CONTROL 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

By

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AN ABSTRACT OF A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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ABSTRACT

This dissertation 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. Critical parameters included pressure, temperature, exposure times, modified atmosphere conditions, and days of simulated retail display.

1500 psi $_{CP}CO_2$ for 15 min achieved 0.83, 0.96, 1.00, and 1.06 log reductions for Total Plate Count (TPC), Generic *E. coli* (GEC), *E. coli* O157:H7 (O157), and *Salmonella* spp. (SS), respectively. Bacterial reductions in ground beef and beef trimmings were similar (P≥0.05).

CIE L^*, a*, and b^* values in raw patties showed no differences (P≥0.05) immediately after $_{CP}CO_2$ application on beef trimmings. Nevertheless, significant (P<0.05) interactions were found in pressure by packaging for L^*, in pressure by packaging by days of simulated retail display for a^*, and in packaging by days of simulated retail display for b^* scores. Nevertheless, after 5 days of simulated retail display, L^*, a^*, and reflectance (630/580nm) ratios were similar for all treatments (P≥0.05), and b^* scores were most acceptable with 1500 $_{CP}CO_2$ (P≥0.05), regardless of the packaging conditions.

After 5 days of display, cooked patties showed similar (P≥0.05) values for crude protein (%CP) and crude fat (%CF), the extent of lipid oxidation (TBARS), was higher (P< 0.05) in aerobic trays than flushed packages with 100% CO_2.

Ground beef patties manufactured from beef trimmings treated with $_{CP}CO_2$scored higher values for tenderness (P< 0.05) than other treatments. In addition, no differences

($P<0.05$) for juiciness, beef flavor intensity, or off flavor intensity were found between non-treated and the 1500 psi $_{CP}CO_2$ treated patties.

Microbial control of spoilage organisms and foodborne pathogens in ground beef patties with $_{CP}CO_2$ application in beef trimmings was effective (0.6 to 1.2 logs). Lethality levels are comparable to other intervention strategies. Discoloration of beef trimmings after $_{CP}CO_2$ application may not be a concern for grinding purposes. Further packaging with 100% CO_2 is viable for controlling spoilage and pathogenic microorganisms after packaging and during refrigerated storage, although discoloration of raw ground beef patties packaged with 100% CO_2 may be a concern for product marketing.

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DEDICATION

This work is dedicated to my most valuable treasures in life:

Susana, **K**arim, and **O**mar, my beloved family:

I always had:

Your everlasting and unconditional love,

your indestructible loyalty and unrestricted support,

your absolute faith and devoted trust.

You have made this possible…

Thanks…

This is for you. I owe it to you.

Introduction

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 implemented 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 of utmost importance 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) in an enclosed system. Controlled Phase Carbon Dioxide ($_{CP}CO_2$) in gaseous state dissolves in water to form carbonic acid, thus lowering pH between 3 and 4. The pH of the meat can be continuously maintained at <3.5 when

pressures around 1000 pounds per square inch (psi) 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 properties 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.

CHAPTER 1.

Literature Review

1.1 Microbiology of Beef Trimmings and Ground Beef

1.1.1 Sources of Microbial Contamination

Beef muscle becomes contaminated with pathogenic microorganisms present on the exterior of the live animal during skinning, and/or from the environment, where they are transferred to the product surface (Sofos et al., 1999). Some of the most common sources of bacterial contamination are the hide, hair, and hooves of the animals (Mies et al., 1999).

Microbial contamination of beef carcasses frequently occurs during processing, when muscle tissue is converted to meat. The inner layers of muscle tissue in a healthy animal are free of contamination; however, a large number of microorganisms are regularly transferred to the carcass by contact with equipment and various surfaces (Guerrero and Taylor, 1994) as well as by human contact (Gorman et al., 1995a, 1995b; Castillo et al., 1998a, 1998b, 1999).

Dorsa et al. (1998) reaffirmed that the microbial quality of raw materials used for grinding is the most important factor contributing to the level of microbial contamination in ground beef. He stressed that the method in which ground beef is processed could determine the susceptibility for microbiological contamination on the final product.

1.1.2 Predominant Endogenous Microbial Populations on Fresh Meat

Different bacterial groups are prevalent on meat, and this prevalence is dependent on several factors, among others: the characteristics of the meat (fat, protein, moisture, pH),

the storage environment (refrigerating, freezing, vacuum or modified atmosphere packaging, etc.), and the type of processing operations (processing in the same site of slaughtering).

Gram-negative bacteria comprise the greatest pathogenic and spoilage risk for meat and meat products. The greatest risk is observed in fresh meat chill-stored aerobically, where some members of the genera *Acinetobacter*, *Psychrobacter*, *Pseudomonas*, and *Moraxella* display the fastest growth rates and hence the greatest spoilage. Other species, such as *Shewanella* and *Enterobacteriaceae*, need favorable conditions to develop and produce spoilage metabolites (Davies and Board, 1998).

Depending upon the specific conditions, fresh meat shelflife may be in the range of days before showing any signs of spoilage (off-odors and slime). Additional bacterial groups of relative concern may be Micrococci, lactic acid bacteria, *Brochothrix thermosphacta*, other saprophytic Gram-positive bacteria, (e.g. *Kurthia* and non-toxigenic staphylococci), and pathogenic and toxigenic representatives (e.g. *Staphylococcus aureus*, *Listeria monocytogenes*, and several *Clostridium* species) (Davies and Board, 1998).

Along the same lines of study, Arthur et al. (2004) demonstrated that the prevalence of indicator organisms, such as aerobic bacteria (APC) and *Enterobacteriaceae* (EBC) showed a strong correlation to other pathogenic microorganisms like *Escherichia coli* O157:H7. Particularly, they established that samples containing higher levels of APC and EBC were more likely to contain detectable levels of *E. coli* O157:H7.

Psychrotrophic pseudomonads may typically dominate the microbial population of fresh and unprocessed meats under aerobic conditions and refrigeration temperatures, due to their higher growth rate. Gram-positive bacteria usually show higher survival rate and

longer persistence than most Gram-negative bacteria, since they show higher tolerance against limiting factors like refrigeration temperatures, water activity (a_w), and pH variations. Furthermore, within the typically mixed microbial population of a meat ecosystem, lactic acid bacteria may also have a strong competitive advantage when the product is vacuum packaged, emulsified, and cured (Davies and Board, 1998).

During chilling-storage, the number of microorganisms on fresh meat surfaces changes following a typical microbial growth pattern. Bacterial populations in meat have 2-5 log CFU/cm^2, but only around 10% have been shown to be able to initiate growth (Nychas et al., 1988). When numbers exceed 7 log CFU/cm^2, off-odors are detected as the first spoilage sign. Bacterial slime, another characteristic spoilage indicator is also perceptible when cell numbers have reached this level (Gill, 1982). Nevertheless, dominance of a single microbial group starts shortly after the growing phase declines and the stationary phase is reached, due to a higher growth rate under specific conditions, and because of metabolism advantages or higher tolerance to influencing factors (Gill and Molin, 1991).

Other important groups of microorganisms commonly prevalent in meat are some species of psychrotrophic *Enterobacteriaceae*, with higher occurrence on chilled meat products because their growth is favored at temperatures of 2-4°C (Blickstad and Molin, 1983). However, Gram-negative motile and non-motile aerobic rods and coccobacilli belonging to the genera *Pseudomonas*, *Moraxella*, *Psychrobacter,* and *Acinetobacter* are the main concerns regarding the spoilage flora of raw meat stored aerobically under refrigeration (Garcia-Lopez et al., 1998).

In order to limit or reduce microbial growth in ground beef, vacuum packaging and modified atmosphere packaging (MAP) have been two of the preferred methods commonly used in wholesale marketing. Conventional over-wrapped aerobic trays are also normally used in retail marketing (Hood and Mead, 1993). In addition, carbon dioxide (CO_2), generally in combination with nitrogen (N_2) and/or oxygen (O_2), is used in the MAP of meats because it enhances microbial inhibition. Gas mixtures in MAP had been used in levels varying from 10% to 40% in the case of CO_2 and 90% to 60% for O_2. Gill and Molin (1991) concluded that, in general, higher concentrations of CO_2 present in the mixture correlated directly to the level of spoilage inhibition, with longer shelflife attained at 100% CO_2; however, they expressed a concern of products possibly experiencing chemical changes detrimental to meat quality.

Gill and Penney (1988) determined that the most efficient gas-to-meat ratio for MAP products was about 2 liters of gas per kilogram of meat. They found that the level of residual oxygen in the headspace is important because when various oxygen concentrations were included in packs having different headspace volumes, an initial level of >0.15% oxygen compromised the color of beef and lamb but not pork, which rendered acceptable organoleptic scores to levels up to l.0%. They also determined that increasing headspace volumes counteracted this problem but at a cost in terms of pack size (Penney and Bell, 1993). This problem has been approached with the inclusion of oxygen scavengers such that the full potential of MAP is achieved. These scavengers contain loose, finely divided iron powder useful to reduce and even eliminate residual oxygen by the formation of nontoxic iron oxide; other scavengers absorb oxygen with the concurrent production of equal volumes of carbon dioxide, thereby preventing pack collapse (Smith et al., 1990).

Since all films in commercial use have certain oxygen permeability, absolute anaerobic conditions are seldom achieved. Vacuum packaging is the preferred method for large pieces of chilled primals or wholesale cuts. In vacuum packs, the residual oxygen may rapidly be consumed (below 1%) by tissue and microbial respiration, and CO_2 increases to about 20%. Therefore, during storage, aerobic Gram-negative bacteria are slowly overgrown by Gram-positive bacteria. Within this group, lactic acid bacteria have been the most frequently isolated bacteria from this kind of products due to their tolerance to CO_2 and low temperatures (Egan and Roberts, 1987; Dainty and Mackey, 1992).

The types of meat and fat source also determine the initial level of contamination, trimmings from fed-beef are usually found to be a source of fat in ground beef formulation. These are commonly generated in large amounts from table trimmings resulting from wholesale/retail fabrication, ventral thin meats, and external carcass trimmings containing substantial proportions of subcutaneous fat. Emswiler et al. (1976) reported that when beef carcasses are processed into retail cuts, any bacterial population not removed by trimming or washing at slaughter may spread and attach to newly exposed surfaces.

Scanga et al. (2000) confirmed that trimmings with higher fat content had higher Aerobic Plate Counts (APC) and trimmings that had 30% fat had the highest APC, regardless of trim type; however, no differences in *E. coli* Counts (ECC), Total Coliform Counts (TCC) or *Staphylococcus aureus* (SA) counts were found across the different fat percentages. It is important to note that in this study, levels of TCC, ECC and SA counts were relatively low, and often below the detection limits (1 log CFU/g) of the microbiological analyses. The trend for raw materials with higher fat content to have higher bacterial counts was believed to be influenced by the location on the carcass from which

the trimmings were derived (ventral thin meats compared to whole muscle cuts) and the amount of surface area represented in a core sample.

In support of these observations, Eisel et al. (1997) discovered that other portions of the carcass were not as highly contaminated as the brisket and the skirt areas. Additionally, they reported that in some facilities, trimmings of higher fat content generated from the wholesale and/or retail-steak fabrication process came from product that had been aged, contained a greater amount of surface area, or exposed to extensive handling and cross contamination. Therefore, conclusions could be drawn that microbial contamination is not only a result of tissue type (fat vs. lean), but also of the surface area (i.e. subcutaneous fat) found in higher fat content raw materials.

Beef trimmings were also analyzed by Scanga et al. (2000), who sampled product at facilities that utilize fresh, frozen or both forms of raw materials. Their research objective was to determine which state of cold storage, during holding and transportation of raw materials, was able to maintain lower levels of bacterial contamination. This study revealed that higher APC counts were found in fresh beef trimmings when compared to frozen trimmings, but there were no differences in TCC, ECC or SA counts between fresh and frozen trim. They suggested that these differences in APC counts were found due to freezing, ice formation or increased ionic strength of the bacteria during a sudden drop in temperature. As these counts were generally lower than the detection limit (<10 CFU/g), Rosset (1982) suggested that similarities between TCC, ECC and SA counts were most likely due to the sensitivity of the analysis. He reported combo-bins of fresh trimmings as having a higher incidence of *Salmonella* spp. He suggested that Gram-negative bacteria (coliforms, *Salmonella*) are more sensitive to freezing and cold shock, than are Gram-

positive bacteria (*Staphylococcus* spp. and *Listeria*), and hypothesized that this is due to the inclusion of sugars and diaminoacids that protect Gram-positive cell membranes from denaturation, making them less susceptible to cold shock due to low-molecular weight metabolite leakage through the cell membrane. This may cause the loss of vital cellular components such as inorganic phosphate and ribose. Additionally, he reported that Gram-negative bacteria lose their ability to use inorganic nitrogen to synthesize protein following freezing and must rely solely on peptides, as they cannot utilize amino acids directly.

The complexity of bacterial attachment to skin and meat has been also a topic of research, as reflected by the work of Thomas and McMeekin (1981) and Lillard (1986), who documented that bacteria could become entrapped in tissue crevices, protecting them from subsequent aerobically applied antimicrobial treatments. Dorsa et al. (1996) suggested that the presence of additional moisture provided by fecal inoculations could affect collagen, lipids, and proteins on tissue surfaces, therefore providing an additional level of protection. He considered of great importance to develop antimicrobial treatments and application methods designed to provide better tissue surface penetration in order to destroy surface and subsurface microorganisms. Bacterial attachment to meat surfaces has been shown to increase bacterial resistance to decontamination methods for fresh meat surfaces. Selgras et al. (1993) reported that bacterial attachment to meat surfaces might be affected by numerous factors, such as type of meat surface, pH, temperature, surface charge, and chemical residues.

1.1.3 Pathogens of Concern on Beef Trimmings and Ground Beef

Foodborne illness causes an estimated 76 million cases annually (CDC, 2005) resulting in billions of dollars in economic and productivity losses every year. The annual cost in medical treatment, productivity loss, and cost of premature death, only for five foodborne pathogens (*Campylobacter*, *Salmonella*, *Escherichia coli* O157:H7, non-O157 *E. coli* and *Listeria monocytogenes*) is estimated to be 6.9 billion dollars (ERS, 2004).

E. coli O157:H7 causes approximately 73,000 cases of illness each year and 61 deaths. *Salmonella,* on the other hand, had 40,000 reported cases with the estimated actual number of cases being 20 times more than the reported amount. More than 1000 deaths occur each year due to *Salmonella* infections, making it the deadliest food-borne pathogen. Ground beef products are commonly implicated in outbreaks of *Salmonella* and *E. coli* O157:H7 (CDC, 2005).

Members of the genus *Salmonella* and *Escherichia* belong to the family *Enterobacteriaceae* that includes other pathogens, such as *Shigella* and *Yersinia*. They are Gram-negative, non-spore forming, aerobic, and facultative anaerobic bacilli. *Salmonella* and *E. coli* are motile by peritrichous flagella. Like all other *Enterobacteriaceae*, their cell wall is a complex structure composed of lipids, polysaccharides, protein, and lipoproteins (Luderitz et al., 1966). The lipopolysaccharide portion of the cell wall (although some protein may also be present) functions as an endotoxin, with lipid A (the lipid moiety of the endotoxin) being responsible for its biological effects (Morrison and Ulevitch, 1978). The common core monosaccharides and polysaccharides of the endotoxin are also called somatic O antigens (Luderitz et al., 1966). Additionally, there are several different flagella

(H) antigens that are identified by numbers and letters, based on a strongly reacting somatic antigen (major determinant) and one or more minor somatic antigens.

1.1.3.1 *Salmonella* spp.

The present name for the genus *Salmonella* was proposed by Lignieres in 1900, for D. E. Salmon, who with Theobald Smith isolated *Salmonella* Choleraesuis for the first time. This nomenclature was officially adopted in 1933 (Hornick, 1974).

The genus *Salmonella,* with close to 2400 serotypes, is considered a single species named *Salmonella enterica*. Serotyping differentiates the strains, and these are referred to by name (i.e. *Salmonella enterica* serotype Typhimurium or *Salmonella* Typhimurium) (Hohmann, 2001). Salmonellae are Gram-negative, motile (with a few exceptions), facultative anaerobic bacteria (D'Aoust, 1997). Salmonellae grow between 8°C and 45°C, and at a pH of 4 to 8. With the exception of a limited number of human-host-adapted serotypes (also referred to as the typhoidal salmonellae), the members of the genus *Salmonella* are denominated as zoonotic or potentially zoonotic (Acha and Szyfres, 2001).

There are approximately 60 "O" antigens for *Salmonella*, which are designated by numbers. Salmonellae may be separated into major groups using specific antisera. Most salmonellae that cause human disease belong to Kauffman-White scheme groups A, B, C, C2, D, and E14. For specific serotype identification, flagellar antigens must be identified. Some serotypes of *Salmonella* (i.e. *S.* Typhi), possess a capsular antigen called the virulence (Vi) antigen, which envelops the cell wall (Koneman et al., 1997).

The symptoms of Salmonellosis range from nausea, vomiting, abdominal cramps, diarrhea, headache, and fever, with an onset time of six to eight hours. Depending on the ingested dose and strain characteristics, acute symptoms typically last for one to two days

but may be prolonged. Chronic cases of Salmonellosis can be characterized by arthritic symptoms manifesting three to fours weeks after the onset of acute symptoms. The infective dose ranges from 15-20 cells depending upon a series of health and host factors, and strain differences. The organism colonizes the host by penetrating the intestinal lumen into the epithelium of the small intestine where symptomatic inflammation is often observed (CFSAN, 2001).

Salmonella spp. isolation and enumeration in foods commonly uses Difco® Xylose Lysine Desoxycholate (XLD) agar, which provides excellent identification and enumeration capabilities. Basis of identification on this medium is the ability of *Salmonella* spp. to decarboxylate lysine creating an alkaline environment, and to produce hydrogen sulfide, which appears as a black precipitate, because of the reaction with ferric ammonium citrate and sodium thiosulfate. Confirmation can then be completed with PCR, rapid test kits or by serum agglutination tests. In cases where sample components may bind to the ferric ammonium citrate and limit XLD agar's effectiveness, an alternative differential agar such as Difco® Brilliant Green (BG) could be used for correct identification based on the formation of deep red colonies with a halo due to the fermentation of lactose in the presence of basic fuchsin. Coliforms like *Salmonella* spp. that do not ferment lactose produce colorless to faint pink colonies allowing for presumptive identification and enumeration (Difco, 1998).

Heat-injured *Salmonella* may be inhibited from growing in XLD, whereas nonselective media, such as Tryptic Soy Agar (TSA), is favorable for their recovery. Based on these facts, Kang and Fung (2000) developed a thin agar layer (TAL) procedure designed for higher rates of heat-injured *Salmonella* cell recovery, while providing

selectivity of isolation from other bacteria in the sample. This procedure, tested with excellent results, consists of preparing a thin agar layer plate by overlaying 14 ml of non-selective medium, such as TSA, onto prepoured and solidified selective or differential media, such as XLD, in an 8.5 cm diameter Petri dish. During the first few hours of incubating the plate, the injured *Salmonella* cells will repair and grow on the TSA. After the injured cells have been resuscitated, the selective agents from XLD will diffuse through the TSA top layer, allowing targeted cells to produce a typical reaction (black color) while other microorganisms will be inhibited by the selective agents.

1.1.3.2 Generic *Escherichia coli* and Serotype O157:H7

Escherichia coli, originally known as *Bacterium coli commune*, was identified in 1885 by a German pediatrician named Theodor Escherich (BAM, 2001). Normally *E. coli* serves a useful function in the body by suppressing the growth of potentially harmful bacterial species and by synthesizing appreciable amounts of vitamins. Some strains of *E. coli* are capable of causing human illness by several different mechanisms. One of four rare varieties of *E. coli* that produces large quantities of toxins is *E. coli* serotype O157:H7, which severely damages the lining of the intestine and other internal organs. The pathogenic groups of *E. coli* include enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), and perhaps others that are not yet well characterized (Levine, 1987; Nataro and Kaper, 1998). Of these, only the first four groups have been implicated in food or water borne illness. The toxins produced by these pathogens are called verotoxins, which are closely related to those toxins produced by *Shigella dysenteriae* (CFSAN, 2001)

Illness is characterized by severe abdominal cramping and bloody diarrhea. Vomiting occurs occasionally, with fevers being low-grade or absent. It is usually self-limited and lasts for an average of eight days with some individuals only exhibiting watery diarrhea. In some cases, deaths have been reported from the immuno-compromised sector. The infective dose is around 10 cells, most likely similar to that of *Shigella* spp. The organism has also demonstrated the ability to be passed from person-to-person in day-care settings and nursing homes (DBMD, 2002).

Some of the methods used for isolation and enumeration of *E. coli* O157:H7 in food utilize MacConkey Sorbitol Agar (MSA), an excellent medium for isolation and enumeration of *E. coli* O157:H7 from food. MacConkey Sorbitol Agar bases its use as isolating and differential medium for *E. coli* O157:H7 because it contains D-Sorbitol rather than lactose. *E. coli* O157:H7 does not ferment sorbitol or does it very slowly, appearing as colorless colonies on the media, whereas generic *E. coli,* due to the fermentation of sorbitol, will appear from pink to red. Bile salts and crystal violet are usually added to inhibit the growth of Gram-positive microorganisms (Difco, 1998). Thin Agar Layer (TAL) has also been evaluated for the recovery of *E. coli* injured cells. Results indicated that TAL recovered a higher number of cells when compared with conventional plating (Crozier-Dodson and Fung, 2002)

1.1.4 Pathogen Control on Beef Trimmings and Ground Beef

The use of antimicrobial interventions on animal tissues has been widely researched including the use of hide-on decontamination, chemical de-hairing, knife trimming, hot water washing, and steam pasteurization. Trimming beef carcasses has shown little effect on the overall microbial safety of the tissue when compared to other methods available to

the industry (Acuff et al., 1997). Therefore, new methods of decontamination are currently being investigated that may prove to be more successful at elimination of pathogens. Although using a single method of intervention on carcasses have been shown to be effective, these can be used individually or as part of a multi-hurdle system (Ransom et al., 2003). Some other chemical compounds (i.e. Lactoferrin) and organic acids (acidified chlorine, acidified sodium chlorate, peroxyacetic acid, cetylpyridinium chloride, and others) have also being investigated.

1.1.5 Intervention Strategies for Pathogen Control

Hide-on decontamination practices have been shown to reduce bacteria on hides by about 5 log CFU/100 cm^2, and decrease the bacterial load of carcasses by 1 log CFU/100 cm^2. This in turn dramatically reduces the prevalence of *E. coli* O157:H7 on carcasses (Bosilevac et al., 2005).

Chemical de-hairing is another extremely effective practice at virtually eliminating carcass decontamination from the de-hiding process (Nou et al., 2003). In a study by Castillo et al. (1998c), chemical de-hairing was found to be extremely effective on reducing contamination on cattle hides, as this practice was able to reduce *E. coli* O157:H7 populations on the hides by almost 5 log.

Ellebracht et al. (1999) applied hot water and lactic acid treatments to decontaminate fresh beef trim from young and mature beef cattle; reports show prevalent levels of 4.3 and 4.3 log CFU/g of *E. coli* O157:H7, and 3.8 and 3.9 log CFU/g of *Salmonella* Typhimurium, respectively. They found that hot water treatment alone significantly reduced the level of *E. coli* O157:H7 by 0.5 log CFU/g and *Salmonella* Typhimurium by 0.7 log CFU/g. Hot water followed by lactic acid produced an additional

reduction of 1.1 and 1.8 log CFU/g for both *E. coli* O157:H7 and *Salmonella* Typhimurium, respectively.

Phebus et al. (1997) compared the effectiveness of steam pasteurization (S) in reducing pathogenic bacterial populations of *Listeria monocytogenes* Scott A, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium on surfaces of freshly slaughtered beef versus other standard methods used commercially. They included practices such as knife trimming (T), water washing at 35°C (W), hot water/stream vacuum spot cleaning (V), and spraying with 2% vol/vol lactic acid at 54°C with a pH of 2.25 (L). Treatment combinations (in order of application) evaluated were TW, TWS, WS, VW, VWS, TWLS, and VWLS. All combinations showed average reductions from 3.5 to 5.3 log CFU/cm^2 in all three pathogenic groups. Knife trimming in combination with other treatments were equally effective as those followed by steam pasteurization, resulting in reductions ranging form 4.2 to 5.3 log CFU/cm^2. When used individually, trimming, vacuum spot cleaning, and steam pasteurization resulted in 2.5 to 3.7 log reductions; however, steam pasteurization consistently provided numerically greater pathogen reductions than all other individual treatments.

Conner et al. (1997) reported that spraying 1 ml combinations of both acetic and lactic acid at 55°C at beef trimmings, slightly reduced *E. coli* O157:H7 populations in fresh ground beef by 0.1 and 0.2 log CFU/g for 2% and 4% mixtures, respectively; however, counts still remained high after the application of a combination of both acids. They suggested that higher reductions of pathogens would be more difficult to achieve due to extensive handling and grinding associated with ground beef operations.

Lactoferrin is another product used to limit and control bacterial populations as it binds to the outer membrane of Gram-negative bacteria, causing membrane blisters (Yamauchi et al., 1993), which effect morphological changes in the cell (Bellamy et al., 1993). Lactoferrin B also prevents attachment of bacteria to carcass surfaces; in addition, it has been shown that within one-half hour, initial populations of *E. coli* O157:H7 were reduced by 3.0 log, and within one hour levels were below detection limits (<1.3 log CFU/ml) (Shin et al., 1998).

Peroxyacetic acid, also used as an antimicrobial, has been shown to achieve as high as 3.3 log reductions in *E. coli* O157:H7 when used as a sanitizer on equipment surfaces (Farrell et al., 1998). Peroxyacetic acid was also shown to reduce the percentage of positive samples taken from stainless steel chips (1 cm^2) that were glued to the auger-housing portion of a meat grinder. Stopforth et al. (2003) reported that peroxyacetic acid was more effective at inactivating *E. coli* O157:H7 in biofilms on equipment than a quaternary ammonium compound solution used alone. Although these findings support the possibility that peroxyacetic acid may be effective against enteric pathogens on fresh beef trim, King et al. (2005) indicated peroxyacetic acid sprayed on carcass surfaces was minimally effective at reducing pathogen levels.

Powerful oxidants such as ozone and chlorine dioxide have been used as potential antimicrobial treatments to decontaminate beef tissues (Kochevar et al., 1997; Reagan et al., 1996), because their mechanism of action is based in an irreversible damage to the fatty acids in the cell membrane and to cellular proteins of the microorganisms (Luck and Jager, 1998). Emswiler et al. (1976) achieved a 1.64 log CFU/cm^2 reduction on aerobic plate counts of beef carcass tissues by using 200 ppm chlorine dioxide. Kochevar et al. (1997)

also reported a reduction of 2.64 log CFU/cm^2 on lamb adipose tissues in aerobic plate count using 35°C water in combination with 0.003% chlorine dioxide. Similarly, Unda et al. (1989) found that 100 ppm chlorine dioxide reduced by 1 log CFU/cm^2 the total plate count on fresh beef steaks, but had negative effects on color. Gorman et al. (1995b) was able to achieve reductions of *Escherichia coli* populations using a combination of 35°C water and 0.5% ozone by 1.84 log CFU/cm^{2}on beef brisket fat and 1.49 log CFU/cm^2 using 35°C water and 0.5% ozone on beef adipose tissue. Finally, Reagan et al. (1996) showed that 2.3 ppm of ozone applied on beef carcasses was able to reduce APC by 1.3 log CFU/cm^2.

Stivarius et al. (2002) reviewed the impact of some antimicrobial treatments commonly applied to beef trimmings. Antimicrobial treatments for this study included 1% ozonated water bath at 7.2°C for 15 min (15O); 1% ozonated water bath at 7.2°C for 7 min (7O); 200 ppm (vol/vol) chlorine dioxide solution (CLO), and an untreated control (C). Chlorine dioxide (CLO) was effective against all bacterial types as it reduced *E. coli* (EC), coliforms (CO), *Salmonella* Typhimurium (ST), and aerobic plate counts (APC) by 0.71, 0.57, 0.61, and 0.72 log CFU/g, respectively. These results are in agreement with Emswiler et al. (1976) which applied the same concentration of CLO by spraying beef carcasses, obtaining a 1.64 log reduction on APC. Unda et al. (1989) tested beef rib eye steaks dipped in 100 ppm of CLO, showing 1 log CFU/cm^2 reductions on APC. The 15O treatment was able to achieve 0.44, 0.78 and 0.57 log CFU/g for CO, ST and APC, respectively in ground beef, with pH values of 5.55 for C, 5.34 for CLO, 5.29 for 7O, and 5.77 for 15O, respectively. The slightly lower antimicrobial effectiveness of the 15O treatment was caused by pH differences within the treatments when compared with CLO. Application of

7O treatment on beef trimmings achieved reductions of 0.45 and 0.32 log CFU/g for ST and APC, respectively in the ground beef. This treatment was not as effective against EC and CO perhaps due to a shorter duration of treatment.

Meat scientists and food microbiologists have performed an enormous amount of research to promote safety in the meat processing operations. Nevertheless, progress towards safer meat products needs to consider the development of preharvest and postharvest intervention strategies at the same time, because safety cannot be totally achieved in a single step in the processing system from farm to table (Fung et al., 2000).

1.1.6 Organoleptic Concerns

The ideal method for pathogen and spoilage control on meat would be to maximize bacterial control, minimize discoloration of meat, and cause no discernable off-flavors, especially in cooked meat (Bell et al., 1986), because the visual appearance of meat is the attribute that has been most critically considered by consumers as the basis for product selection or rejection.

Meat color depends on the concentration and the oxidative state of myoglobin, a water-soluble protein responsible for muscle pigmentation. When myoglobin is oxygenated, it forms oxymyoglobin, which is stable and not easily oxidized to metmyoglobin, and is responsible for the normal color of beef meat (bright, cherry-red color).

Formation of oxymyoglobin occurs spontaneously when meat is exposed to air, but the oxidative metabolism is regulated by oxygen dependant enzymatic activity, hence its stability depends on a continuing supply of oxygen. Upon binding to water, myoglobin turns to deoxymyoglobin, which causes meat to have a purplish color.

Deoxymyoglobin is often found in vacuum packaged products, it is highly unstable, and when meat is exposed to air, it will readily combine with oxygen to form oxymyoglobin or metmyoglobin. Lastly, metmyoglobin, responsible for the brown color of meat, is the oxidized state of myoglobin. Metmyoglobin production is of particular concern to the beef industry in regards to color stability and length of time meat can be placed on the retail shelf before becoming discolored. Mason (2004) explained that external factors can cause metmyoglobin formation. Given that metmyoglobin is very stable, meat remains brown in color presence of oxygen. Only by depriving metmyoglobin from oxygen and developing reducing conditions will revert meat to a desirable color.

