THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN LOW-FAT GROUND BEEF PATTIES

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CHAPTER I

INTRODUCTION

Consumers in the 1990's seem to be preoccupied with two aspects of their diet. The first is maintaining a low-fat diet and the second is the safety of the food that they consume. The recent outbreak of *Escherichia coli* O157:H7 in the pacific Northwest infected over 400 people and four children ultimately died (Dorn, 1993). This outbreak has received national media attention and has refocused consumer attention to food safety issues. A majority of the outbreaks of *E. coli* O157:H7 have been traced back to undercooked ground beef (Riley et al., 1983; Wells et al., 1983; Ryan et al., 1986; Ostroff et al., 1990; Pavia et al., 1990; Belongia et al., 1991; O'Brien et al., 1993).

It has been estimated that in the United States over 1.36 metric tons of ground beef is consumed each year. Retail ground beef has an average of 20-30% fat while ground beef patties sold through large fast food chains is usually between 20 and 25% fat (Hoogenkamp, 1991). Health conscious consumers are requiring a reduced fat ground beef (approximately 10% fat) that still exhibits all the characteristics of a 20-30% fat ground beef formulation. It seems that the easiest solution should be to remove part of the fat by using leaner cuts of meat. Unfortunately, this seemingly simple solution, results in a higher priced product that does not have the same sensory properties of 20-30% fat ground beef. (Berry et al., 1984; Berry and Leddy, 1984; Trout et al., 1992a; Berry, 1994). Therefore, food scientists have responded by developing applications for ingredients such as soy proteins, starches, gums, carrageenan, sucrose etc., that can be used in ground beef to replace fat and still maintain organoleptic properties and yields (Keeton, 1991).

Although a significant amount of research has been conducted into the sensory aspects and yields of low-fat ground beef formulations, very little research has been

conducted into the effect that these low-fat formulations will have on the survival of spoilage microorganisms and pathogens. Shipp (1992) found that *E. coli* O157:H7 survived in 10 and 20% fat ground beef patties and not in 30% fat formulations. Shipp concluded that the survival of *E. coli* O157:H7 in 10% fat ground beef may have been due to a decreased rate of heat transfer through lean verses fat tissue. Although, the primary objective of Shipp's research was to evaluate the survival of *E. coli* O157:H7 in ground beef with varied fat levels, they also found that *E. coli* O157:H7 survived in ground beef patties made with carrageenan when cooked to 60°C. It is unknown how new low-fat formulations, that include ingredients such as carrageenan, soy, salt and water, may influence the survival of *E. coli* O157:H7. Craven et al. (1983) found that D-values were significantly increased for *Salmonella* species when ground beef was supplemented with soy protein. Increased resistance to heat by the *Salmonella* appeared to be caused by an increase in the pH of the beef upon the addition of the soy proteins. Further research is needed to determine the survival of *E. coli* O157:H7 in these new low-fat formulations.

The first objective of this literature review is to provide an overview of *E. coli* O157:H7 as a pathogenic microorganism. Understanding the occurrence in foods allows for the establishment of food safety guidelines that can help control *E. coli* O157:H7. Accurate, sensitive, and rapid laboratory methods must be reviewed so that *E. coli* O157:H7 can be identified in samples and researched in the laboratory. The second objective is to review available information on ground beef, fat replacers, and methods of cooking. These three elements can work together to determine the fate of *E. coli* O157:H7 and ultimately the safety of ground beef products. Food manufactures can not afford to risk associating new low-fat product lines with a pathogen such as *E. coli* O157:H7. Therefore, the objective of this study was to determine the temperature and holding times necessary to inactivate *Escherichia coli* O157:H7 in low-fat, precooked ground beef patties.

CHAPTER II

REVIEW OF LITERATURE

Escherichia coli

Background

Escherichia coli is a coliform gram negative rod, found throughout the world, that can live in a symbiotic relationship with different species of organisms in which neither is harmed. However, a pathogenic subgroup of *E. coli* exists that are associated with life threatening infections such as meningitis, pneumonia, and septicemia. This subgroup includes enteropathogenic *E. coli*, enteroinvasive *E. coli*, enterotoxigenic *E. coli*, and enterohemorrhagic *E. coli* (Padhye and Doyle, 1992). The enterohemorrhagic *E. coli* which includes O157:H7, has four specific traits: 1) cause hemorrhagic colitis, 2) produce a *Shiga*-like toxin, 3) do not produce either a heat-stable toxin or a heat-labile toxin and 4) are not invasive. Clinical symptoms include: abdominal pain, gross bloody diarrhea, and absent or low grade fever (Dorn, 1993). *E. coli* O157:H7 appears to be the most common and therefore the most studied.

