 1	*M2*	*1700*	*3*	*36*	*E. coli O157:H7*	*6.33*
Study 1	*M1*	*1700*	*3*	*36*	*Generic E. coli*	*5.70*
Study 1	*M4*	*1700*	*3*	*36*	*L. monocytogenes*	*5.04*
Study 1	*M3*	*1700*	*3*	*36*	*Salmonella spp.*	*5.81*
Study 2 Part 3	*F*	*1800*	*7*	*36*	*Generic E. coli*	*1.07*
Study 2 Part 1	*B1*	*2000*	*7.5*	*36*	*Aerobic Plate Count*	*1.97*
Study 2 Part 1	*B2*	*2000*	*15*	*36*	*Aerobic Plate Count*	*1.88*
Study 2 Part 4	*H*	*2100*	*3*	*36*	*E. coli O157:H7*	*0.60*
Study 1	*N2*	*2100*	*3*	*36*	*E. coli O157:H7*	*5.16*
Study 1	*N1*	*2100*	*3*	*36*	*Generic E. coli*	*4.04*
Study 1	*N4*	*2100*	*3*	*36*	*L. monocytogenes*	*5.60*
Study 1	*N3*	*2100*	*3*	*36*	*Salmonella spp.*	*5.81*
Study 2 Part 3	*E*	*2400*	*3*	*36*	*Generic E. coli*	*1.22*
Study 2 Part 1	*C1*	*3000*	*5*	*29*	*Aerobic Plate Count*	*0.67*
Study 2 Part 1	*C2*	*3000*	*5*	*36*	*Aerobic Plate Count*	*1.83*
Study 2 Part 1	*D*	*5000*	*7.5*	*36*	*Aerobic Plate Count*	*2.23*

In the next study, the effects of $_{CP}CO_2$ application in beef trimmings was evaluated in relation to some of the organoleptic and proximate characteristics of raw and cooked beef patties manufactured from treated beef trimmings.

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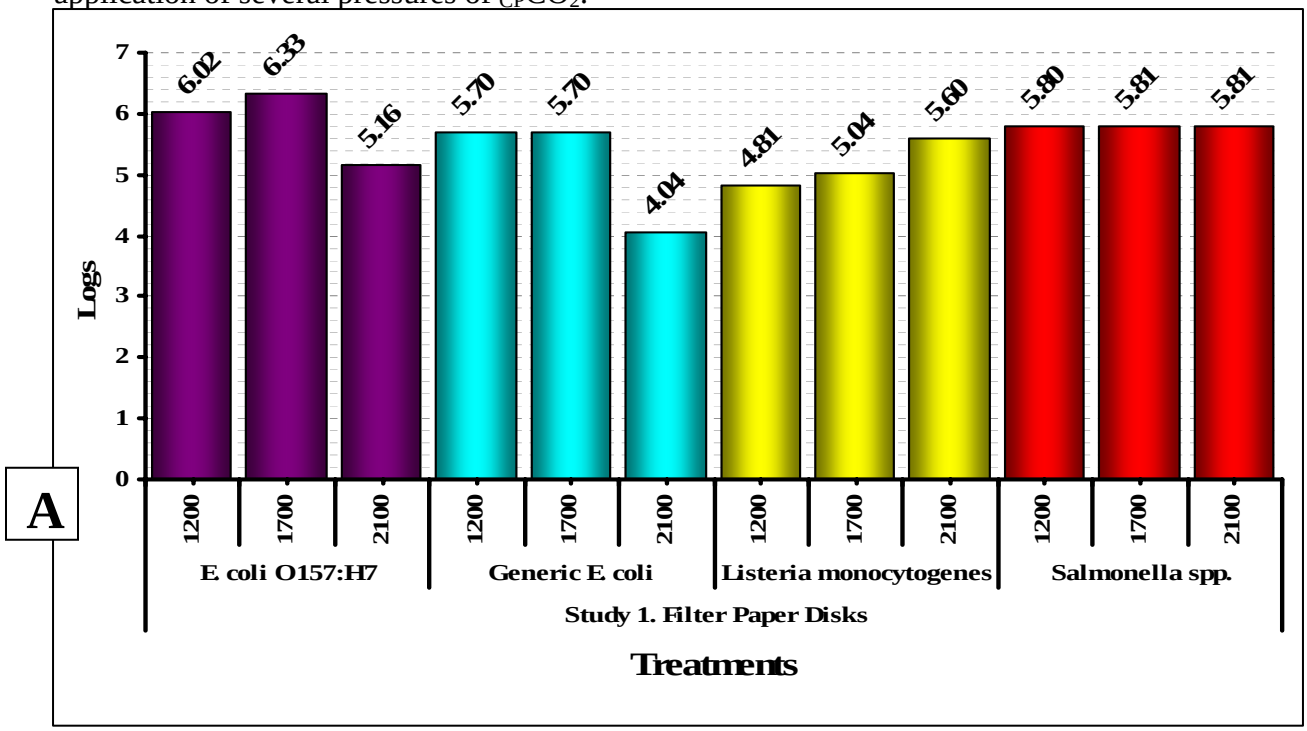
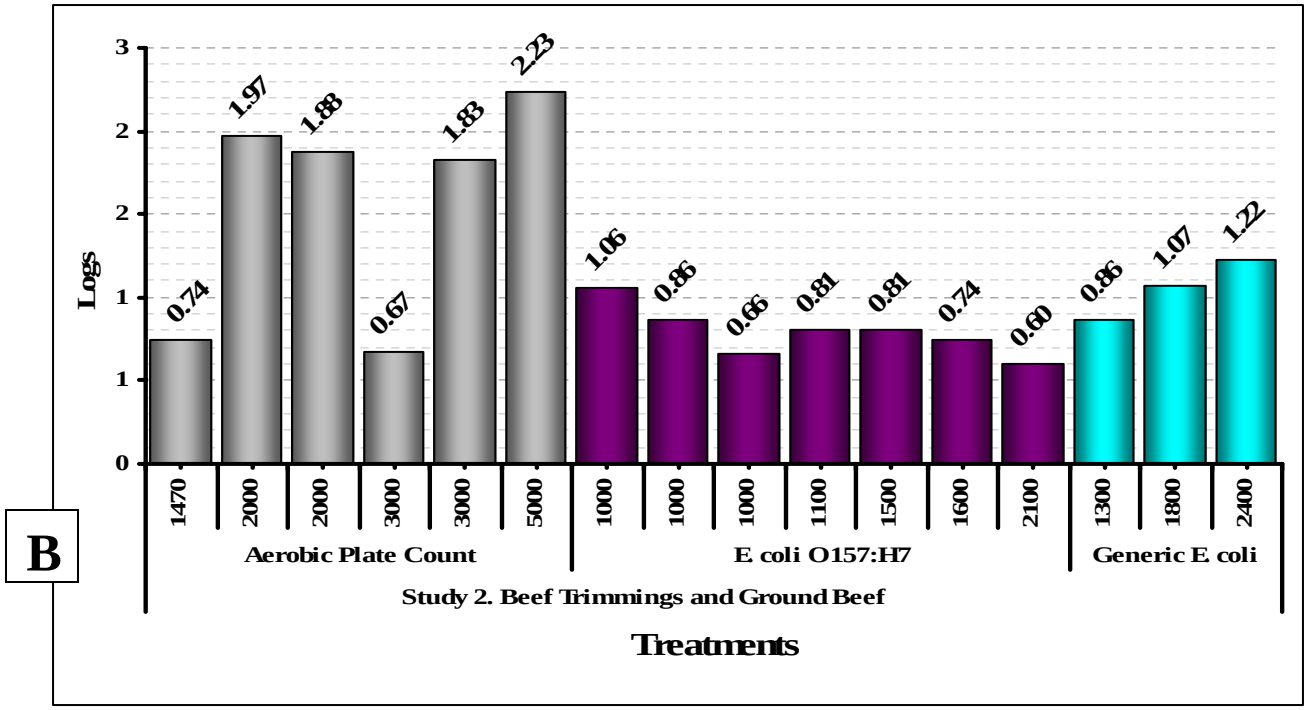
Figure 8. Summary of Bacterial Reductions from preliminary studies, sorted by microorganism, performed in filter paper disks (A) and in beef trimmings and ground beef (B) obtained by application of several pressures of $_{CP}CO_2$.





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Quality and Shelf Life Effects of Controlled Phase Carbon Dioxide ($_{CP}CO_2$) Application on Beef Trimmings in Ground Beef

Objective

This experiment was designed to evaluate the quality and shelf life effects of controlled phase carbon dioxide ($_{CP}CO_2$) application on beef trimmings in further ground beef used alone or in combination with different packaging atmospheres. Organoleptic and sensory effects on treated raw and cooked ground beef patties manufactured with the treated trim were analyzed.

Materials and Methods

Preparation of Samples

85/15 lean/fat beef trimmings stored at 4°C were obtained from the Meats Laboratory at Kansas State University (KSU). Beef trimmings were cut into ca. 1 in. cubes and vacuum packaged in 1000 g batches and stored at 0°C until treated.

Treatment of Samples

Meat samples were treated with $_{CP}CO_2$ inside an experimental vessel (Appendix A), according to the general protocol (Appendix B), a non-treated control (CTRL) was included in the design to be compared with the following treatments:

- 750 psi for 15 min at 36°C
- 1500 psi for 15 min at 36°C

Experimental Protocol

Due to the volume constrictions of the CO_2 reactor, which only allowed for 1000 g to be treated at a time, each treatment was applied in two separate experimental runs (RUN 1, & RUN 2) for every treatment (B1, & B2). See Figure 9.

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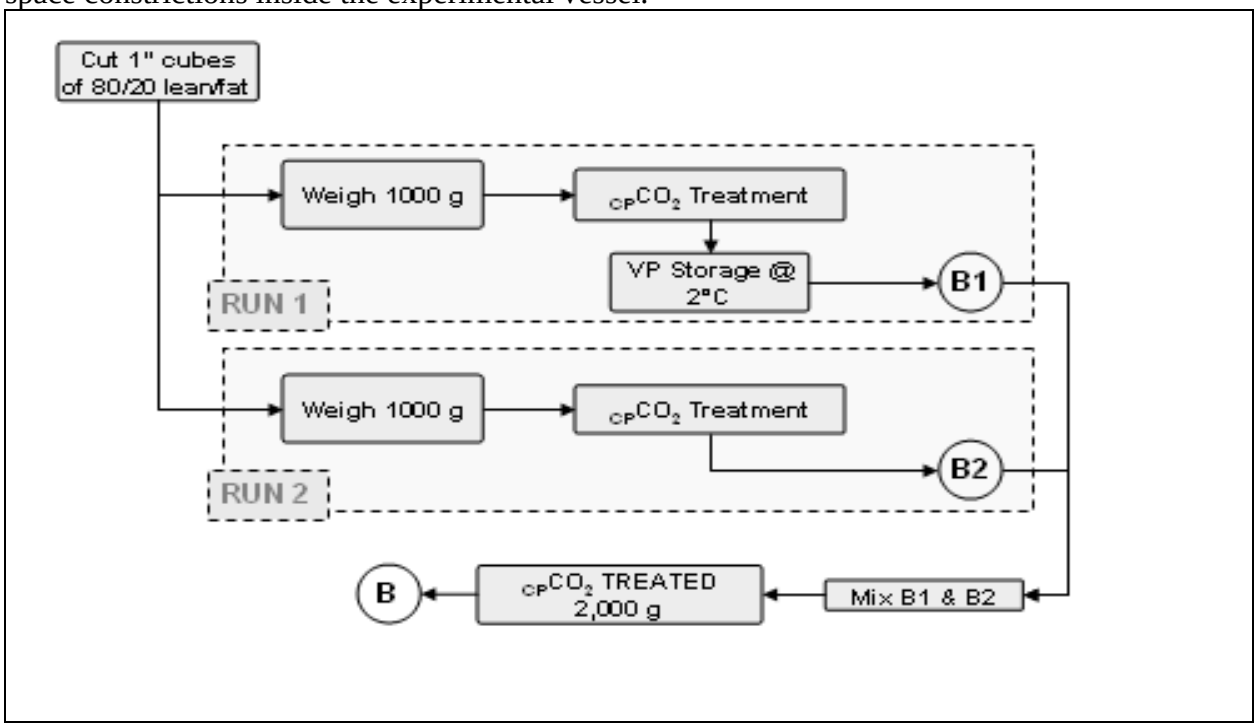
Figure 9. Flow Diagram of Experimental Runs. 85/15 lean/fat beef trimmings from the same batch cut into ca. 1 in. cubes were exposed to $_{CP}CO_2$ in two separate experimental runs due to space constrictions inside the experimental vessel.



After the first experimental run of beef trimmings was exposed to $_{CP}CO_2$ (RUN 1), treated samples were vacuum packaged and stored at 2°C in order to preserve the anaerobic conditions and the cold temperature of the beef trimmings (B1).

Same procedure was followed to conduct the second experimental run. After the second experimental run (RUN 2) was finished and the second batch of beef trimmings had been treated (B2), beef trimmings from both experimental runs (B1 & B2) were placed inside a containment chamber flushed to saturation with CO_2, where they were thoroughly mixed to obtain a total weight of 2000 g of treated beef trimmings (B).

Treated beef trimmings from each treatment inside the chamber were immediately ground two consecutive times through a 3/8" plate in a ½ HP Cabella's Grinder while the chamber was saturated with CO_2.

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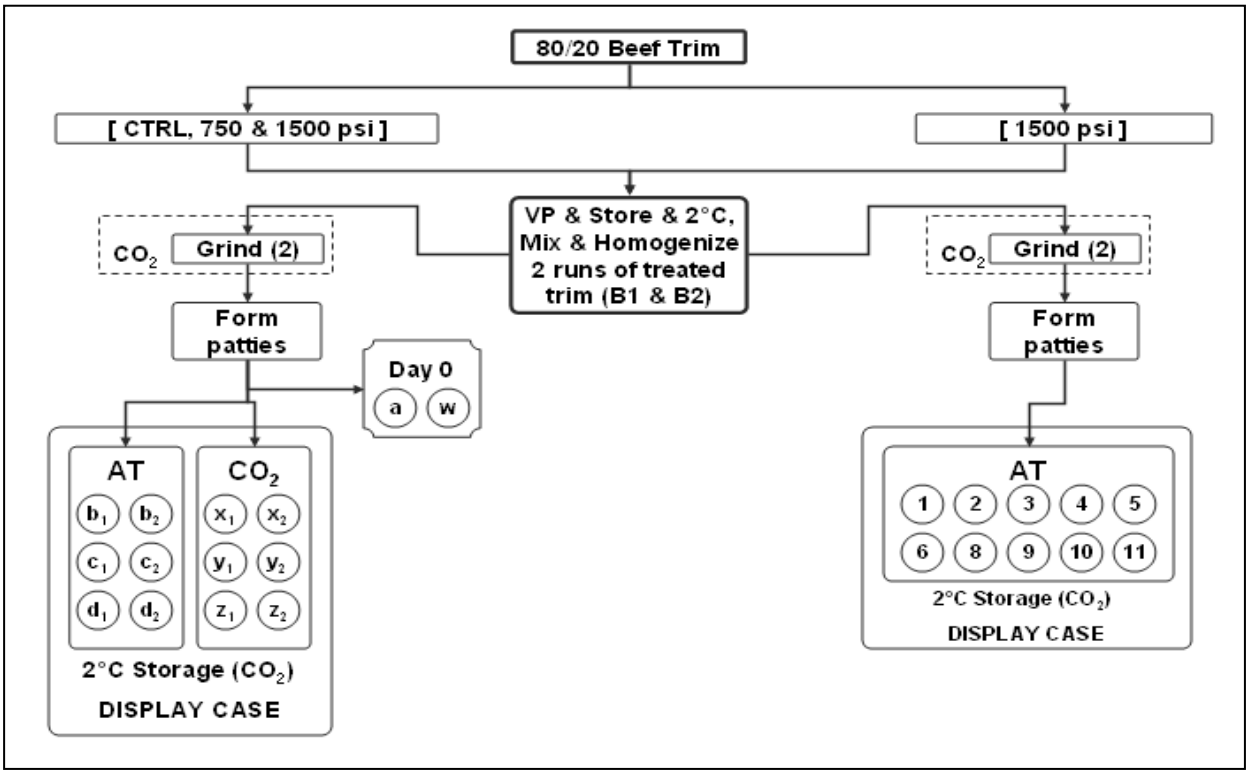
Figure 1. Flow Diagram of Experimental Design. Patties manufactured with beef trimmings exposed to various pressure treatments of $_{CP}CO_2$ (Control [CTRL], 750 psi, 1500 psi) were allocated to two types of packaging atmospheres under refrigerated display storage.