Visual appraisals of color have been closely related to consumer evaluations and set the benchmark for instrumental measurement comparison. The American Meat Science Association has found consumer evaluations difficult to perform by using trained or consumer panels, because repeatability of human judgments is difficult to achieve and personal preference, lighting, visual deficiencies of the eye and appearance factors other than color may influence their observations (AMSA, 1991).

Color of uncooked meat and meat products is usually described as pink or red, but colors range from nearly white to dark red. Discoloration of these products often involves tan, brown, gray, green or yellow colors. Hunt and Kropf (1985) recommend the measurement of the lack of redness or other normal color for meat, because brown colors are difficult to quantify instrumentally. Researchers have used L^*, a^*, and b^* values to report color evaluations (See Figure 1). For discoloration studies, ratios of a/b, hue angle or saturation index have been used traditionally, and reflectance values have been used often to measure meat color, to follow color changes and to quantify myoglobin forms.

The Hunter Lab color space, one of the most common methods use for color evaluation, is organized in a cube form. *L* values range from 0 to 100, from bottom to top, giving a numerical value of 0 for black (no reflectance) to a 100 for white (highest reflection). The *a* and *b* axes have no specific numerical limits, but values would range from negative values to positive values, giving values for green to red, and from blue to yellow, respectively for *a* and *b*. (AMSA, 1991). Below is a diagram of the Hunter Lab color space.

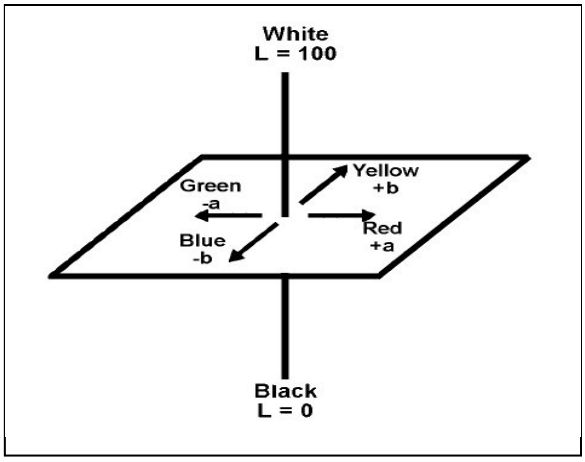


Figure 1. Hunter *L a b* Color Space is Organized in a Cube Form with *L* Values Ranging From 0 for Black (No Reflectance) to 100 for White (Highest Reflection); *a* and *b* Values Range from Negative Values to Positive Values, Giving Values for Green to Red, and from Blue to Yellow, Respectively for *a* and *b* (AMSA, 1991).

Hot water, organic acids in high concentrations, or other decontamination treatments have shown adverse effects on the color or odor of beef tissue (Bell et al., 1986; Unda et al., 1989). Applying organic acids to the surface of retail cuts can cause undesirable discoloration and sensory attributes.

Many studies have tested the effects on organoleptic attributes and palatability of beef cuts. Bell et al. (1986) evaluated dipping one-centimeter cubes of semimembranosus and adductor muscles for 1, 10, and 100 sec in solutions of 0.6%, 1.2%, 1.8% or 2.4% acetic acid or a mixture of 0.6% acetic acid and 0.046%, 0.092%, 0.184% or 0.230% formic acid. Only dipping for one minute in 0.6% acetic acid did not adversely affect sensory color scores, and as the concentration of acetic acid increased, the effects were more detrimental for the color scores. Jeremiah et al. (1972) found that Gardner a^* values between 29.06 and 23.05 are preferred by meat consumers, which is neither extremely dark nor extremely pale.

Stivarius et al. (2002) also summarized the impact of the antimicrobial treatments discussed in the previous section to beef trimmings on ground beef instrumental color and sensory characteristics. Ground beef from the CLO treatment was lighter (*L**), less red (*a**), contained less oxymyoglobin (630 nm/580 nm), and was less orange (hue angle) in color, but was not different in yellowness (*b**) compared to the untreated control (C).

Along these lines, Unda et al. (1989) found that 100 ppm of CLO caused lower *a** values when used on rib eye steaks. In addition, beef trimmings treated with CLO were less vivid in color (saturation index), when compared to C. The 7O treatment was also lighter (*L**), less red (*a**) possessed less oxymyoglobin (630 nm/ 580 nm), was less yellow (*b**) and less vivid (saturation index) in color compared to C, however, hue angle did not differ between the 7O and C treatments. Likewise, ground beef from the 15O treatment was also lighter (*L**) and more yellow in color (*b**), and contained less oxymyoglobin (630 nm/580 nm) than C, yet was no different in redness (*a**) when compared to C. Differences in the CIE b*value translated into a larger hue angle for the 15O treatment compared to C;

however, saturation index did not differ between these two treatments. Sensory panelists evaluated color and odor characteristics of ground beef through simulated retail display. Although all treatments were slightly less bright when compared to C, no differences were observed between C and any other treatment for percentage discoloration, beef odor or off-odor characteristics.

Another study conducted by Garcia-Zepeda et al. (1994) compared aroma profiles of beef chuck rolls sprayed with chlorine (200 ppm) and lactic acid (3%) for 2 min at a temperature of 20°C. Decontaminated beef chuck rolls had higher acceptability scores than those treated with lactic acid but not a significant effect on microbial reductions.

Graves-Delmore et al. (1998) concluded that antimicrobial applications were more effective for reducing microbial contamination on beef adipose tissue than were individual decontamination treatments. Pohlman et al. (2002a, 2002b) reported that the use of trisodium phosphate, cetylpyridinium chloride, chlorine dioxide or lactic acid as multiple antimicrobial interventions prior to grinding, These chemical intervention strategies can reduce microbial loads and reduce bacterial contamination when used closer to ground beef packaging. In these studies, it was found that ground beef treated with cetylpyridinium chloride followed by trisodium phosphate (CT), and ground beef treated with chlorine dioxide and followed by cetylpyridinium chloride (CLC), maintained similar redness (a^*) as the control on days 1, 2 and 3 of display. However, on day 7 of display the CT treatment showed redder color than the untreated inoculated control.

The effect on processing characteristics, instrumental and sensory color, and lipid stability of ground beef patties, when these antimicrobials are used as multiple interventions on ground beef patties produced from non-inoculated beef trimmings, is still

unknown. Since any discoloration or off odors caused by antimicrobial treatments might result in consumer rejection, it is important to determine, control, and optimize the effects of any novel decontamination systems, used alone or in combination, on the microbial stability, instrumental color, and sensory characteristics of ground beef.

1.2 Carbon Dioxide

1.2.1 History

Carbon dioxide was one of the first air gases described as an individual substance. In the 17th century, Jan Baptist van Helmont, a Belgian chemist, observed that the original mass of charcoal burned in a closed vessel was much higher than the mass from the resulting ash. His interpretation was that an invisible substance, he denominated “wild spirit” (spiritus sylvestre) or “gas”, was the result of a transmutation of charcoal. (Wikipedia, 2006).

During the 1750’s, Joseph Black, a Scottish physician, also studied the properties of CO_2 finding that a gas he denominated "fixed air", which was denser than air and did not support combustion or animal life (Williams, 1904), could be produced by heating or treating limestone with acids. He also found that an aqueous solution of lime (calcium hydroxide), when bubbled through, it would precipitate calcium carbonate, and used this phenomenon to illustrate the production of CO_2 by animal respiration and microbial fermentation (Wikipedia, 2006). The French chemist, Antoine Lavoisier, proved that the gas obtained by the combustion of charcoal is identical in its properties with the “fixed air” obtained by Black, showing that CO_2 is an oxide of carbon (Encarta, 2005).

The first information of an artificially carbonated drink is dated 1772, when Joseph Priestley used CO_2, produced from the action of sulfuric acid on limestone, to prepare soda water (Wikipedia, 2006).

In 1823, Humphrey Davy and Michael Faraday achieved carbon dioxide liquefaction for the first time. Charles Thilorier, reported the earliest description of solid carbon dioxide in 1834, when opened a pressurized container of liquid carbon dioxide accidentally forming a "snow" of solid CO_2, produced by the cooling from the rapid evaporation of the liquid (Wikipedia, 2006).

1.2.2 Physical and Chemical Properties

Carbon dioxide is a chemical compound, formed by combining in a double-bonded structure one atom of carbon with two oxygen atoms with no electrical dipole, O=C=O, expressed by the chemical symbol CO_2. Figure 2 shows the molecular structure of the gas.

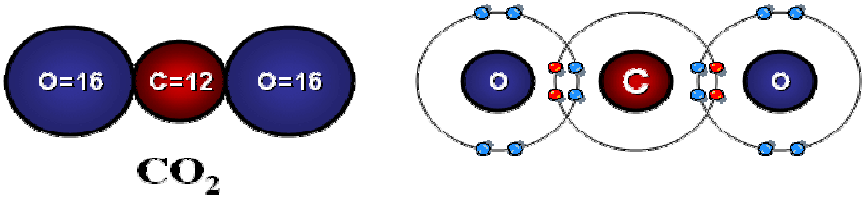


Figure 2. Molecular Structure of Carbon Dioxide (CO_2) with a Double Bonded Structure One Atom of Carbon with Two Oxygen Atoms with no Electrical Dipole.

Carbon dioxide is an odorless, colorless gas at room temperatures (20-25°C) and normal atmospheric pressure (1 atm, 14.7 psi), but can exist, as a gas, a liquid or as a solid. Not very reactive and in particular not flammable because it is completely oxidized, CO_2 is one of the several gases of the atmosphere, being uniformly distributed over the earth's

surface. In addition to being a component of the atmosphere, CO_2 also dissolves in water. Table 1 illustrates some of the relevant properties of CO_2 (Witteman, 2005).

The normal concentration of CO_2 in the atmosphere is approximately 0.03% (300 PPM), and is not usually considered a toxic gas (Witteman, 2005). CO_2 plays an important role on vital functions, such as respiratory stimulation, regulation of blood circulation, and acidity of body fluids. CO_2 concentration in the air affects all of these. Under extended exposure conditions, high concentrations could be dangerous due to increased breathing, heart rate acceleration, and a change in the body acidity.

Table 1. Properties of CO_2.

PROPERTY	US Units
Molecular Weight	44.01
Density (Gas) @ 70°F (21°C) and 14.7 psi @ 32°F (0°C) and 1 atm (101 kPa abs)	 0.114 lb/ft^3 0.123 lb/ ft^3
Density (Saturated Liquid) @ 70°F (21°C) [Cylinder] @ 32°F (0°C) @ 2°F (-17°C) [refrigerated liquid] @ -20°F (-29°C) @ -69.9°F(-56.6°C) [triple point)	 47.6 lb/ ft^3 58.0 lb/ ft^3 63.3 lb/ ft^3 66.8 lb/ ft^3 73.5 lb/ ft^3
Density (Solid Dry Ice) @ 14.7 psi and -109.3°F (101 kPa abs and -78.5°C)	 97.5 lb/ ft^3
Sublimation Temperature (at 14.7 psi or 1 atm)	-109.3°F (-42.9°C)
Critical Temperature	87.8 °F(31°C)
Critical Pressure	1066 psi
Critical Density	29.2 lb/ ft^3
Triple Point	-69.9°F (-21°C) / 75.1 psi
Solubility of gas in water, vol/vol @ 32°F (0°C) and 1 atm (14.7 psi) @ 60°F (16°C) and 1 atm (14.7 psi) @ 32°F (0°C) and 4.1 atm (60 psi)	 1.7 1.0 8.6

From: Witteman Company, LLC. (Witteman, 2005)

The maximum concentration of CO_2 and the time weighted average exposure to CO_2 is regulated by the OSHA (Occupational Safety and Health Act). This U.S. National

Institute for Occupational Safety and Health considers a concentration exceeding 4% as "immediately dangerous to life and health", and has established limits of 0.5% CO_2 for prolonged and 3% for brief exposures (up to ten minutes). Breathing 5% CO_2 for more than half an hour causes acute hyperapnea, while breathing 7%–10% CO_2 renders unconsciousness in only a few minutes. CO_2, either in gas or dry ice form, should be handled only in well-ventilated areas (Wikipedia, 2006).

Presence of CO_2 is evident to the human senses at concentrations over about 2% (20,000 ppm), at lower concentrations is difficult to perceive unless associated with the odor of other materials (automobile exhaust or fermenting yeast, for instance). Above 2%, CO_2 causes a feeling of heaviness in the chest; therefore, exposed individuals experience more frequent and deeper respirations. After several hours of exposure at this level, minimal "acidosis" (an acid condition of the blood) may occur (Wikipedia, 2006).

1.2.3 Solubility

Crawford et al. (1963) and Jarrell et al. (2002) estimated the solubility of CO_2 from experimental work, but the basic principle is that the solubility of CO_2 in water increases with increasing pressure. Figure 3 shows solubility curves used to generate a relational table at different pressure and temperature combinations (Kansas Geological Survey, 2003). Under normal conditions, 14.7 psi at 60°F (15.5°C), one volume of CO_2 will completely dissolve in an equal volume of water, and it also dissolves readily in most liquids, but it remains dissolved only as long as pressure is applied, otherwise it escapes to the atmosphere in the form of bubbles. As solubility is increased by pressure, the greater the pressure, the more carbon dioxide a liquid can hold in solution. Almost 53% heavier than air, CO_2 will settle to the bottom of a container displacing the air to the top (Toromont

Energy Systems, 1995). Shakhashiri (2006) explained through a series of chemical equations this process.

First, in aqueous solution, $CO_2(g)$ in gaseous form simply dissolves:

$$CO_2(g) \longrightarrow CO_2(aq)$$

Then, equilibrium is established between the dissolved $CO_2(aq)$ and carbonic acid (H_2CO_3). Only about 1% of the dissolved CO_2 exists as H_2CO_3.

$$CO_2(aq) + H_2O(l) \rightleftharpoons H_2CO_3(aq)$$

Carbonic acid is a weak acid, which dissociates in two steps:

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$$

The aqueous carbonic acid dissociates, producing gaseous $CO_2(g)$:

$$H_2CO_3(aq) \rightleftharpoons H_2O(l) + CO_2(g)$$

In nature, surface water often becomes acidic because atmospheric CO_2 dissolves in it. The sour taste in the mouth and stinging sensation in the nose and throat are a consequence from the gas dissolving in the mucous membranes and saliva, resulting from a weak solution of carbonic acid (Wikipedia, 2006).

CO_2 is also highly soluble in both muscle and fat tissue (Gill and Penney, 1988; Jakobsen and Bertelsen, 2003). The absorption capacity is related to biological factors like pH, water, and fat content (Gill, 1982; Jakobsen and Bertelsen, 2002), but also largely to the packaging and storage conditions, specifically CO_2 partial pressure, headspace to meat volume ratio and storage temperature (Jakobsen and Bertelsen, 2002; Zhao et al., 1995).

The effect of these factors has been studied in a number of experiments. The results show that rising pH levels increase the solubility by 0.36 L/kg per pH unit in the interval

from pH 5.4–6.9 (Gill and Penney, 1988). In water and fat phases, CO_2 absorption has been shown to be directly proportional to temperature (Gill and Penney, 1988; Jakobsen and Bertelsen, 2003), while other researchers concluded solubility could not be calculated solely on knowledge of the water content (Fava and Piergiovanni, 1992).

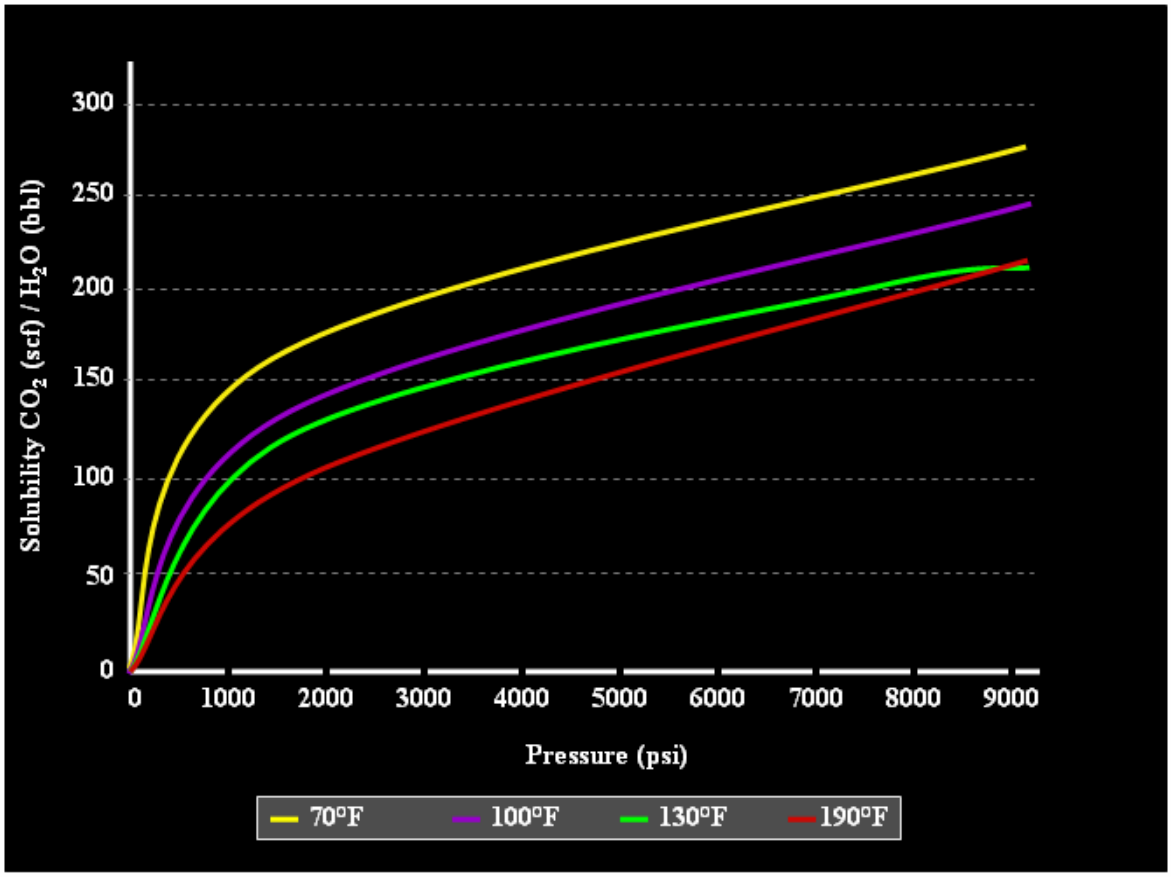


Figure 3. CO_2 Solubility in Water at Different Combinations of Pressures and Temperatures. Modified from Kansas Geological Survey (KGS, 2003)

Ledward (1970), Piergiovanni and Fava (1992), and Zhao et al. (1995) suggested that CO_2 solubility is linearly correlated with partial pressure. Zhao et al. (1995) also found that increased headspace to meat volume ratio also correlates directly to solubility. Studies also show that increasing the temperature decreases solubility for lean meat by approximately 0.02 L/kg per 1°C in the interval from 1.5 to 10°C (Gill and Penney, 1988;

Jakobsen and Bertelsen, 2003) or 0.01 L/kg per 1°C in the interval 3–13°C (Zhao et al., 1995).

However, it is probably more important to consider the relative influence of fat content, pH, temperature, headspace volume, and CO_2 partial pressure in the absorption of CO_2 in meat products. Devlieghere et al. (1998b) demonstrated that the initial headspace concentration (10–100%CO_2) and the headspace to meat volume ratio (0.5–4) were the main factors, but pH (4.5–7.5) was also shown to have a significant effect. In the other hand, lard content (0–40%) and temperature (4–10°C) were shown to produce only minor effects when compared to the other factors.

The different factors affecting solubility, in order to obtain the full preservative effect of carbon dioxide and to ensure an attractive package appearance, need further investigation. However, several models have been developed and used as a valuable tool to predict the amount of absorbed CO_2 for a specific product composition, packaging, and storage conditions. These models can also be used to correlate those attributing factors to bacterial inhibition in modified atmosphere packed meat (Devlieghere et al., 1998a; Devlieghere et al., 2001).

As stated before, the CO_2 solubility in the lean meat/water phase of the meat decreases with increasing temperature (Jakobsen and Bertelsen, 2003). Furthermore, when determining the headspace volume to prevent package collapse, it is necessary to consider the solubility of CO_2 in the fat phase, as it depends on the total amount of fat, fatty acid composition and storage temperature (Jakobsen and Bertelsen, 2003). Recent results suggest that an increase in surface area merely increases the rate of CO_2 absorption rather than influencing the total amount of CO_2 absorbed. Jakobsen and Bertelsen (2003) found

that when maximum CO_2 was absorbed in the lean meat samples (approximately 1 L/kg meat), pH tended to decrease, even at higher rates with a higher initial pH of the meat. These findings support the results of Rousset and Renerre (1991), and might be due to the larger solubility of CO_2 at higher pH. Nevertheless, Dixon and Kell (1989) believe that it is clear that pH decreases because of the production of carbonic acid from absorbed CO_2 and not as a result of the food matrix absorbing the CO_2.

Jakobsen and Bertelsen (2003) established that, in determining CO_2 absorption in meat, partial pressure and headspace from the gas to meat volume ratio were more important. They demonstrated that temperature, pH, and fat content were of little practical importance, and a partial pressure above 30% CO_2 and a volume ratio of at least 2 L gas/kg meat was necessary to prevent snug down of the package. However, partial pressure of CO_2 at the time of packaging or equilibrium/end of storage time was sufficient to predict the amount of absorbed CO_2.

1.2.4 Controlled Phase Carbon Dioxide

CO_2 can exist in different forms or phases. The term "controlled phase carbon dioxide" ($_{CP}CO_2$) it is used in this dissertation to describe the liquid or supercritical regions of carbon dioxide in the phase diagram (Figure 4). In these regions carbon dioxide remains as a fluid or dense gas with respect to gaseous CO_2. As shown in the figure, $_{CP}CO_2$ has a range of densities that are within the range of liquid-like or near-liquid substances, exists as a low-density gas at standard temperature and pressure conditions, and possesses phase boundaries with a triple point (solid, liquid, and gas co-exist in equilibrium) and a critical point, where liquid and gas have identical densities. Through pressure or temperature

modification, CO_2 can be compressed into a dense gas state. In addition, CO_2 in the supercritical region displays lower viscosity and higher diffusivity (Rizvi et al., 1986).

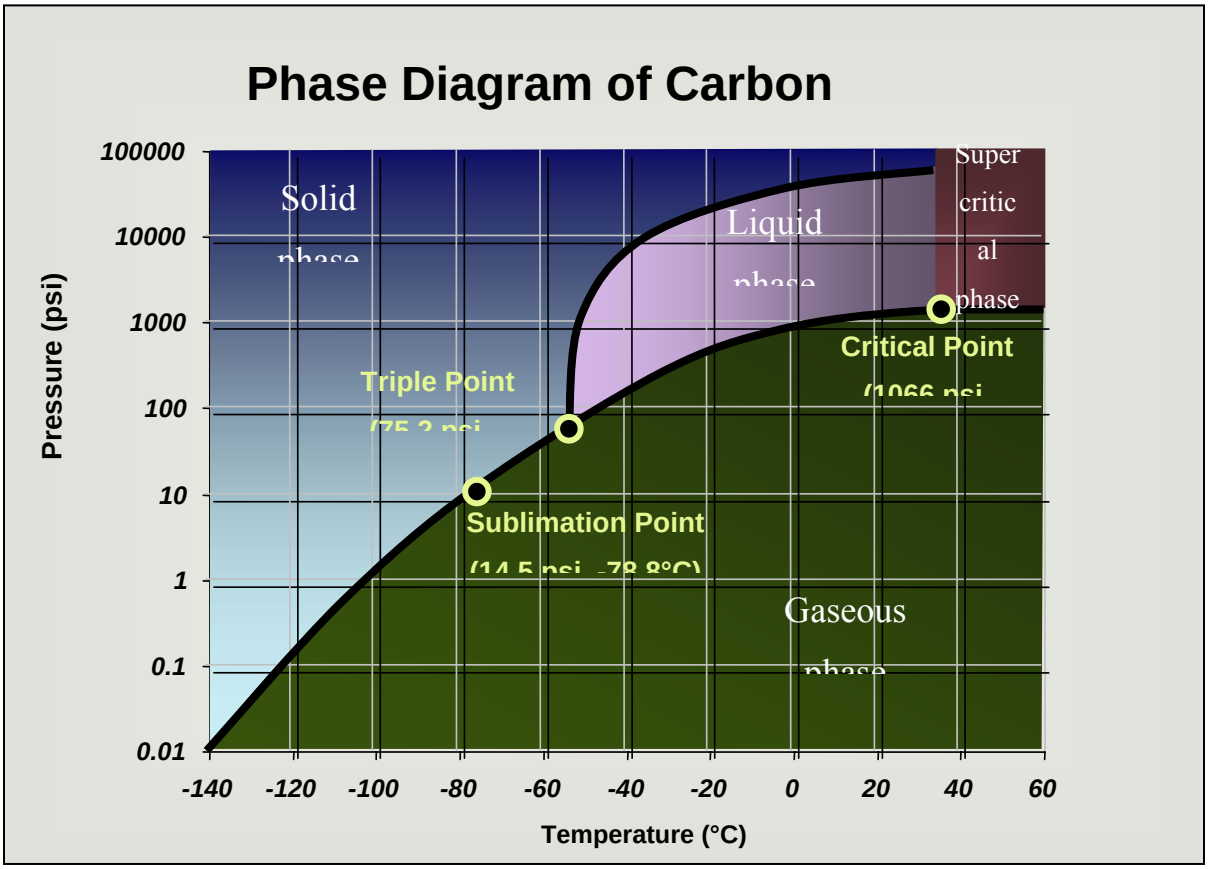


Figure 4. Pressure-Temperature Phase Diagram of CO_2. Modified from Science is Fun. Chemical of the Week. "Carbon Dioxide" (Shakhashiri, 2006).

The "Triple Point" is the pressure-temperature combination at which carbon dioxide can exist simultaneously in its three states as gas, liquid, and solid. When carbon dioxide is compressed over 1066 psi at a temperature below its critical temperature of 87.8°F (31.1°C), the gas is liquefied. Above the critical temperature and above the critical pressure (1066 psi) it is impossible to liquefy the gas by increasing the pressure, retaining the density and the solvent properties of its liquid form. If the pressure is reduced from the triple point, the liquid evaporates to gas. If the temperature is reduced, the liquid freezes. If the temperature is increased, the liquid boils, which generates gas. The normal temperature

of solid CO_2 (dry ice) is -109°F (-78.5°C). The solid will change directly back to the gaseous, subliming slowly at ambient temperatures and atmospheric pressure. At the critical point, the density of CO_2 is approximately 0.47 g/ml. At or above this point CO_2 is termed a supercritical fluid (SCF), neither a liquid state nor a gas state substance.

A supercritical fluid is a substance above its critical temperature and critical pressure. Under these conditions, it is impossible to distinguish between gas and liquid; ultimately, the chemical properties become identical at the critical point, the distinction between liquid and gas disappears, and the substance can only be described as a "supercritical fluid". Supercritical carbon dioxide ($_{SC}CO_2$) can be compressed to a range of liquid-like densities, yet it will retain the diffusivity of a gas. Continued compression of $_{SC}CO_2$ increases fluid density, approaching that of its liquid phase.

Figure 5 shows the transition between the separate phases of CO_2 inside an enclosed container. The meniscus is easily observed in the first frame. In the second frame, with an increase in temperature, the meniscus begins to diminish. Increasing the temperature further causes the gas and liquid densities to become more similar, as shown in the third frame, where the meniscus is less easily observed but still evident. Finally, once the critical temperature and pressure have been reached, the two distinct phases of liquid and gas are no longer visible. The meniscus can no longer be seen. One homogenous phase called the "supercritical fluid" phase occurs which shows properties of both liquids and gases.

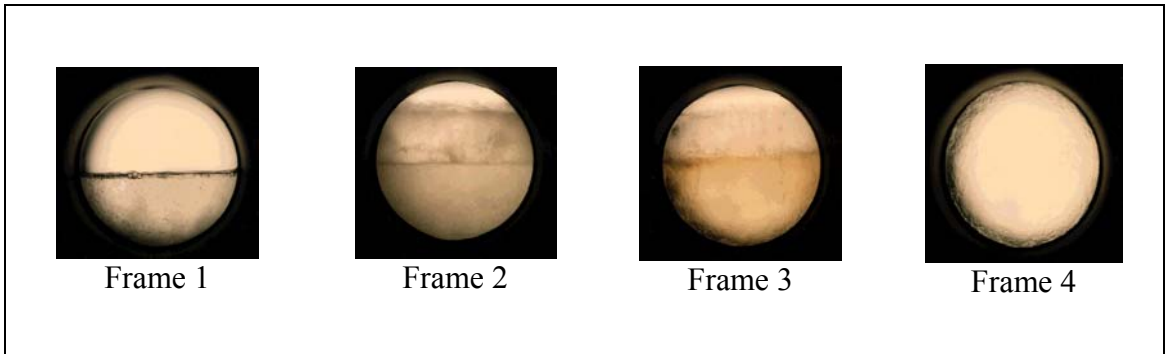


Figure 5. Transition of CO_2 phases. From Harvesting Mars (Miller and Phillips, 2005).

1.2.5 Uses and Applications of CO_2 in the Food Industry

The versatility of CO_2 in any of its three forms—as a gas, a liquid, or a solid— has encouraged its application in many industrial areas. CO_2 has become a valuable tool as a refrigerant in production processes and transportation due to its ability to offer precisely controlled fast cooling.

However, for the past three decades, high investment costs and unfamiliar operation has limited the commercial application of supercritical fluid technology to very few products. Not until recent years, technological advances in process, equipment, and product design, have opened new opportunities for supercritical fluid technology (Sihvonen et al., 1999). Conventionally, the extraction process with this technology is conducted under high-pressure conditions by exposing the material from which a desirable product is to be separated to the supercritical solvent. The supercritical solvent, saturated with the extracted product, is expanded to atmospheric conditions and the solubilized product is recovered in separation vessels, and the supercritical solvent is recycled for further use.

Supercritical fluid technology also can be applied for the qualitative and quantitative identification of constituents of naturally occurring products and heat-labile

compounds, as a recognized and effective analytical technique with favorable and comparable efficiencies to existing chemical analysis methods (Dionisi et al., 1999; Ibañez et al., 2000; De Castro and Jimenez-Carmona, 2000; Moret and Conte, 2000).

In addition, the reduction of liquid solvent waste, and the substitution of some undesirable organic substances, is another advantage of supercritical fluid analytical techniques. Extraction with supercritical fluids is also a unit operation employed for applications including: extraction and fractionation of edible fats and oils, purification of solid matrices, separation of tocopherols and other antioxidants, purification of herb medicines and food products from pesticides, detoxification of shellfish, and concentration of fermentation broth and fruit juices (Eggers et al., 2000; Lang and Wai, 2001; Gonzalez et al., 2002; Ibañez et al., 2000).