The intestines of warm blooded animals, water, fruits, and vegetables contaminated by feces, raw milk and asymptomatic carriers can serve as reservoirs for *E. coli* O157:H7 (Dorn, 1993). Infection may occur by the ingestion of contaminated food or by person to person contact. *E. coli* O157:H7 has been associated with sporadic cases of gastrointestinal illness. This strain of *E. coli* infects both children and adults, but particularly the very young and the very old, and has led to the death of some patients that were infected. Outbreaks of *E. coli* O157:H7 have been documented in the United States, Canada, Great Britain, Wales, Belgium, Argentina, Australia, and Japan (Ryan et al., 1986; Pai et al., 1984; Morgan et al., 1988; Vasavada et al., 1988). The symptoms include

severe abdominal cramps, non-bloody and bloody diarrhea, little to no fever, evidence of colonic mucosal edema, erosion, or hemorrhage (hemorrhagic colitis) in the absence of conventional enteric pathogens in stools such as *Salmonella, Shigella, Campylobacter, Yersinia,* or parasites (Riley et al., 1983). A hemolytic uremic syndrome (HUS) may also develop which can lead to hemolytic anemia and renal failure, especially in children and a thrombotic thrombocytopenic purpura (TTP) may develop in children and the elderly. Patients may require dialysis and blood transfusions and may also suffer from heart failure, seizures, and coma (Griffin and Tauxe, 1991). TTP involves more frequent and severe neurologic involvement while HUS involves more frequent and severe renal failure; however, these do not clearly distinguish the two syndromes.

Pathogenicity

Pathogenicity of *E. coli* O157:H7 is not completely understood and an infective dose for humans has not been established. However, reports have shown that as few as 12-15 *E. coli* O157:H7 bacteria may cause infection (Doyle, 1993; Whipp, 1994). Such a low infective dose requires a critical understanding of the bacteria's pathogenicity. Virulence appears to be mediated by two properties of the bacteria, toxin production and adherence/attachment. The first virulence factor was initially described in 1977 by Konowalchuk et al.(1977). Initially, Konowalchuk found that certain strains of *E. coli* were able to produce a cytotoxin that was cytotoxic to Vero (African green monkey kidneys) cell culture. However, in 1977, Konowalchuk did not realize the pathogenic significance of his findings. It was Karmali et al. (1985) and Gransden et al. (1986) that found significant evidence to associate idiopathic HUS and verotoxin producing *E. coli*. In 1985, Karmali et al. examined 40 pediatric patients with hemolytic uremic syndrome (HUS) to see if HUS was due to an infection by a Verotoxin-producing *E. coli* (VTEC). Karmali et al. concluded that a significant association exists between HUS and infection by

VTEC; of the 40 patients with HUS 12 had VTEC isolated, 3 of which were identified as the *E. coli* O157:H7 serotype. Levine et al. (1978) reported that certain strains of *E. coli* caused diarrhea but did not produce heat-labile or heat-stabile enterotoxins and were non-invasive. He concluded that the possibility existed that certain strains of *E. coli* were distinct from heat-labile or heat stable enterotoxins.

E. coli toxin production is very similar in its structure and activity to that of *Shigella* dysenteriae type I, which was first described in 1903 (Karmali, 1989). Eventually researchers discovered that there were actually two toxins that could be produced by *E. coli* O157:H7 (Strockbine et al., 1985; Scotland et al., 1985). This has led some researchers to refer to the toxin as *Shiga*-like toxin I and *Shiga*-like toxin II while others still adhere to the original Konowalchuk, et al. (1977) nomenclature and refer to the toxins as Verotoxin I and Verotoxin II. This thesis will adhere to the original nomenclature.