Immediately after grinding, ground meat was formed into fourteen 114 g patties with a manual patty former. Figure 19 shows the experimental design for this study. Two patties were immediately placed on a Styrofoam tray with PVC overwrap and labeled as Day 0 (D0) (a,w). Twelve patties were placed on a Styrofoam trays and randomly subdivided into two groups; six of them were packaged with 100% flushed CO_2 (CO_2) with an oxygen scavenger (Multisorb Technologies), and six more were placed in a Styrofoam tray with PVC overwrap (AT).

For every treatment (CTRL, and 750 &1500 psi $_{CP}CO_2$), paired patties from each package type (AT & CO_2) were labeled Day 1, Day 3 and Day 5 (D1, D3, D5). Patties were stored in a lighted display case (Model DMF8, Tyler Refrigeration, Niles, MI) with continuous lighting (intensity 1,614 lux; Philips Deluxe Warm White 40-W fluorescent lights; Philips Lighting, Salina, KS) at 2°C.

One set of the paired raw patties (a, b_1, c_1, d_1, x_1, y_1, z_1) was analyzed for instrumental color, proximate analysis (Fat Crude Protein, Crude Fiber and Moisture), thiobarbituric acid reactive substances (TBARS), pH, and microbiological counts after completion of each designated storage time.

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Table 7. Analyses Conducted in Raw and Cooked Patties Treated with $_{CP}CO_2$.

Patties	*Subgroup*	*Analyses*
a	*[Raw] AT D0*	*I-Color, Headspace, Proximate, TBARS, pH & Micro*
w	*[Cooked] AT D0*	*I-Color, Proximate, TBARS*
b_1	*[Raw] AT D1*	*I-Color, Headspace, Proximate, TBARS, pH & Micro*
b_2	*[Cooked] AT D1*	*I-Color, Proximate, TBARS*
c_1	*[Raw] AT D3*	*I-Color, Headspace, Proximate, TBARS, pH & Micro*
c_2	*[Cooked] AT D3*	*I-Color, Proximate, TBARS*
d_1	*[Raw] AT D5*	*I-Color, Headspace, Proximate, TBARS, pH & Micro*
d_2	*[Cooked] AT D5*	*I-Color, Proximate, TBARS*
x_1	*[Raw] CO_2 D1*	*I-Color, Headspace, Proximate, TBARS, pH & Micro*
x_2	*[Cooked] CO_2 D1*	*I-Color, Proximate, TBARS*
y_1	*[Raw] CO_2 D3*	*I-Color, Headspace, Proximate, TBARS, pH & Micro*
y_2	*[Cooked] CO_2 D3*	*I-Color, Proximate, TBARS*
z_1	*[Raw] CO_2 D5*	*I-Color, Headspace, Proximate, TBARS, pH & Micro*
z_2	*[Cooked] CO_2 D5*	*I-Color, Proximate, TBARS*

The other set of paired patties (w, b_2, c_2, d_2, x_2, y_2, z_2) were cooked on a George Foreman Grill to an internal temperature of 160°F (71.1°C). Internal temperature was monitored with a digital thermometer. Following cooking, ground beef patties were allowed to cool for approximately 3 minutes and were cut in half to be evaluated for internal instrumental color, proximate analysis and thiobarbituric acid reactive substances (TBARS). Patties were analyzed according specifications in the next table.

The last set of 11 patties prepared from the 1500 psi $_{CP}CO_2$ treatment were placed on a Styrofoam tray with PVC overwrap, labeled as Day 3, and placed under refrigerated storage to be cooked prior to sensory analysis.

pH and Microbiological Analysis

Raw patties were analyzed for natural occurring microflora and pH from was conducted at 0, 1, 3, and 5 d of display. From every treatment, 10 g of each sample was diluted with 90 ml of deionized water for microbiological analysis. A second 5 g sample was diluted with 5 ml of 0.1% sterile peptone water (PW) for pH measurement. Both samples were homogenized in a stomacher for one minute. After homogenization, microbiological samples were serially diluted in sterile PW and plated according to the dilution scheme shown in Figure 11.

Samples were plated in duplicates onto APC Petrifilm and ECC Petrifilm (3M Microbiology, St. Paul, MN) and incubated at 35°C for 48 h for Total Aerobic Plate Count (APC) and *E. coli*/coliforms (ECC), respectively. In addition, duplicate DeMan Rogosa Sharp (MRS; Difco, Detroit, MI) agar overlayed plates were incubated in anaerobic conditions at 30°C for 5 days to enumerate Lactic Acid Bacteria (LAB).

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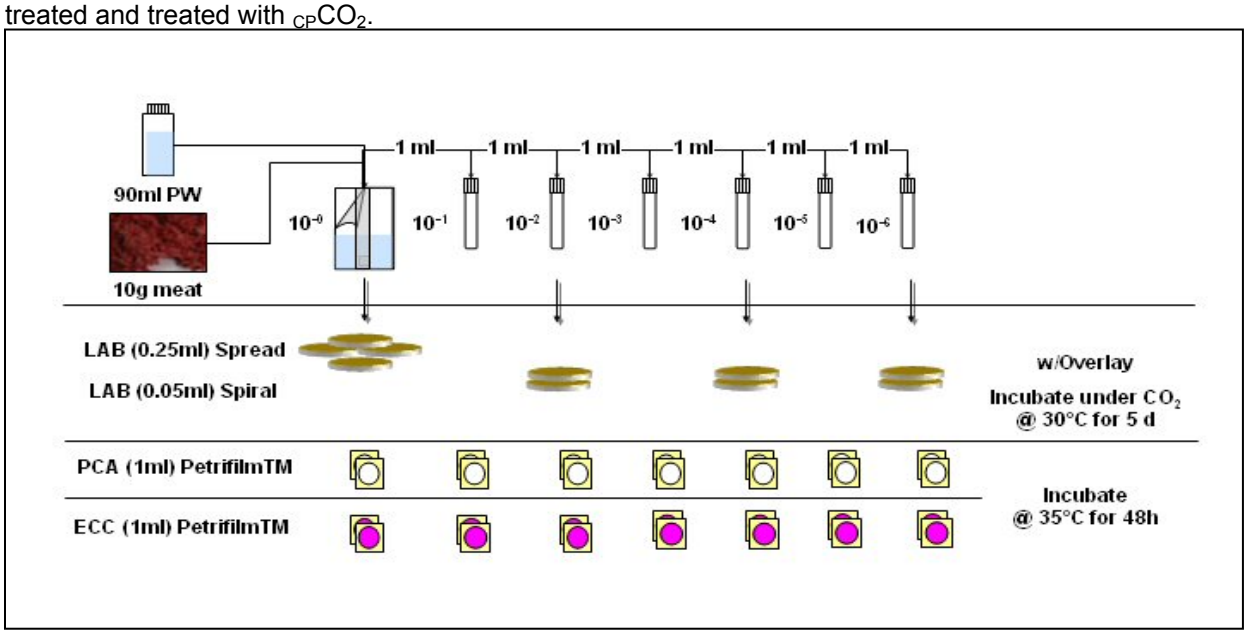
The colony forming units were enumerated using a spiral plate dark field Quebec colony counter (Model 330; American optical company, Buffalo, NY). Colony forming units were converted into log and reductions were calculated as the difference between respective non-treated controls and the average of their three replicates. The study was conducted at the KSU Food Safety Laboratory at Call Hall.

Figure 2. Dilution Scheme for Microbiological Samples analyzed for normal microflora in Raw Patties non-treated and treated with $_{CP}CO_2$.



Instrumental Color

On days 0, 1, 3 and 5 of simulated retail display, instrumental color was evaluated using a HunterLab MiniScan XE Spectrocolorimeter, Model 4500L (Hunter Associates Laboratory Inc., Reston, West Virginia, USA). Samples were read using illuminant A/10◦ observer and evaluated for CIE (L^* a^* and b^*) color values.

In addition, reflectance measurements were taken in the visible spectrum from 580 to 630 nm. The reflectance ratio of 630 nm/580 nm was calculated and used to estimate the oxymyoglobin proportion of the myoglobin pigment (Hunt et al., 1991; Strange et al., 1974).

Before use, the Spectrocolorimeter was standardized using white tile, black tile, and working standards. Three measurements were taken of each sample and averaged for statistical analysis.

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Proximate and CO_2/O_2 Headspace Analysis

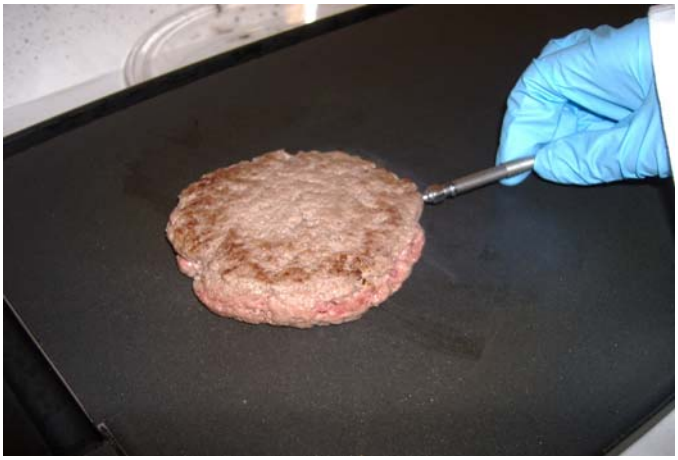
Cooked patties manufactured with beef trimmings treated with $_{CP}CO_2$ were analyzed for proximate analysis as % Moisture, % Crude Protein, and % Crude Fat. In addition, the extent of lipid oxidation was measured as thiobarbituric acid reactive substances (TBARS) as determined by the extraction method of Witte et al. (1970). Duplicate scores from each sample were averaged and expressed as milligrams of malonaldehyde per kilogram of Dry Matter (DM). Finally, CO_2 and O_2 headspace concentrations inside the packages and inside the chamber were measured by a Pack Check™ 650 (Mocon, Minneapolis, MN) CO_2/O_2 Headspace analyzer.

Sensory Analysis

An additional set of 11 patties were prepared from the each $_{CP}CO_2$ treatment and placed on a Styrofoam tray with PVC overwrap. Patties were immediately cooked on a George Foreman Grill (Salton Inc., Lake Forest, IL), since Wheeler and Koohmaraie (1994) found contact grilling to be a highly repeatable cooking method. Patties were cooked to an internal temperature of 160°F (71.1°C). Internal temperature was monitored with a digital thermometer.

Following cooking, ground beef patties were allowed to cool for approximately 3 minutes. After cooling, six samples were cut into equal sizes, then placed on individually coded plates and arranged randomly on a serving tray, which was then taken to another room where the sensory test was conducted in individual booths. The ordering of the plates on each serving tray was random such that each panelist tasted the samples in a different order. The samples were also arranged in a matrix to lessen biases due to the position effect (Eindhoven et al., 1964).

Figure 12. Patties were cooked in a George Foreman Grill to an internal temperature of 160°F (71.1°C). Internal temperature was monitored with a digital thermometer.



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Sensory analysis was conducted on cooked patties by a seven-member trained sensory panel. Samples from each treatment (CTRL, 750 psi, and 1500 psi) were evaluated for Overall Tenderness (OT, 8=extremely tender, 1=extremely tough), Juiciness (J, 8=extremely juicy, 1=extremely dry), Beef Flavor Intensity (BFI, 8=extremely intense, 1=extremely bland), and Off Flavor Intensity (OFI, 8=non, 1=abundant).

For panelists not to experience sensory fatigue, six samples of each product, which is the maximum number of samples that the American Meat Science Association recommends (AMSA 1991), were given to each panelist in a single sitting. Each descriptive flavor profile panelist had a minimum of 120 h of flavor and texture profile training, more than 2,000 h of sensory experience, and extensive experience in testing meat products.

Sampling was conducted in an environmentally controlled room (temperature and relative humidity were controlled at levels of 21 ± 1°C and 55 ± 5%, respectively) partitioned into booths and lighted by a mixture of red (<107.64 lumens) and green (<107.64 lumens) light, at the KSU Sensory Analysis Lab at Weber Hall.

Statistical Analysis

Individual controls for the experiments consisted of non-treated meat samples (CTRL). Statistical Analysis was conducted in a Split Plot Design in a complete randomized design with three replications using the General Linear Model from SAS (SAS, 2003). Treatments were blocked by replication then analyzed for the main effects of antimicrobial treatment, day of display and main effect interactions by the LSMEANS statement. Means and least square means were generated and separated using the PDIFF option of SAS.

Results and Discussion

Impact of $_{CP}CO_2$ Application of Beef Trimmings Prior to Grinding on Normal Microbial Populations of Ground Beef.

Bacteria may become entrapped in meat crevices, which subsequently offer protection against antimicrobial treatments (Lillard, 1988). This dissertation hypothesized that applying pressurized carbon dioxide to meat pieces would cause an expansion of the outer surfaces of meat and would allow further penetration of the gas into the muscle, thereby increasing bacterial reductions in ground beef processed from these trimmings.

Theoretically, the expansion of the meat structure should allow for a greater population of entrapped bacteria to be exposed to the antimicrobial treatment, causing a reduction in bacterial numbers.

Statistical analysis of the results (Table 8) showed $_{CP}CO_2$ at 1500 psi the most effective antimicrobial treatment with the lowest numerical immediate recoveries for the Aerobic Plate

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Count (APC) and Lactic Acid Bacteria (LAB) with 1.45 and 1.02 log CFU/g, respectively ($P<0.05$).

Total Coliform Counts and generic *E. coli* were not recovered in any samples (Detection Limit= 0.4 Log CFU/g). Regardless of packaging type, refrigerated storage time, microorganism tested or any interaction, only pressure showed significance in the model ($P<0.05$).

Table 8. Least Square Means of Average Bacterial Recoveries (Log CFU/g) of Normal Microflora (Aerobic Plate Count and Lactic Acid Bacteria) in Ground Beef Patties Manufactured from $_{CP}CO_2$ Treated Beef Trimmings (1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5).