Supercritical fluid extraction has proved effective in the separation of essential oils and its derivatives for use in food, cosmetics, pharmaceutical and other related industries, producing high-quality essential oils with commercially more satisfactory compositions than obtained with conventional hydro-distillation (Ehlers et al., 2001; Diaz-Maroto et al., 2002; Ozer et al., 1996).

Recent investigations have demonstrated the potential of CO_2 in the commercial decaffeination of coffee and black tea, and recovery of alkaloids such as theophylline, theobromine, and pilocarpine, among others (Saldaña et al., 2002; Saldaña et al., 2000).

Similarly, the risk association of high blood cholesterol levels with heart diseases or cancer has been a motivating factor to propose supercritical extraction for fat and cholesterol extraction in eggs and dairy products (Greenwald, 1991), as cholesterol was shown to be soluble in $_{SC}CO_2$. Extraction with supercritical fluids requires higher

investment but can be highly selective and more suitable for food products. These results clearly indicate the great potential of supercritical fluid extraction in the recovery of meat products with acceptable cholesterol and fat contents.

1.2.6 Effects on CO_2 on Enzymatic Activity

Thermal processing also has been used to inactivate enzymes in food products, mainly because enzymes in food are largely responsible for the quality of a finished product. Some of the major negative effects that enzymes have in some food products involve breakdown of cloud in juices, browning of cut produce, lipid oxidation and hydrolysis proteins. However, research on the effects of $_{CP}CO_2$ on enzymatic activity is scarce, and it suggests the use of $_{CP}CO_2$ as a potential technology to reduce some of the detrimental effects of enzymes in a food, by causing irreversible damages to the secondary structure of proteins above 45000 psi at 25°C. Pressures below this level generally involve no damage to proteins or minor reversible changes upon depressurization (Hendrickx et al., 1998). Others believe that inactivation begins at 30000 psi, causing the tertiary structure of proteins to break down (Balny and Masson, 1993); this may be reversible depending on external sources such as temperature, pH, and presence of sugars, salts, or other additives.

On the other hand, Knorr et al. (1998) questions this technology as being suitable for enzyme inactivation because the pressures normally used in $_{CP}CO_2$ are much lower than those proven to inactivate enzymes at room temperature (30000 – 80000 psi).

All enzymes have the ability to modify their capacity to accept a substrate, as the pH promotes changes in the degree of ionization of the amino acid side groups at its active site. Therefore, as pH decreases by the addition of $_{CP}CO_2$, enzymes may also be inactivated, affecting both the kinetics and the stability of an enzyme, but stability and optimal pH are

not related (Schwimmer, 1981). Individual enzymes exhibit a range of pH stabilities from a pH of below 2 to above 13 (Fennema, 1996); nevertheless, because enzymes exhibit a range of acid stabilities and optimal pH, it is difficult to predict the effect of acidification on its reaction rate.

$_{CP}CO_2$ may also affect the enzymatic activity of a food product by changing the substrate structure. Since substrates require a suitable conformation for reaction with an enzyme, any alteration of the substrate's conformation or orientation could inhibit or stimulate the rate activity of the enzyme. For example, fractures in the microstructure of a substrate generated by high shear produced in this process would increase the surface area of the substrate, increasing the target sites for a particular enzyme, thereby increasing the rate of enzymatic activity.

Taniguchi et al. (1987) reported that when CO_2 at 2900 psi was applied as a mixture suspended in a 3% ethanol solution to a non-aqueous mixture of enzymes at 35ºC for one hour, $_{CP}CO_2$ had little effect in treatments without water across several enzyme types. In addition, the presence of other substances such as ethanol may increase the effectiveness of $_{CP}CO_2$as an enzyme inhibitor, as illustrated in Table 2.

Kamihira (1987) reported mixed results for the use of $_{CP}CO_2$ to inactivate enzymatic activity, as the enzymatic activity for two different enzymes, lipase and α-amylase, treated with CO_2 at 3,046 psi and 35°C for 2 hours, resulted in 88% and 121%, respectively, when compared to their original activities.

Table 2. Residual Enzyme Activity after $_{SC}CO_2$ Treatment at 2900 psi and 35°C for 1 hour.

Enzyme	CO_2	CO_2+ 3% Ethanol
Amylase	94	96
glucoamylase	102	96
B-Galactosidase	98	98
Glucose Oxidase	97	93
Glucose Isomerase	102	95
Lipase	96	88
Thermolysin	101	96
Alcohol Dehydrogenase	97	87
Catalase	90	96

(Taniguchi et al., 1987)

Other findings suggest that higher enzyme activities can be achieved by increasing the buffering capacity of the enzyme preparations (pH range 4-9) when compared to non-buffered controls (Tejdo and Eshtiaghi, 2000).

1.2.7 Antimicrobial Effects and Mechanism of Action of CO_2.

CO_2 has been used as an antimicrobial agent because it is an ultra-pure product, free from toxicity, generally recognized as safe, and can be easily removed from food products. Extensive studies on the microbial inhibition by CO_2 near atmospheric pressure, and changes in the function of the cell membrane, have been reviewed (Jones and Greenfield, 1982; Eklund, 1984; Dixon and Kell, 1989; King and Nagel, 1967). More recently, it has been found that if CO_2 is pressurized, the process is no longer bacteriostatic, but bactericidal (Arreola and Balaban, 1991; Nakamura et al., 1994; Enomoto et al., 1997b; Erkmen, 2000a, 2000b, 2000c, 2001a; Calvo and Balcones, 2001).

$_{CP}CO_2$ may stimulate or inhibit cell development. This inhibitory action has been the recent interest and focus of various researchers intensively looking to improve the safety of foodstuffs and protecting them from bacterial contamination (Dixon and Kell, 1989; Donald et al., 1924; Haas et al., 1989; Wei et al., 1991). Spoilage prevention has

been another area of opportunity for the application of $_{CP}CO_2$, as shown by Blickstad et al. (1981) achieving longer shelf life in pork meat, by increasing the partial pressure of carbon dioxide on the microflora of pork and adding it to the packaging atmosphere.

$_{CP}CO_2$ processing in food has shown effective reduction of bacterial populations in a variety of products including mozzarella, Parmesan, strawberries, onions, and fresh herbs (Haas et al., 1989). The University of Florida used the technology on liquid food products including orange juice and whole egg (Wei et al., 1991). Work on juice has been subsequently continued by other investigators as well (Kincal et al., 2006; Park et al., 2002).

More recently, $_{CP}CO_2$ has been used as a bactericidal (Calvo and Balcones, 2001; Erkmen 2001a, 2001b; Erkmen and Karaman, 2001) and also as a mean to precipitate casein proteins from milk (Jordan et al., 1987; Tomasula et al., 1995, 1997, 1998; Hofland et al., 1999).

The bactericidal effect of $_{CP}CO_2$ has been studied by several researchers (Ballestra et al., 1996; Frazer, 1951; Haas et al., 1989; Isenschmid et al., 1995; Ishikawa et al., 1995; Kamihira et al., 1987; Lin et al., 1991, 1992a, 1992b, 1993; Shimoda et al., 1998). Their results show a wide range of inhibitory activity of pressurized CO_2 caused by the concentration of dissolved CO_2 in the cell suspension and the susceptibility to pressure treatments of the pathogenic population tested.

The degree of bacterial efficacy from the application of CO_2 varies widely, this inconsistency mainly due to the parameters that can be modified during experimentation, such as CO_2 pressure, pH, temperature in the system, exposure time, organoleptic

characteristics of the food matrix, bacteria type, reactor type (continuous or batch), number of pressure cycles, decompression rates, etc.

Kamihira et al. (1987) 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, suggesting that yeast is more resistant to the antimicrobial action of $_{CP}CO_2$. Nevertheless, others believe that yeast cells may not be more resistant (Wei et al., 1991; Arroyo and Sanz; 1997; Enomoto et al., 1997a). Other studies indicate that both bacteria and yeast cells require appreciable water content for pressurized CO_2 inactivation (Haas et al., 1989; Nakamura et al., 1994; Kumagai et al., 1997).

Other researchers were able to reduce 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 (Haas et al., 1989). Higher pressures and temperatures increased the effectiveness of the treatment.

Nakamura et al. (1994) observed 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. Arreola and Balaban. (1991) used pressures between 1200-4,800 $_{CP}CO_2$ at temperatures between 35°C and 60°C in a batch system on abused orange juice samples to determine effect on total plate count. The lowest D value (12.7 minutes) was obtained with the highest temperature and pressure. However, an acidic environment like the orange juice could cause endogenous microorganisms to be more tolerant to low pH values.

The effect of $_{CP}CO_2$ on bacterial spores has been reviewed extensively (Kamihira et al., 1987; Arroyo and Sanz, 1997; Spilimbergo et al., 2002), and most of them determined the ineffectiveness of the methodology, possibly due to their low water content or higher pressure resistance. In order to destroy spores effectively (>7 log), $_{CP}CO_2$ needs to be applied at temperatures over 60°C and much longer periods of exposure, which makes it impossible to apply for fresh products. Enomoto et al. (1997a) theorized that at pressures below 870 psi, treatments were not vigorous enough, and higher pressures caused spores to flocculate, providing a protective effect. A 30 hour treatment is too long for most food applications, but may be suitable for pharmaceuticals.

Fungal spores appear to be more sensitive to $_{CP}CO_2$ treatment than bacterial spores, as demonstrated by Shimoda et al. (2001) who treated *Aspergillus niger* spores with 1450 psi at 52ºC, obtaining a D value of 0.16 minutes. In the absence of $_{CP}CO_2$, same treatment was incapable of eliminating the spores.

Microbial inactivation by $_{CP}CO_2$ is dependent on many parameters. Some of the experimental conditions, such as temperature, pressure, and moisture, contribute to a more effective treatment by increasing the diffusivity of $_{CP}CO_2$. Exposure time is another experimental variable that could be modified for better results. Microbial resistance to $_{CP}CO_2$ also depends on the type of microorganism, the phase of growth, and the food matrix type, especially as in some high-protein food systems, can inhibit the bactericidal effect of $_{CP}CO_2$, (Wei et al., 1991).

Several mechanisms of action for the antimicrobial activity of $_{CP}CO_2$ have been proposed. Lin et al. (1991, 1992a) suggested that supercritical carbon dioxide penetrates cells and ruptures them when high pressure is suddenly released. This effect was improved

by decompressing repeated times. Fraser (1951) observed that the best results were obtained when the $_{CP}CO_2$ pressure was released as rapidly as possible.

In food with high water content, CO_2 dissolves in the water to form carbonic acid, lowering the pH of the medium, and the resulting acidity leads to lower the interstitial pH of bacterial cells. It was therefore suggested that microbial inhibition was due to an alteration in the properties of the cell (membrane, cytoplasm, enzymes, etc.) (Dixon and Kell, 1989). $_{CP}CO_2$ in a dense phase penetrates the bacterial cell wall easier than in its gaseous state, and it has been said to be more efficient near its critical point (Rizvi et al., 1986).

Additional research endorses an opposing theory, that the antimicrobial activity of $_{CP}CO_2$ cannot be accounted solely by a reduction in the pH of the system, since stronger acids used to lower medium acidity (hydrochloric acid, phosphoric acid, etc.) exhibit lower inhibitory effects when compared to the same pH conditions offered by the application of $_{CP}CO_2$. (Becker, 1933; Haas et al., 1989; King and Mabitt, 1982; Lin et al., 1993).

Hong and Pyun (1999, 2001) established that bacteria can survive in an acidic environment, compensating for acidic conditions by maintaining a pH gradient between the internal and external environment, but keeping the internal pH close to neutral by actively pumping hydrogen ions from the interstitial fluid to the outside of the cell. However, this buffering process is unable to be sustained because of the constant flow and high permeation of $_{CP}CO_2$ through the cell membrane. Therefore, the interstitial pH is finally reduced and this acidification results in the destruction of the cell, probably by the denaturing of cellular enzymes (Ballestra et al., 1996) and/or affecting the rate at which

particular reactions proceed via induction or repression of cytoplasmic enzyme synthesis (Bowien and Leadbeater, 1984; Dixon et al., 1988).

Some researchers claim that the reduction of internal pH is likely to be the main source of inactivation, citing that pressure alone (up to17400 psi) is *per se* incapable of bacterial inactivation without $_{CP}CO_2$ (Dillow, 1999).

Another theory is that the rapid depressurization compromises the structural integrity of the bacterial cell, causing ruptures in the cell wall when abruptly releasing the gas. (Enomoto et al., 1997a).

$_{CP}CO_2$ application to bacterial cells has been shown to yield a non-linear death curve (Ballestra et al., 1996; Shimoda and Osajima, 1998). These curves consist of a short stationary response followed by a logarithmic phase. The short stationary phase can be shortened with a faster rate of $_{CP}CO_2$ diffusing into the cell by increasing the temperature and pressure, thereby accelerating the rate of the death phase. Ballestra et al. (1996) theorized that the stationary phase was caused by the accumulation of $_{CP}CO_2$ within the cell wall during the sub-lethal stressing of cells. When the amount of accumulated $_{CP}CO_2$ within the cell reached a critical value, the pH change was sufficient to denature the interstitial proteins causing the death of the cell.

Nakamura et al. (1994) suggested that the death of microorganisms occurred mainly due to mechanical rupture while rapidly depressurizing. Most of their evidence from disrupting *Saccharomyces cerevisiae* by the use of rapid depressurization of $_{CP}CO_2$ cited electron micrographs that show the presence of ruptured cells after treatment with 725 psi at 40°C for 4 hours followed by a sudden depressurization step.

The idea of an inactivation caused only by pressure rupture of cells has been increasingly disfavored in recent years. Enomoto et al., (1997a) established not significant differences in lethality when slow depressurization was compared to flash depressurization at pressures of 580 psi. This would suggest that at pressures below 580 psi, the death due to explosive decompression was minimal. However, the amount of protein released from the treated yeast cells as an indicator of protein denaturation was three times higher than the incubated control. Therefore, he suggested the highest antimicrobial activity took place during pressurization, not during the pressure release. This, however, does not completely disqualify the rapid decompression theory because higher-pressure differentials could demonstrate higher lethality. In addition, the decompression step took nearly a minute to complete. Faster decompression times could also increase the lethality effects of $_{CP}CO_2$.

Ballestra et al. (1996), using electron microscopy, observed significant destruction in *E. coli* cell walls before and after treatment with 725 psi $_{CP}CO_2$, with more than 75% of the cells being visibly ruptured and only 25% of the cells with intact cell walls. Nevertheless, only 1% of the sample was recovered by conventional culturing. This suggests that either the holes in the cell wall were not detected by electron microscopy, or another variable was contributing to the effect. In the other hand, Dillow et al. (1999) found no visible differences in the electron microscope photographs, although achieving higher lethality effects with pressurization and depressurization cycles treatments.

Lin et al. (1991; 1992a) pressurized yeast cells up to 4900 psi and proposed a synergistic effect of the solvent properties of $_{CP}CO_2$ and the depressurization theory. He measured the amount of cellular proteins released from the cells after treatment and established that the $_{CP}CO_2$ was able to partially dissolve the cell proteins. Lin et al. (1991)

suggested that this solvent mechanism was responsible for cell damage but was most effective during depressurization, when the expansion of $_{CP}CO_2$ at pressure release was able to transfer the extracted intercellular proteins out of the bacteria very rapidly. A significant increase in protein release and rate of bacterial inactivation was observed using repeated cycling.

Shimoda et al. (1998) found that *Lactobacillus brevis* was completely inactivated with $_{CP}CO_2$ at 0.16 g/cm^3 in a continuous reactor and 0.9 g/cm^3 in a batch reactor. The increased lethality was attributed to $_{CP}CO_2$ dispersing more rapidly in the continuous reactor than the batch system, leading to a higher concentration of dissolved $_{CP}CO_2$, and a more rapid decompression than the batch system, a force that has been mentioned as a mechanism for inactivation. Additionally, it is possible to assume that the continuous flow of $_{CP}CO_2$ was sufficient to overcome the buffering capacity of the product.

Decontamination of beef trimmings destined for ground beef represent a challenge in the beef industry because ground beef is considered a single ingredient. The advantage of $_{CP}CO_2$ as an antimicrobial for beef trimmings is that $_{CP}CO_2$ evaporates after application, leaving no residue in the product, therefore maintaining its integrity as a wholesome single product. Consequently, it is necessary to establish new lines of investigation and further research to evaluate $_{CP}CO_2$ as an antimicrobial intervention process. Furthermore, $_{CP}CO_2$ may offer additional attributes for quality control improvement, such as color enhancement, spoilage prevention, and shelf-life extension.

CHAPTER 2.
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, because currently there are no validated decontamination technologies to control foodborne pathogens on beef trimmings while providing acceptable quality in ground beef.

This investigation was initiated by performing a series of preliminary studies. The first study, described in Chapter 3, was conducted to evaluate the effect of $_{CP}CO_2$ as an antimicrobial on sterile filter paper disks challenged with multiple strains of generic *Escherichia coli, E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. Different $_{CP}CO_2$ pressures were evaluated to determine their effects on bacterial populations.

Subsequently, a second study, discussed in Chapter 4, 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.

Chapter 5 explains a third study conducted to confront these organoleptic 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, Chapter 6 describes the last study designed to evaluate the effects of $_{CP}CO_2$ as an antimicrobial intervention process for beef trimmings destined for grinding.

CHAPTER 3.

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

3.1 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.

3.2 Materials and Methods

3.2.1 Preparation of Bacterial Cultures

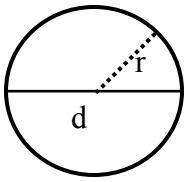
Bacterial cultures were obtained from the Food Microbiology Laboratory stock culture collection at Kansas State University (KSU). The following cultures were utilized: *Listeria monocytogenes* 101M, 109, and 108M (Larry Beuchat, UGA); Generic *E. coli* ATCC 14763 (Jackie Staats, KSU), ATCC 23740 (Microb. 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.* Senftenburg 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^{o}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^{o}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.

3.2.2 Inoculation of Filter Paper Disks

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. The total surface area was calculated with the formula for the surface area of a disk ($2*\Pi*r^2$):



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.

3.2.3 $_{CP}CO_2$ Treatment of Filter Paper Disks

After inoculation, a non-treated sample was analyzed for bacterial populations as a control and 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).

3.2.4 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 treated samples.

3.3 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 ($P \geq 0.05$) in bacterial recoveries (Table 3) among any of the pressures tested, challenged pathogens or the interactions between the effects.

No bacterial colonies forming units were recovered after application of $_{CP}CO_2$ at 1200 psi or 1700 psi for generic *E. coli*, considering a detection limit of 0.80 log CFU/cm^2, providing the highest numerical reductions (5.7 log) for this pathogen (Table 4); however, both treatments 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 similar reductions for *Salmonella* spp. as the 1700 psi $_{CP}CO_2$ for 3 min treatment; 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. All treatments were equally effective ($P\geq0.05$) in reducing bacterial populations (Figure 6).

Reduction levels demonstrated $_{CP}CO_2$ as an extremely efficient antimicrobial treatment, as all the pressures tested consistently achieved at least a 4.04 log reduction over the inoculated controls across the challenging microorganisms, similar to those reported in a study by Kamihira et al. (1997).

Table 3. Effect of $_{CP}CO_2$ on Bacterial Recoveries in Filter Paper Disks Challenged with Generic *E. coli*, *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp.

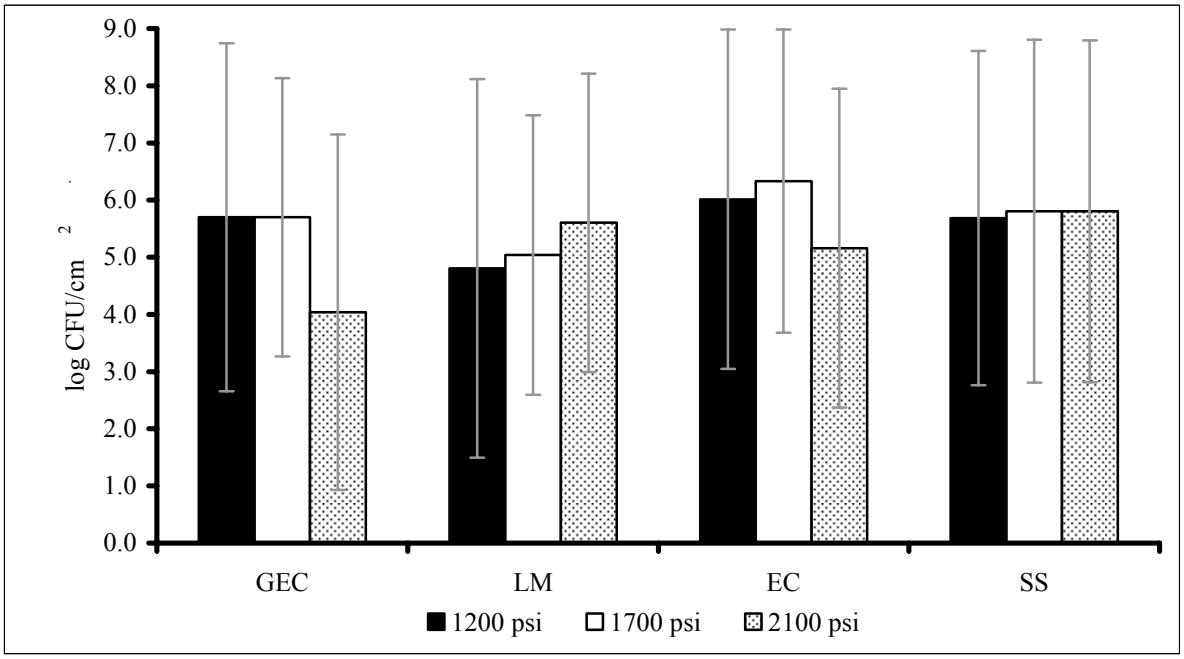
TREATMENT	Bacterial Recoveries (log CFU/cm^2)			
	Generic *Escherichia coli*	*Listeria monocytogenes*	*Escherichia coli* O157:H7	*Salmonella* spp.
CTRL	6.50	6.86	7.13	6.61
1200 psi	ND 0.80	2.05	1.12	0.92
1700 psi	ND 0.80	1.82	ND 0.80	ND 0.80
2100 psi	2.46	1.25	1.97	ND 0.80

ND= Non-detected. Detection Limit was calculated as 0.8 CFU/cm^2

Table 4. Effect of $_{CP}CO_2$ on Bacterial Reductions in Filter Paper Disks Challenged with Generic *E. coli*, *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp.

TREATMENT	Bacterial Reductions (log CFU/cm^2)			
	Generic *Escherichia coli*	*Listeria monocytogenes*	*Escherichia coli* O157:H7	*Salmonella* spp.
1200 psi	5.70	4.81	6.01	5.69
1700 psi	5.70	5.04	6.33	5.81
2100 psi	4.04	5.61	5.16	5.81

No statistical differences ($P\geq0.05$) were found in bacterial reductions among treatments or microorganisms.



No statistical differences (P≥0.05) were found in bacterial reductions among treatments or microorganisms.

Figure 6. Effect of $_{CP}CO_2$ on Bacterial Reductions in Filter Paper Disks Challenged with Generic *E. coli* (GEC), *Listeria monocytogenes* (LM), *E. coli* O157:H7 (EC) and *Salmonella* spp (SS).

CHAPTER 4.

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

4.1 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 establish differential criteria between supercritical and subcritical conditions, in order to determine the most important factors to be included as the main independent variables of one final study, such as pressures, temperatures, exposure times, types of modified atmosphere packaging conditions, and days of refrigerated storage.

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). For CO_2, supercritical conditions are when gas is exposed to pressures over 1066 psi at temperatures above 31°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 refrigerated storage (at 4°C for 4 days) under modified atmospheric packaging conditions (vacuum packaging and flushing with 100% CO_2).

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 refrigerated storage (at 4°C for 10 days) under modified atmospheric packaging conditions (vacuum packaging and flushing with 100% CO_2).

Finally, during the fourth part of this study, fresh meat core samples challenged with *E. coli* O157:H7 were exposed to supercritical $_{CP}CO_2$ at 1100 psi for 5 min, 1500 psi for 1 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 refrigerated storage (at 4°C for 10 days) under modified atmospheric packaging conditions (vacuum packaging and flushing with 100% CO_2).

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

4.2.1 Materials and Methods

4.2.1.1 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.

4.2.1.2 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 each 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).

4.2.1.3 Microbiological Analysis

After treatment, samples were weighed, placed in 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).

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 (CFU) were converted into log and reductions were calculated as the difference between respective non-treated controls and the average of their treated duplicates.

4.2.2 Results and Discussion

$_{CP}CO_2$ pressurized under supercritical conditions at 36°C provided higher lethality ($P<0.05$) when compared to subcritical conditions at 29°C, regardless of the pressure and time of exposure combination utilized.

As shown in Table 5, samples treated with $_{CP}CO_2$ under supercritical conditions at 36°C showed similar ($P\geq0.05$) recovery levels. 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.

On the other hand, samples treated with $_{CP}CO_2$ under subcritical conditions at 29°C, yielded similar ($P\geq0.05$) recoveries 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 5. Effect of $_{CP}CO_2$ on Endogenous Bacterial Populations in Non-Challenged Ground Beef Exposed to 1470, 2000, 3000, and 5000 psi $_{CP}CO_2$ in a Supercritical Fluid Extractor.

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

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

No 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 total plate count recoveries within both of the temperatures tested (Figure 7).

Results from this experiment also demonstrated that $_{CP}CO_2$ under subcritical conditions may not be suitable as an antimicrobial treatment, as reductions obtained under subcritical conditions (29°C) were substantially lower (P<0.05) than those obtained under supercritical conditions (36°C), regardless of the pressure and time of exposure combination applied.

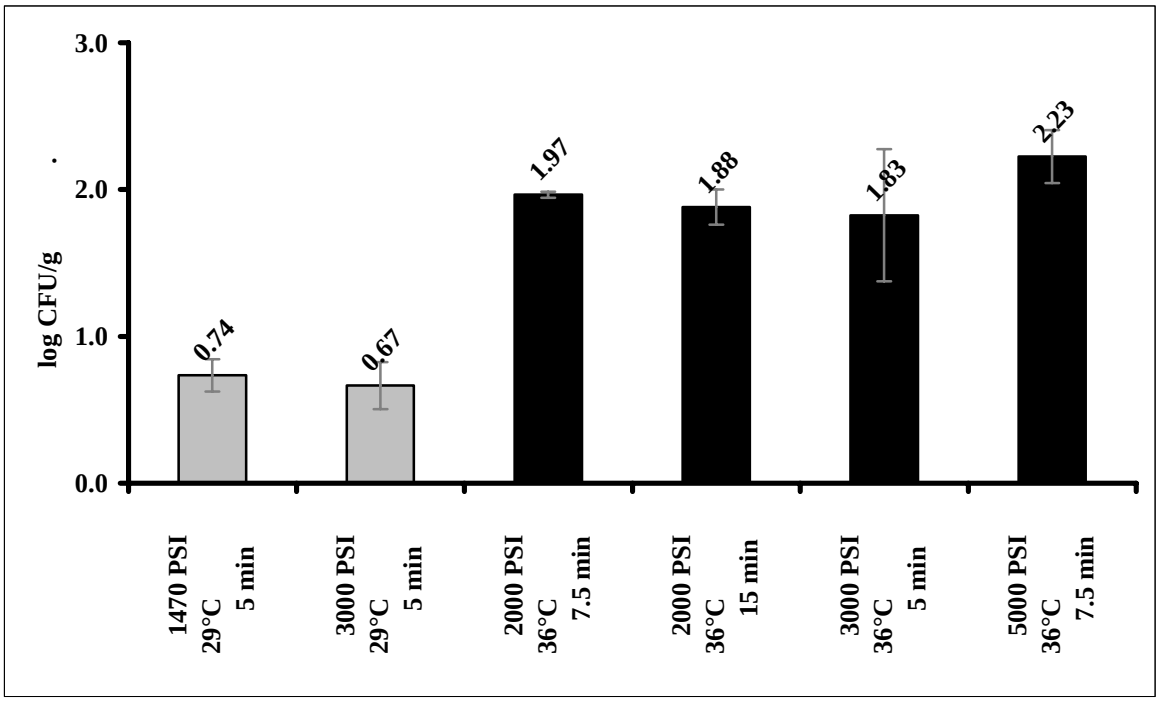


Figure 7. Effect of $_{CP}CO_2$ under Subcritical (29°C) and Supercritical (36°C) Conditions on Endogenous Bacterial Reductions in Non-Challenged Ground Beef.

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

4.3.1 Materials and Methods

4.3.1.1 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 a 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 37°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 h at 37°C. Cultures were confirmed by cultivation on selective and differential media, and biochemical analysis of presumptive colonies using API 20E kits.

4.3.1.2 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 with a total surface area of approximately 38.7 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 $_{CP}CO_2$ at 36°C inside the experimental vessel (Appendix A), according to the general protocol (Appendix B), with the following parameters:

- 1000 psi for 5 min
- 1000 psi for 10 min
- 1000 psi for 4 h

A non-treated inoculated meat sample was microbiologically analyzed as the control. The 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 under refrigeration 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).

4.3.1.3 Microbiological Analysis

After treatment, samples were weighted, placed in 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). CFU were converted into log and reductions were calculated as the difference between non-treated controls and their respective treated samples.

4.3.2 Results and Discussion

E. coli O157:H7 recoveries in treated samples exposed to 1000 psi at 36°C during 5, 10, and 240 min yielded 6.71, 6.92, and 7.11 log CFU/g, respectively (Table 6). Statistical analysis confirmed that bacterial recoveries as a function of the exposure time were not statistically different ($P \geq 0.05$). 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 (Figure 6).

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, considering that the pressure utilized was very close to the critical pressure of 1066 psi (1000 psi). These findings are in agreement with the Rizvi et al. (1986) in the sense that $_{CP}CO_2$ in a dense phase (under supercritical conditions) penetrates the bacterial cell wall easier than in its gaseous state (as in subcritical conditions), thus providing a higher lethality effect in that phase.

Table 6. Effect of $_{CP}CO_2$ on *E. coli* O157:H7 Populations on Challenged Fresh Meat Cubes Exposed to 1000 psi $_{CP}CO_2$ for 5, 10, and 240 min.

Treatments	Time	*E. coli* O157:H7	
		Recoveries (log CFU/g)	Reductions (log CFU/g)
Control	Non treated	7.78	
1000 psi	5 min	6.71	1.06[b]
1000 psi	10 min	6.92	0.86[b]
1000 psi	240 min	7.11	0.66[b]
+ 100% CO_2 day 4	4 days	4.06	3.71[a]

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

Modified atmosphere packaging flushed with 100% CO_2 and immediate storing for 4 days at 4°C further reduced (P<0.05) bacterial populations to 3.71 log CFU/g, providing an additional 2.65 log reductions to that obtained by pressurization. This data suggested the opportunity of further investigation of modified atmosphere packaging with flushed 100% 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). Therefore, it became logical to include vacuum packaging as a variable in order to compare its performance versus modified atmosphere packaging flushed with 100% CO_2 in subsequent experimental designs. In addition, sampling by surface area was considered a more consistent method to eliminate variability due to the different weight of the meat samples.