The second virulence property of *E. coli* O157:H7 is adherence and attachment. *E. coli* O157:H7 possesses a 60-megadalton plasmid that encodes a new fimbrial antigen that promotes attachment to intestinally derived epithelial cells which will ultimately result in destruction of the microvilli in that localized area (Karch et al., 1987). However, the precise mode of bacterial attachment to intestinal cells is still not completely understood. Knutton et al. (1989) reported that actin microfilaments may play a role in the adhesion of enteropathogenic and enterohemorrhagic *E. coli* to cells in culture. He found that *E. coli* localized their concentration in cell culture with dense concentrations of actin microfilaments. Because actin is a component of muscle, further research needs to be conducted to determine if there is a correlation between the occurrence of *E. coli* O157:H7 in beef and the associated actin microfilaments. The receptor for both of the verotoxins is a globotriosyle ceramide containing a galactose alpha-(1-4)-galactose-beta-(1-4) glucose ceramide (Gb3), which is present in large quantities in the kidney (Lindberg et al., 1987). Understanding this receptor for the toxin produced by *E. coli* O157:H7, explains the kidney's role in the development of HUS in infected patients.

Another potential pathogenic characteristic of *E. coli* O157:H7 is its ability to form heat shock proteins (HSP). Yamamori and Yura (1980), Kusakawa and Yura (1988) and Squires et al. (1991) provided evidence that *E. coli* cells somehow "recognize" a temperature change from 30°C to 40°C which activates transcription of several distinct operons and produces temperature-induced protein synthesis or HSP. HSP are an acquired thermotolerance which result after cells have been exposed to a mild heat stress or heat shock. Murano and Pierson (1993) found that heat shocked *E. coli* O157:H7 cells were better able to survive, or repair themselves, after exposure to heat when compared to controls. Therefore, they concluded that heat shock did not protect the cells against injury, but instead enhanced the cells ability to recover. Regardless of the mechanism both theories show that *E. coli* O157:H7 has the ability to recover or protect itself after a heat treatment and thus increasing its pathogenicity. Research performed at Iowa State University by Collins and Murano (1993) attempted to determine if varying the rate of heating ground beef and pork would trigger a heat response in *E. coli* O157:H7. They found that 20g of ground meat samples exposed to the slowest rate of heat resulted in the highest number of survivors after a 55°C temperature treatment. Previous work by Shipp (1992) had found that *E. coli* O157:H7 produced heat shock proteins similar to proteins observed in other strains of *E. coli*. Shipp found that the greatest concentration of heat shock proteins were observed in ground beef patties that were cooked to 60°C and held at that temperature for up to 300 seconds. This finding may indicate that patties cooked to lower temperatures and held are enhancing the bacteria's pathogenicity and may have a detrimental effect of the safety of ground beef patties.

Isolation and Detection

E. coli O157:H7, the O refers to the somatic antigen and the H refers to the flagellar antigen. *E. coli* O157:H7, like many other bacteria, grow best at 37°C, but will also increase in numbers when incubated at 12-21°C. *E. coli* O157:H7 can survive

freezing and refrigeration but their populations decrease when temperatures are below 5°C (Hao and Bracket, 1993; Abdul-Raouf, et al., 1993a). However, *E. coli* O157:H7 does not grow well above 41°C. Therefore, it may not be detected in standard fecal coliform procedures (44-45.5°C) used to test food and water. This indicates that *E. coli* O157:H7 must have a separate isolation and identification procedure (Doyle et al., 1984; Raghubeer et al., 1990). Hao and Bracket (1993) determined the effect of 5, 10, and 20°C storage temperatures on *E. coli* O157:H7. Their results conclude that *E. coli* O157:H7 can survive but not grow at low temperatures (4°C). In this study, *E. coli* O157:H7 grew at 10°C which differs from the results published by Doyle and Schoeni (1984). The difference in the results may be due to the different strains used in each test. Both papers agree that *E. coli* O157:H7 will grow at 20°C. In the same research project, Hao and Bracket found that the use of modified atmosphere packaging utilizing $CO_2/O_2/N_2$ in the following concentrations: 0/5/95, 0/10/95, 5/10/90, 5/10/85, 5/20/75, 10/5/85, and 10/20/70 did not significantly affect the growth of *E. coli* O157:H7. *E. coli* O157:H7 can be differentiated from other *E. coli* serotypes by its inability to ferment sorbitol within 48 hours and the absence of B-glucuronidase which hydrolyzes 4-methylumbelliferyl-B-D-glucuronide (Wells et al., 1983). *E. coli* 0157:H7 infections have been linked primarily to undercooked ground beef; however, the organism has been difficult to isolate from ground beef samples due to its presence in low numbers and its occurrence with competitive normal flora. The inability of *E. coli* O157:H7 to ferment sorbitol was used by March and Ratnam (1986) to develop a differential medium MacConkey sorbitol agar (MSA) for the detection of the bacteria. *E. coli* O157:H7 colonies appear colorless while sorbitol fermenting bacteria appear pink on MSA. Okrend, et al. (1990a) used ground beef suspensions of 1:10 in a modified *E. coli* broth (MEC) with 1.5g/L of bile salts and 20mg/L of novobiocin as an enrichment step. The enrichment step is necessary so that the numbers of *E. coli* may be amplified in the mixed microorganism culture and is a step that is included in most identification procedures. MacConkey sorbitol agar (MSA) incubated