TREATMENT			Aerobic Plate Count	Lactic Acid Bacteria	Total Coliform	E. coli	pH
Pressure	Atmospheres	2°C Storage					
CTRL	AT	Day 0	2.10^{bc}	1.77^{de}	<0.40	<0.40	5.65
		Day 1	2.10^{bc}	1.97^{bcde}	<0.40	<0.40	5.58
		Day 3	2.23^{abc}	2.00^{abcd}	<0.40	<0.40	5.54
		Day 5	2.33^{a}	2.00^{abcd}	<0.40	<0.40	5.67
	CO_2	Day 1	2.13^{abc}	1.96^{bcde}	<0.40	<0.40	5.69
		Day 3	2.17^{abc}	2.10^{ab}	<0.40	<0.40	5.50
		Day 5	2.03^{c}	2.13^{ab}	<0.40	<0.40	5.62
750 psi	AT	Day 0	2.13^{abc}	2.03^{abc}	<0.40	<0.40	5.64
		Day 1	2.13^{abc}	2.03^{abc}	<0.40	<0.40	5.59
		Day 3	2.20^{abc}	2.10^{ab}	<0.40	<0.40	5.51
		Day 5	2.23^{abc}	2.23^{a}	<0.40	<0.40	5.71
	CO_2	Day 1	2.13^{abc}	1.93^{bcde}	<0.40	<0.40	5.70
		Day 3	2.17	1.93^{bcde}	<0.40	<0.40	5.52
		Day 5	2.27^{ab}	2.07^{ab}	<0.40	<0.40	5.64
1500 psi	AT	Day 0	1.45^{e}	1.03^{h}	<0.40	<0.40	5.60
		Day 1	1.50^{de}	1.50^{fg}	<0.40	<0.40	5.58
		Day 3	1.54^{de}	1.73^{ef}	<0.40	<0.40	5.53
		Day 5	1.67^{e}	1.80^{cde}	<0.40	<0.40	5.65
	CO_2	Day 1	1.46^{de}	1.37^{g}	<0.40	<0.40	5.80
		Day 3	1.50^{de}	1.40^{g}	<0.40	<0.40	5.43
		Day 5	1.43^{d}	1.50^{fg}	<0.40	<0.40	5.56

Total coliforms and Generic E. coli were not detected. Detection Limit = 0.4 log CFU/g
Average of 3 replications.
[ab] *Least square means within a column bearing different superscript letters are different ($P<0.05$).*

Furthermore, lactic acid bacteria counts clearly showed an increase on bacterial populations during refrigerated storage in treated ground beef packaged in aerobic trays. On the other hand, lactic acid bacteria growth in ground beef packages flushed with 100% CO_2 occurred at a slower rate (Figure 12). Specific atmospheric packaging conditions tested as an additional lethality step after 1, 3 and 5 days of refrigerated storage showed only marginal reductions (Figure 13).

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Microbial reductions in ground beef manufactured with treated beef trimmings at $_{CP}CO_2$ at 1500 psi, when compared to those treated with $_{CP}CO_2$ at 750 psi, which were minimal or non-existent (Table 9), showed a better control in reducing bacterial populations immediately after treatment achieving immediate bacterial reductions of 0.65 and 0.77 log on APC and LAB, respectively.

Figure 12. Interaction of $_{CP}CO_2$ exposure (750 psi and 1500 psi for 15 min) at 36°C with refrigerated storage (Day 0, 1, 2, 3, and 4) by Type of Packaging (Not Packaged [NP], 100% Flushed CO_2 [100% CO2], and Vacuum Packaged [VP]) of Beef Trimmings on pH and Average Bacterial Recoveries in Ground Beef (Log CFU/g).

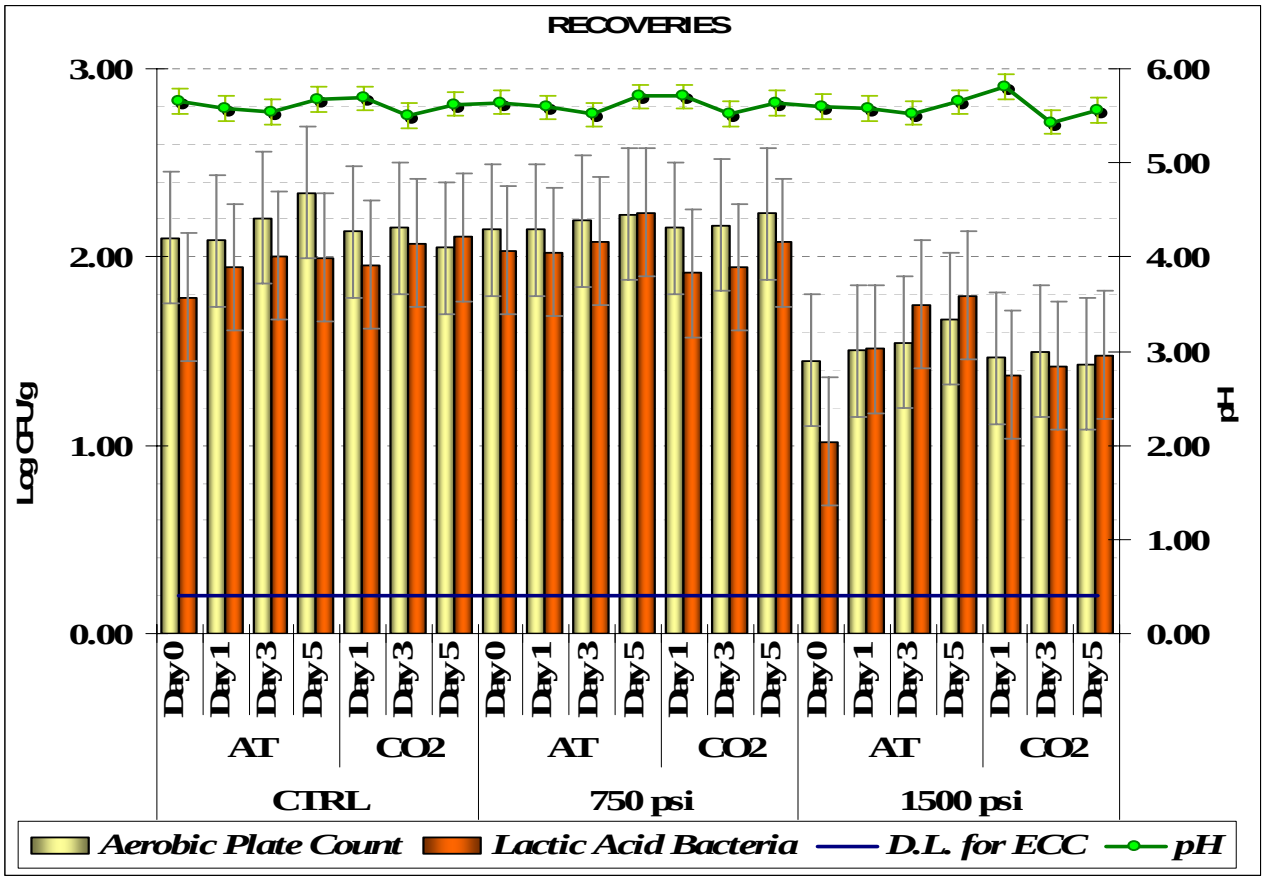

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Figure 13. Interaction of $_{CP}CO_2$ exposure (750 psi and 1500 psi for 15 min) at 36°C with refrigerated storage (Day 0, 1, 2, 3, and 5) by Type of Packaging (Not Packaged [NP], 100% Flushed CO_2 [100% CO2], and Vacuum Packaged [VP]) of Beef Trimmings on Average Bacterial Reductions in Ground Beef (Log CFU/g).

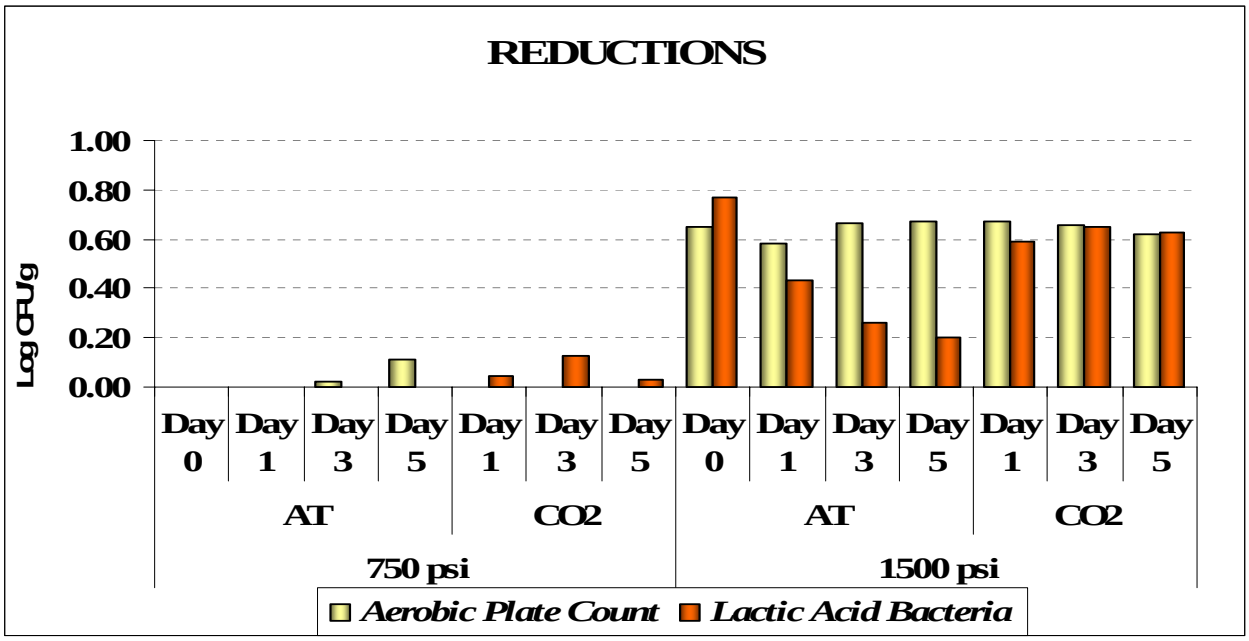

Table 9. Least Square Means of Average Bacterial Reductions (Log CFU/g) of Normal Microflora (Aerobic Plate Count and Lactic Acid Bacteria) in Ground Beef Patties Manufactured from $_{CP}CO_2$ Treated Beef Trimmings (1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5).

TREATMENT			Aerobic Plate Count	Lactic Acid Bacteria	Total Coliform	E. coli
	Atmospheres	2°C Storage				
750 psi	AT	Day 0	-0.05	-0.25	0.00	0.00
		Day 1	-0.06	-0.08	0.00	0.00
		Day 3	0.02	-0.08	0.00	0.00
		Day 5	0.11	-0.24	0.00	0.00
	CO_2	Day 1	-0.02	0.05	0.00	0.00
		Day 3	-0.01	0.13	0.00	0.00
		Day 5	-0.18	0.03	0.00	0.00
1500 psi	AT	Day 0	0.65	0.77	0.00	0.00
		Day 1	0.58	0.43	0.00	0.00
		Day 3	0.67	0.26	0.00	0.00
		Day 5	0.67	0.20	0.00	0.00
	CO_2	Day 1	0.67	0.59	0.00	0.00
		Day 3	0.65	0.65	0.00	0.00
		Day 5	0.62	0.63	0.00	0.00

Average of 3 replications.
Total coliforms and Generic E. coli were not detected. Detection Limit = 0.4 log CFU/g

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Although further packaging under modified atmospheres appeared not to have a significant additional antimicrobial effect, it is clear that $_{CP}CO_2$ applied at 1500 psi was able to maintain normal bacterial populations under 1.8 log CFU/g. Furthermore, while LAB and APC populations in aerobic trays were steadily increasing over time (Figure 12), $_{CP}CO_2$ applied at 1500 psi hindered bacterial growth in ground beef packaged with 100% flushed CO_2, maintaining lower bacterial counts than those shown by the control by approximately 0.5 log CFU/g.

Impact of $_{CP}CO_2$ Application of Beef Trimmings Prior to Grinding on Instrumental Color (CIE $L^* a^* b^*$) of Raw Ground Beef Patties.

On days 0, 1, 3 and 5 of simulated retail display, instrumental color was measured in raw ground beef patties packaged under normal atmospheric conditions in aerobic trays (AT) or flushed with 100% CO_2 (CO_2). The impact $_{CP}CO_2$ application in beef trimmings on instrumental color and reflectance values of raw ground beef patties is shown in Table 10 and Figure 14.

Table 1. Least Square Means of Instrumental Color Values Obtained in Raw Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5).

	Package Type	Refrigerated Storage	Color L^*	Color a^*	Color b^*	630/580[1]
CTRL	AT	Day 0	45.12 ^{bcde}	27.50 ^{ab}	19.45 ^{bd}	4.06 ^{b}
		Day 1	45.17 ^{bcde}	24.82 ^{bc}	19.73 ^{abd}	3.11 ^{bcd}
		Day 3	45.79 ^{abcd}	21.09 ^{cdef}	18.06 ^{bde}	2.58 ^{cdef}
		Day 5	47.00 ^{abc}	19.27 ^{efgh}	15.90 ^{g}	2.26 ^{defg}
	CO_2	Day 1	44.75 ^{cde}	16.07 ^{fghi}	13.47 ^{g}	2.33 ^{cdefg}
		Day 3	45.38 ^{bcde}	19.71 ^{defg}	14.13 ^{g}	2.48 ^{cdefg}
		Day 5	45.27 ^{bcde}	14.55 ^{hi}	11.77 ^{g}	1.70 ^{fg}
750 psi	AT	Day 0	46.99 ^{abc}	29.94 ^{a}	22.20 ^{a}	5.47 ^{a}
		Day 1	45.08 ^{bcde}	25.14 ^{abc}	20.53 ^{ab}	3.21 ^{bc}
		Day 3	45.90 ^{abcd}	21.37 ^{cde}	17.53 ^{deg}	2.59 ^{cdef}
		Day 5	45.80 ^{abcd}	20.34 ^{cdefg}	16.64 ^{g}	2.45 ^{cdefg}
	CO_2	Day 1	42.96 ^{de}	14.05 ^{hi}	13.56 ^{g}	2.27 ^{cdefg}
		Day 3	42.45 ^{e}	18.03 ^{efghi}	14.66 ^{g}	2.28 ^{cdefg}
		Day 5	42.42 ^{e}	13.56 ^{hi}	11.51 ^{g}	1.59 ^{g}
1500 psi	AT	Day 0	47.88 ^{abc}	27.71 ^{ab}	20.64 ^{ab}	3.94 ^{bc}
		Day 1	48.30 ^{ab}	24.35 ^{bcd}	19.99 ^{abd}	2.87 ^{cde}
		Day 3	48.87 ^{a}	21.40 ^{cde}	18.20 ^{bde}	2.58 ^{cdef}
		Day 5	48.14 ^{ab}	19.50 ^{defgh}	17.04 ^{deg}	2.26 ^{defg}
	CO_2	Day 1	44.03 ^{cde}	13.52 ^{hi}	13.56 ^{g}	2.44 ^{cdefg}
		Day 3	44.75 ^{cde}	22.84 ^{bcde}	17.02 ^{eg}	3.10 ^{cd}
		Day 5	44.64 ^{cde}	16.03 ^{ghi}	13.36 ^{g}	1.99 ^{efg}

Average of 3 replications.