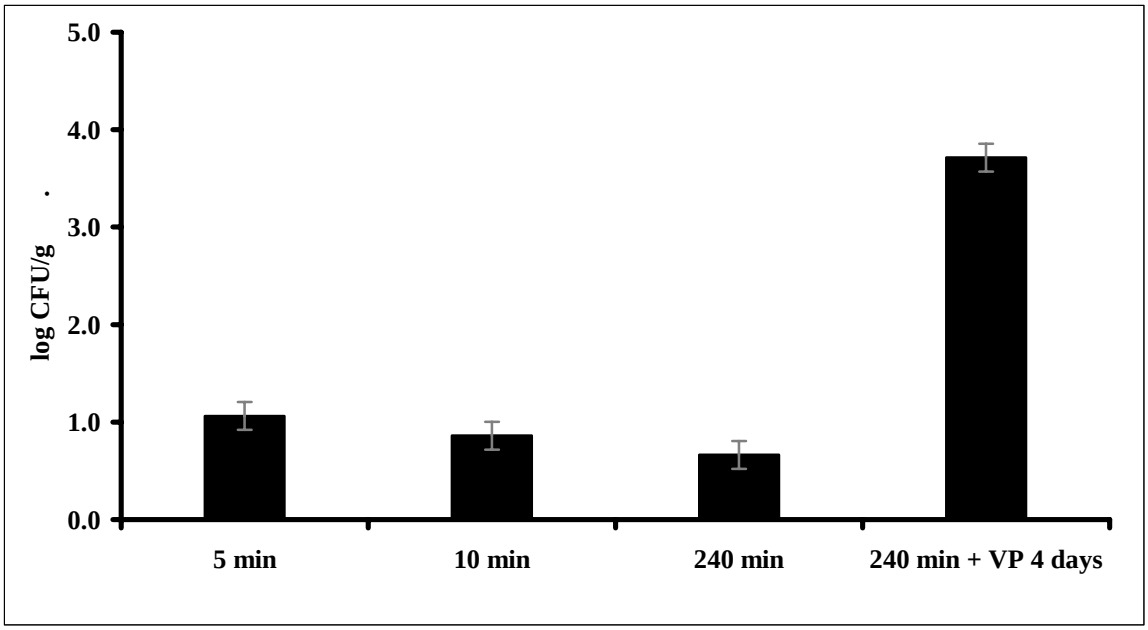


Figure 8. Effect of $_{CP}CO_2$ on *E. coli* O157:H7 Reductions in Challenged Ground Beef by Pressure (1000 psi), Time (5, 10, and 240 min) and Vacuum Package (VP)

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

4.4.1 Materials and Methods

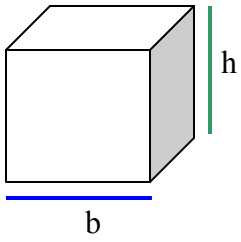
4.4.1.1 Preparation of Samples

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 37^{o}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^{o}C. Following incubation, samples were centrifuged (13,300 x g at 4^{o}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^{o}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^{o}C for 48 hours. Cultures were confirmed by cultivation on selective and differential media, and biochemical analysis of presumptive colonies using API 20E kits.

4.4.1.2 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 an approximate surface area of 38.7 cm^2. The total surface area was calculated with the formula for the external surface of a cube (6*h*b):



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 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
- 1800 psi for 7 min
- 2400 psi for 3 min

All of these treatments, conducted at 36°C, were additionally tested for increased lethality effects immediately after treatment (non-packaged) and after storage under modified atmospheric packaging conditions. Immediately after CO_2 treatment, for the 2400 psi treatment only, a sample was microbiologically analyzed immediately after treatment

without further packaging, while five additional 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 each treatment, a sample was microbiologically analyzed immediately after treatment without further packaging, 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. CFU were converted into log and reductions were calculated as the difference between respective non-treated controls and the average of their treated duplicates.

4.4.1.3 Microbiological Analysis

After exposure and storage times, samples were placed in 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 24 hours. The CFU were enumerated manually using a spiral plate dark field Quebec colony counter (Model 330; American optical company, Buffalo, NY).

4.4.2 Results and Discussion

Application of $_{CP}CO_2$ at 36°C yielded similar recoveries for generic *E. coli* populations in non-packaged (NP) 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 (Table 7). The 1800 psi $_{CP}CO_2$ for 7 min treatment showed the lowest level of recoveries 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 ($P<0.05$) lethality level among non-packaged samples, reducing generic *E. coli* populations by 1.21 log cycles (Table 8). These results suggest that greater pressures have a larger bactericidal effect when compared with time of exposure.

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 1.73 log cycles reduction for a total log reduction of 2.94. In all cases, flushing with 100% CO_2 prior to storage at 4°C contributed to further reductions after 1, 2, 4, 6, and 10 days of storage. However, there were no statistical differences ($P\geq0.05$) in reduction levels attributable to refrigerated storage time within any of the treatments for the 100% CO_2 flushed packages.

Flushing with 100% CO_2 was more effective ($P<0.05$) when compared to vacuum packaging, as shown in Table 8. 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 9). After application of 2400 psi $_{CP}CO_2$ for 3 min, packages flushed with 100% CO_2 achieved 2.94 log reductions after 6 days of storage. Vacuum packaging (VP) reached approximately the same level of reductions (2.90 log) only after 10 days of storage.

Vacuum packaging (VP) was only tested after the 2400 psi $_{CP}CO_2$ for 3 min treatment (Table 8). VP yielded additional lethality effects to those originally exerted by the pressure treatment, but only with a marginal ($P<0.05$) reduction of 0.20 log cycles after 1 day of refrigerated storage, achieving the highest ($P<0.05$) additional lethality after 10 days of refrigerated storage with an additional reduction of 1.69 log cycles, for a total reduction of 2.9 log.

Table 7. Effect of $_{CP}CO_2$ on Generic *E. coli* Recoveries in Challenged Fresh Meat Cubes by Pressure Treatment (1300 psi for 5 min, 1800 psi for 7 min, 2400 psi for 3 min), by Packaging Type (Vacuum Packaged [VP] and Flushed with 100% CO_2 [100% CO_2]), and by Time of Refrigerated Storage (Day 0, 1, 2, 4, 6, and 10).

Packaging Type and Time of Refrigerated Storage	Generic *E. coli* (log CFU/g)		
	1300 psi 5 min	1800 psi 7 min	2400 psi 3 min
Control	7.76	7.75	8.13
Non-packaged	6.90	6.69	6.92
+ 100% CO_2 day 1	6.32	6.45	6.26
+ 100% CO_2 day 2	5.82	6.09	5.45
+ 100% CO_2 day 4	5.73	5.84	5.33
+ 100% CO_2 day 6	5.59	5.75	5.19
+ 100% CO_2 day 10	5.47	5.76	5.28
+ VP day 1			6.72
+ VP day 2			Lost *
+ VP day 4			5.92
+ VP day 6			5.82
+ VP day 10			5.23

**During depressurization of the vessel, sample was expelled through the exhaust outlet.*

Table 8. Effect of $_{CP}CO_2$ on Generic *E. coli* Reductions in Challenged Fresh Meat Cubes by Pressure (1300 psi for 5 min, 1800 psi for 7 min, 2400 psi for 3 min), by Packaging Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Refrigerated Storage (Day 0, 1, 2, 4, 6, and 10).

Packaging Type and Time of Refrigerated Storage	Generic *E. coli* (log CFU/g)		
	1300 psi 5 min	1800 psi 7 min	2400 psi 3 min
Non-packaged	0.85^{bz}	1.07^{bz}	1.21^{az}
+ 100% CO_2 day 1	1.43^{by}	1.31^{by}	1.87^{ay}
+ 100% CO_2 day 2	1.93^{bx}	1.67^{by}	2.68^{ax}
+ 100% CO_2 day 4	2.02^{bx}	1.92^{bx}	2.80^{ax}
+ 100% CO_2 day 6	2.16^{bx}	2.01^{bx}	2.94^{ax}
+ 100% CO_2 day 10	2.28^{bx}	2.00^{cx}	2.85^{ax}
+ VP day 1			1.41^{z}
+ VP day 2			Lost *
+ VP day 4			2.21^{y}
+ VP day 6			2.31^{y}
+ VP day 10			2.90^{x}

**During depressurization of the vessel, sample was expelled through the exhaust outlet.*
[a-c] *Least square means within a row bearing different letters are different (P<0.05).*
[x-z] *Least square means within a column bearing different letters are different (P<0.05).*

Based on informal observations during this experiment, 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. Therefore, the interior of several samples was exposed by cutting the cubes in half. A cross section from treated trimmings revealed a normal purplish color with a brown layer next to the surface of the meat (Figure 10). In uncut meat, the deoxymyoglobin form predominates inside. Upon deoxymyoglobin exposure to air, oxygen binds rapidly to the free sixth co-ordination binding site induced by the ferrous state, forming oxymyoglobin (bright cherry red). 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 the meat. Meat cubes, however, bloomed to a red cherry color after being exposed to air for a few minutes (Figure 11).

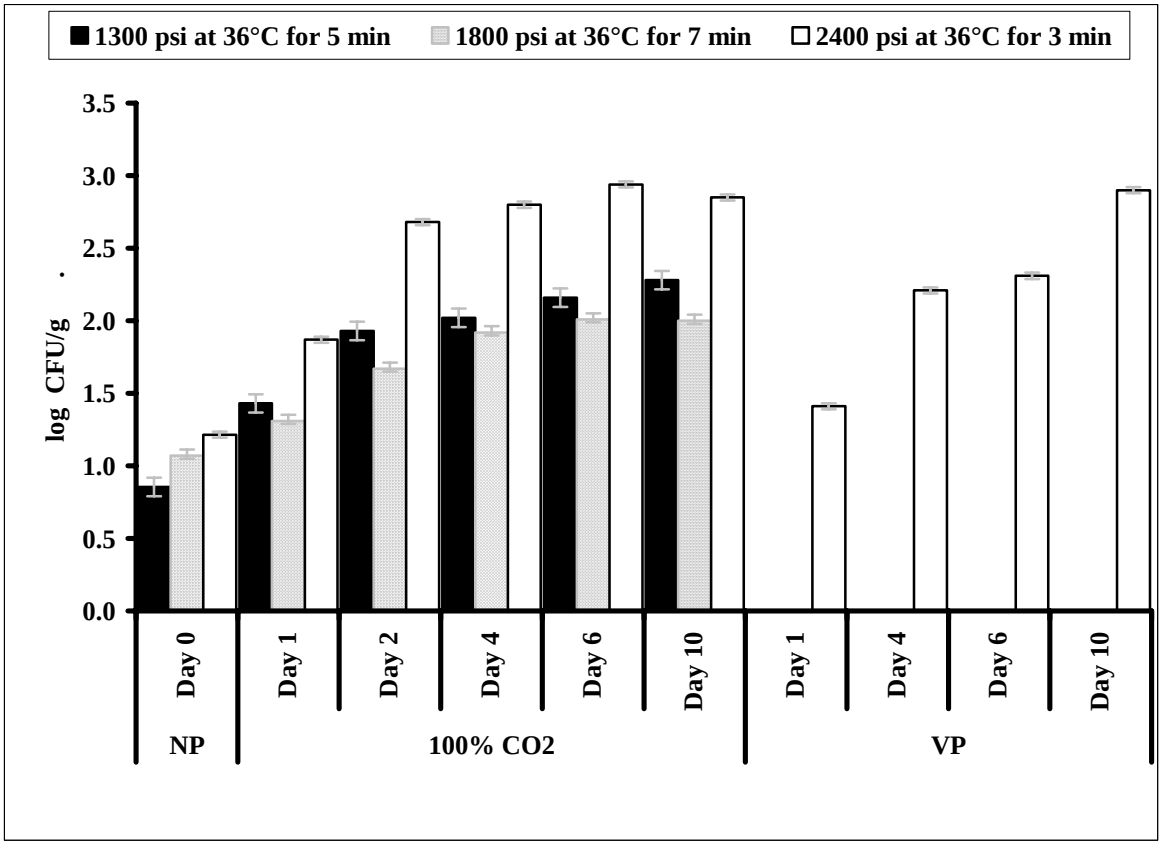


Figure 9. Effect of $_{CP}CO_2$ on Generic *E. coli* Reductions in Challenged Fresh Meat Cubes by Pressure (1300 psi for 5 min, 1800 psi for 7 min, 2400 psi for 3 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Refrigerated Storage (Day 0, 1, 2, 4, 6, and 10).

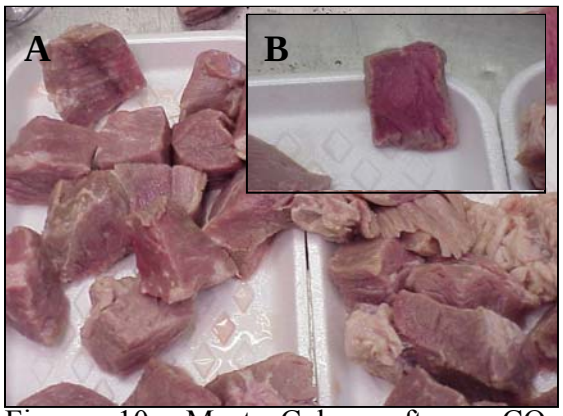


Figure 10. Meat Cubes after $_{CP}CO_2$ Application (A). Cross Section Revealed a Normal Purplish Color with a Brown Layer Next to the Surface of the Meat (B).

Figure 11. Bloomed Meat Cubes. After $_{CP}CO_2$ Application, Meat Cubes Bloomed to a Red Cherry Color after Being Exposed to Air for Few Minutes.

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

4.5.1 Materials and Methods

4.5.1.1 Preparation of Samples

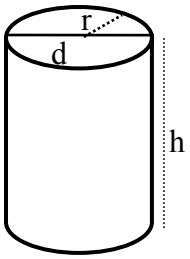
Bacterial cultures included three strains of *Escherichia coli* O157:H7 obtained from the Food Microbiology Laboratory stock culture collection at Kansas State University (KSU). Cultures used included *E. coli* O157:H7 ATCC 43890 (Jackie Staats, KSU), ATCC 43895, and KSU01 (CDC).

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 37^{o}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^{o}C. Following incubation, samples were centrifuged (13,300 x g at 4^{o}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^{o}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 h at 37^{o}C. Cultures were confirmed by cultivation on selective and differential media, and biochemical analysis of presumptive colonies using API 20E kits.

4.5.1.2 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) with a total surface area of 38.5 cm^2. The total surface area was calculated with the formula for the external surface of a cylinder $(2*Pi*r^2)+(Pi*r*h)$:



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 the sample received the same exposure to the inoculum all throughout the surface of the meat. 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 at 36°C as follows:

- 1100 psi for 5 min
- 1500 psi for 1 min
- 1600 psi for 7 min
- 2100 psi for 3 min

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 6 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).

4.5.1.3 Microbiological Analysis

After treatment, samples were placed in 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 24 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.

4.5.2 Results and Discussion

The $_{CP}CO_2$ application resulted in an 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 were statistically significant. The highest lethality was achieved by

pressurizing at 1100 psi for 5 min, reaching 0.81 log (Table 9). 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 ($P \geq 0.05$) 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 12) 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 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. Storage 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 9. Effect of $_{CP}CO_2$ on Generic *E. coli* Recoveries in Challenged Fresh Meat Cubes Packaged with Flushed 100% CO_2 [100% CO_2]) by Pressure Treatment (1100 psi for 5 min, 1500 psi for 1 min, 1600 psi for 7 min, 2400 psi for 3 min) and Time of Refrigerated Storage (Day 0, 1, 2, 4, and 6).

Packaging Type and Time of Refrigerated Storage	Generic *E. coli* (log CFU/g)			
	1100 psi 5 min	1500 psi 1 min	1600 psi 7 min	2400 psi 3 min
Control	7.60	7.34	6.93	6.93
Non-packaged	6.80	6.54	6.19	6.33
+ 100% CO_2 day 1	6.23	6.29	5.72	6.67
+ 100% CO_2 day 2	5.50	5.50	5.90	6.84
+ 100% CO_2 day 4	5.46	5.39	5.37	5.89
+ 100% CO_2 day 6	5.47	5.96	5.54	5.59

Table 10. Effect of $_{CP}CO_2$ on Generic *E. coli* Reductions in Challenged Fresh Meat Cubes Packaged with Flushed 100% CO_2 [100% CO_2]) by Pressure (1100 psi for 5 min, 1500 psi for 1 min, 1600 psi for 7 min, 2400 psi for 3 min), and by Time of Refrigerated Storage (Day 0, 1, 2, 4, and 6).

Packaging Type and Time of Refrigerated Storage	Generic *E. coli* (log CFU/g)			
	1100 psi 5 min	1500 psi 1 min	1600 psi 7 min	2400 psi 3 min
Non-packaged	0.80^{ax}	0.80^{az}	0.74^{ay}	0.60^{ax}
+ 100% CO_2 day 1	1.37^{ax}	1.05^{ay}	1.21^{ay}	0.26^{bx}
+ 100% CO_2 day 2	2.10^{ax}	1.84^{bx}	1.03^{cxy}	0.09^{dx}
+ 100% CO_2 day 4	2.14^{ax}	1.95^{bx}	1.56^{cx}	1.04^{dx}
+ 100% CO_2 day 6	2.13^{ax}	1.38^{bx}	1.39^{bx}	1.34^{bx}

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

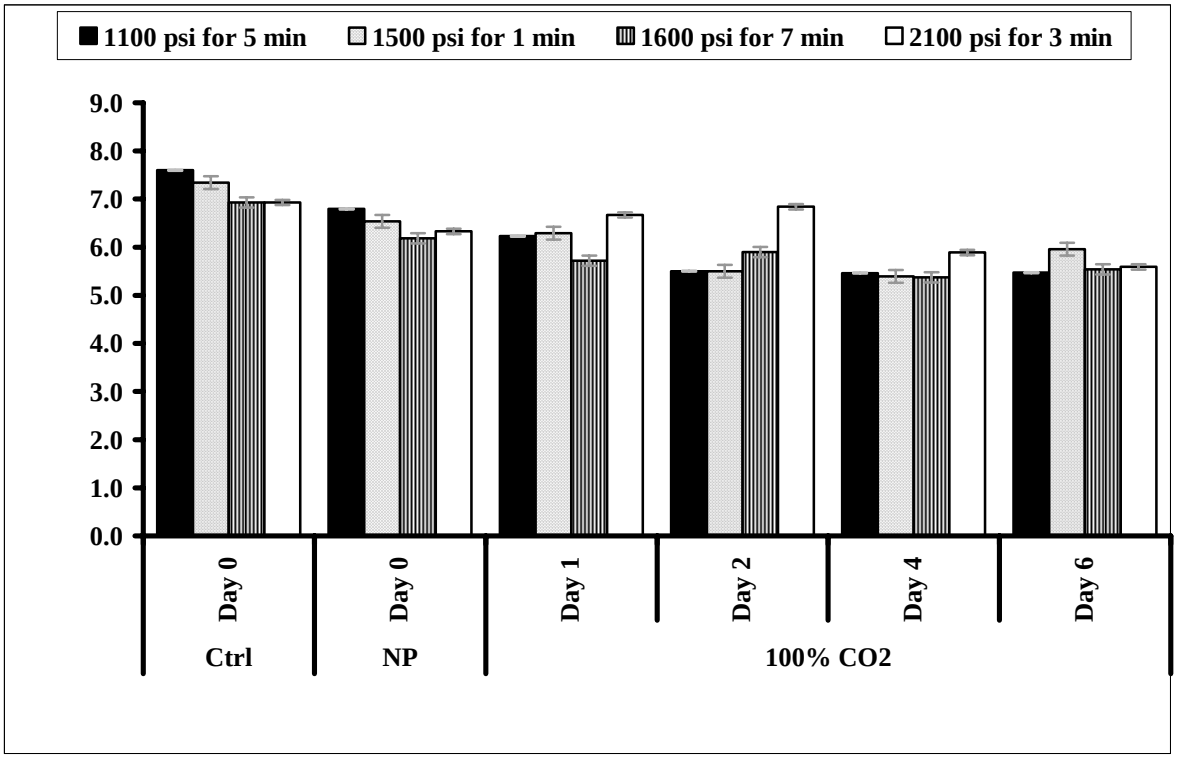


Figure 12. Effect of $_{CP}CO_2$ on Generic *E. coli* Recoveries in Challenged Fresh Meat Cubes Packaged with Flushed 100% CO_2 [100% CO_2]) by Pressure Treatment (1100 psi for 5 min, 1500 psi for 1 min, 1600 psi for 7 min, 2400 psi for 3 min), and by Time of Refrigerated Storage (Day 0, 1, 2, 4, and 6).

Table 11 illustrates a summary for the lethality obtained from all previous studies. Figure 13 illustrates the results of the studies performed in filter paper disks (A) and in beef trimmings and ground beef (B).

Table 11. Summary of Bacterial Reductions Obtained by Application of Several Pressures of $_{CP}CO_2$.from Previous Studies.

Studies	Microorganism	Pressure (psi)	Time of Exposure (min)	Temp. (°C)	REDUCTIONS (log)
Filter Paper Disks	*E. coli* O157:H7	1200	3	36	6.02
		1700	3	36	6.33
		2100	3	36	5.16
	Generic *E. coli*	1200	3	36	5.70
		1700	3	36	5.70
		2100	3	36	4.04
	Listeria monocytogenes	1200	3	36	4.81
		1700	3	36	5.04
		2100	3	36	5.60
	Salmonella spp.	1200	3	36	5.80
		1700	3	36	5.81
		2100	3	36	5.81
Beef Trimmings and Ground Beef	Aerobic Plate Count	1470	5	29	0.74
		2000	7.5	36	1.97
		2000	15	36	1.88
		3000	5	29	0.67
		3000	5	36	1.83
		5000	7.5	36	2.23
	E. coli O157:H7	1000	5	29	1.06
		1000	10	29	0.86
		1000	240	29	0.66
		1100	5	36	0.81
		1500	1	36	0.81
		1600	7	36	0.74
		2100	3	36	0.60
	Generic *E. coli*	1300	5	36	0.86
		1800	7	36	1.07
		2400	3	36	1.22

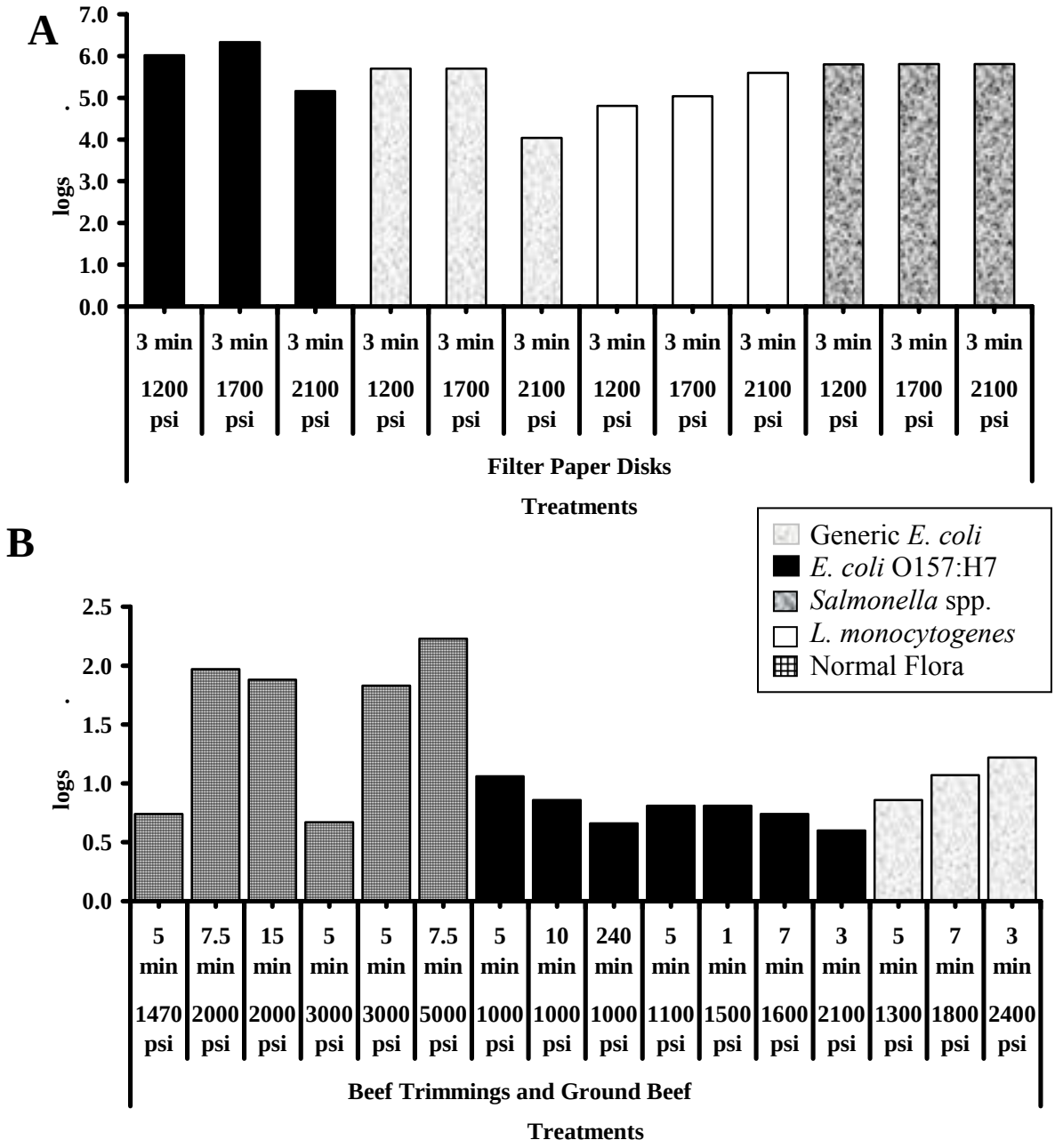


Figure 13. Summary of Bacterial Reductions Obtained by Application of Several Pressures of $_{CP}CO_2$.from Previous Studies on Filter Paper Disks (A), and Beef Trimmings and Ground Beef (B).

CHAPTER 5.

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

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

5.1 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 under simulated retail display conditions. Organoleptic and sensory effects on treated raw and cooked ground beef patties manufactured with the treated trim were analyzed.

5.2 Materials and Methods

5.2.1 Preparation of Samples

The 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.

5.2.2 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
- 1500 psi for 15 min

5.2.3 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), as shown in Figure 14.

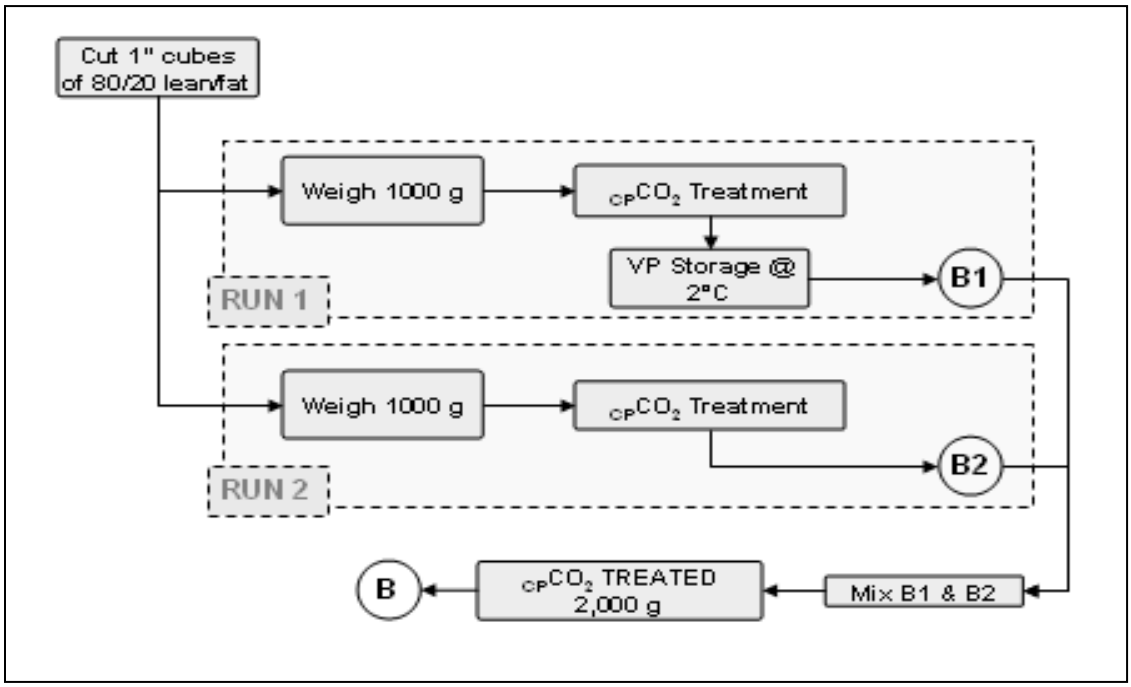


Figure 14. Flow Diagram of Experimental Runs. 85/15 lean/fat Beef Trimmings From the Same Batch Cut Into ca. 1 in. Cubes Were Exposed to $_{CP}CO_2$ in Two Separate Experimental Runs Due to Space Constrictions Inside the Experimental Vessel.

After the first experimental run of beef trimmings was exposed to $_{CP}CO_2$ (RUN 1) at 36°C, 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.

Immediately after grinding, ground meat was formed into fourteen 114 g patties with a manual patty former. Figure 15 shows the experimental design for this study. Two patties were immediately placed on a Styrofoam tray with PVC overwrap (AT) and labeled as Day 0 (D0) (Referred as "a" and "w" in Figure 15). Twelve patties were placed on a Styrofoam trays and randomly subdivided into two groups; six were packaged and flushed with 100% CO_2 (CO_2) with an oxygen scavenger (Multisorb Technologies), and six more were placed in a Styrofoam tray with PVC overwrap (PVC).

For every treatment (CTRL, 750, and 1500 psi $_{CP}CO_2$), paired patties from each package type (AT and 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 to simulate retail display conditions.

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. Table 12 shows the analyses conducted on raw and cooked patties treated with $_{CP}CO_2$.