^{ab} *Least square means within a column bearing different letters are different (P<0.05).*

[1]. *Ratios of 630/580 nm approach 1.0 for metmyoglobin and >4.0 for oxymyoglobin.*

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No differences were found among treatments in raw patties for any of the interactions of pressure, packaging conditions and days of storage on CIE L^* values, (P≥0.05), except the interaction of pressure by packaging conditions (L^*, P<0.05). Before refrigerated storage, raw patties packaged under aerobic conditions, in most cases, were lighter (L^*, P<0.05) than those exposed to the same pressure packaged under flushed CO_2, and this effect was most apparent for the 1500 psi pressure treatment. However, after 5 days of refrigerated storage, there was no difference among treatments for CIE L^* values (P≥0.05).

CIE a^* values in raw patties also showed significance (a^*, P<0.05) for the interactions of pressure by packaging conditions by days of storage, and pressure by storage. Raw patties packaged under aerobic conditions consistently showed higher redness scores (a^*, P<0.05) throughout the refrigerated storage when compared to those packaged under flushed CO_2 conditions. All treatments under aerobic conditions showed declining a^* values trough refrigerated storage, whereas flushed CO_2 packages showed a peak in redness values (a^*, P<0.05) after three days. Redness scores of flushed CO_2 packages after refrigerated storage were similar after 1 and 5 days. In addition, all redness scores for all treatments were similar after 5 days of refrigerated storage, regardless of $_{CP}CO_2$ pressure or packaging condition applied (a^*, P≥0.05).

CIE b^* scores obtained from raw patties exhibited very similar behavior as those obtained for CIE a^* scores. CIE b^* scores only showed significance for the packaging conditions by storage interaction (b^*, P<0.05). Values declined in raw patties packaged under aerobic conditions and showed a peak in raw patties packaged under flushed CO_2 conditions after three days of refrigerated storage (b^*, P<0.05). However, CIE b^* scores of flushed CO_2 packages after refrigerated storage were similar after 1 and 5 days. Interestingly, raw patties exposed to the 1500 $_{CP}CO_2$ treatment showed the best CIE b^* scores after 5 days of refrigerated storage, regardless of the packaging conditions (b^*, P≥0.05).

Figure 15 shows two patties manufactured from manufactured from $_{CP}CO_2$ treated beef trimmings after 3 days of refrigerated storage; in the left side a patty packaged under flushed CO_2 conditions, and in the right side a patty packaged inside an aerobic tray under normal atmospheres. Figure 16 shows the prevalent atmospheric conditions inside the packages during simulated retail display of raw patties.

Figure 15. Raw Patties Manufactured from $_{CP}CO_2$ Treated Beef Trimmings Packaged under Flushed CO_2 Conditions (CO2) (left) and under Aerobic Conditions in Aerobic Tray (AT) after 3 Days of Refrigerated Storage.

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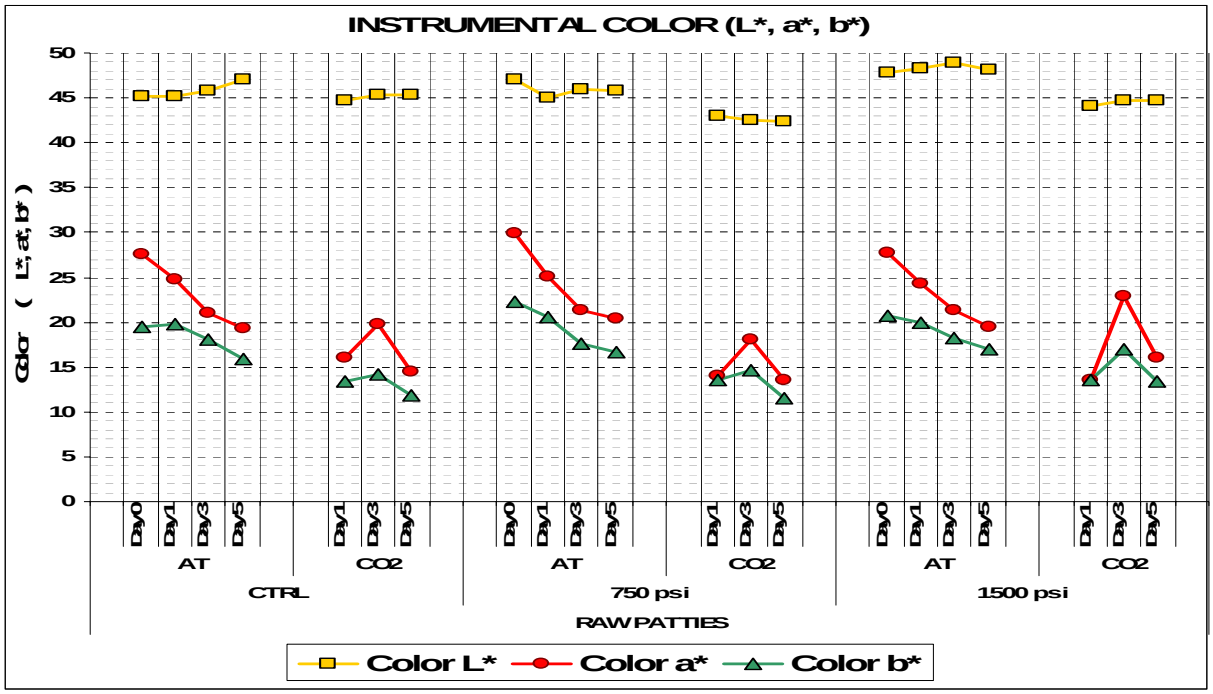
Figure 14. Interaction of $_{CP}CO_2$ Exposure (750 psi and 1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5) by Type of Packaging (Not Packaged [NP], 100% Flushed CO_2 [CO2], and Aerobic Trays [AT]) of Beef Trimmings on Instrumental Color (CIE L*, a*, b*) in Raw Ground Beef Patties.



Reflectance ratios from the 630 and 580 nm wavelengths in raw patties (Table 10) used as an estimation of the oxymyoglobin proportion of the myoglobin pigment, showed significance only for the packaging conditions by refrigerated storage interaction (630/580nm, $P<0.05$).

In ground beef patties packaged under aerobic conditions, as expected, reflectance ratios showed average initial values in the range of 3.9-5.5 before refrigerated storage, declining to 2.3-2.5 after 5 days of refrigerated storage. Values in the order of 2.3-2.4 were recorded for ground beef patties packaged under flushed CO_2 before refrigerated storage, declining to 1.6-2 after 5 days of simulated retail display. Oxymyoglobin steadily converted to either metmyoglobin or deoxymyoglobin in both types of packaging conditions (aerobic and flushed with CO_2), but apparently at a higher rate in aerobic conditions. Nevertheless, after 5 days of refrigerated storage, scored values for reflectance ratios from the 630 and 580 nm wavelengths in raw patties were statistically similar for all treatments ($P\geq0.05$), as it can be observed in Figure 17.

Furthermore, application of $_{CP}CO_2$ in beef trimmings prior to grinding, and maintaining anaerobic conditions all through refrigerated storage, by flushing the packages with CO_2 immediately after grinding, represents a viable option for enhancing color stability during refrigerated storage of ground beef. Therefore, under the conditions in which this study was conducted, results suggest that $_{CP}CO_2$ application on beef trimmings had no apparent detrimental effects in ground beef.

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Figure 16. Packaging Atmospheres in Raw Ground Beef Patties Manufactured from Beef Trimmings Exposed to $_{CP}CO_2$ (750 psi and 1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5).

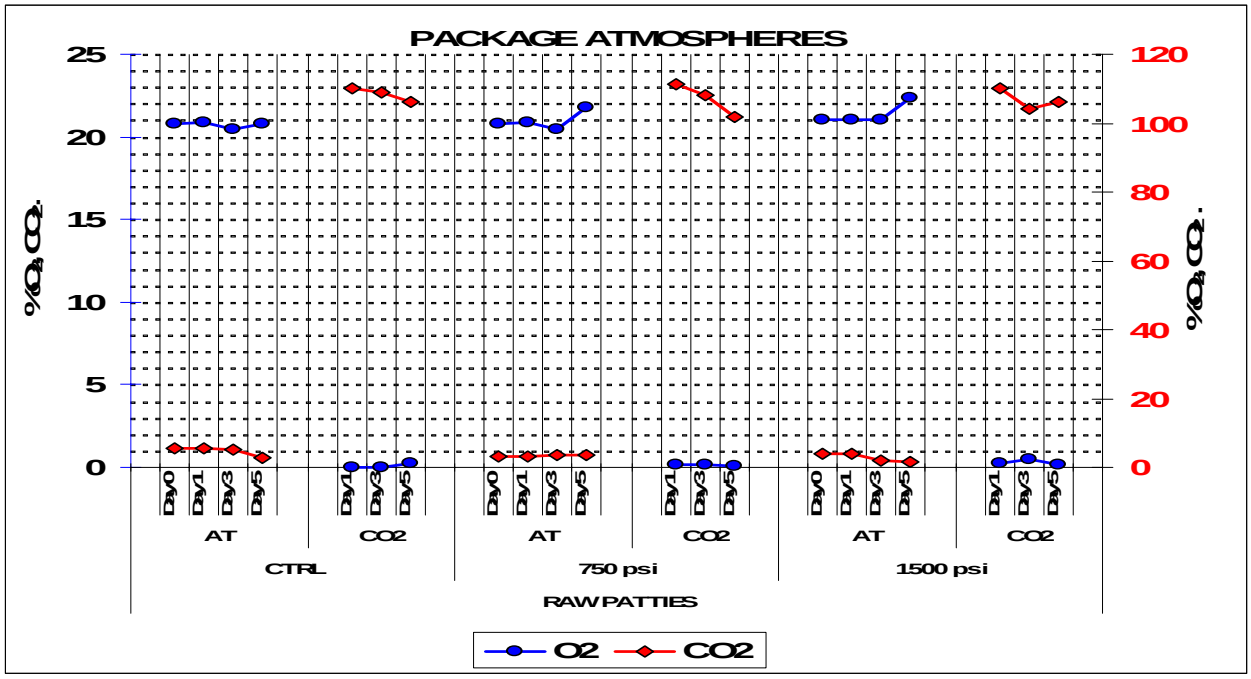
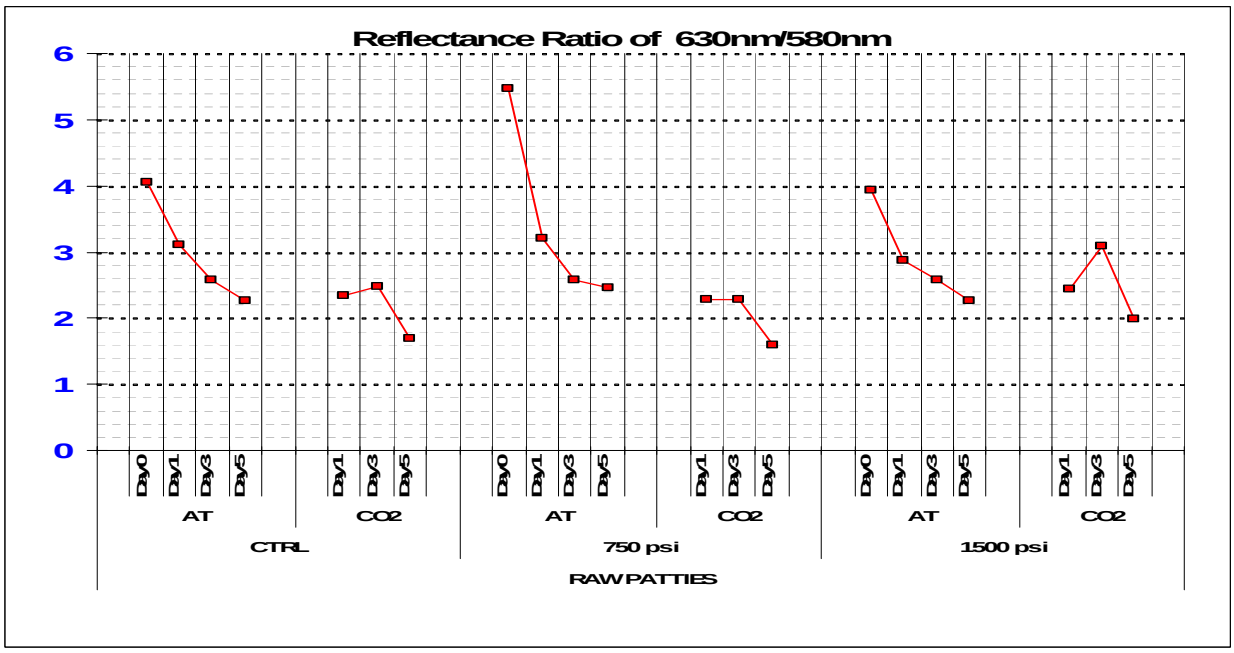


Figure 17. Reflectance Ratios of 630nm/580nm in Raw Ground Beef Patties Manufactured from Beef Trimmings Exposed to $_{CP}CO_2$ (750 psi and 1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5).



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Impact of $_{CP}CO_2$ Application of Beef Trimmings Prior to Grinding on Instrumental Color (CIE L^* a^* b^*), Proximate and Sensory Analyses of Cooked Ground Beef Patties.

Table 11 shows the impact of 0, 1, 3 and 5 days of simulated retail display, packaged under 100% flushed CO_2 (CO2) and normal atmospheric conditions in aerobic trays (AT), after $_{CP}CO_2$ application in beef trimmings on instrumental color and reflectance values of ground beef cooked patties.

As it can be observed in Figure 18, CIE L* values for all the treatments were similar (P≥0.05) when comparing cooked ground beef patties under 100% flushed CO_2 (CO2) and normal atmospheric conditions in aerobic trays (AT). Similar trends were exhibited in all treatments for each type of package during simulated retail display, showed by a decrease in lightness (L^*, P<0.05) over storage time. However, after 5 days of refrigerated display, CIE L* values were slightly higher for cooked patties packaged under 100 flushed CO_2 conditions.

CIE a^* and b^* values exhibited very similar trends when compared to each other. Similar values on both scores (a^*, b^*, P≥0.05) were found after the 5 days or simulated retail display for all the treatments, with the exception of the 1500 psi CO_2 cooked patties from 100% flushed CO_2 (CO2), which showed less redness (a^*, P<0.05) than the rest of the treatments. In cooked ground beef patties scored values for reflectance ratios from the 630 and 580 nm wavelengths in raw patties were statistically similar for all treatments (P≥0.05), with the exception of the 1500 psi CO_2 cooked patties from 100% flushed CO_2 (CO2), which showed less redness (a^*, P<0.05) than the rest of the treatments. as it can be observed in Figure 19.

Table 11. Least Square Means of Instrumental Color Values Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5).

	Package Type	Refrigerated Storage	Color L^*	Color a^*	Color b^*	630/580[1]
CTRL	AT	Day 0	54.76 ^{a}	13.57 ^{fh}	17.03 ^{efghi}	1.65 ^{bcdef}
		Day 1	50.95 ^{cdef}	18.99 ^{ade}	20.23 ^{abcd}	2.35 ^{abcd}
		Day 3	49.80 ^{ef}	19.40 ^{ad}	20.15 ^{abcde}	2.32 ^{abcde}
		Day 5	49.72 ^{f}	20.77 ^{a}	22.39 ^{ab}	2.75 ^{a}
	CO_2	Day 1	54.73 ^{a}	12.82 ^{fh}	16.83 ^{fghi}	1.58 ^{cdef}
		Day 3	53.67 ^{abcd}	10.93 ^{h}	16.18 ^{hi}	1.32 ^{f}
		Day 5	52.06 ^{abcdef}	18.14 ^{adef}	21.05 ^{abc}	2.19 ^{abcdef}
750 psi	AT	Day 0	54.63 ^{ab}	10.03 ^{h}	14.81 ^{i}	1.28 ^{f}
		Day 1	52.54 ^{abcdef}	18.78 ^{ade}	19.51 ^{bcdefg}	2.31 ^{abcde}
		Day 3	50.08 ^{ef}	15.12 ^{defh}	17.02 ^{efghi}	1.79 ^{bcdef}
		Day 5	50.15 ^{ef}	20.22 ^{a}	22.77 ^{a}	2.47 ^{abc}
	CO_2	Day 1	54.60 ^{ab}	10.88 ^{h}	15.31 ^{hi}	1.39 ^{ef}
		Day 3	52.75 ^{abcdef}	19.13 ^{ad}	19.87 ^{abcdef}	2.37 ^{abcd}
		Day 5	52.00 ^{abcdef}	17.18 ^{adef}	20.91 ^{abc}	2.05 ^{abcdef}

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Table 11. Least Square Means of Instrumental Color Values Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5) Cont.

	Package Type	Refrigerated Storage	Color L*	Color a*	Color b*	630/5801
1500 psi	AT	Day 0	54.40 ^{ab}	12.45 ^{fh}	16.35 ^{ghi}	1.56 ^{cdef}
		Day 1	53.74 ^{abcd}	21.05 ^{a}	19.98 ^{abcdef}	2.58 ^{ab}
		Day 3	51.41 ^{bcdef}	14.09 ^{efh}	17.11 ^{defghi}	1.64 ^{bcdef}
		Day 5	50.69 ^{def}	17.47 ^{adef}	20.87 ^{abc}	2.09 ^{abcdef}
	CO_2	Day 1	53.96 ^{abc}	12.00 ^{h}	16.61 ^{ghi}	1.44 ^{def}
		Day 3	53.74 ^{abcd}	21.05 ^{a}	19.98 ^{abcdef}	2.58 ^{ab}
		Day 5	53.01 ^{abcde}	12.55 ^{fh}	18.33 ^{cdefgh}	1.49 ^{def}

Average of 3 replications.
^{ab} *Least square means within a column bearing different letters are different (P<0.05).*
^{1}. *Ratios of 630/580 nm approach 1.0 for metmyoglobin and >4.0 for oxymyoglobin.*

Figure 18. Interaction of $_{CP}CO_2$ exposure (750 psi and 1500 psi for 15 min) at 36°C with refrigerated storage (Day 0, 1, 2, 4, 6, and 10) by Type of Packaging (Not Packaged [NP], 100% Flushed CO_2 [CO2], and Aerobic Trays [AT]) of Beef Trimmings on Instrumental Color (CIE L*, a*, b*) in Cooked Ground Beef Patties.

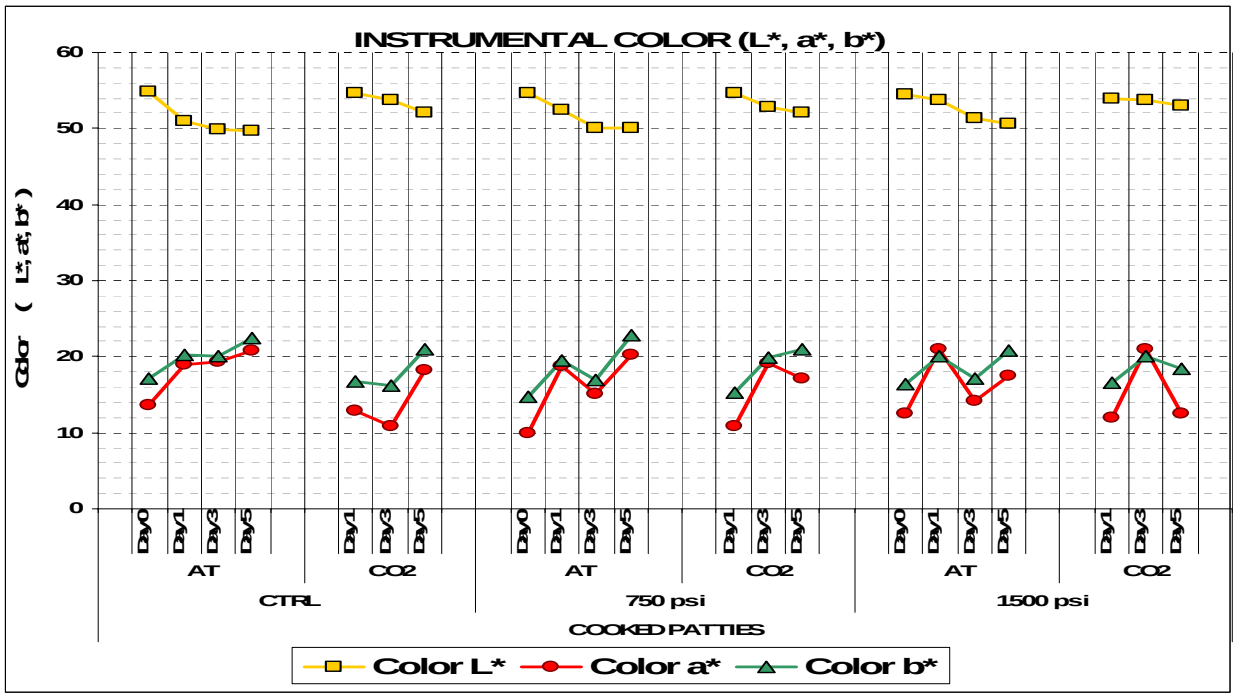

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Figure 19. Reflectance Ratios of 630nm/580nm in Cooked Ground Beef Patties Manufactured from Beef Trimmings Exposed to $_{CP}CO_2$ (750 psi and 1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 4, 6, and 10).

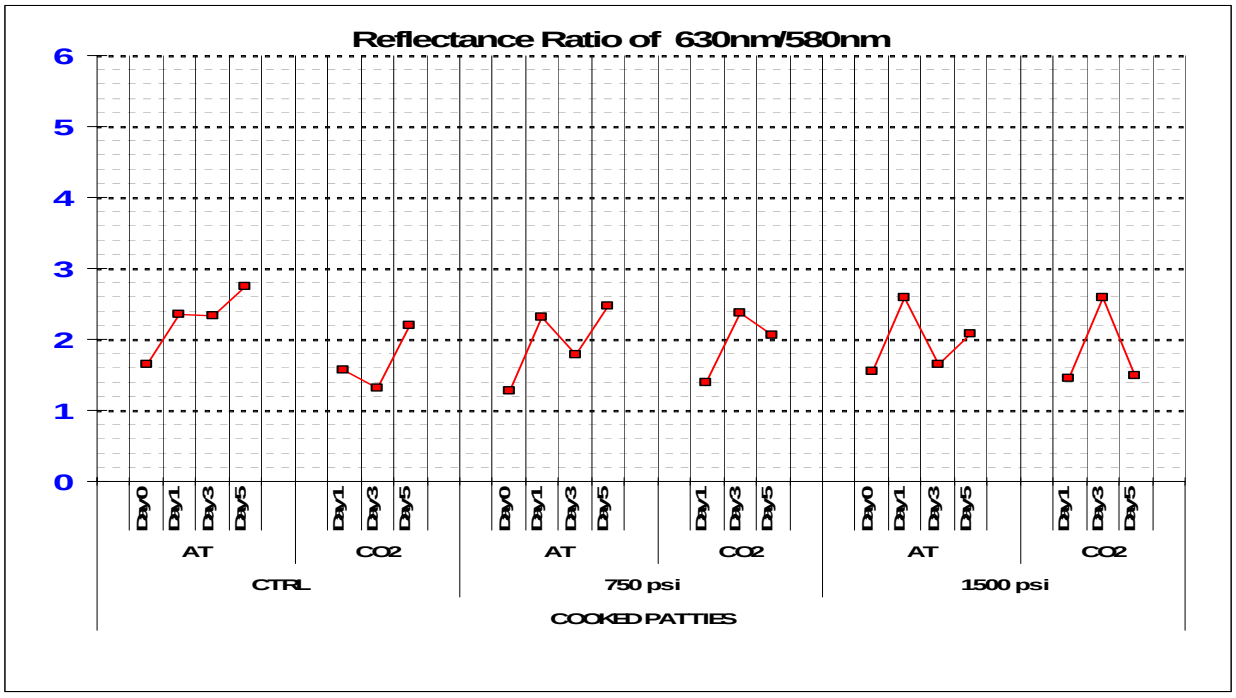

Table 12 shows scores for proximate analysis in cooked patties analyzed as % Moisture, % Crude Protein, and % Crude Fat. The extent of lipid oxidation was measured as thiobarbituric acid reactive substances (TBARS) and expressed as milligrams of malonaldehyde per kilogram of DM.

However, little data exists on cooking losses related to CO_2 storage of meat. Holley, Gariépy, Delaquis, Doyon, and Gagnon (1994) compared cooking losses in pork packaged in 50% and 100% CO_2, and found no differences. Jeremiah, Gibson, and Arganosa (1996) found no differences between non-CO_2 storage and the different CO_2 ratios on the cooking loss in stored pork at –1.5 °C in different ratios from 0 to 5 l CO_2 gas per kg meat. Bentley et al. (1989) found that cooking losses were similar, but higher than in vacuum from ground beef stored in 100% CO_2 and 100% N_2. In our experiment, as it can be observed in Figure 20, moisture (% H_2O) values exhibited very similar trends with the exception of the 1500 psi CO_2 cooked patties from 100% flushed CO_2 (CO2), which showed higher dryness (%H_2O, $P<0.05$) than the rest of the treatments.

No perceivable differences were observed in cooked ground beef patties scored values for crude protein (%CP) and crude fat (%CF) after 5 d of simulated retail display (%CP, %CF, $P\geq0.05$). The extent of lipid oxidation (shown in Figure 21), after 5 days of simulated retail display, scored higher values in all patties packaged under aerobic conditions (AT) when compared to those flushed under 100% CO_2 (CO2), regardless the $_{CP}CO_2$ treatment applied, with the highest values detected in the 1500 psi $_{CP}CO_2$ treatment ($P<0.05$). Nevertheless, TBAR

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values in patties flushed with 100% CO_2 were able to maintain similar values all throughout refrigerated storage (P≥0.05), showing that 100% flushed CO_2 could be considered as a valid technology to prevent lipid oxidation.

Table 12. Least Squeare Means of Proximate Values (% Moisture [H_20], % Crude Protein [CP], % Crude Fat [CF]) and thiobarbituric Acid Reactive Substances (TBARS, mg/Kg malonaldehyde) Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5).

			H_20 (%)	*CP (%)*	*CF (%)*	*TBARS malonaldehyde (mg/Kg DM)*
CTRL	AT	Day 0	*58.98 abcde*	*27.56 fgh*	*12.43 bcdefg*	*0.12 g*
		Day 1	*60.19 ab*	*27.91 defg*	*10.66 fgh*	*0.20 defg*
		Day 3	*60.71 a*	*28.54 abcdef*	*9.16 gh*	*0.28 cd*
		Day 5	*60.18 ab*	*28.83 abcde*	*10.04 gh*	*0.37 b*
	CO_2	Day 1	*56.56 efg*	*27.88 defg*	*14.73 abc*	*0.17 fg*
		Day 3	*57.46 cdefg*	*28.96 abcd*	*12.34 bcdefg*	*0.18 fg*
		Day 5	*58.66 abcdef*	*28.51 abcdef*	*12.80 bcdefg*	*0.20 defg*
750 psi	AT	Day 0	*58.23 bcdefg*	*25.93 j*	*14.38 abcd*	*0.13 g*
		Day 1	*58.99 abcd*	*27.70 efgh*	*11.34 efgh*	*0.18 fg*
		Day 3	*58.01 bcdefg*	*28.92 abcd*	*11.53 defgh*	*0.27 cde*
		Day 5	*59.19 abc*	*28.28 bcdefg*	*11.93 cdefgh*	*0.37 b*
	CO_2	Day 1	*56.79 cdefg*	*26.57 hij*	*15.87 a*	*0.20 defg*
		Day 3	*56.67 defg*	*28.94 abcd*	*13.39 abcdef*	*0.18 fg*
		Day 5	*57.58 cdefg*	*29.48 a*	*13.39 abcdef*	*0.17 fg*
1500 psi	AT	Day 0	*58.09 bcdefg*	*27.26 ghi*	*13.27 abcdef*	*0.15 fg*
		Day 1	*56.87 cdefg*	*26.39 ij*	*15.18 ab*	*0.21 def*
		Day 3	*56.91 cdefg*	*28.06 cdefg*	*13.97 abcde*	*0.30 bc*
		Day 5	*56.94 cdefg*	*28.21 bcdefg*	*12.66 bcdefg*	*0.50 a*
	CO_2	Day 1	*58.28 bcdef*	*25.64 j*	*15.11 ab*	*0.20 efg*
		Day 3	*55.82 g*	*29.09 abc*	*13.26 abcdef*	*0.19 efg*
		Day 5	*56.50 fg*	*29.35 ab*	*13.59 abcdef*	*0.20 defg*

Average of 3 replications.

[ab] Least square means within a column bearing different letters are different (P<0.05).

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Figure 3. Proximate Values (% Moisture [H_20], % Crude Protein [CP], % Crude Fat [CF]) Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5).