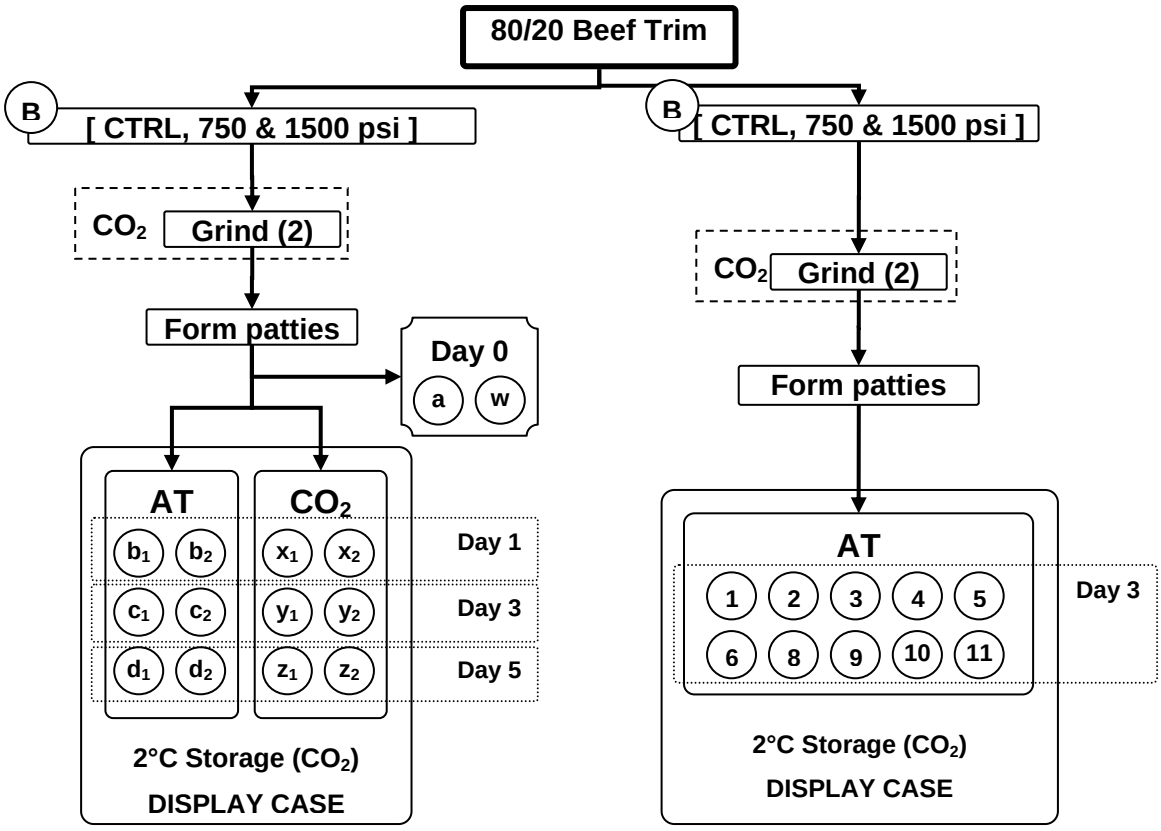


Figure 15. Flow Diagram of Experimental Design. Patties Manufactured with Beef Trimmings Exposed to Various $_{CP}CO_2$ Pressure Treatments (Control [CTRL], 750 psi, 1500 psi) Were Allocated to Two Types of Packaging Atmospheres under Simulated Retail Display.

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.

Table 12. Analyses Conducted in Raw and Cooked Patties Treated with $_{CP}CO_2$.

Patties	Subgroup	Analyses
a	Raw, PVC, Day 0	I-Color, Headspace, Proximate, TBARS, pH & Micro
w	Cooked, PVC, Day 0	I-Color, Proximate, TBARS
b_1	Raw, PVC, Day 1	I-Color, Headspace, Proximate, TBARS, pH & Micro
b_2	Cooked, PVC, Day 1	I-Color, Proximate, TBARS
c_1	Raw, PVC, Day 3	I-Color, Headspace, Proximate, TBARS, pH & Micro
c_2	Cooked, PVC, Day 3	I-Color, Proximate, TBARS
d_1	Raw, PVC, Day 5	I-Color, Headspace, Proximate, TBARS, pH & Micro
d_2	Cooked, PVC, Day 5	I-Color, Proximate, TBARS
x_1	Raw, CO_2, Day 1	I-Color, Headspace, Proximate, TBARS, pH & Micro
x_2	Cooked, CO_2, Day 1	I-Color, Proximate, TBARS
y_1	Raw, CO_2, Day 3	I-Color, Headspace, Proximate, TBARS, pH & Micro
y_2	Cooked, CO_2, Day 3	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 last set of 11 patties prepared from each $_{CP}CO_2$ treatment were placed on a Styrofoam tray with PVC overwrap, labeled as Day 3, and placed under refrigerated storage.

5.2.4 pH and Microbiological Analysis

Raw patties were analyzed for natural occurring microflora and pH after 0, 1, 3, and 5 d of simulated retail 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 1 minute. After homogenization, microbiological samples were serially diluted in sterile PW and plated according to the dilution scheme shown in Figure 16.

Samples were plated in duplicates onto APC Petrifilm and ECC Petrifilm (3M Microbiology, St. Paul, MN) and incubated at 37°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).

The CFU were enumerated using a spiral plate dark field Quebec colony counter (Model 330; American optical company, Buffalo, NY). CFU were converted into log values 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.

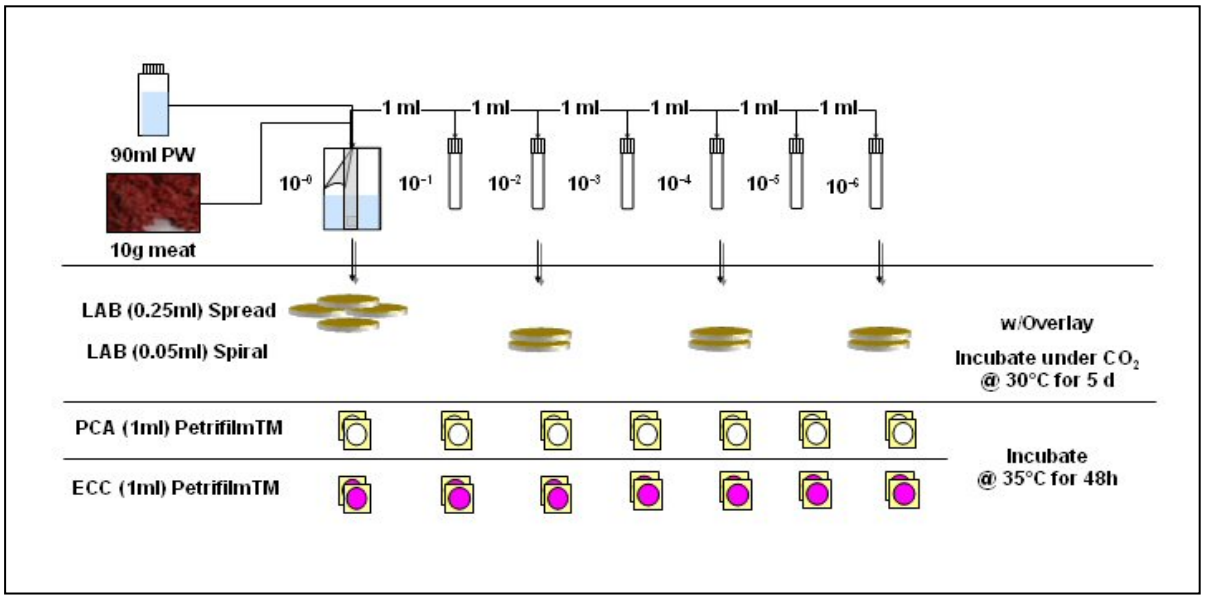


Figure 16. Dilution Scheme for Microbiological Samples Analyzed for Normal Microflora in Raw Patties Non-Treated and Treated with $_{CP}CO_2$.

5.2.5 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.

5.2.6 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^{TM} 650 (Mocon, Minneapolis, MN) CO_2/O_2 Headspace analyzer.

5.2.7 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 (Figure 17).

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).

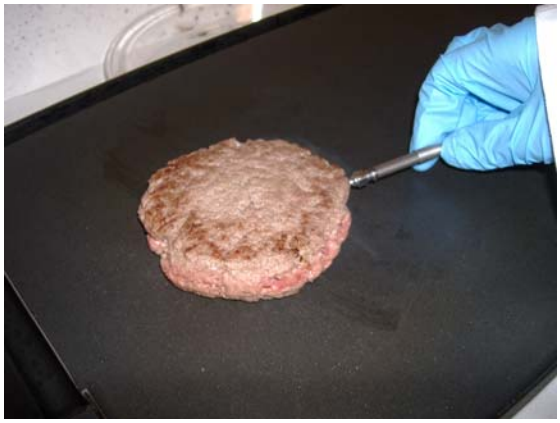


Figure 17. Internal Temperature Monitored with a Digital Thermometer to an Internal Temperature of 160°F (71.1°C).

Sensory analysis was conducted 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.

5.2.8 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.

5.2.9 Results and Discussion

5.2.9.1 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 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.

Results (Table 13) demonstrated $_{CP}CO_2$ at 1500 psi was the most effective treatment with the lowest numerical immediate recoveries for the Aerobic Plate 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, days of simulated retail display, microorganism tested or any interaction, only pressure showed significance in the model ($P<0.05$).

Table 13. Effect of $_{CP}CO_2$ on Endogenous Bacterial Recoveries in Ground Beef Patties Manufactured from Non-Challenged Treated Beef Trimmings, by Pressure (750 and 1500 psi for 15 min) at 36°C and by Time of Simulated Retail Display (Day 0, 1, 3, and 5).

TREATMENT			Aerobic Plate Count (log CFU/g)	Lactic Acid Bacteria (log CFU/g)	Total Coliform (log CFU/g)	*E. coli* (log CFU/g)	pH
Pressure	Package Type	Display					
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
[a-g] Least square means within a column bearing different superscript letters are different (P<0.05).

Furthermore, LAB counts clearly showed an increase on bacterial populations during refrigerated storage in treated ground beef packaged in aerobic trays. On the other hand, LAB growth in ground beef packages flushed with 100% CO_2 occurred at a slower rate (Figure 18). Specific atmospheric packaging conditions tested as an additional lethality step after 1, 3, and 5 days of simulated retail display showed only marginal reductions (Figure 19).

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 14), 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.

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. Additionally, while LAB and APC populations in aerobic trays were steadily increasing over time (Figure 19), $_{CP}CO_2$ applied at 1500 psi hindered bacterial growth in ground beef packaged flushed with 100% CO_2, maintaining lower bacterial counts than those shown by the control by approximately 0.5 log CFU/g.

Table 14. Effect of $_{CP}CO_2$ on Endogenous Bacterial Reductions in Ground Beef Patties Manufactured from Non-Challenged Treated Beef Trimmings, by Pressure (750 and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Simulated Retail Display (Day 0, 1, 3, and 5).

TREATMENT			Aerobic Plate Count (log CFU/g)	Lactic Acid Bacteria (log CFU/g)	Total Coliform (log CFU/g)	*E. coli* (log CFU/g)
Pressure	Package Type	Display				
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
	100% 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
	100% 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

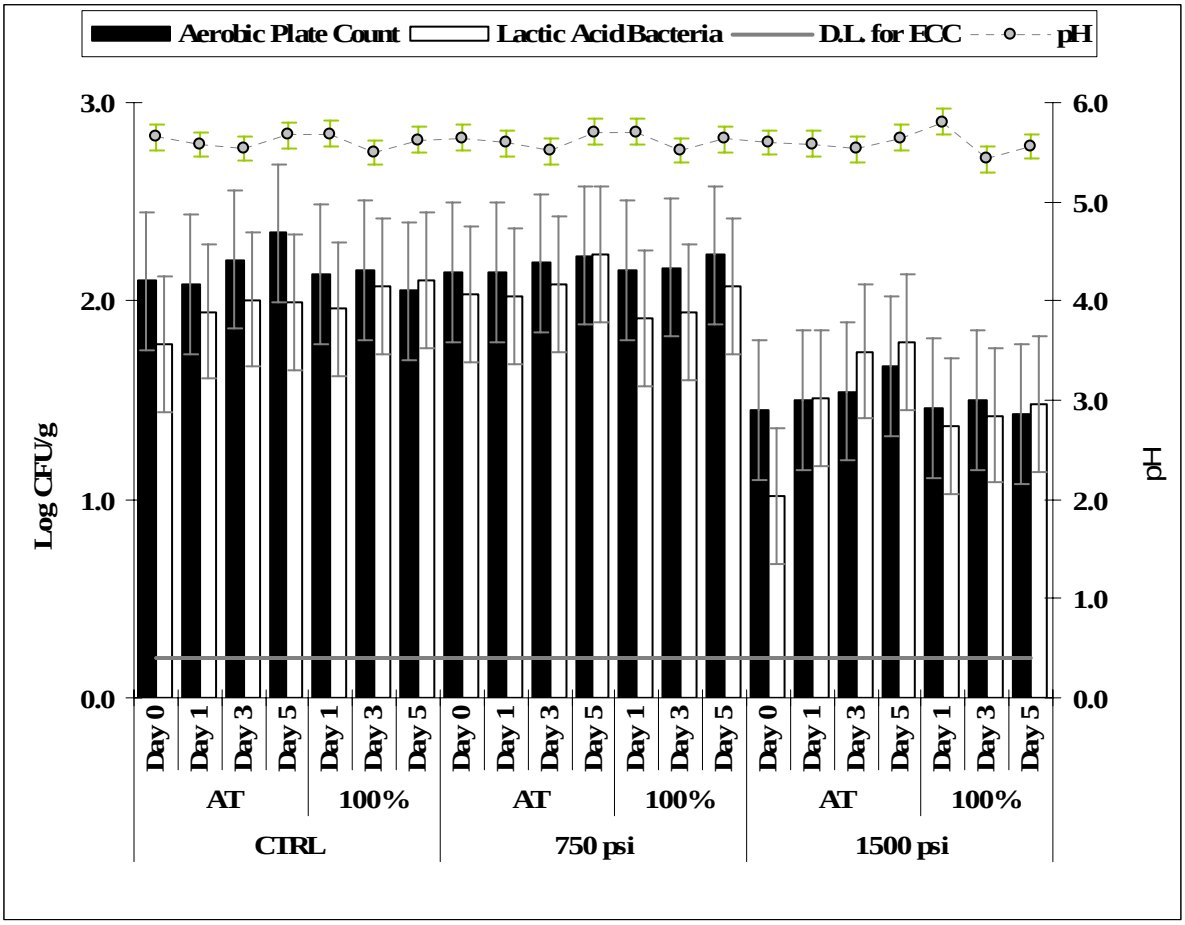


Figure 18. Effect of $_{CP}CO_2$ on Endogenous Bacterial Recoveries in Ground Beef Patties Manufactured from Non-Challenged Treated Beef Trimmings, by Pressure (750 and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Simulated Retail Display (Day 0, 1, 3, and 5).

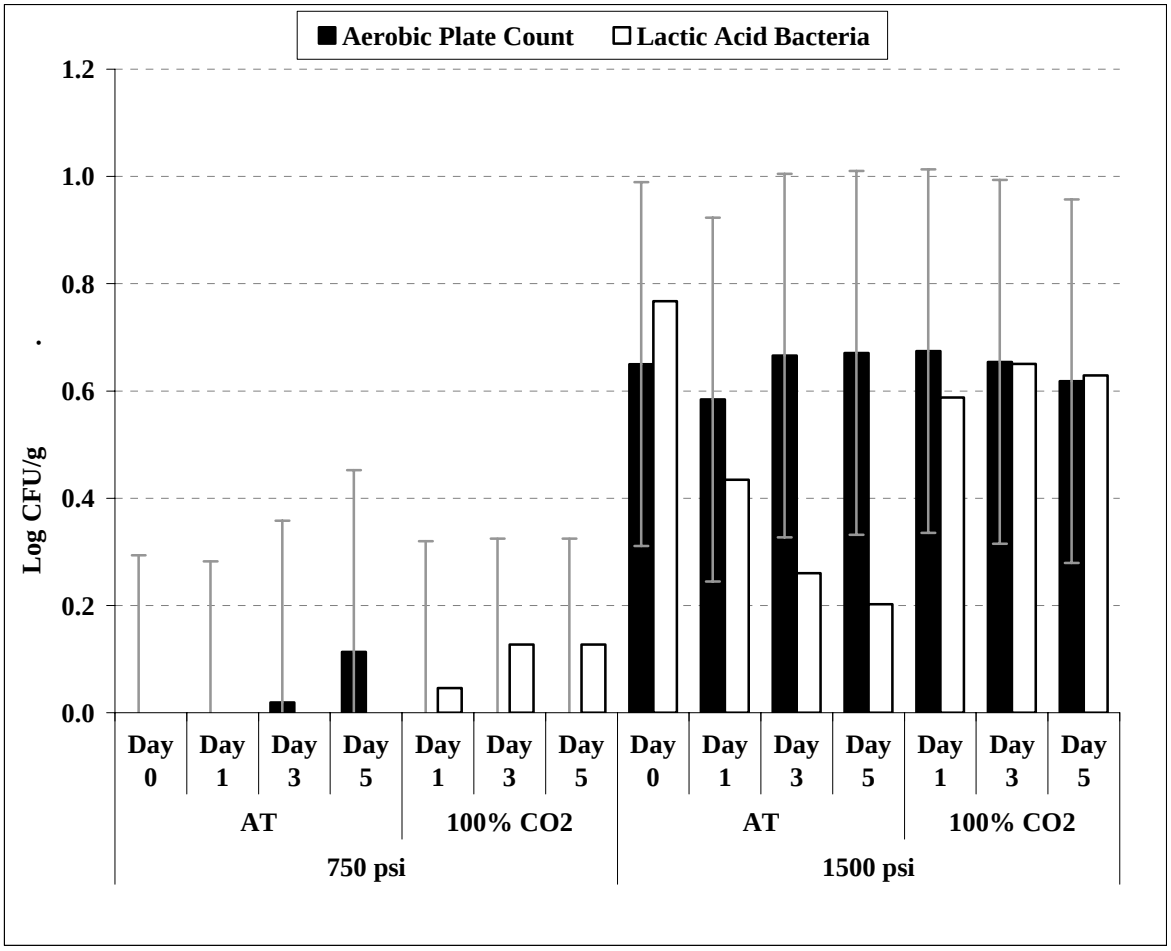


Figure 19. Effect of $_{CP}CO_2$ on Endogenous Bacterial Reductions in Ground Beef Patties Manufactured from Non-Challenged Treated Beef Trimmings, by Pressure (750 and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]),and by Time of Simulated Retail Display (Day 0, 1, 3, and 5).

5.2.9.2 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 (100% CO_2). The impact of $_{CP}CO_2$ application in beef trimmings on instrumental color and reflectance values of raw ground beef patties is shown in Table 15. Ratios of 630/580 nm approach 1.0 for metmyoglobin and >4.0 for oxymyoglobin.

Table 15. Effect of $_{CP}CO_2$ on Instrumental Color Values Obtained in Raw Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings, by Pressure (750 and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Simulated Retail Display (Day 0, 1, 3, and 5).

TREATMENT			Instrumental Color			
Pressure	Package Type	Display	Color L^*	Color a^*	Color b^*	630/580 Ratio
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}
	100% 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}
	100% 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}
	100% 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}

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

As shown in Figure 20, no differences were found among treatments in raw patties for any of the interactions of pressure, packaging conditions and days of simulated retail display on L^* values, (P≥0.05), except the interaction of pressure by packaging conditions for L^* (P<0.05). Before refrigerated storage, raw patties packaged under aerobic conditions, in most cases, were lighter (P<0.05) than those exposed to the same pressure packaged and flushed with 100% CO_2, and this effect was most apparent for the 1500 psi pressure treatment. However, after 5 days of simulated retail display, there was no difference (P≥0.05) among treatments for L^* values.

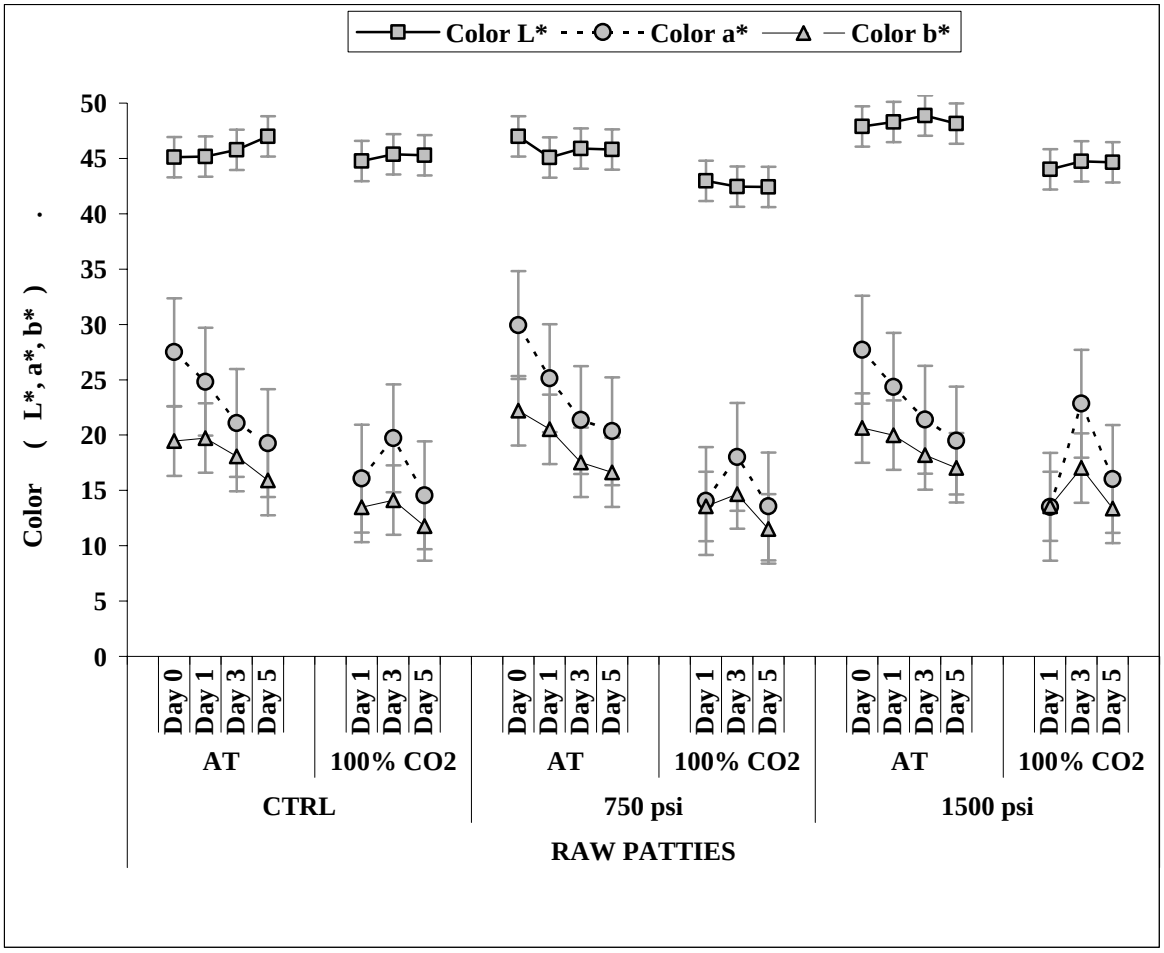


Figure 20. Effect of $_{CP}CO_2$ on Instrumental Color Values Obtained in Raw Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings, by Pressure (750 and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Simulated Retail Display (Day 0, 1, 3, and 5).

Raw patties also showed significance ($P<0.05$) for a^* values in the interactions of pressure by packaging conditions by days of display, and pressure by days of display. Raw patties packaged under aerobic conditions consistently showed higher ($P<0.05$) redness scores throughout display when compared to those packaged with 100% CO_2 conditions. All treatments under aerobic conditions showed declining a^* values through display, whereas patties flushed with 100% CO_2 showed a peak in redness values (a^*) after three days. Redness scores of patties flushed with 100% CO_2 after display were similar ($P\geq0.05$) after 1

and 5 days. In addition, all redness scores for all treatments were similar ($P \geq 0.05$) after 5 days of display, regardless of $_{CP}CO_2$ pressure or packaging condition.

The b^* scores obtained from raw patties exhibited a very similar trend as a^* scores. Only for the b^* scores by days of display was shown to be significant ($P < 0.05$). Values for b^* declined ($P < 0.05$) in raw patties packaged under aerobic conditions and showed a peak in raw patties flushed with 100% CO_2 conditions after three days of display. However, b^* scores of patties flushed with 100% CO_2 were similar ($P \geq 0.05$) after 1 and 5 days of display. Interestingly, raw patties exposed to the 1500 $_{CP}CO_2$ treatment showed the highest ($P < 0.05$) scores for b^* after 5 days of display for both types of packaging conditions.

Figure 21 shows two patties manufactured from $_{CP}CO_2$ treated beef trimmings after 3 days of display; in the left side, a patty in a vacuum bag flushed with 100% CO_2, and in the right side, a patty in an aerobic tray under normal atmospheres. Figure 22 shows the prevalent atmospheric conditions inside the packages during s display of raw patties.

Reflectance ratios from the 630 and 580 nm wavelengths in raw patties (Table 15) used as an estimation of the oxymyoglobin proportion of the myoglobin pigment, only showed a significant ($P < 0.05$) interaction for packaging conditions by days of display interaction. The evaluation of reflectance ratios immediately (Day 0) after application of $_{CP}CO_2$ at 1500 psi showed no differences ($P \geq 0.05$) in corresponding values when compared to the control.

Figure 21. Raw Patties Manufactured from $_{CP}CO_2$ Treated Beef Trimmings Packaged under Flushed CO_2 Conditions (CO2) (left) and Under Aerobic Conditions in an Aerobic Tray (AT) After 3 Days of Simulated Retail Display.

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 display, declining to 2.3-2.5 after 5 days of simulated retail display. Values in the order of 2.3-2.4 were recorded for ground beef patties packaged under flushed CO_2 before display, declining to 1.6-2 after 5 days of 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 display, scored values for reflectance ratios from the 630 and 580 nm wavelengths in raw patties were statistically similar for all treatments ($P \geq 0.05$), as can be observed in Figure 23.

Furthermore, application of $_{CP}CO_2$ in beef trimmings prior to grinding, and maintaining anaerobic conditions all through display, by flushing the packages with CO_2 immediately after grinding, represents a viable option for enhancing color stability during

display 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, as all lightness (L^*) and redness scores (a^*) were similar ($P \geq 0.05$) immediately after application (Day 0). Furthermore, raw patties exposed to the 1500 $_{CP}CO_2$ treatment showed the lowest ($P < 0.05$) brown discoloration (b^*) after 5 days of display.

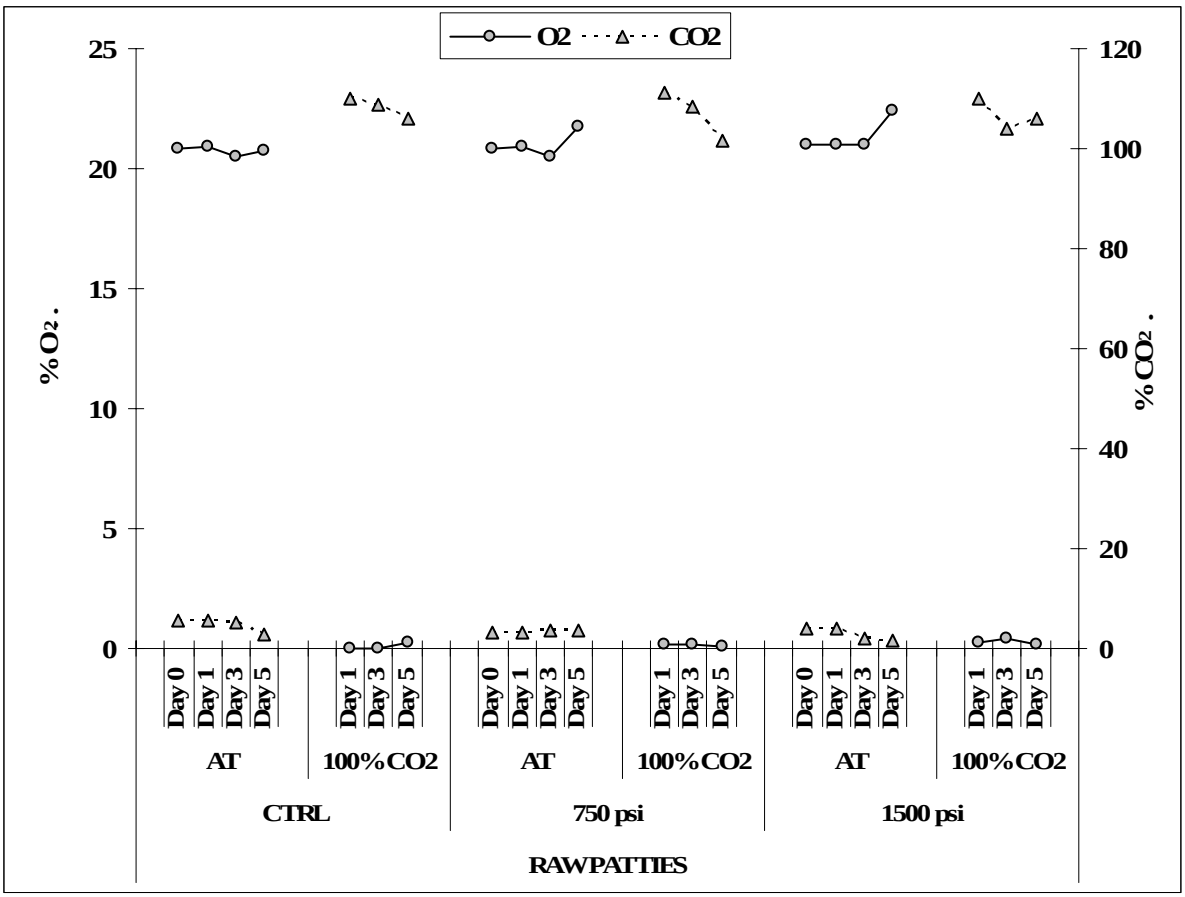


Figure 22. Packaging Atmospheres (Carbon Dioxide [CO2] and Oxygen [O2]) in Raw Ground Beef Patties Manufactured from Beef Trimmings Exposed to $_{CP}CO_2$ (750 psi and 1500 psi for 15 min), by Pressure (750 and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Simulated Retail Display (Day 0, 1, 3, and 5) During Simulated Retail Display (Day 0, 1, 3, and 5).