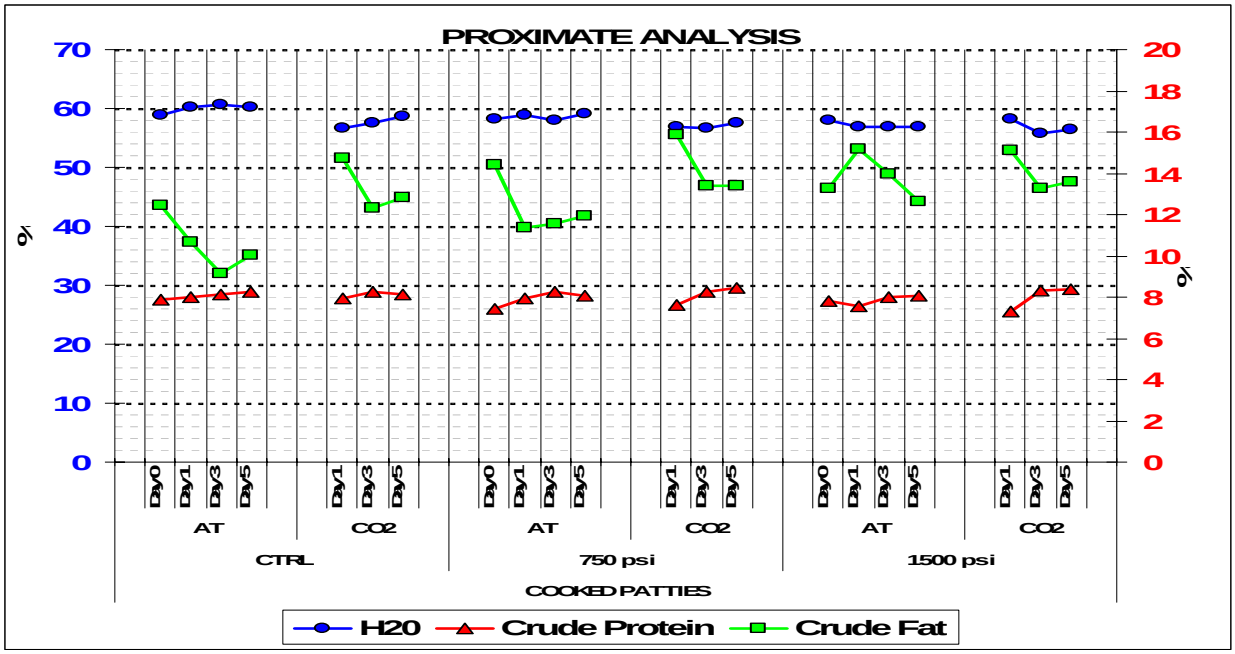
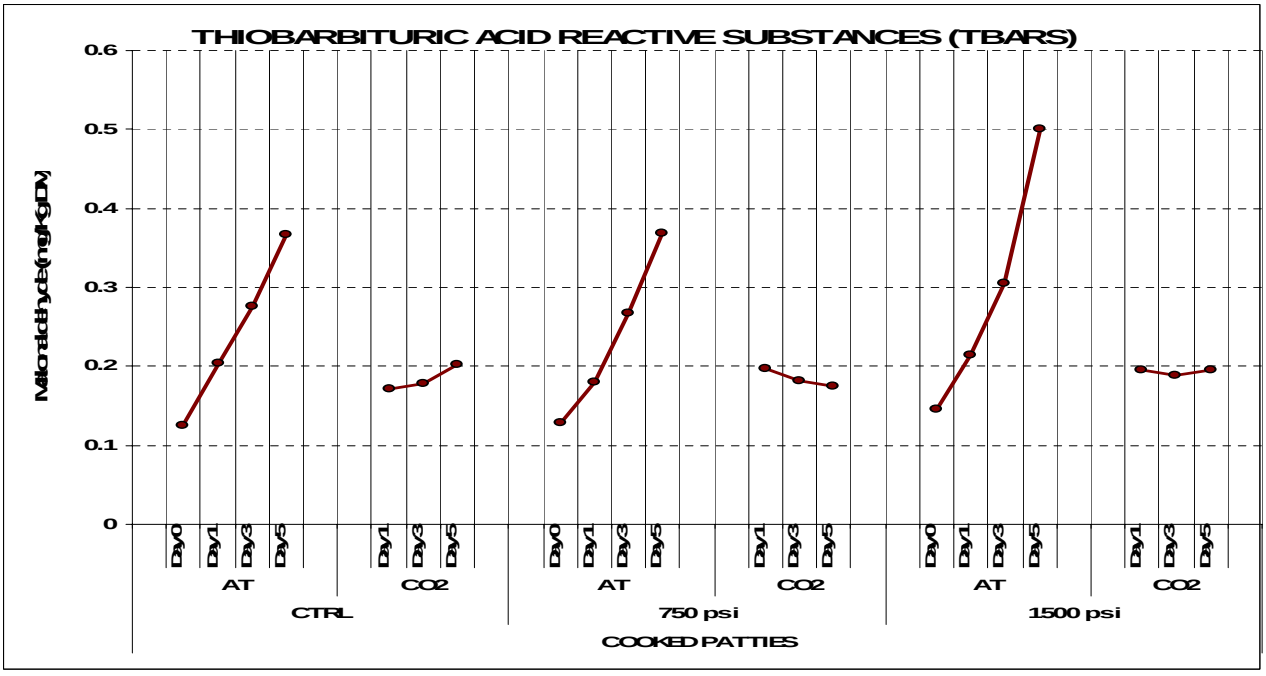


Figure 21. Thiobarbituric Acid Reactive Substances (TBARS, mg/Kg malonaldehyde) Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min) at 36°C During Refrigerated Storage (Day 0, 1, 2, 3, and 5).



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Sensory analysis was conducted on cooked patties from each treatment (CTRL, 750 psi, and 1500 psi). A seven-member trained sensory panel evaluated the samples for Overall Tenderness (OT, 8=extremely tender, 1=extremely tough), Juiciness (J, 8=extremely juicy, 1=extremely dry), Beef Flavor Intensity (BFI, 8=extremely intense, 1=extremely bland), and Off Flavor Intensity (OFI, 8=non, 1=abundant). Table 13 show the scales used to measure these traits. Two replications were performed.

Table 2. Hedonic Scale for Sensory Analysis of Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min) at 36°C

OVERALL TENDERNESS (OT)		BEEF FLAVOR INTENSITY (BFI)		JUICINESS (J)		OFF FLAVOR INTENSITY (OFI)	
8	Extremely tender	8	Extremely intense	8	Extremely juicy	8	Abundant
7	Very tender	7	Very intense	7	Very juicy	7	Moderately abundant
6	Moderately tender	6	Moderately intense	6	Moderately juicy	6	Slightly abundant
5	Slightly tender	5	Slightly intense	5	Slightly juicy	5	Moderate
4	Slightly tough	4	Slightly bland	4	Slightly dry	4	Slightly bland
3	Moderately tough	3	Moderately bland	3	Moderately dry	3	Traces
2	Very tough	2	Very bland	2	Very dry	2	Practically none
1	Extremely tough	1	Extremely bland	1	Extremely dry	1	None

Mean trained sensory panel scores for cooked beef patties from control and $_{CP}CO_2$ treated beef trimmings are presented in Table 14.

Table 3. Mean Trained Sensory Panel Scores for Cooked Beef Patties from Control and $_{CP}CO_2$ Treated Beef Trimmings after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min) at 36°C.

SAMPLE	Overall Tenderness (OT)	Juiciness (J)	Beef Flavor Intensity (BFI)	Off Flavor Intensity (OFI)
Control	6.6 [c]	5.8 [a]	6.0 [a]	0.5 [a]
750 ppm	7.4 [a]	5.9 [a]	5.4 [b]	2.0 [b]
1500 ppm	7.1 [b]	5.9 [a]	6.1 [a]	0.9 [a]

Average of 2 replications.

[ab] *Least square means within a column bearing different letters are different (P<0.05).*

Ground beef patties manufactured from the $_{CP}CO_2$ beef trimmings were freshly cooked and evaluated by the descriptive flavor profile panel. Panelist evaluated them as having more (*P*<.05) tenderness when compared to the control (Figure 22). There were no differences detected for juiciness (J, P≥0.05), beef flavor intensity (BFI, P≥0.05) and off flavor intensity (OFI, P≥0.05) when comparing the 1500 psi $_{CP}CO_2$ treatment to the control. Nevertheless, 750 psi $_{CP}CO_2$ appeared to have the worse scores for these two traits (BFI, OFI; P<0.05).

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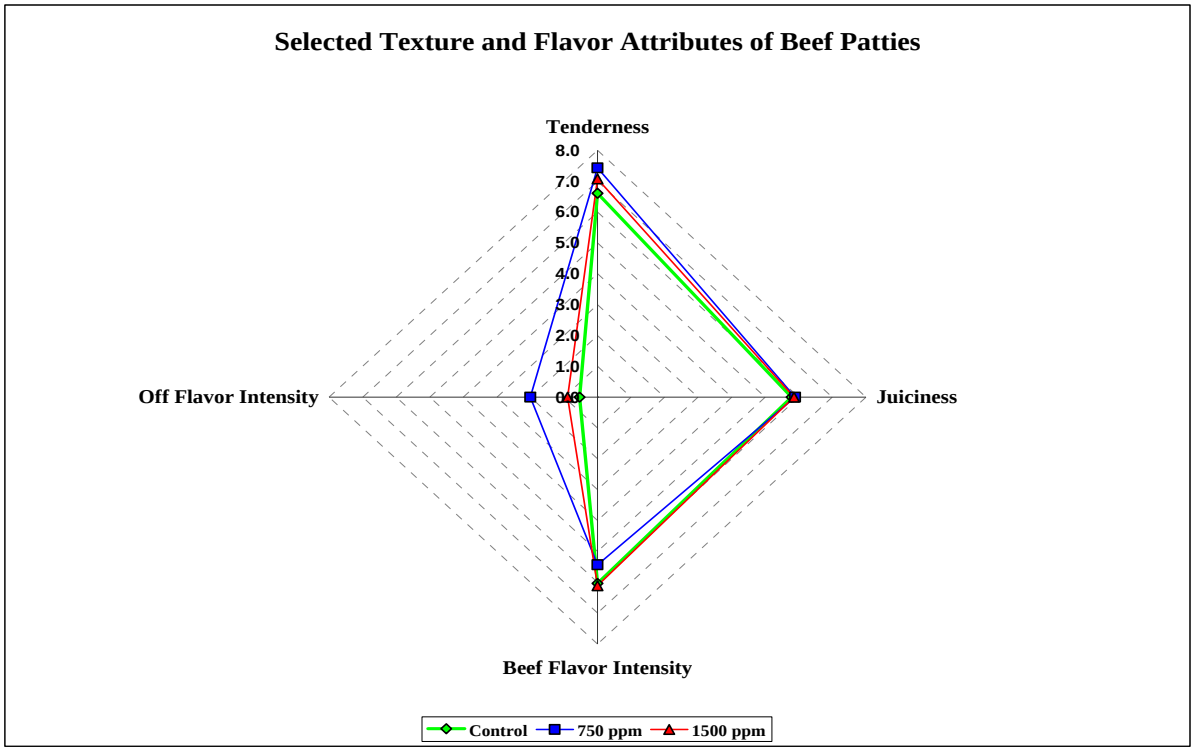
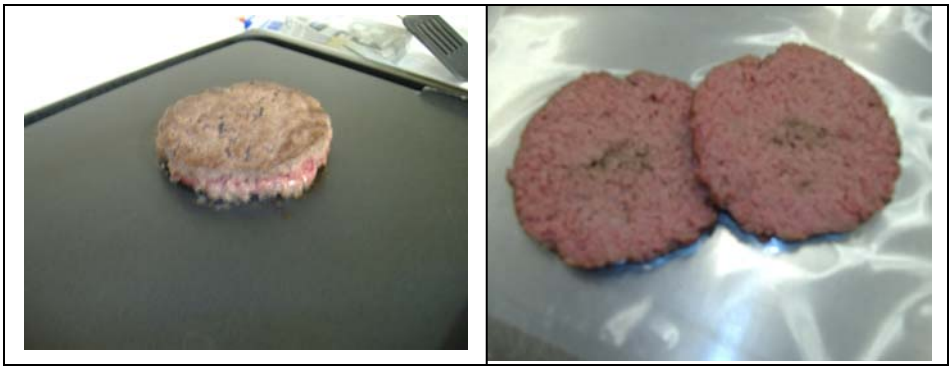
Figure 22. Selected Texture and Flavor Attributes (Tenderness, Juiciness, Beef Flavor Intensity, and Off Flavor Intensity) Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min) at 36°C



It has been reported that beef, which had been stored in 100% CO_2, developed visible pores and fissures upon cooking caused by a rapid release of CO_2 gas from the meat on heating (Bruce et al., 1996). These finding are in agreement with visual observations obtained in this dissertation, and it is still subject to further research weather these CO_2 related pores and fissures may have an impact on the functional properties of meat (Figure 23).

Figure 23. Image of a cooked patty (after $_{CP}CO_2$ Application on Beef Trimmings) with visible pores and fissures upon cooking caused by a rapid release of CO2 gas from the meat on heating (left). A cross section of a cooked patty for instrumental color measurement (right).



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These findings suggest that application of high pressures of $_{CP}CO_2$ in beef trimmings prior to grinding, and maintaining anaerobic conditions all through refrigerated storage, by flushing the packages with CO_2 immediately after grinding, under the conditions in which this study was conducted, had no major concerns on detrimental effects in cooked ground beef patties.

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Antimicrobial Effects of Controlled Phase Carbon Dioxide ($_{CP}CO_2$) Application on Beef Trimmings in Ground Beef

Objective

This experiment was designed to evaluate the antimicrobial effects of controlled phase carbon dioxide ($_{CP}CO_2$) application on beef trimmings in further ground beef.

Materials and Methods

Preparation of Samples

Bacterial cultures in this study included five different strains of generic *Escherichia coli,* five different strains of *Escherichia coli* O157:H7, and five different strains of *Salmonella enteritidis*. All cultures were obtained from the Food Microbiology Laboratory stock culture collection at Kansas State University (KSU). Generic *E. coli* cultures utilized were ATCC 14763, FSSL-007 (Food Safety and Security Lab, KSU, Manhattan, KS), ATCC 35421, and ATCC 25922. *E. coli* O157:H7 cultures included ATCC 43890, ATCC 43895, FSSL-012 (Larry Beuchat, University of Georgia, Griffin, GA), FSSL-013 (Food Safety and Security Lab, KSU, Manhattan, KS), and FSSL-014 (Food Safety and Security Lab, KSU, Manhattan, KS). *Salmonella* spp. cultures included Serotype Montevideo FSSL-042 (Larry Beuchat, University of Georgia, Griffin, GA), ATCC 13311, Newport FSSL-043 (Food Safety and Security Lab, KSU, Manhattan, KS), Enteritidis ATCC 4931, Enteritidis ATCC 13076.

Stock cultures were cultivated by placing one impregnated bead into a 5 ml solution of Difco® Tryptic Soy Broth (TSB) and incubating for 24 h at 35°C. Next, a 0.05 ml loop of the respective culture was inoculated into a 5 ml solution of TSB and incubated for 24 h at 37°C. Following incubation, samples were mixed together to create a 45 ml cocktail containing 10^9 to 10^{10} CFU/ml of generic *E. coli.* The cell density of this suspension was determined by plating appropriate dilutions on selective plates incubated at 37°C for 48 hours. Cultures were confirmed by cultivation on selective and differential media, and biochemical analysis of presumptive colonies using API 20E kits.

Inoculation and Treatment

Fresh beef meat was obtained from the Meats Laboratory at Kansas State University (KSU). A select top round roast stored at 4°C was aseptically cut into ca. 1 in. cubes. 450g were weighted and individual pieces were aseptically placed in a previously sterilized tray covered with butcher paper. Samples were inoculated inside a “bio-containment” chamber by “misting” all surface of the meat with approximately 45 ml of the inoculum. This was done ensuring that all sides of each piece of meat received the same exposure to the inoculum. Samples were held for 30 min at room temperature in a sterile laminar flow cabinet (SterilGARD II ®, The Baker Company, Sanford, ME) to allow proper bacterial attachment to the surface of the meat.

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Meat samples were treated with $_{CP}CO_2$ inside an experimental laboratory model of a vessel (Appendix A). After safely closing the vessel, meat samples were treated with the general procedure (Appendix B) with the following specifications:

- 750 psi for 5 min at 36°C
- 750 psi for 15 min at 36°C
- 1500 psi for 5 min at 36°C
- 1500 psi for 15 min at 36°C

Pressure and temperatures during the study were measured in psi and °C, respectively. These parameters were recorded by an OM-CP-Quadprocess 4 channel 4-20 mA Data Logger (Omega Engineering, Inc.; Stamford, CT) and electronically stored with the Omega® Data Logging Software Ver. 2.00.43c for Windows®. The study was conducted at the KSU Food Safety Processing Laboratory. Statistical Analysis was conducted in three replications with a Split Plot Design using the General Linear Model from SAS (SAS, 2003).