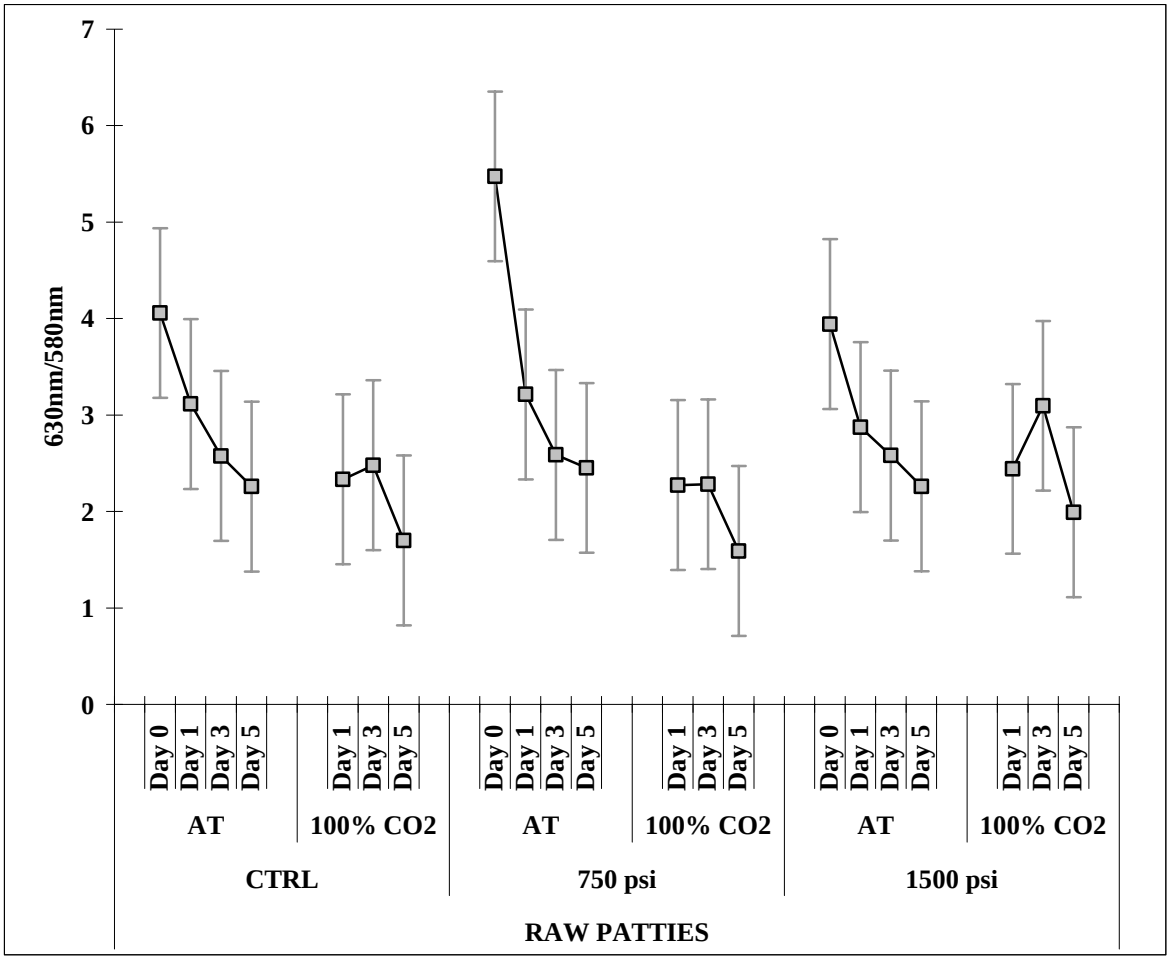


Figure 23. Effect of $_{CP}CO_2$ on Reflectance Ratios of 630nm/580nm Obtained in Raw Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings, by Pressure (750 and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Simulated Retail Display (Day 0, 1, 3, and 5).

5.2.9.3 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.

The evaluation of L^*, a^* or b^* immediately (Day 0) after application of $_{CP}CO_2$ at 1500 psi showed no differences ($P \geq 0.05$) in corresponding values when compared to the control. Table 16 shows the impact of 0, 1, 3 and 5 days of display, packaged under 100% flushed CO_2 (100% 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. *L** values for all the treatments were similar (P≥0.05) when comparing cooked ground beef patties flushed with 100% CO_2 (100% CO2) with cooked ground beef patties in aerobic trays (AT). Similar trends were exhibited in all treatments for each type of package during display, showed by a decrease (P<0.05) in lightness (L^*). However, after 5 days, *L** values were similar (P≥0.05) for cooked patties flushed with 100% CO_2.

Table 16. Effect of $_{CP}CO_2$ on Instrumental Color Values Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings, by Pressure (750 and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Simulated Retail Display (Day 0, 1, 3, and 5).

TREATMENT			Instrumental Color			
Pressure	Package Type	Refrigerated Storage	Color L^*	Color a^*	Color b^*	630/580 Ratio
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}
	100% 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}
	100% 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}
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}
	100% 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}

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

The a^* and b^* values exhibited very similar trends when compared to each other (Figure 24). Similar (P≥0.05) values were found on both scores after 5 days of display for all

treatments, with the exception of cooked patties flushed with 100% CO_2 from the 1500 psi CO_2 treatment, which showed less (P<0.05) redness (a^*) than the other treatments.

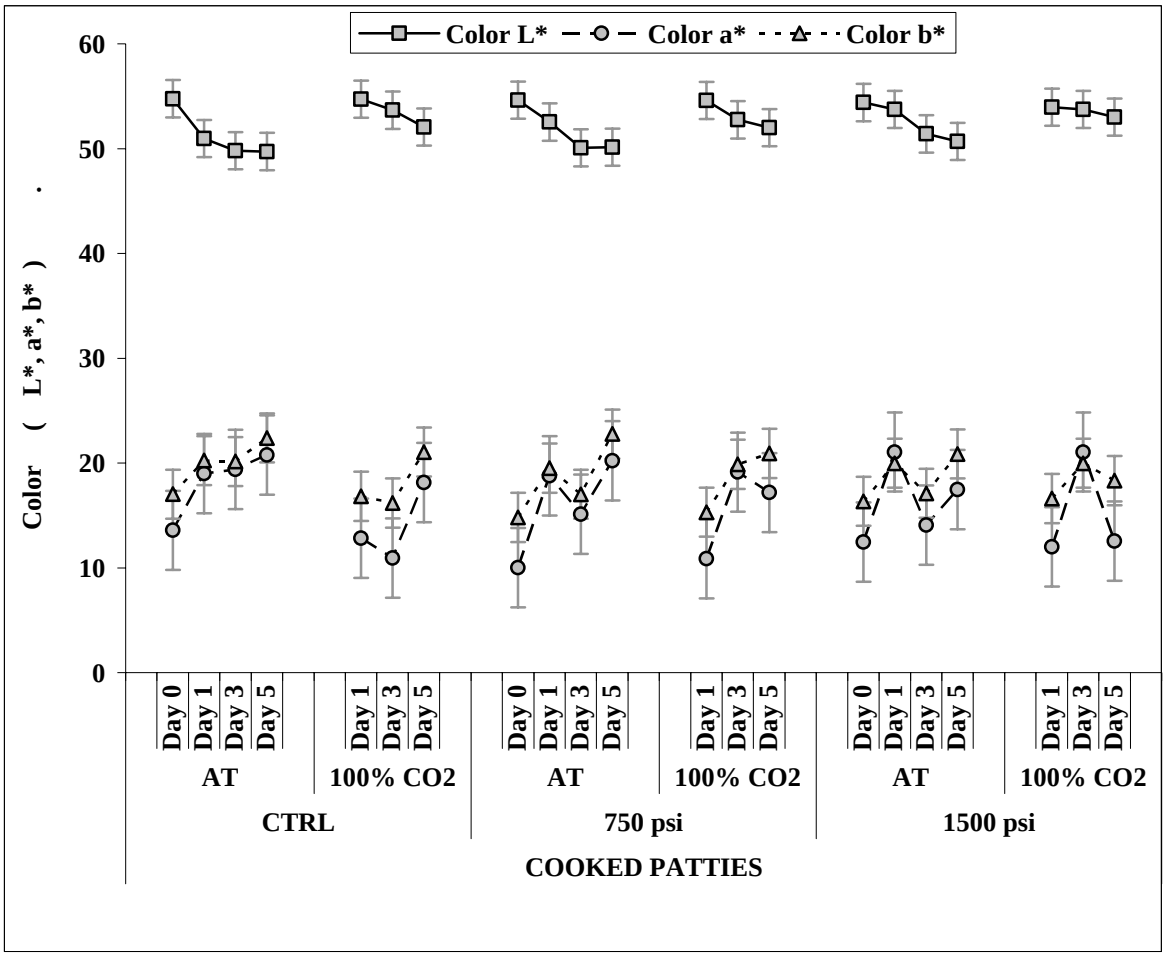


Figure 24. Effect of $_{CP}CO_2$ on Instrumental Color Values Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings, by Pressure (750 and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Simulated Retail Display (Day 0, 1, 3, and 5).

Reflectance ratios from the 630 and 580 nm wavelengths in cooked ground beef patties were similar for all treatments (P≥0.05), with the exception of cooked patties flushed with 100% CO_2 from the 1500 psi CO_2 treatment, which showed less (P<0.05) redness (a^*) than the rest of the treatments, as shown in Figure 25. The evaluation of reflectance ratios

immediately (Day 0) after application of $_{CP}CO_2$ at 1500 psi showed no differences ($P \geq 0.05$) in corresponding values when compared to the control.

Table 17 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.

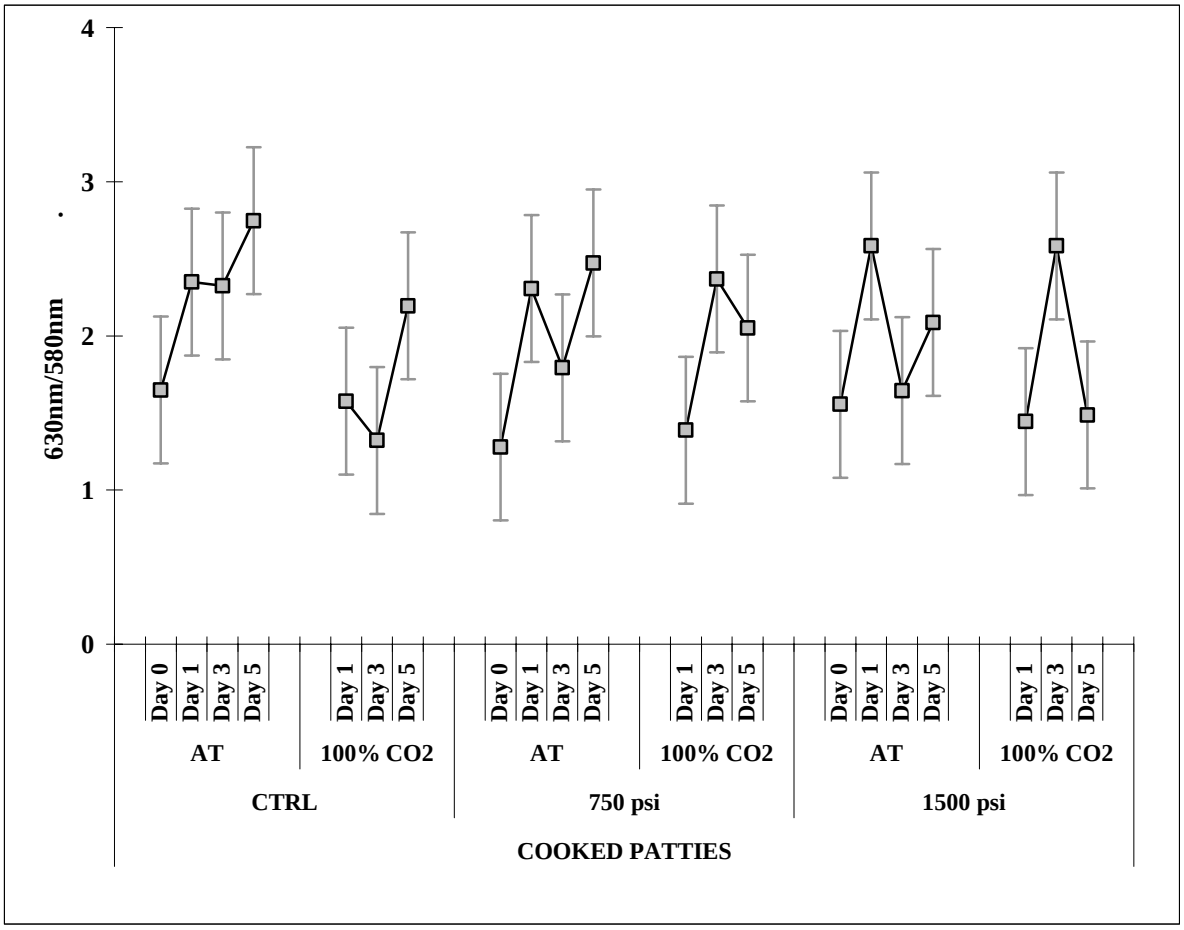


Figure 25. Reflectance Ratios of 630nm/580nm in Cooked Ground Beef Patties Manufactured from Beef Trimmings Exposed to $_{CP}CO_2$ (750 psi and 1500 psi for 15 min) at 36°C During Simulated Retail Display (Day 0, 1, 2, 3, and 5).

Table 17. Effect of $_{CP}CO_2$ on 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, by Pressure (750 and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), and by Time of Simulated Retail Display (Day 0, 1, 3, and 5).

TREATMENT			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}
	100% 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}
	100% 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}
	100% 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}

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

Little data exists on cooking losses related to CO_2 storage of meat; however, Holley et al. (1994) compared cooking losses in pork packaged in 50% and 100% CO_2, and found no differences. Jeremiah et al. (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 packaged with 100% CO_2 and 100% N_2.

The evaluation of Proximate Values (% Moisture [H_20], % Crude Protein [CP], % Crude Fat [CF]), immediately (Day 0) after application of $_{CP}CO_2$ at 1500 psi showed no differences ($P \geq 0.05$) in corresponding values when compared to the control. In the experiment, as it can be observed in Figure 26, moisture (% H_2O) values exhibited very similar trends with the exception of the 1500 psi CO_2 cooked patties flushed with 100% CO_2 (100% CO2), which showed higher ($P<0.05$) dryness (%H_2O) than the rest of the treatments.

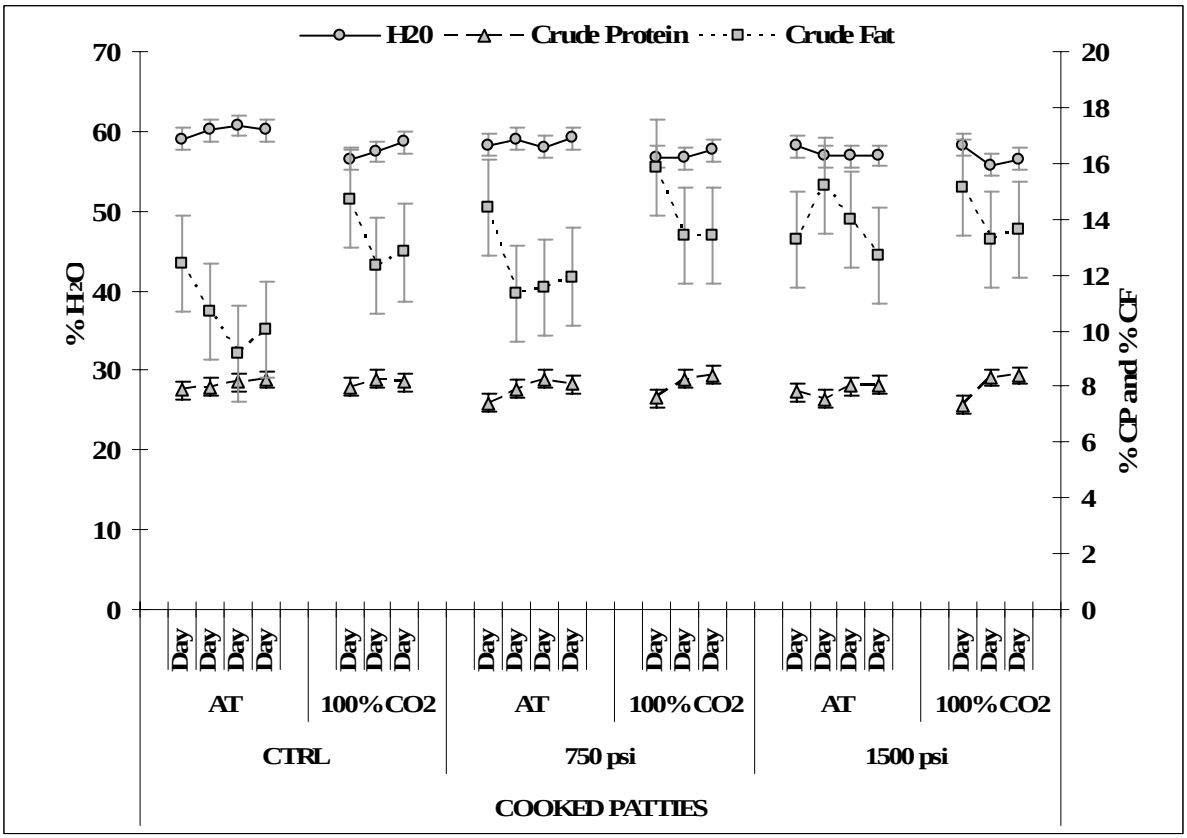


Figure 26. Proximate Values (% Moisture [H_20], % Crude Protein [CP], % Crude Fat [CF]) Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), During Simulated Retail Display (Day 0, 1, 3, and 5).

No perceivable differences were observed for crude protein (%CP) and crude fat (%CF) in cooked ground beef patties after 5 days of display (P≥0.05). The extent of lipid oxidation (shown in Figure 27), after 5 days of display, was shown to be higher in all patties packaged under aerobic conditions (AT) when compared to those flushed under 100% CO_2 (100 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 values immediately after application of $_{CP}CO_2$ and 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 effective in preventing further lipid oxidation.

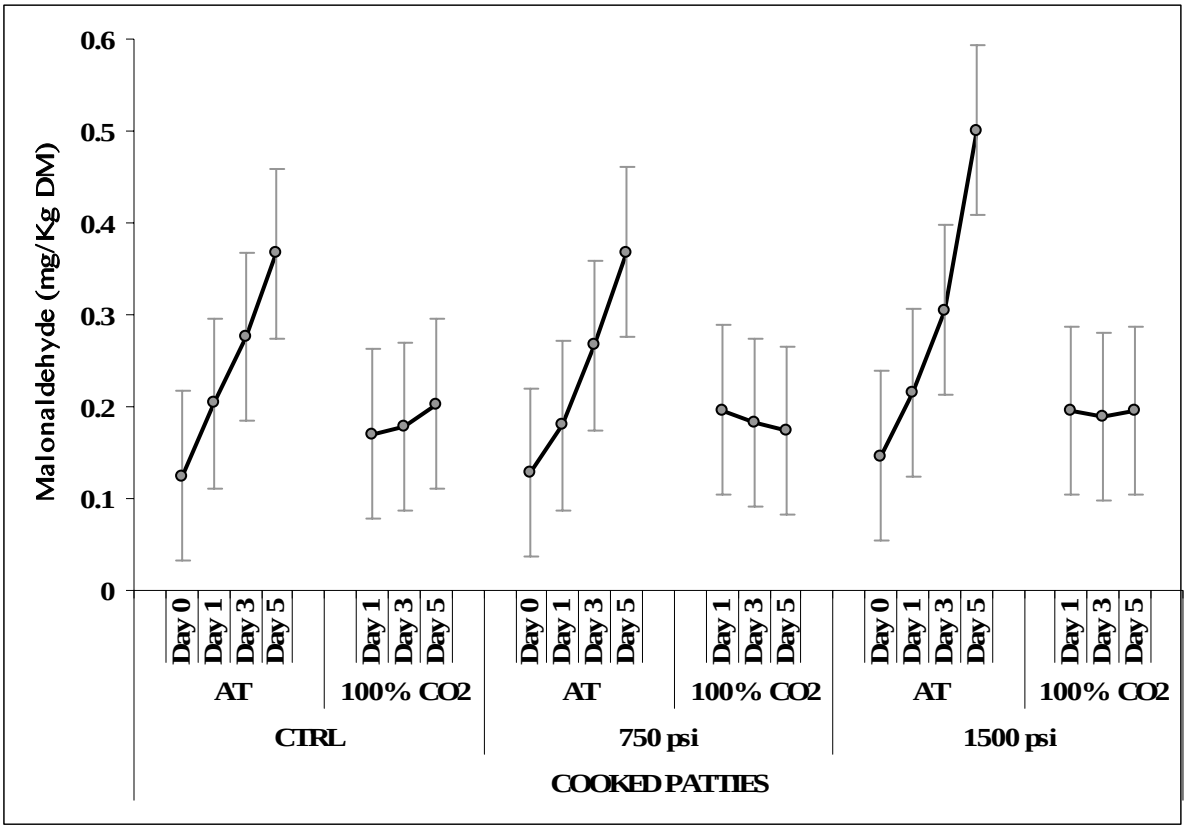


Figure 27. Thiobarbituric Acid Reactive Substances (TBARS, mg/Kg malonaldehyde) Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min), by Package Type (Vacuum Packaged [VP] and Flushed with CO_2 [100% CO_2]), During Simulated Retail Display (Day 0, 1, 3, and 5).

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 18 show the scales used to measure these traits. Two replications were performed.

Table 18. 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 19.

Table 19. Trained Sensory Panel Average 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).

TREATMENT	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 psi	7.4 [a]	5.9 [a]	5.4 [b]	2.0 [b]
1500 psi	7.1 [b]	5.9 [a]	6.1 [a]	0.9 [a]

[a-c] *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 ($P< 0.05$) tenderness when compared to the control (Figure 28). 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). Nevertheless, for OT, BFI, and OFI, a consumer panel probably would be incapable of detecting the differences between treatments as values for these traits were observed to be very close to each other.

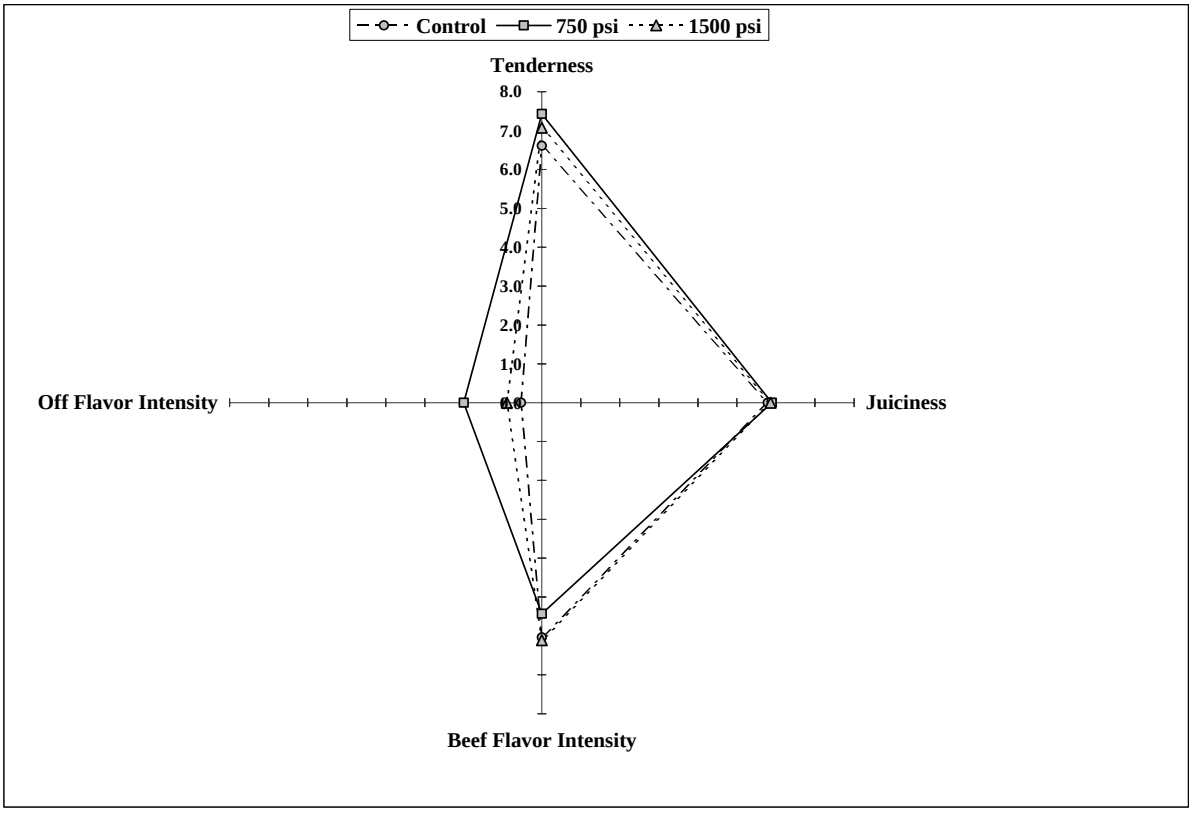


Figure 28. Selected Texture and Flavor Attributes (Tenderness, Juiciness, Beef Flavor Intensity, and Off Flavor Intensity) Obtained in Cooked Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings (750 psi and 1500 psi for 15 min)

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. These finding are in agreement with visual observations obtained in this study, and it

is still subject to further research as to weather these CO_2 related pores and fissures may have an impact on the functional properties of meat (Figure 29).

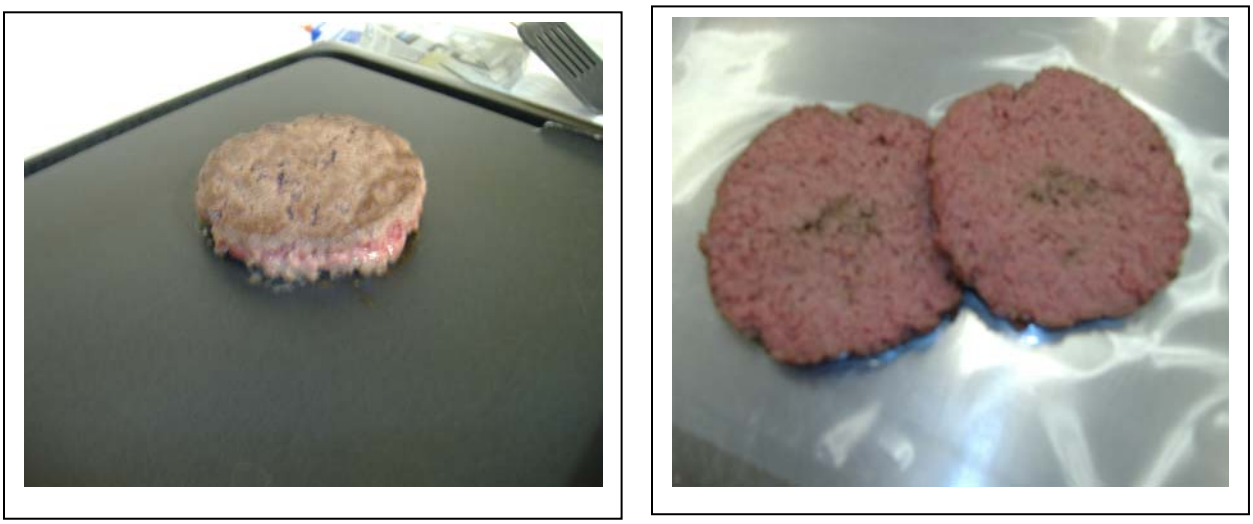


Figure 29. Image of a Cooked Patty (after $_{CP}CO_2$ Application on Beef Trimmings) with Visible Pores and Fissures upon Cooking Caused by a Rapid Release of CO_2 Gas from the Meat upon Heating (Left). A Cross Section of a Cooked Patty for Instrumental Color Measurement (Right).

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, resulted in no major concerns or detrimental effects on cooked ground beef patties.

CHAPTER 6.

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

6.1 Objective

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

6.2 Materials and Methods

6.2.1 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 37°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.

6.2.2 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.

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) at 36 °C with the following specifications:

- 750 psi for 5 min
- 750 psi for 15 min

- 1500 psi for 5 min
- 1500 psi for 15 min

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).

6.2.3 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 weighing 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 30). 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 24 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.

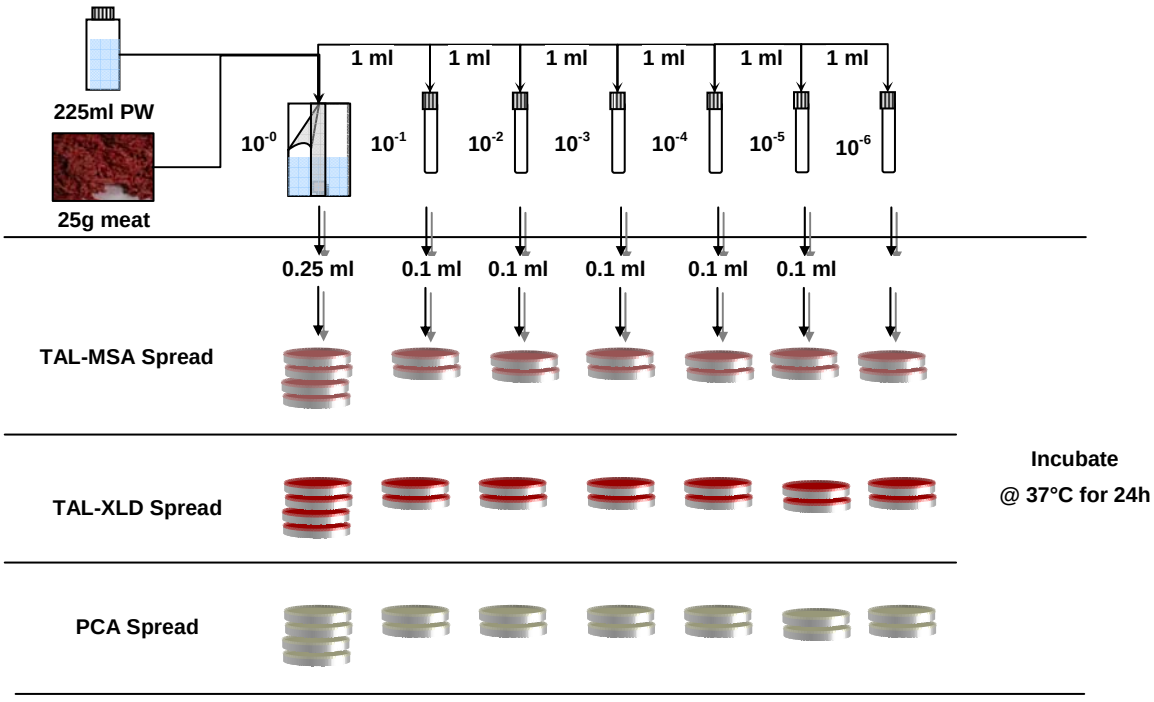


Figure 30. Dilution Scheme for Microbiological Samples Analyzed for Challenging Pathogenic Microorganisms in Raw Patties Treated with $_{CP}CO_2$.

6.2.4 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 through the adipose tissue and muscle crevices of the trim, extending its antimicrobial effects to the interior of the trim. As illustrated in Table 20, the highest lethality achieved immediately after $_{CP}CO_2$ application was by pressurizing at 1500 psi for 15 min. With this treatment, 0.83, 0.93, 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.

Table 20. Bacterial Reductions (Total Plate Count [TPC], Generic *Escherichia coli* [GEC], *E. coli* O157:H7 [O157], and *Salmonella* Spp. [SS]) in Raw Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings, by Pressure (750 and 1500 psi for 15 min), by Exposure Time (5 and 15 min), and by Type (Beef Trimmings [TR] and Ground Beef [GR]).

Food Pathogens	TREATMENTS							
	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]

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

Reductions obtained for TPC, GEC, and O157 in all treatments were similar ($P\geq0.05$), but lower when compared to those obtained for SS with 1500 psi for 15 min in TR and GR (Figure 31). 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.

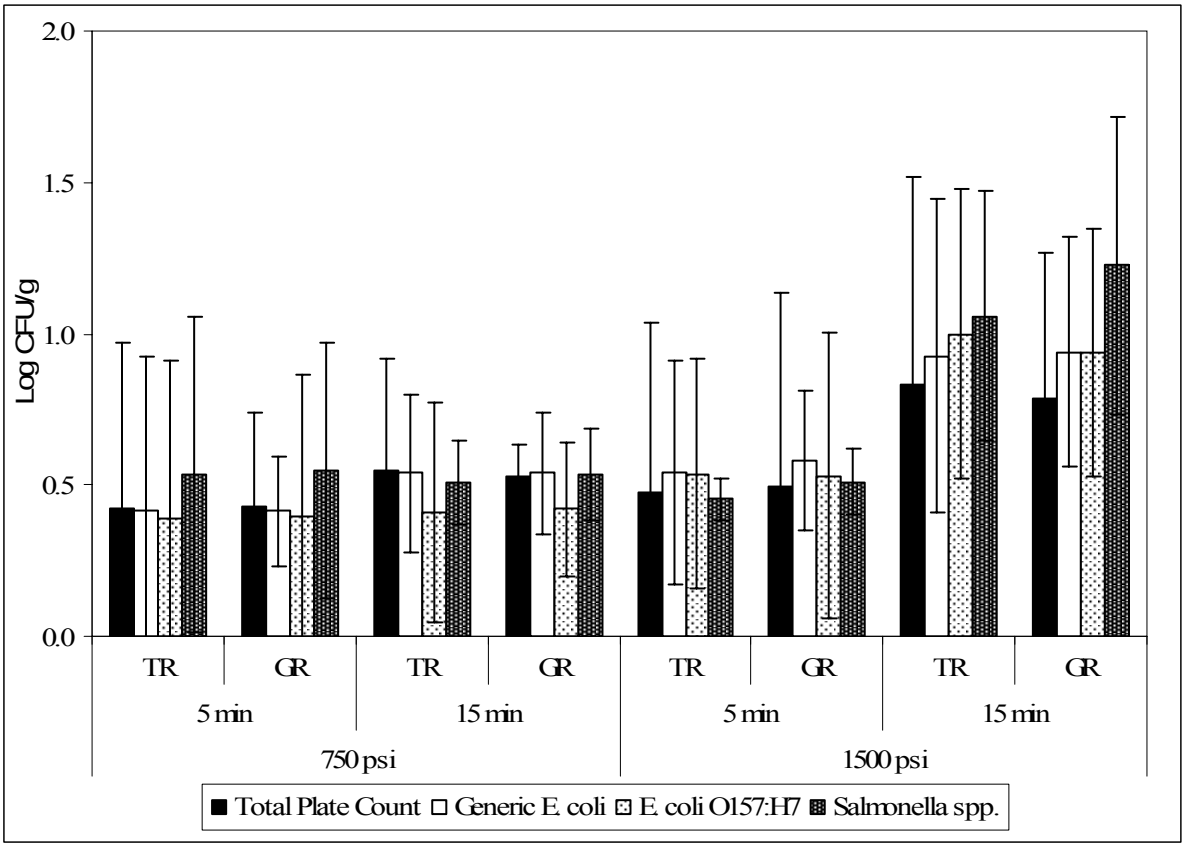


Figure 31. Bacterial Reductions (Total Plate Count [TPC], Generic *Escherichia coli* [GEC], *E. coli* O157:H7 [O157], and *Salmonella* Spp. [SS]) in Raw Ground Beef Patties after $_{CP}CO_2$ Application on Beef Trimmings, by Pressure (750 and 1500 psi for 15 min), by Exposure Time (5 and 15 min), and by Type (Beef Trimmings [TR] and Ground Beef [GR]).

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. 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. These

findings are also in agreement with results reported from other studies in which it was established that pressures under 870 psi were not vigorous enough to have an effective bactericidal effect (Enomoto et al., 1997b).

CHAPTER 7.
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, 2000a; King and Mabitt, 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., 1997a; 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).

Microbial inactivation with high-pressure CO_2 has been shown to be more effective when performed under a continuous treatment (sudden decompression) rather than in a batch treatment (slow decompression), and this effect has been attributed to the sudden expansion of compressed CO_2 in the cells, which induces bursting of the cells. On the other hand, similarities in viability have been also observed with different decompression times under subcritical CO_2. It has been shown, for example, that the antimicrobial effect of CO_2 was not related only to the pressure but to the concentration of dissolved CO_2 as well (Shimoda and Osajima, 1998).

Additional reactor designs that offer gas flow and concentration measurement and controls, will enable further research to determine optimal parameters for different food matrices. Along these lines, enhancement of contact times and mechanical action during $_{CP}CO_2$ application will probably increase the lethality effects of this technology. Nevertheless, 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 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 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 was conducted under supercritical conditions, the inactivation of tested food pathogens was substantially increased.

Published studies have been focused mainly to evaluate the effectiveness of the use of $_{CP}CO_2$ as an antimicrobial as a reasonable goal for a new application. However, this approach does not clarify the individual effects of various parameters such as pressure, carbonation, level and other critical parameters involved in this technology, much less when these parameters are used in factorial design. Although there has been a generalized effort on the study of this technology, the fundamental mechanisms of microbial inactivation by $_{CP}CO_2$ are not yet fully explained. Further studies need to be conducted to determine what mechanism dominates under specific process conditions for a comprehensive understanding of the unique and complicated antimicrobial effects of $_{CP}CO_2$.

Intensive research of $_{CP}CO_2$ treatments are further necessary to demonstrate their effectiveness to control microorganisms in foods. Food processing applications with antimicrobial systems based on the use of $_{CP}CO_2$ are becoming more popular, as they evolved as economically viable alternatives to heat treatments. Potential applications for $_{CP}CO_2$ show promising future in non-thermal processing, aiming mainly at products that are sensitive to heat and pressure such as fresh produce, fruit juices and beverages, fresh and smoked fish, fresh meats, ground beef and many other.

Results from this study show that it is possible to utilize $_{CP}CO_2$ on beef trimmings before grinding not only as a mechanism to reduce and eliminate spoilage organisms and enhance color and shelf life of ground beef, but also as an intervention control for generic *E. coli*, *E. coli* O157:H7, *Salmonella spp.,* coliforms, and aerobic bacteria in ground beef. From the results of the studies in this dissertation, we can conclude:

- Microbial control of spoilage organisms and foodborne pathogens in beef trimmings prior to grinding with $_{CP}CO_2$ is effective (0.6 to 1.2 logs).
- Lethality levels are comparable to those obtained with other intervention strategies. However, this technology does not cause heat denaturation of the product.
- Discoloration of beef trimmings after $_{CP}CO_2$ application may not be a concern for grinding purposes.
- Further packaging with 100% CO_2 is a viable venue to controlling spoilage and pathogenic microorganisms after packaging and during refrigerated storage.
- Discoloration of ground beef patties caused by packaging with 100% CO_2 may be a concern for product marketing.

The use of $_{CP}CO_2$ on beef trimmings before grinding s 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.

APPENDIX A.

Description of the $_{CP}CO_2$ System

The basic parts of the experimental vessel (see Figure 32), manufactured by Atlas/Parker (Des Plaines, IL), consist of two basic parts, a custom stainless steel base end and a rod end head (Figure 33). 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 ∅5 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 34, 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.

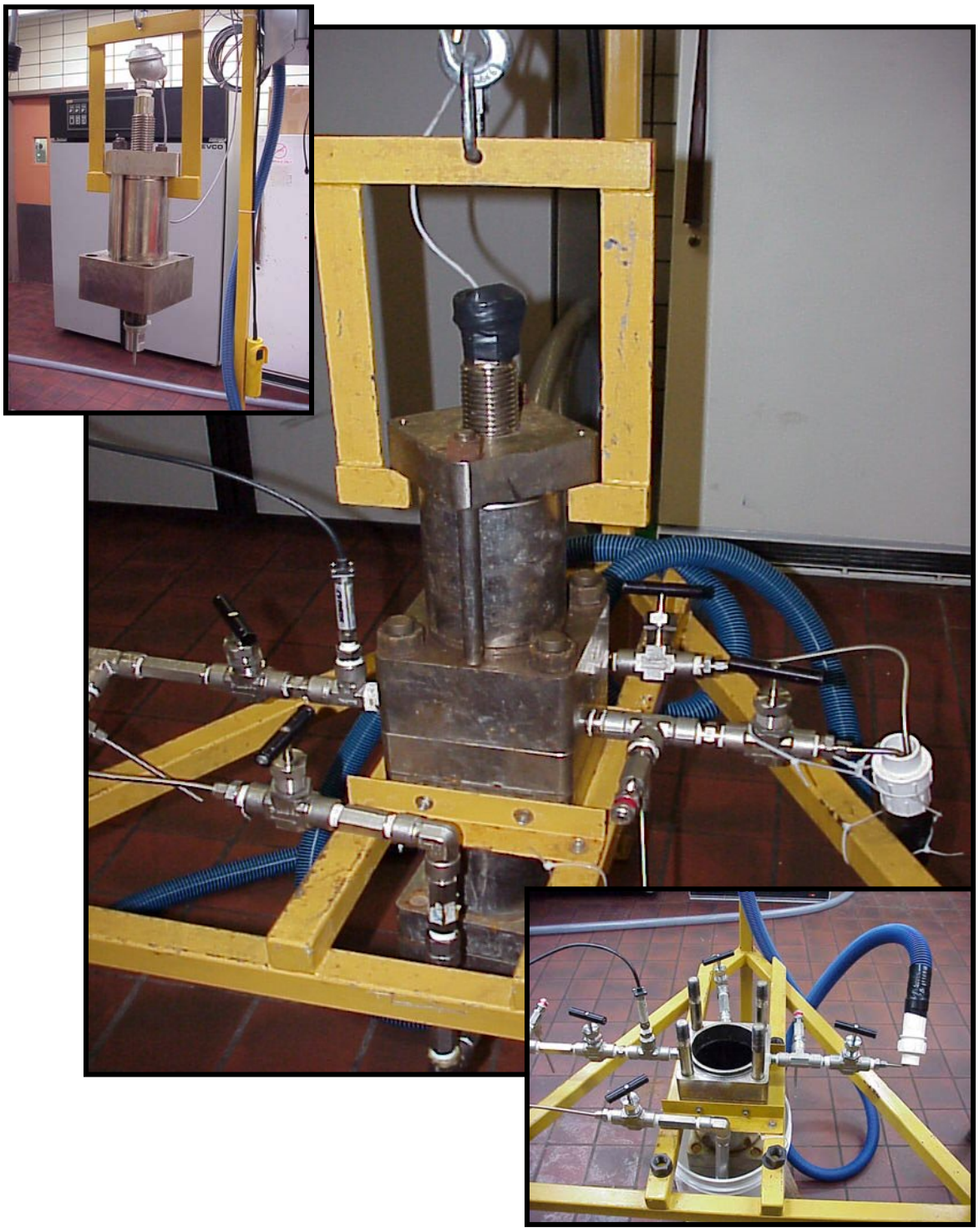


Figure 32. Experimental Vessel.

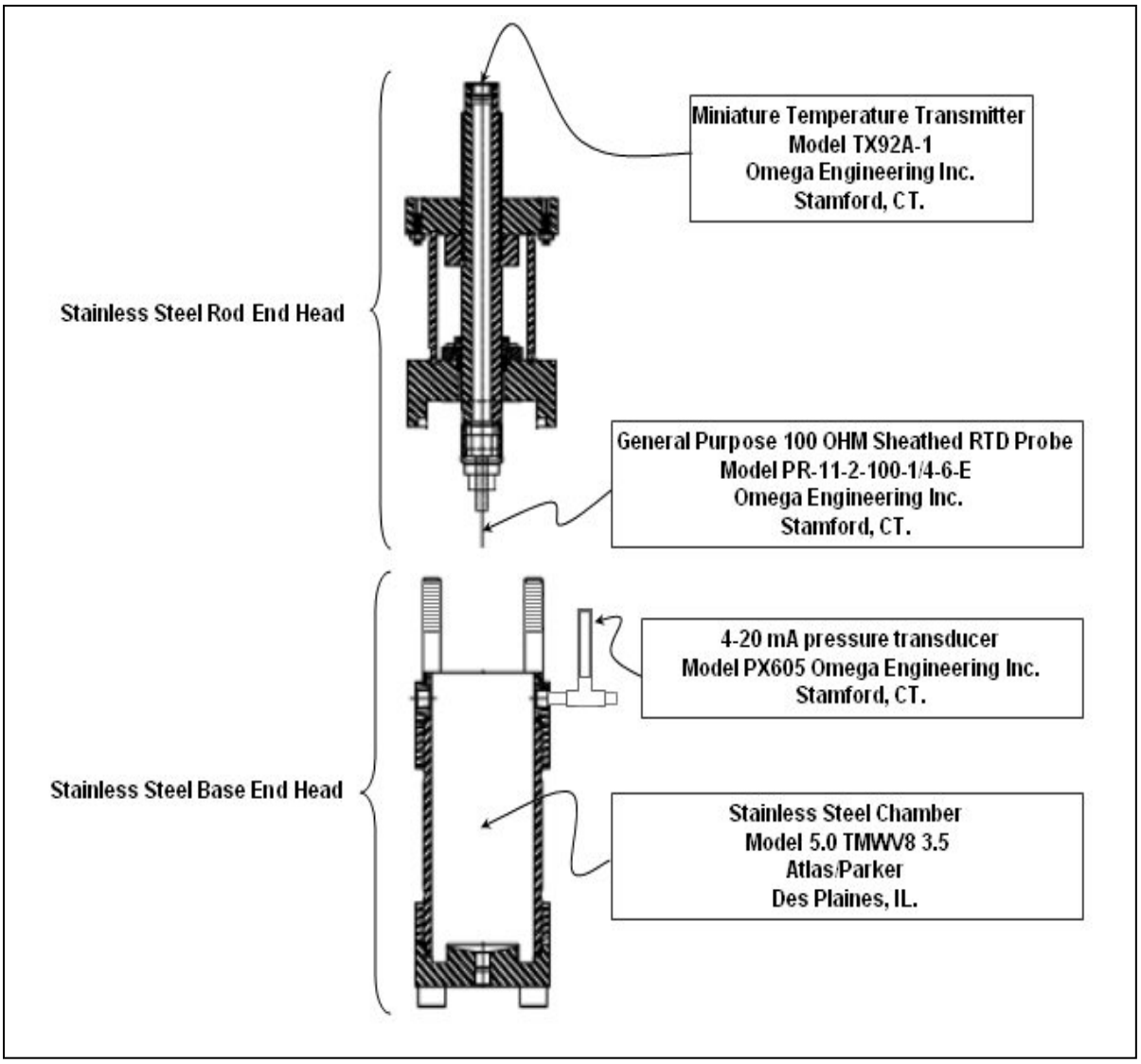


Figure 33. Basic Parts of the $_{CP}CO_2$ Vessel.

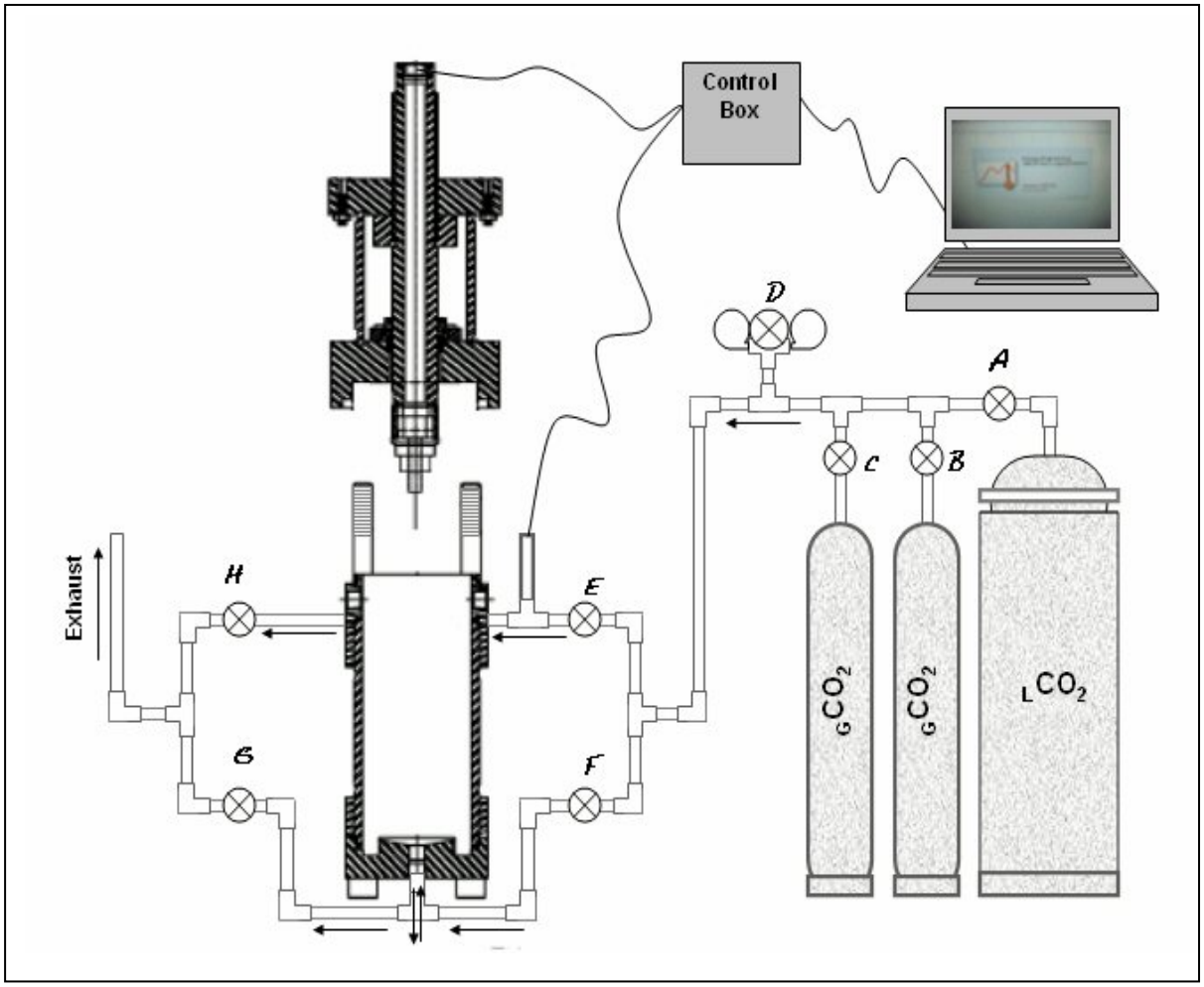


Figure 34. Schematic of the cpCO_2 System.

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 35 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***).

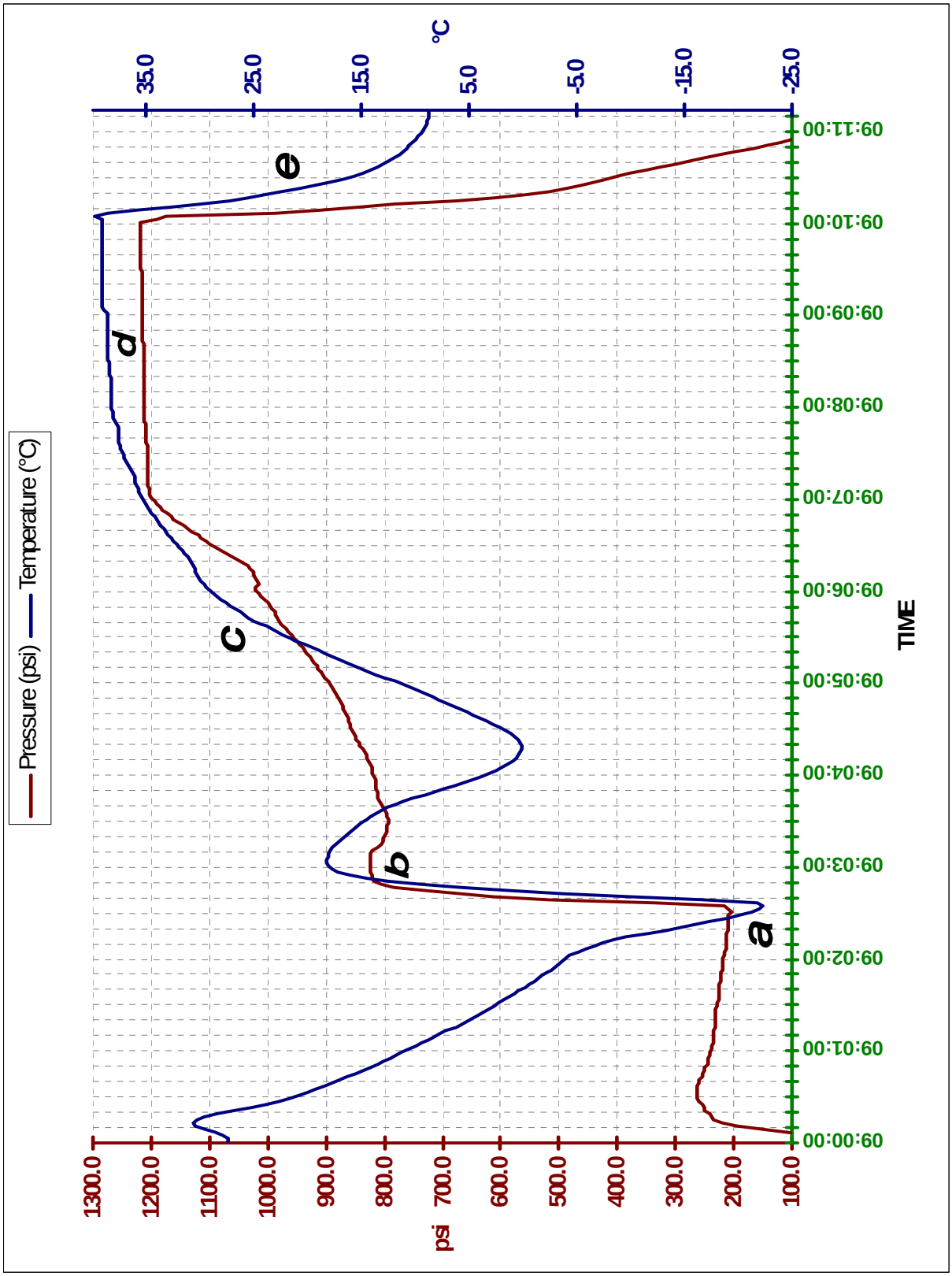


Figure 35. Typical Operational Chart for 3 min Exposure at 1200 psi

References

Acha, P.N. and Szyfres, B. 2001. Salmonellosis. In: Zoonoses and communicable diseases common to man and animals. Pan American Health Organization, Washington, DC. Scientific and Technical Publication. 580:223–246.

Acuff, G.R., Castillo, A. and Savell, J.W. 1997. Hot water rinses. Proc. Reciprocal Meat Conference. 49:125-128.

American Meat Science Association (AMSA). 1991. Guidelines for Meat Color Evaluation. www.meatscience.org. Retrieved 3/23/06

Arreola, A.G. and Balaban, M.O. 1991. Effect of Supercritical Carbon-Dioxide on Microbial-Populations in Single Strength Orange Juice. J. Food Qual. 14:275-284.

Arroyo, G. and Sanz, P.D. 1997. Effect of high pressure on the reduction of microbial populations in vegetables. J. Appl. Microbiol. 82:735-742.

Arthur, T.M., Bosilevac, J.M., Nou, X., Shackelford, S.D., Wheeler, T.L., Kent, M.P., Jaroni, D., Pauling B., Allen D.M. and Koohmaraie M. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. J. Food Prot. 67:658–665.

Bacteriological Analytical Manual (BAM), 2001. Food and Drug Administration. U.S Government Printing Office. Washington. D.C.

Ballestra, P., Abreu Da Silva, A. and Cuq, J.-L. 1996. Inactivation of *Escherichia coli* by carbon dioxide under pressure. J. .Food Sci. 61:829–831, 836.

Balny, C. and Masson, P. 1993. Effects of High-Pressure on Proteins. Food Rev. Int. 9:611-628.

Becker, Z.E. 1933. A comparison between the action of carbonic acid and other acids upon the living cell. Protoplasma 25:161–175.

Bell, M.F., Marshall, R.T. and Anderson, M.E. 1986. Microbiological and sensory tests of beef treated with acetic and formic acids. J. Food Prot. 49:207–210.

Bellamy, W., Wakabayashi, H., Takase, M., Kawase, K., Shimamura, S. and Tomita, M. 1993. Killing of *Candida albicans* by lactoferrin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. Med. Microbiol. Immunol. 182:97-105.

Bentley, D,S., Reagan, J.O. and Miller, M.F. 1989. Effects of gas atmosphere, storage temperature and storage time on the shelflife and sensory attributes of vacuum packaged ground beef patties. J. Food Sci. 54:284–286.

Blickstad, E. and Molin, G. 1983. Carbon dioxide as a controller of the spoilage flora of pork, with special reference to temperature and sodium chloride. J. Food Prot. 46:756-763.

Blickstad, E., Enfors, S.O. and Molin, G. 1981. Effect of hyperbaric carbon dioxide pressure on the microbial flora of pork stored at 4 or 14°C. J. Appl. Bacteriol. 50:493–504.

Bosilevac, J.M., Nou, X., Osborn, M.S., Allen, D.M. and Koohmaraie, M. 2005. Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. J. Food Prot. 68:265-272.

Bowien, B. and Leadbeater, L. 1984. Molecular and cellular regulation of carbon dioxide assimilation in bacteria. In Microbial Growth on C1 Compounds. Crawford R.L. and Hanson, R.S. Eds. American Society for Microbiology. Washington, D.C. pp. 9-13.

Calvo, M. M. and Balcones, E. 2001. Inactivation of microorganisms and changes of proteins during treatment of milk with sub-critical carbon dioxide. Milchwissenschaft-Milk Science International 56:366-369.

Castillo, A., Lucia, L.M., Goodson, K.J., Savell, J.W. and Acuff, G.R. 1998a. Comparison of water wash, trimming and combined hot water and lactic acid treatments for reducing bacteria of fecal origin on beef carcasses. J. Food Prot. 61:823-828.

Castillo, A., Lucia, L.M., Goodson, K.J., Savell, J.W. and Acuff, G.R. 1998b. Use of hot water for beef carcass decontamination. J. Food Prot. 61:19-25.

Castillo, A., Dickson, J.S., Clayton, R.P., Lucia, L.M. and Acuff, G.R. 1998c. Chemical de-hairing of bovine skin to reduce pathogenic bacteria and bacteria of fecal origin. J. Food Prot. 61:623-625

Castillo, A., Lucia, L.M., Goodson, K.J., Savell, J.W. and Acuff, G.R. 1999. Decontamination of beef carcass surface tissue by steam vacuuming alone and combined with hot water and lactic acid sprays. J. Food Prot. 62:146-151.

Centers for Disease Control and Prevention (CDC). 2005. Foodborne and Diarrheal Diseases. CDC. http://www.cdc.gov/foodborne. Retrieved October 2005.

Center for Food Safety and Applied Nutrition (CFSAN), 2001 "Foodborne Pathogenic Microorganisms and Natural Toxins Handbook; Salmonella spp." U.S. Food & Drug Administration. http://www.cfsan.fda.gov/~mow/chap1.html Retrieved March 2006.

Conner, D.E., Kotrola, J.S., Mikel, W.B. and Tamblyn, K.C. 1997. Effects of acetic-lactic acid treatments applied to beef trim on populations of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in ground beef. J. Food Prot. 60:1560–1563.

Crawford, H.R., Neill, G. H., Bucy, B. J. and Crawford, P. B. 1963. Carbon Dioxide, a Multipurpose Additive for Effective Well Stimulation, J. Petroleum Technol. pp. 237.

Crozier-Dodson, B.A. and Fung, D.Y.C. 2002. Comparison of Recovery of Airborne Microorganisms in a Dairy Cattle Facility Using Selective Agar and Thin Agar Layer Resuscitation Media. J. Food Prot. 65:1488–1492.

Dainty, R.H. and Mackey, B.M. 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. J. Appl. Bacteriol. 73, Symp. Suppl. 21:103-114.

D'Aoust, J.Y. 1997. *Salmonella* Species. In: Doyle, M.P., Beuchat, L.R., and Montville, T.J. (Eds.). Food Microbiology: Fundamentals and Frontiers. Washington, DC: American Society for Microbiology Press. Washington, D.C. pp. 138-139.

Davies, A. and Board, R. 1998. The Microbiology of Meat and Poultry. Blackie Academic and Professional. 1st. Edition. Springer – Verlag Ed., Thomson Science, NY, USA. 346 p.

Debs-Louka, E., Louka, N., Abraham, G., Chabot, V. and K. Allaf. 1999. Effect of compressed carbon dioxide on microbial cell viability. Appl. Environ. Microbiol. 65**:**626-631.

De Castro, M.D.L. and Jimenez-Carmona, M.M. 2000. Where is supercritical fluid extraction going? Trac-Trends Anal. Chem. 19:23-228.

Devlieghere, F., Debevere, J. and Van Impe, J. 1998a. Effect of dissolved carbon dioxide and temperature on the growth of *Lactobacillus sake* in modified atmospheres. Int. J. Food Microbiol. 41:231-238.

Devlieghere, F., Debevere, J. and Van Impe, J. 1998b. Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. Int. J. Food Microbiol. 43:105-113.

Devlieghere, F., Geeraerd, A.H., Versyck, K.J., Vandewaetere, B., Van Impe, J. and Debevere, J. 2001. Growth of *Listeria monocytogene*s in modified atmosphere packed cooked meat products: A predictive model. J. Food Microbiol. 18:53-66.

Díaz-Maroto, M.C., Pérez-Coello, M.S. and Cabezudo, M.D. 2002. Supercritical carbon dioxide extraction of volatiles from spices – comparison with simultaneous distillation – extraction. J. Chrom. A. 947:3-29.