Microbiological Analysis

Before Inoculation a random 25 g sample of beef trimmings was microbiologically analyzed as a non-inoculated non-treated control (CTRL), Immediately after inoculation, a random non-treated 25 g sample of inoculated beef trimmings was microbiologically analyzed as individual control for each treatment (NT). After every CO_2 treatment was completed, beef trimmings were aseptically extracted from the vessel and randomized. A 25 g sample was labeled as “Trim” (TR). A second sample was prepared by weighting 25 g of beef trim, aseptically grinding it in a sterile food processor through a fine plate (1/8”) and labeling it “Ground” (GR). Each sample was microbiologically analyzed by placing it in 225 ml of 0.1% sterile peptone water (PW) and homogenizing in a stomacher for one minute. After homogenization, each sample was serially diluted in sterile PW. An aliquot of 0.25 ml from the initial dilution and 0.1ml of next six dilutions were spread plated onto duplicate plates of selective-resuscitating media (Figure 34). Thin Agar Layer MacConkey Sorbitol Agar (TAL-MSA) was utilized to enumerate residual populations of generic *E. coli* (GEC) and *E. coli* O157:H7 (O157); Plate Count Agar (PCA), to enumerate Aerobic Plate Count (APC); and Thin Agar Layer Xylose Desoxycholate Agar (TAL-XLD), to enumerate Salmonella spp. TAL-MSA and TAL-XLD plates were prepared from pre-poured commercial plates by aseptically adding 14 ml of Tryptic Soy Agar (TSA) as an overlay. Plates were incubated at 37°C for 48 hours. The colony forming units were enumerated manually using a spiral plate dark field Quebec colony counter (Model 330; American optical company, Buffalo, NY). Colony forming units were converted into Log and reductions were calculated as the difference between the averages of non-treated controls and the average of their respective treated replicates. The study was conducted at the KSU Food Safety Laboratory at Call Hall.

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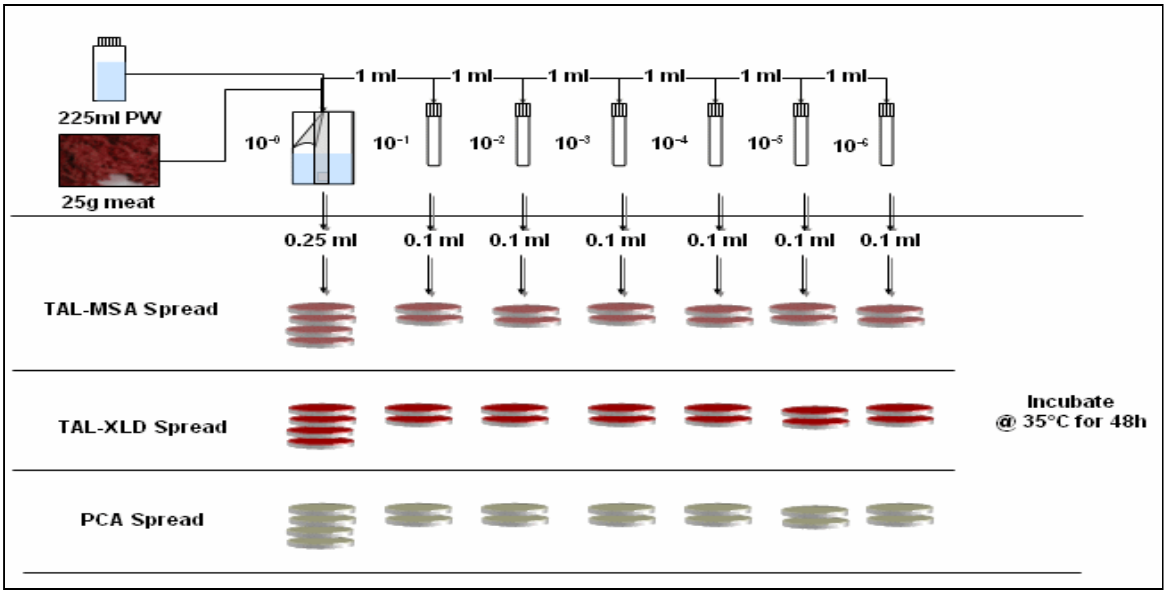
Figure 24. Dilution Scheme for Microbiological Samples analyzed for challenging pathogenic microorganisms in Raw Patties treated with $_{CP}CO_2$.



Results and Discussion

Results from this experiment indicate that after $_{CP}CO_2$ application, reductions in ground beef (GR) were similar when compared to beef trimmings (TR) within the same treatment (P□0.05) for all bacterial populations tested. This result suggests that $_{CP}CO_2$ was able to diffuse effectively trough the adipose tissue and muscle crevices of the trim, extending its antimicrobial effects to the interior of the trim.

As illustrated in Table 15, the highest lethality achieved immediately after $_{CP}CO_2$ application was by pressurizing at 1500 psi for 15 min. With this treatment, 0.83, 0.96, 1.00, and 1.06 log reductions were achieved in beef trimmings for Total Plate Count (TPC), Generic *E. coli* (GEC), *E. coli* O157:H7 (O157), and Salmonella spp. (SS), respectively. Reductions obtained for TPC, GEC, and O157 in all treatments were similar (P≥0.05), but lower when compared to those obtained for SS with 1500 psi for 15 min in TR and GR (Figure 24).

The degree of bacterial efficacy from the application of $_{CP}CO_2$ varies widely, this inconsistency mainly due to the parameters that can be modified during experimentation, such as CO_2 pressure, temperature in the system, exposure time, proximate and organoleptic characteristics of the food matrix, bacteria type, reactor type (continuous or batch), number of pressure cycles, decompression rates, etc.

Nevertheless, results obtained in this study agree with Kamihira (1987), who reported a 4-6 log reduction in *E. coli* populations after treating an aqueous suspension with 580-2900 psi $_{CP}CO_2$ for 2 hours at 20°-35ºC, and only 1 log for baker's yeast. Haas et al. (1989) also reported

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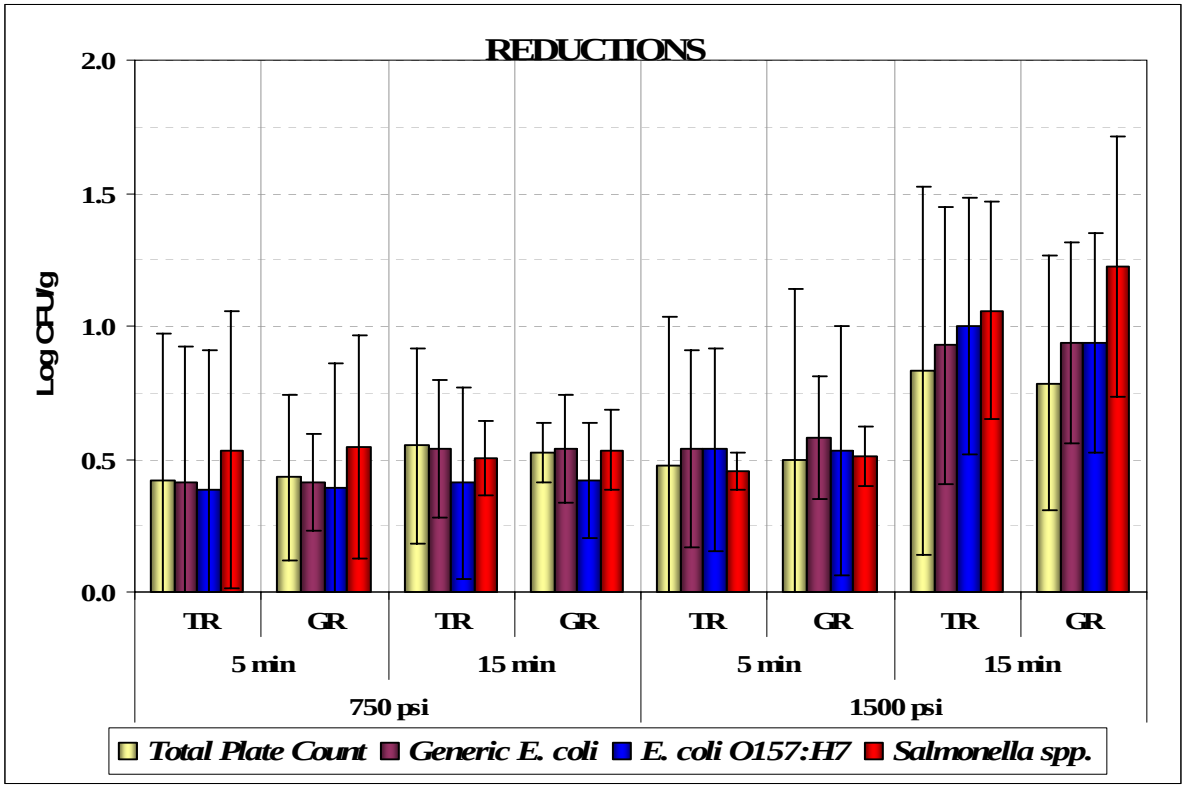
bacterial counts between 2 and 5 log in a variety of products (from cheese to herbs) with pressures between 190-870 psi and times in excess of 2 hours. Nakamura et al. (1994) reported an 8.0 log reduction in *Saccharomyces cerevisiae* populations in distilled water under 580 psi $_{CP}CO_2$ exposure at 40ºC for 5 hours, 2.0 log reduction with 580 psi at 30ºC, 4 log reduction with 435 psi at 40ºC, and 2 log with 430 psi at 40ºC for 1 hour). Below 20ºC, minor antimicrobial effects were achieved at any time or temperature combination.

Table 4. Least Square Means of Bacterial Reductions (Log CFU/g) of Food Pathogens in Beef Trimmings (TR) Treated with $_{CP}CO_2$, and Ground Beef (GR) Manufactured with Treated Beef Trimmings.

Food Pathogens	*750 psi*				*1500 psi*			
	5 min		*15 min*		*5 min*		*15 min*	
	TR	*GR*	*TR*	*GR*	*TR*	*GR*	*TR*	*GR*
TPC	*0.42 [a]*	*0.43 [a]*	*0.55 [a]*	*0.53 [a]*	*0.48 [a]*	*0.50 [a]*	*0.83 [a]*	*0.78 [a]*
GEC	*0.42 [a]*	*0.41 [a]*	*0.54 [a]*	*0.54 [a]*	*0.54 [a]*	*0.58 [a]*	*0.93 [a]*	*0.94 [a]*
O157	*0.39 [b]*	*0.39 [b]*	*0.41 [b]*	*0.42 [b]*	*0.54 [ab]*	*0.53 [ab]*	*1.00 [a]*	*0.94 [ab]*
SS	*0.53 [b]*	*0.55 [b]*	*0.51 [b]*	*0.53 [b]*	*0.45 [b]*	*0.51 [b]*	*1.06 [ab]*	*1.23 [a]*

Average of 3 replications.
[ab] Least square means within a row bearing different letters are different (P<0.05).

Figure 25. Average Reductions (Log CFU[a]/g) of Food Pathogens in Beef Trimmings (TR) Treated with $_{CP}CO_2$ (750 psi, 1500 psi) for 5 and 15 min, and Ground Beef (GR) Manufactured with Treated Beef Trimmings.



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CONCLUSIONS

To explain the antimicrobial effects of CO_2, it will be necessary to consider that by the penetration of CO_2 into the cells and by its dissociation within the cells, a decrease of intracellular pH as well as toxic effect due to the accumulation of CO_2 in the cytoplasmic membrane are induced. These inactivation mechanisms could be attributed to the specific effects of CO_2 compared to the effects of other organic acids used in conjunction as acidulates (Erkmen, O. 2000; King and Mabbit, 1982; Molin, 1983).

The antimicrobial effects of $_{CP}CO_2$ have been extensively studied and several investigators have explained them as follows:

The extraction of intracellular substances such as hydrophobic compounds in the cell wall and cytoplasmic membrane may result in microbial death (Kamihira et al., 1987).

The inactivation of key enzymes related to the essential metabolic process of microorganisms, caused by acidification of the system due to diffused CO_2 (Ballestra et al., 1996; Dixon and Kell, 1989; Donald et al., 1924; Enomoto et al., 1997; Hong and Pyun, 1999; Kamihira et al., 1987).

The expansion of CO_2 within the cells may induce loss of viability due to cell rupture (Debs-Louka, et al., 1999, Shimoda et al, 1998).

The compression of CO_2 may damage the cell membrane or kill the microorganism due to swelling, or may induce the inhibition of metabolic systems (Hong and Pyun, 1999; Isenschmid, et al., 1995, Shimoda et al, 2001).

Other authors have demonstrated that the microbial inactivation power of high-pressure CO_2 was more effective in a continuous treatment (sudden decompression) than in a batch treatment (slow decompression) attributed to cell bursting due to sudden expansion of compressed CO_2 in the cells. Nevertheless, similarities in viability were observed with different decompression times under subcritical CO_2.

Recently, it was shown that the antimicrobial effect was not related only to the pressure of carbon dioxide (CO_2) but to the concentration of dissolved CO_2 as well (Shimoda and Osajima, 1998).

Inactivation kinetics may be optimized further with additional reactor designs that offer gas flow measurement, more reliable pressure controls, and enhancement of contact times between the food matrix and the $_{CP}CO_2$.

The time required for pathogen inactivation with $_{CP}CO_2$ application is significantly less with this technology than other methods, and it is similar to steam autoclaving. These advantages could be improved with innovating designs that permit repeated compression and

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decompression cycles when $_{CP}CO_2$ is applied. In addition, the microbial disruption rates are sensitive to process temperature and pressure. Higher temperatures appear to enhance the transfer rate of CO_2 and also relax the cell walls to ease the penetration of $_{CP}CO_2$ inside the microorganisms. Hence, proper and reliable devices need to be included in future designs to control the increase in two utmost important factors for microbial disruption, temperature and/or pressure, both of which have been shown to facilitate the antimicrobial effect upon penetration of CO_2 into cells. Cell breakage, as a result of gas expansion within the microbial cells when the vessel pressure is suddenly released, may be strengthened under higher pressures.

There were minimal differences in microbial inactivation between gaseous CO_2 and liquid CO_2 despite the differences in temperatures. However, when the process conditions were elevated to supercritical conditions, the inactivation of tested food pathogens increased. Nevertheless, under the experimental conditions of these studies, it was difficult to determine if the addition of CO_2 or the higher-pressure level caused the greater inactivation.

Most published studies have been focused toward effectiveness evaluation as a reasonable goal for a new application. However, this approach does not clearly separate the effects of various parameters such as pressure, carbonation, level and other critical parameters involved in this technology. Despite the generalized effort spent on the study of this technology, the fundamental mechanisms of microbial inactivation by $_{CP}CO_2$ are not yet fully understood. For a satisfactory explanation of the complex antimicrobial effects of $_{CP}CO_2$, further studies need to be conducted to determine what mechanism dominates under various process conditions.

In summary, intensive research of $_{CP}CO_2$ treatments are further necessary to demonstrate their effectiveness to control microorganisms and enzymes in foods. Food processing applications of $_{CP}CO_2$ are becoming more popular as economically viable alternatives to heat treatments. Potential applications for$_{CP}CO_2$ shows promise in the realm of non-thermal processing, with products that are sensitive to heat and pressure such as fresh produce, fruit juice and beverages, fresh fish and smoked fish, fresh meats, and others.

Results from this study show that it is possible, using $_{CP}CO_2$ on beef trimmings before grinding to reduce *E. coli*, coliforms, *Salmonella spp.* and aerobic bacteria in ground beef. In addition, the use of $_{CP}CO_2$ on beef trimmings before grinding had little effect on ground beef instrumentally evaluated redness, oxymyoglobin content and sensory characteristics.

The use of $_{CP}CO_2$ in ground beef production systems can be effective for reducing microbial pathogens in beef trimmings with minimal effects on color or odor characteristics of ground beef. Additional work might focus on $_{CP}CO_2$ concentration, exposure times, exposure temperature, compression/decompression cycles, and other variables necessary to optimize its antimicrobial properties. This technology should not only improve the microbial safety of ground beef, but also promote extended ground beef color shelf-life stability.

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APPENDIX A.

Description of the $_{CP}CO_2$ System

The basic parts of the experimental vessel (see Figure 36), manufactured by Atlas/Parker (Des Plaines, IL), consist of two basic parts, a custom stainless steel base end and a rod end head. The vessel is equipped with a 4-20 mA pressure transducer Model PX605 (Omega Engineering Inc. Stamford, CT) with a range of 0-3000 psi mounted on the side of the steel base end. The rod end head is a stainless steel screw equipped with a General Purpose 100 OHM Sheathed RTD Probe Model PR-11-2-100-1/4-6-E (Omega Engineering Inc. Stamford, CT) connected to a Miniature Temperature Transmitter Model TX92A-1 (Omega Engineering Inc. Stamford, CT) which transmits to a computer system through an RTD cable. The steel base end is a stainless 12 in. x 15 in. steel cylinder Model 5.0 TMWV8 3.5, with an approximate volume of 415 cubic in. The free volume of the reactor when closed is approximately 200 cubic in., which allows for ca. 1.6 lbs of lean meat (considering a specific gravity of 60 lbs/cubic in.) and slightly over 100 cubic in of CO_2. The system is designed to withstand pressures up to 3000 psi.

A full schematic of the $cpCO_2$ system is shown in Figure 37, where it can be observed how the Base End Head is connected to the necessary fittings for gas recirculation. The carbon dioxide in gaseous form is supplied to the system by two 60 lb cylinders ($_GCO_2$) serially connected to one 300 lb cylinder providing the liquid carbon dioxide ($_LCO_2$). The 60lb gas cylinders are individually covered with custom electronically controlled heating jackets set at 36°C.

Figure 26. Basic Parts of the $_{CP}CO_2$ Vessel.

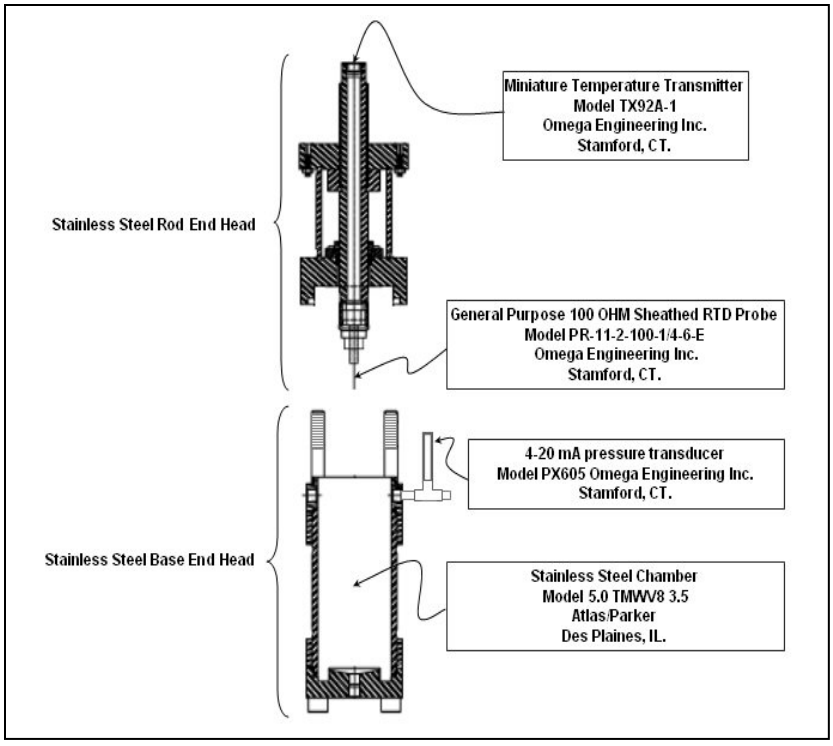

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Figure 27. Schematics of the $cpCO_2$ System.

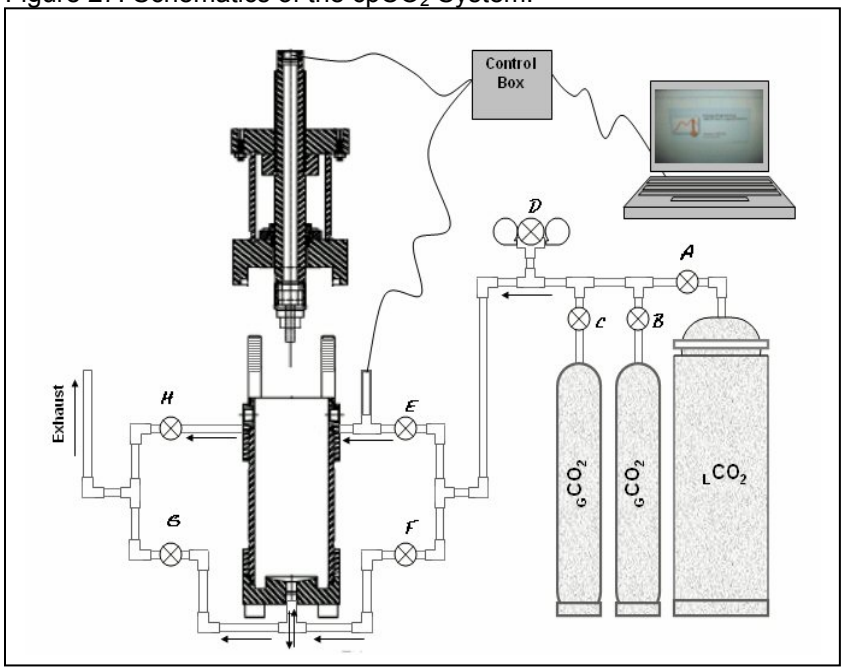
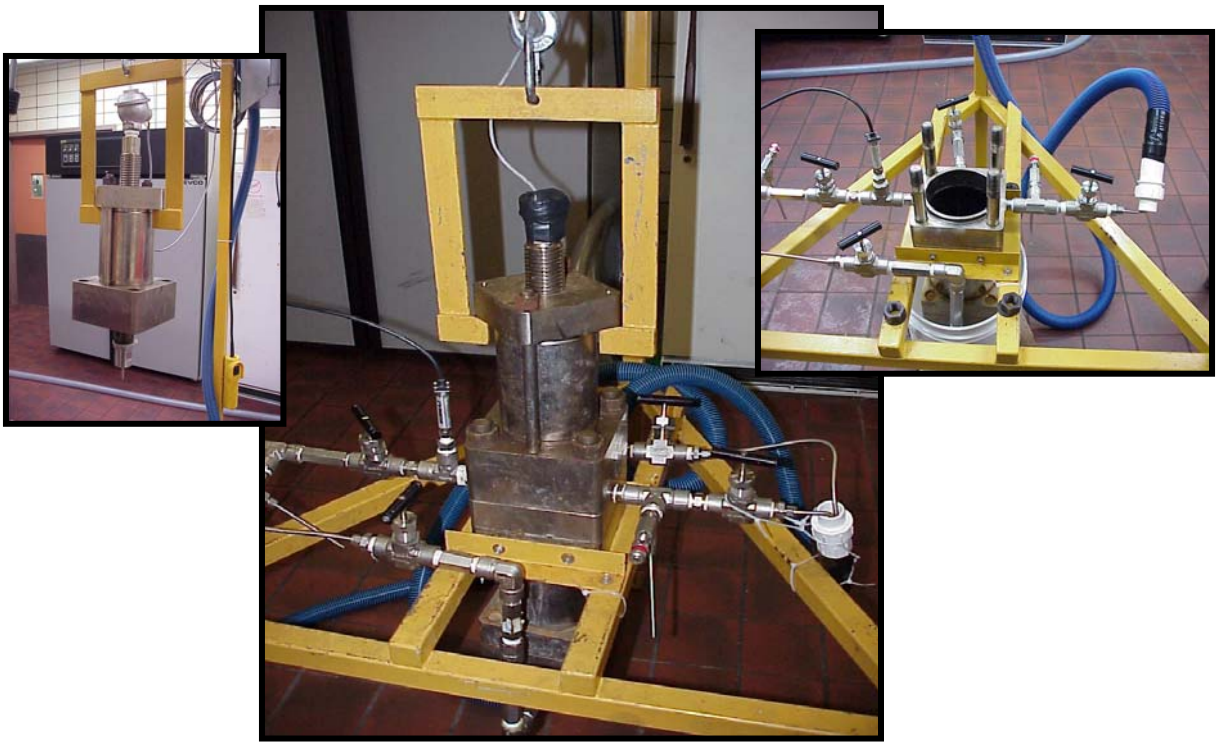


Figure 28. Experimental Vessel.



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APPENDIX B.

$_{CP}CO_2$ Application Protocol

Omega software was installed in a desktop computer and initialized. Pressure inducer and RTD cable were properly connected to the computer and calibrated through the software, according to manufacturer instructions. Software and device were verified for proper functionality.

Figure 38 shows an example of a typical operational chart, generated by the Omega® Data Logging Software Ver. 2.00.43c, with the sections of the protocol referred for an example exposure of 3 m to a targeted pressure of 1200 psi.

In order to be able to reach pressures over 2000 psi inside the chamber, the pressure inside the cylinders containing gaseous carbon dioxide needed to be increased to at least 1500 psi from its nominal pressure (600 psi); therefore, cylinders ($_GCO_2$) were covered with a heating jacket. Heating jackets were turned on 24 hours in advanced in order to pre-heat the cylinders.

All valves in the system were closed before start. Exhaust valve (H) was opened ¼ of a turn. Pressure was checked for 0 psi (<4.0 mA) in the system. The liquid carbon dioxide ($_LCO_2$) inlet valve (F) and the $_LCO_2$ control valve (A) were both fully opened.

To begin $_LCO_2$ application, the main control valve (D) was fully opened and $_LCO_2$ was applied at a constant flow until an internal temperature and pressure inside the vessel reached -20°C and 250-350 psi, respectively (***a***). At -20°C, $_LCO_2$ valve (A) was closed.

The top exhaust (H) and the $_LCO_2$ inlet valve (A) were closed and $_LCO_2$ application was suspended. Immediately, $_GCO_2$ was initiated by opening inlet valves (B&C) until internal pressure equilibrated with the $_GCO_2$ cylinders (***b***).

At equilibrium, the chamber was completely isolated by closing all the inlet and exhaust valves. In order to increase the pressure inside the chamber, the hot water (50°C) inlet valve (I) was opened, causing the CO_2 inside the chamber to expand, increasing the pressure inside the vessel, until an internal temperature of 36°C is reached (***c***). When the targeted pressure was lower than the pressure at equilibrium, heating of the chamber with hot water was not necessary.

At 36°C the hot water valve (I) was closed, suspending the heat application to the vessel in order to maintain a temperature fluctuation between 36°C and 38°C (***d***). The hot water exhaust (J) valve was opened and the water exhausted or re-supplied as needed, in order to control the temperature inside the vessel below 38°C and to achieve targeted pressure.

When necessary, system pressure was regulated to desired pressures by 1 sec releases of $_GCO_2$ through the exhaust valve (H).

At completion of exposure time, exhaust valve (H) was opened and the chamber was slowly decompressed to avoid formation of dry ice and freezing of samples, during approximately 1 min, until atmospheric pressure (0 psi) inside the chamber was reached (***e***).

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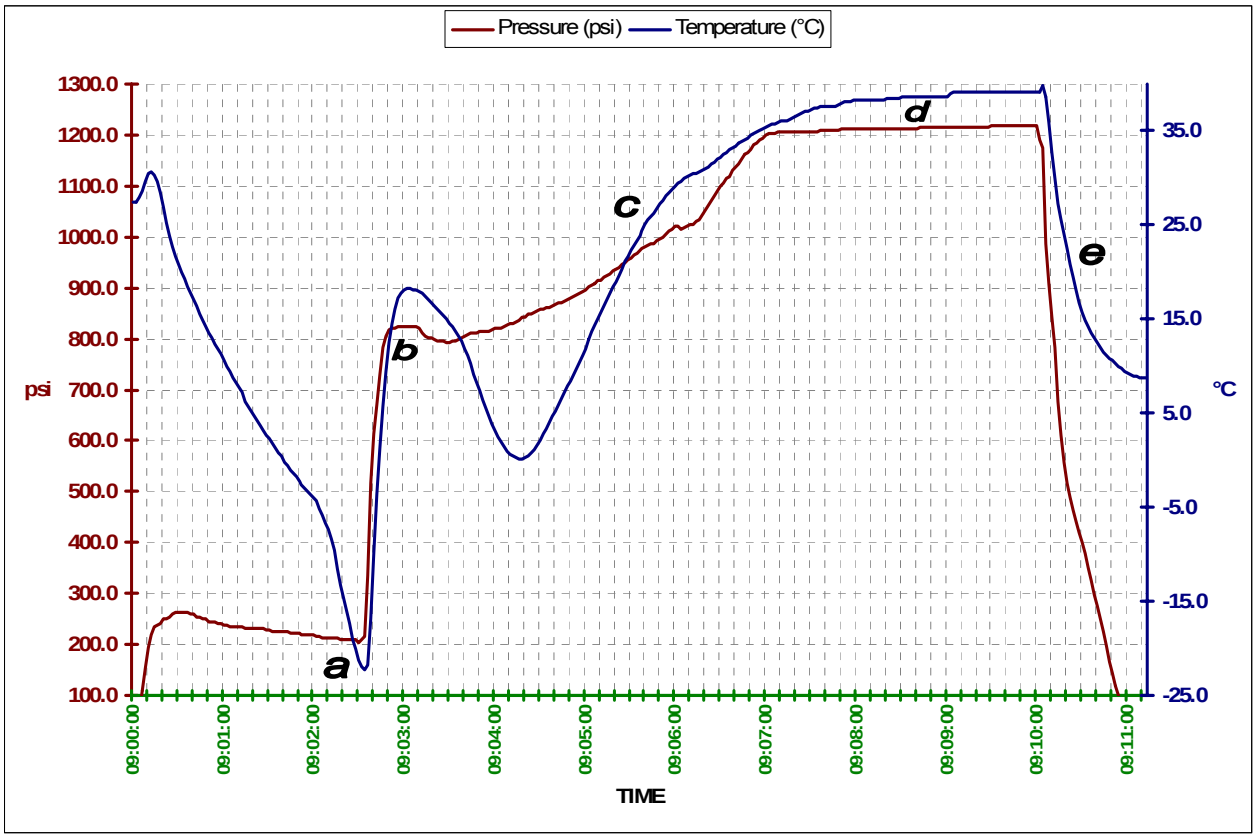
Figure 29. Typical Operational Chart for 3 min Exposure at 1200 psi



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