Difco. Difco & BBL Manual, 2003. 1st. edition. Difco Laboratories. Becton and Dickson Company. Sparks. MD. Pp. 340-341, 625-628.

Dillow, A.K., Dehghani, F., Hrkach, J.S., Foster, N.R. and Langer, R. 1999. Bacterial inactivation by using near and supercritical carbon dioxide. Proceedings of the National Academy of Sciences of the United States of America 96:10344-10348.

Dionisi, F., Hug, B., Aeschlimann, J. and Houlemar M. 1999. A. Supercritical CO_2 extraction for total analysis of food products. J. Food Sci. 64:612-615.

Division of Bacterial and Mycotic Diseases (DBMD). 2002. National Center for Infectious Diseases. Centers for Disease Control and Prevention. Atlanta, Georgia. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_t.htm. Retrieved March 2006.

Dixon, N.M., Lovitt, R.W., Morris, J.G. and Kell, D.B. 1988. Growth energetics of *Clostridium sporogenes* NCIB 8053: Modulation by CO_2. J. Appl. Bacteriol. 65:09-119-133

Dixon, N.M. and Kell, D.B. 1989. The inhibition by CO_2 of the growth and metabolism of micro-organisms. J. Appl. Bacteriol. 67:109-136.

Donald, J.R., Jones, C.L. and MacLean, A.R.M. 1924. The effect of carbonation on bacteria in beverages. Am. J. Pub. Health 14:122–128.

Dorsa, W.J., Cutter, C.N. and Siragusa, G.R. 1998. Bacterial profile of ground beef made from carcass tissue experimentally contaminated with pathogenic and spoilage bacteria before being washed with hot water, alkaline solution, or organic acid and then stored at 4 or 12°C. J. Food Prot. 61:1109–1118.

Dorsa, W.J., Cutter, C.N., Siragusa, G.R. and Koohmaraie, M. 1996. Microbiological decontamination of beef and sheep carcasses by steam, hot water spray washes, and a steam-vacuum sanitizer. J. Food Prot. 59:127–135.

Economic Research Service (ERS). 2004. Economics of foodborne disease. USDA. http://www.ers.usda.gov/briefing/FoodSafetyPolicy/features.htm. Pp. 1-3. Retrieved March 2006.

Egan, A.F. and Roberts, T.A. 1987. Microbiology of meat and meat products, in Essays in Agricultural and Food Microbiology, J.R. Norris and G.L. Pettipher eds., Wiley, New York, Pp.167-197.

Eggers, R., Ambrogi, A. and von Schnitzler, J. 2000. Special features of SCF solid extraction of natural products: de-oiling of wheat gluten and extraction of rose hip oil. Braz. J. Chem. Eng. 17:29-334.

Ehlers, D., Nguyen, T., Quirin, K.W. and Gerard, D. 2001. Analysis of essential basil oils-CO_2 extracts and steam-distilled oils. Deutsche Lebensmittel-Rundschau, 97:245-250.

Eindhoven, J., Peryam, D., Heiligman, F. and Hamman, J.W.. 1964. Effect of sample sequence on food preferences. J. Food Sci. 29:520-524.

Eisel, W.G., Linton, R.H. and Muriana, P.M. 1997. A survey of microbial levels for incoming raw beef, environmental sources, and ground beef in a red meat processing plant. Food Microbiol. 14:273-282.

Eklund, T. 1984. The effect of carbon dioxide on bacterial growth and on uptake processes in bacterial membrane vesicles. Int. J. Food Microbiol. 1:179-185.

Ellebracht, E.A., Castillo, A., Lucia, L.M., Miller R.K. and Acuff, G.R. 1999. Reduction of Pathogens Using Hot Water and Lactic Acid on Beef Trimmings. J. Food Sci. 64:1094-1099.

Emswiler, B.S., Kotula, A.W. and Rough, D.K. 1976. Bactericidal effectiveness of three chlorine sources used in beef carcass washing. J. Anim. Sci. 42:1445–1450.

Encarta. 2005. “Carbon Dioxide”. Microsoft® Encarta® Online Encyclopedia http://encarta.msn.com © 1997-2005 Microsoft Corporation. All Rights Reserved. Retrieved 3/1/06

Enomoto, A., Nakamura, K., Nagai, K., Hashimoto, T. and Hakoda, M. 1997a. Lethal effect of high-pressure carbon dioxide on a bacterial spore. J. Ferm. and Bioeng. 83:305-307.

Enomoto, A., Nakamura, K., Nagai, K., Hashimoto, T. and Hakoda, M. 1997b. Inactivation of food microorganisms by high-pressure carbon dioxide treatment with or without explosive decompression. Biosci. Biotechnol. Biochem. 61:1133-1137.

Erkmen, O. 2000a. Antimicrobial effect of pressurized carbon dioxide on *Enterococcus faecalis* in physiological saline and foods. J. Sci. Food and Agric. 80:465-470.

Erkmen, O. 2000b. Effect of carbon dioxide pressure on *Listeria monocytogenes* in physiological saline and foods. J. Food Microbiol. 17:589-596.

Erkmen, O. 2000c. Inactivation of *Salmonella* Typhimurium by high pressure carbon dioxide. J. Food Microbiol. 17: 225-232.

Erkmen, O. 2001a. Effects of high-pressure carbon dioxide on *Escherichia coli* in nutrient broth and milk. Int. J. Food Microbiol. 65:131-135.

Erkmen, O. 2001b. Kinetic analysis of *Listeria monocytogene*s inactivation by high pressure carbon dioxide. J. Food Eng. 47:7-10.

Erkmen, O. 2001c. Mathematical modeling of *Escherichia coli* inactivation under high-pressure carbon dioxide. J. Bioscie. Bioeng. 92:39-43.

Erkmen, O. and Karaman, H. 2001. Kinetic studies on the high pressure carbon dioxide inactivation of *Salmonella* Typhimurium. J. Food Eng. 50:25-28.

Farrell, B.L., Ronner, A.B. and Wong, C.L. 1998. Attachment of *Escherichia coli* O157:H7 in ground beef to meat grinders and survival after sanitation with chlorine and peroxyacetic acid. J. Food Prot. 61:817–822.

Fava, P. and Piergiovanni, L. 1992. Carbon dioxide solubility in foods packaged with modified atmosphere. II: Correlation with some chemical–physical characteristics and composition. Industrie Alimentari, 31:24-430.

Fennema, E. 1996. Food Chemistry. 3d. Edition. Marcel Dekker, Inc. New York, NY. 1067 p.

Fraser, D. 1951. Bursting bacteria by release of gas pressure. Nature. 167:33–34.

Fung, D.Y.C., Hajmeer, M.N., Kastner, C.L., Kastner, J.J., Marsden, J.L., Penner, K.P., Phebus, R.K., Smith, J.S. and Vanier, M.A. 2000. Meat Safety. In Hui, Y.H., Nip, W.K., Rogers, R.W. and Young, D.A. Ed. Meat Science and Applications. Marcel Dekker, Inc. New York. USA. Chapter 8. Pp 171-305.

García-López, M.L., Prieto, M. and Otero, A. 1998. The physiological attributes of Gram-negative bacteria associated with spoilage of meat and meat products. In: Davies, A. and Board, R. 1998. The Microbiology of Meat and Poultry. Blackie Academic and Professional. 1st. Edition. Springer – Verlag Ed., Thomson Science, New York, USA. Pp 1-28.

Garcia-Zepeda, C.M., Kastner, C.L., Kenney, P.B., Campbell, R.E. and Schwenke, J.R. 1994. Aroma profile of subprimals from beef carcasses decontaminated with chlorine and lactic acid. J. Food Prot. 57:674-678.

Gill, C.O. 1982. Microbial Interaction with Meats, in Meat Microbiology. M.H. Brown Ed., Appl. Sci. London. UK. Pp. 225-264.

Gill, C.O. and Molin, G. 1991. Modified atmospheres and vacuum packaging, in Food Preservatives, N.J. Russell and G.W. Gould Editors, Blackie Academic and Professional. Glasgow. UK. Pp. 172-199.

Gill, C.O. and Penney, N. 1988. The solubility of carbon dioxide in meat. Meat Sci. 22:65-71.

Gonzalez, J.C., Fontal, O.I., Vieytes, M.R., Vieytes, J.M. and Botana, L.M. 2002. Basis for a new procedure to eliminate diarrheic shelfish toxins from a contaminated matrix. J. Agric. Food Chem. 50:400-405.

Gorman, B.M., Morgan, J.B., Sofos, J.N. and Smith, G.C. 1995a. Microbiological and visual effects of trimming and/or spray washing for removal of fecal material from beef. J. Food Prot. 58:984–989.

Gorman, B.M., Sofos, J.N., Morgan, J.B., Schmidt, G.R. and Smith, G.C. 1995b. Evaluation of hand trimming, various sanitizing agents, and hot water spray-washing as decontamination interventions for beef brisket adipose tissue. J. Food Prot. 58:899-907.

Graves-Delmore, L.R., Sofos, J.N., Schmidt, G.R. and Smith, G.C. 1998. Decontamination of inoculated beef with sequential spraying treatments. J. Food Sci. 63:890–893.

Greenwald. C.G. 1991. Overview of fat and cholesterol reduction technologies. Chapter 3 In: Fat and Cholesterol Reduced Foods: Technologies and Strategies. Advances in Applied Biotechnology Series. C Haberstroh and CE Morris (Eds.), Gulf Pub. Co., The Woodlands, Texas, USA. 12:1-32.

Guerrero, I. and Taylor, A.J. 1994. Meat surface decontamination using lactic acid from chemical and microbial sources. Food Sci. Technol. 27:201-209.

Haas, G.J., Prescott, J.R., Dudley, E., Dik, R., Hintlian, C. and Keane, L. 1989. Inactivation of microorganisms by carbon dioxide under pressure. J. Food Safety 9:253–265.

Hendrickx, M., Ludikhuyze, L., Van den Broeck, I. and Weemaes, C. 1998. Effects of high pressure on enzymes related to food quality. Trends Food Sci. Technol. 9:197-203.

Hofland, G.W., van Es, M., Luuk, A.M., van der Wielen, A.M. and Witkamp, G-J. 1999. Isoelectric precipitation of casein using high-pressure CO_2. Ind. Eng. Chem. Res. 38:4919-4927.

Hohmann, E.L. 2001. Non-Typhoidal Salmonellosis. Clinical Infectious Diseases, 32:263–269.

Holley, R.A., Gariépy, C., Delaquis, P., Doyon, G. and Gagnon, J. 1994. Static, controlled packaging of retail ready pork. J. Food Sci. 59:1296–1301.

Hong, S.I. and Pyun Y.R., 1999. Inactivation kinetics of *Lactobacillus plantarum* by high pressure carbon dioxide. J. Food Sci. 64:728-733.

Hong, S.I. and Pyun, Y.R. 2001. Membrane damage and enzyme inactivation of *Lactobacillus plantarum* by high pressure CO_2 treatment. Int. J. Food Microbiol. 63:19-28.

Hood. D.E. and Mead, G.C. 1993. Modified atmosphere storage of fresh meat and poultry, in Principles and Applications of Modified Atmosphere Packaging of Food, R.T. Parry ed., Blackie Academic and Professional, London, pp. 269-298.

Hornick, R.B. 1974. Salmonella infections - Newer perspective of an old infection (Jeremiah Metzger Lecture). Soc. Appl. Bacteriol. Symp. Serv. 3:221-228.

Hunt, M. C., Acton, J. C., Benedict, R. C., Calkins, C. R., Cornforth, D. P., Jeremiah, L. E., Olson, D. G., Salm, C. P., Savell, J. W. and Shivas, S. D. 1991. AMSA guidelines for meat color evaluation. In Proceedings 44th Annual Reciprocal Meat Conference (pp. 3–17), Kansas State University, Manhattan, KS. Pp. 3-17

Hunt, M.C. and Kropf, D.H. 1985. Color and appearance. In: Adv. Meat Research. Vol. 3. Restructured Meat and Poultry Products. AVI Publishing Co., Inc., Westport, CT.

Ibáñez, E., Palacios, J., Senorans, F.J., Santa-Maria, G., Tabera, J. and Reglero, G. 2000. Isolation and separation of tocopherols from olive by-products with supercritical fluids. J. Am. Oil Chemists Society. 77:87-190.

Isenschmid, A., Marison, L.W. and von Stockar, U. 1995. The influence of pressure and temperature of compressed CO_2 on the survival of yeast cells. J. Biotechnol. 39:229–237.

Ishikawa, H., Shimoda, M., Shiratsuchi, H. and Osajima, Y. 1995. Sterilization of microorganisms by the supercritical carbon dioxide micro-bubble method. Biosci. Biotechnol. Biochem. 59:949-1950.

Jakobsen, M. and Bertelsen, G. 2002. The use of CO_2 in packaging of fresh red meats and its effect on chemical quality changes in the meat: A review. J. Muscle Foods 13:143-168.

Jakobsen, M. and Bertelsen, G. 2003. Predicting the amount of carbon dioxide absorbed in meat. Meat Sci. 68:603-610.

Jarrell, P.M., Fox, C.E., Stein, M.H. and Webb S.L. 2002. Practical Aspects of CO_2 Flooding. SPE Monograph. Pp. 22, 220.

Jeremiah, L.E., Gibson, L.L. and Arganosa, G.C. 1996. A research note: The influence of CO_2 level on the storage life of chilled pork stored at –1.5°C. J. Muscle Foods, 7:139–148.

Jeremiah, L.E., Carpenter, Z.L. and Smith, G.C. 1972. Beef color as related to consumer acceptance and palatability. J. Food Sci. 37:476–479.

Jones, R.P. and Greenfield, P.F. 1982. Effect of carbon dioxide on yeast growth and fermentation. Enzyme Microbial Technol. 4:210-223.

Jordan, P.J., Lay, K., Ngan., N. and Rodley, G.F. 1987. Casein Precipitation Using High-Pressure Carbon-Dioxide. New Zealand J. Dairy Sci. Technol. 22:247-256.

Kamihira, M., Taniguchi, M. and Kobayashi, T. 1987. Sterilization of microorganisms with supercritical carbon dioxide. Agric. Biol. Chem. 51:407–412

Kang, D.H. and Fung, D.Y.C., 2000. Application of thin agar layer method for recovery of injured *Salmonella* Typhimurium. Int. J. Food Microbiol. 54:127-132.

Kansas Geological Survey. 2003. Open-file Report 2003-33. Online Tools to Evaluate Saline Aquifers for CO_2 Sequestration. http://www.kgs.ku.edu/PRS/publication/2003/ofr2003-33/P1-05.html (2 of 2). Retrieved 3/06/06

Kincal, D., Hill W.S., Balaban, M., Portier, K.M., Sims, C.A., Wei, C.I. and Marshall, M.R. 2006. A Continuous High-Pressure Carbon Dioxide System for Cloud and Quality Retention in Orange Juice. J. Food Sci. 71:338-344

King, J.S., and Mabitt, L.A. 1982. Preservation of raw milk by the addition of carbon dioxide. J. Dairy Res. 49:439-447.

King, A.D. and Nagel, C.W. 1967. Growth Inhibition of a *Pseudomonas* by Carbon Dioxide. J. Food Sci. 32:575-579.

King, D.A., Lucia, L.M., Castillo, A., Acuff, G.R., Harris, K.B. and Savell, J.W. 2005. Evaluation of peroxyacetic acid as a post-chilling intervention for control of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces. Meat Sci. 69:401–407.

Knorr, D., Schlueter, O. and Heinz, V. 1998. Impact of high hydrostatic pressure on phase transitions of foods. Food Technol. 52:42-45.

Kochevar, S.L., Sofos, J.N., LeValley, S.B. and Smith, G.C. 1997. Effect of water temperature, pressure, and chemical solution of removal of fecal material and bacteria from lamb adipose tissue by spray washing. Meat Sci. 45:377–388.

Koneman, E.W., Allen, S.D., Janda, W. M., Schreckenberger, P. C. and Winn, W. C. 1997. Color atlas and textbook of diagnostic microbiology. Chpt. 6. 5th ed. Lippincott, Philadelphia, Pa. 1736 p.

Kumagai, H., Hata, C. and Nakamura, K. 1997. CO_2 sorption by microbial cells and sterilization by high- pressure CO_2. Biosci. Biotechnol. Biochem. 61:931-935.

Lang, Q.Y. and Wai, C.M. 2001. Supercritical fluid extraction in herbal and natural product studies – a practical review. Talanta, 53:771-782.

Ledward, D.A. 1970. Metmyoglobin formation in beef stored in carbon dioxide enriched and oxygen depleted atmospheres. J. Food Sci. 35:33-37.

Levine, M.M. 1987. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J. Inf. Diseases 155:377-389

Lillard, H.S. 1986. Distribution of ‘‘attached’’ *Salmonella* Typhimurium cells between poultry skin and a surface film following water immersion. J. Food Prot. 49:449-454.

Lillard, H.S. 1988. Effect of surfactant or changes in ionic strength on the attachment of *Salmonella* Typhimurium to poultry skin and muscle. J. Food Sci. 53:727-730.

Lin, H.M, Chan, E.Ch, Chen, C. and Chen, L.F 1991. Disintegration of yeast cells by pressurized carbon dioxide. Biotechnol. Prog. 7:201-204.

Lin, H., Yang, Z.Y. and Chen, L.F. 1992a. An improved method for disruption of microbial cells with pressurized carbon dioxide. Biotechnol. Prog. 8:165-166.

Lin, H.M., Yang, Z.Y. and Chen, L.F. 1992b. Inactivation of *Saccharomyces cerevisiae* by Supercritical and Subcritical Carbon-Dioxide. Biotechnol. Prog. 8:458-461.

Lin, H., Yang, Z.Y and Chen, L.F. 1993. Inactivation of *Leuconostoc dextranicum* with carbon dioxide under pressure. Chem. Eng. J. 52:29-34.

Luck, E. and Jager, M. 1998. Antimicrobial food additives. 2nd edition. New York: Springer-Verlag ed. pp. 42–43, 98–101, 116–119, 137–144, 239.

Luderitz, O., Staub, A.M. and Westphal, O. 1966. Immunochemistry of O and R antigen of *Salmonella* and related *Enterobacteriaceae*. Bacteriol. Reviews 30:192-255.

Mason, K.K., 2004. Vitamin E (alpha-tocopherol) and Meat Color. Meat Science Section. Department of Animal Science. Texas A & M University. http://meat.tamu.edu/topics/VitEJuly2004.pdf Retrieved 3/26/06

Mies P.D., Covington, B.R., Harris, K.B., Lucia, L.M., Acuff, G.R. and Savell, J.W. 1999. Commercial and laboratory application of cattle washes with and without antimicrobial agent as decontamination strategies for hides. Department of Animal Science, Texas A&M University. Pp. 114-119. http://animalscience.tamu.edu/ansc/ beef/bcrt/mies.pdf Retrieved 3/26/06

Miller, K. and Phillips, A. 2005. Harvesting Mars. FirstScience.com http://www.firstscience.com/site/articles/harvesting.asp. Retrieved 6/03/06

Mohamed, R.S. and Mansoori G.A. 2002. The Use of Supercritical Fluid Extraction Technology in Food Processing. Featured Article - Food Technology Magazine, June 2002. The World Markets Research Centre, London, UK.

Molin, G. 1983. The resistance to carbon dioxide of some food related bacteria. Eur. J. Appl. Microbiol. Biotechnol. 18:214-217.

Moret, S., Conte, L.S. 2000. Polycyclic aromatic hydrocarbons in edible fats and oils: occurrence and analytical methods. J. Chrom. A. 882:245-253.

Morrison, D.C. and Ulevitch, R.J. 1978. The effects of bacterial endotoxin on host mediation system. Am. J. Pathol. 93:527-617.

Nakamura, K., Enomoto, A., Fukushima, H., Nagai, K. and Hakoda, M. 1994. Disruption of Microbial-Cells by the Flash Discharge of High- Pressure Carbon-Dioxide. Biosci. Biotechnol. Biochem. 58:1297-1301.

Nataro, J.P. and J.B. Kaper. 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11:132-201.

Nou, X., Rivera-Betancourt, M., Bosilevac, J.M., Wheeler, T.L., Shackelford, S.D., Gwartney, B.L., Reagan, J.O. and Koohmaraie, M. 2003. Effect of chemical de-hairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses in a commercial beef processing plant. J. Food Prot. 66:2005-2009.

Nychas, G.J., Dillon, V.M. and Board, R.G. 1988. Glucose, the key substrate in the microbiological changes occurring in meat and certain meat products. Biotechnol. Appl. Biochem. 10:203-231.

Ozer, E.O., Platin, S., Akman, U. and Hortascsu, O. 1996. Supercritical Carbon Dioxide Extraction of Spearmint Oil from Mint-Plant Leaves. Can. J. Chem. Eng. 74:920-928.

Park, S.J., Lee, J.I. and Park., J. 2002. Effects of a combined process of high pressure carbon dioxide and high hydrostatic pressure on the quality of carrot juice. J. Food Sci. 67:1827-1834.

Penney, N. and Bell, R.G. 1993 Effect of residual oxygen on the color, odor, and taste of carbon dioxide-packaged beef, lamb and pork during short term storage at chill temperatures. Meat Sci. 33:245-252.

Phebus, R.K., Nutsch, A.L., Schafer, D.E., Wilson, R.C., Riemann, M.J., Leising, J.D., Kastner, C.L., Wolf, J.R. and Prasai, R.K. 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. J. Food Prot. 60:476-484.

Piergiovanni, L. and Fava, P. 1992. Carbon dioxide solubility in foods packaged with modified atmosphere. I: Measure methods. Industrie Alimentari, 31:97-302, 306.

Pohlman, F.W., Stivarius, M.R., McElyea, K.S., Johnson, Z.B. and Johnson, M. G. 2002a. Reduction of microorganisms in ground beef using multiple intervention technology. Meat Sci. 61:315–322.

Pohlman, F.W., Stivarius, M.R., McElyea, K.S., Johnson, Z.B. and Johnson, M.G. 2002b. The effects of ozone, chlorine dioxide, cetylpyridinium chloride, and trisodium phosphate as multiple antimicrobial interventions on microbiological, instrumental color, and sensory color and odor characteristics of ground beef. Meat Sci. 61:307–313.

Ransom, J.R., Belk, K.E., Sofos, J.N., Stopforth, J.D., Scanga, J.A. and Smith, G.C. 2003. Comparison of intervention technologies for reducing *Escherichia coli* O157:H7 on beef cuts and trimmings. Food Prot. Trends 23:24-34.

Reagan, J.O., Acuff, G.R., Buege, D.R., Buyck, M.R., Dickson, J.S., Kastner, C.L., Marsden, J.L., Morgan, J.B., Nickelson II, R., Smith, G.C. and Sofos, J.N. 1996. Trimming and washing of beef carcasses as a method of improving the microbiological quality of meat. J. Food Prot. 59:751–756.

Rizvi, S.S.H., Benado, A.L., Zollweg, J.A., Daniels, J.A. 1986. Supercritical Fluid Extraction: Fundamental Principles and Modeling Methods. Food Technol. 40:55-64

Rosset, R. 1982. Chilling, Freezing and Thawing. In M. H. Brown, Meat Microbiology. London: Applied Science Publishers Ltd. Pp. 295-318**.**

Rousset, S. and Renerre, M. 1991. Effect of CO_2 or vacuum packaging on normal and high pH meat shelf-life. Int. J. Food Sci. Technol. 26:641-652.

Saldaña, M.D.A., Mohamed, R.S. and Mazzafera, P. 2000. Supercritical carbon dioxide extraction of methylxanthines from maté tea leaves. Braz. J. Chem. Eng. 17:251-259.

Saldaña, M.D.A., Zetzl, C., Mohamed, R.S. and Brunner, G. 2002. Decaffeination of guaraná seeds in a microextraction column using water-saturated CO_2. J. Supercritical Fluids. 22:119-127

SAS. Statistical Analysis Software Ver. 9.1.3. 2003. SAS Institute Inc., Cary, NC. USA. http://www.sas.com/index.html. Retrieved 5/10/06

Scanga, J.A., Grona, A.D., Belk, K.E., Sofos, J.N., Bellinger, G.R. and Smith, G.C. 2000. Microbiological contamination of raw beef trimmings and ground beef. Meat Sci. 56:145-152

Schwimmer 1981. Source Book of Food Enzymology. AVI Publishing Inc. Westport, Connecticut. 967 p.

Selgras, D., Marin, M. L., Pin, C. and Casas, C. 1993. Attachment of bacteria to meat surfaces: a review. Meat Sci. 34:265-273.

Shakhashiri, B.Z. 2006. Science is Fun. Chemical of the Week. “Carbon Dioxide”. University of Wisconsin-Madison. http://scifun.chem.wisc.edu/chemweek/CO2/CO2. html. Retrieved 3/1/06

Shimoda, M., Cocunubo-Castellanos, J., Kago, H. Miyake, M., Osajima, Y. and Hayakawa, I. 2001. The influence of dissolved CO_2 concentration on the death kinetics of *Saccharomyces cerevisiae.* J. Appl. Microbiol. 91:306-311.

Shimoda, M. and Osajima, Y. 1998. Non-heating inactivation of microorganisms and enzymes - Application of supercritical carbon dioxide micro bubble method to food industry. J. Jap. Soc. Food Sci. Technol. 45:334-339.

Shimoda, M., Yamamoto, Y., Cocunubo-Castellanos, J., Tonoike, H., Kawano, T., Ishikawa, H. and Osajima, Y. 1998. Antimicrobial effects of pressured carbon dioxide in a continuous flow system. J. Food Sci. 63:709-712.

Shin, K., Yamauchi, K., Teraguchi, S., Hayassaw, H., Tomita, M., Otsuka, Y. and Yamazaki, S. 1998. Antibacterial activity of bovine lactoferrin and its peptides against enterohemorrhagic *Escherichia coli* O157:H7. Lett. Appl. Microbiol. 26:407-411.

Sihvonen, M., Jarvenpaa, E., Hietaniemi, V. and Huopalahti, R. 1999. Advances in supercritical carbon dioxide technologies. Trends Food Sci. Technol. 10:217-222.

Smith. J.P., Ramaswamy, H.S. and Simpson, B.K. 1990. Developments in food packaging technology. Part II: Storage aspects. Trends Food Sci. Technol. 11:1-18.

Sofos, J.N., Belk, K.E. and Smith, G.C. 1999. Processes to reduce contamination with pathogenic microorganisms in meat. Proc. 45th Intl. Congress of Meat Science and Technology, August 1-6, Yokohama, Japan. 596-605. http://ansci.colostate.edu/dp/msfs/processes.pdf Retrieved 6/03/2006.

Spilimbergo, S., Elvassore, N. and Bertucco, A. 2002. Microbial inactivation by high-pressure. J. Supercritical Fluids 22:55-63.

Stivarius, M.R., Pohlman, F.W., McElyea, K.S. and Apple, J.K. 2002. Microbial, instrumental color and sensory color and odor characteristics of ground beef produced from beef trimmings treated with ozone or chlorine dioxide. Meat Sci. 60:299–305.

Stopforth, J.D., Samelis, J., Sofos, J.N., Kendall, P.A. and Smith, G.C. 2003. Influence of extended acid stressing in fresh beef decontamination runoff fluids on sanitizer resistance of acid adapted *Escherichia coli* O157:H7 in biofilms. J. Food Prot. 66:2258–2266.

Strange, E.D., Benedict, R.C., Gugger, R.E., Metzger, V.G. and Swift, C.E. 1974. Simplified methodology for measuring meat color. J. Food Sci. 39:988–992.

Taniguchi, M., Kamihira, M. and Kobayashi T. 1987. Effect of Treatment with Supercritical Carbon-Dioxide on Enzymatic-Activity. Agric. Biol. Chem. 51:593-594.

Tedjo, W. and Eshtiaghi, M.N. 2000. Impact of supercritical carbon dioxide and high pressure on lipoxygenase and peroxidase activity. J. Food Sci. 65:1284-1287.

Thomas, C.J. and McMeekin, T.A. 1981. Attachment of Salmonella spp. to chicken muscle surfaces. App. Environ. Microbiol. 42:130–134.

Tomasula, P.M., Boswell, R.T. and Dupren, N.C. 1999. Buffer properties of milk treated with high pressure carbon dioxide. Milchwissenschaft-Milk Sci. Int. 54:667-670.

Tomasula, P.M., Craig, J.C. and Boswell, R.T. 1997. A continuous process for casein production using high-pressure carbon dioxide. J. Food Eng. 33:405-419.

Tomasula, P.M., Craig, J.C., Boswell, R.T., Cook R.D., Kurantz, M.J. and Maxwell, M. 1995. Preparation of Casein Using Carbon-Dioxide. J. Dairy Sci. 78:506-514.

Tomasula, P.M., Craig, J.C.and McAloon, A.J. 1998. Economic analysis of a continuous casein process using carbon dioxide as precipitant. J. Dairy Sci. 81:3331-3342.

Toromont Energy Systems. 1995. All about CO_2. http://www.toromontsystems.com/library/All%20About%20CO2.pdf. Retrieved 3/6/06

Unda, J.R., Molins, R. A. and Zamojcin, C.A. 1989. Sanitation of fresh rib eye steaks with chlorine dioxide generating binary systems. J. Food Sci. 54:7–10.

Wei, C.I., Balaban, M.O., Fernando, S.Y. and Peplow, A.J. 1991. Bacterial effect of high-pressure CO_2 treatment on foods spiked with *Listeria* or *Salmonella*. J. Food Prot. 54:189-193.

Wheeler, T.L., and Koohmaraie, M. 1994. Prerigor and postrigor changes in tenderness of ovine longissimus muscle. J. Anim. Sci. 72:1232-1236.

Wikipedia. 2006. "Carbon dioxide". Wikipedia, The Free Enciclopedia. 2006. http://en.wikipedia.org/wiki/Carbon_dioxide. Retrieved 3/1/06.

Williams, H.S. 1904. A History of Science in Five Volumes. Volume IV. Modern Development of The Chemical and Biological Sciences Illustrated Chapter 2 The Beginnings of Modern Chemistry. Joseph Black. http://arthurwendover.com/arthurs/science/hstsci410.html. Retrieved 3/6/06

Witte, V.C., Krause, G.F., and Bailey, M.E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J. Food Sci. 35:582-585.

Witteman. 2005. "Physical properties of carbon dioxide. Witteman LLC. http://www.wittemann.com/techdocs/physical_properties_of_carbon_dioxide.pdf. Retrieved 3/3/06.

Yamauchi, K., Tomita, M., Giehl, T.J. and Ellision, R.T. III. 1993. Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. Infectious Immunology, 61:719-728.

Zhao, Y., Wells, J.H. and McMillin, K.W. 1995. Dynamic changes of headspace gases in CO_2 and N_2 packaged fresh beef. J. Food Sci. 60:571–575, 591.