at 42°C was used for colony distinction. Suspicious colonies on MSA were inoculated onto a plate of phenol red sorbitol agar containing 4-methylumbelliferyl B-D-glucuronide (PRS-MUG) and a plate of Levine eosin methylene blue agar (EMB). Suspicious colonies were confirmed biochemically and serologically. *E. coli* O157:H7 was isolated from 5 of 10 ground beef samples inoculated with 0.6 microorganisms per gram and 10 of 10 samples inoculated with 5 microorganisms per gram. More recently Okrend, et al. (1990b) added 5-bromo-4-chloro-3-indoxyl-B-D-glucuronide (BCIG) at 0.1 g/L concentration to MacConkey sorbitol agar (MSA) and found that this aided in the identification of *E. coli* O157:H7 by differentiating B-glucuronidase positive and B-glucuronidase negative colonies which resulted in a 36% decrease in the number of selected false positive colonies. Both of Okrend's et al. procedures were sensitive; however they can take up to four days of laboratory testing to complete.

The amount of time necessary to identify *E. coli* O157:H7 is critical whether it is in a medical laboratory or in a food laboratory. Realizing that time was of the essence Okrend et al., (1990c) reduced the time necessary for isolation and identification by using an enrichment broth followed by inoculation onto 3M Petrifilm. A single *E. coli* O157:H7 bacteria growing on the Petrifilm *E. coli* count plate will produce a detectable antigenistic signal in the 3M blot Enzyme Linked Immunosorbent Assay (ELISA) test. However, a presumptive positive ELISA must undergo further testing, therefore the 3M blot ELISA is used as reliable negative screen for *E.* coli O157:H7. Using the 3M petrifilm will give presumptive identification of the bacteria within 26-28 hours with a false positive rate of 2%. All positive samples must be confirmed for *E. coli* O157:H7 using additional procedures that take up to 3-4 days.

Another method of identification, for *E. coli* O157:H7, is the use of DNA probes to detect vero-toxin genes. This method is useful when large amounts of *E. coli* colonies need to be screened and allows for the detection of small numbers of bacteria which are present late in an infection. DNA probes appeared to be a highly sensitive and extremely

specific method for identifying *E. coli* O157:H7 strains among large numbers of *E. coli* colonies (Scotland, et al., 1987; Levine, et al. 1987; Samadpour et al. 1990; Smith et al. 1991). Even though DNA probes are sensitive identification systems for *E. coli* O157:H7 in the research laboratory they are not practical for identification in a food company's laboratory.

The hydrophobic grid membrane filter-immunoblot (HGMF) is an additional procedure used to isolate *E. coli* O157:H7 from foods (Doyle and Schoeni, 1987; Szabo et al. 1986, Todd, et al. 1988). Enriched cultures are filtered through hydrophobic grid membranes, and the membranes are then incubated on nitrocellulose paper on selective agar or enumerated by an enzyme-labeled antibody assay to detect *E. coli* O157:H7. Recovery in the meat samples using this method had a sensitivity of less than 1 CFU/g, which Szabo et al. reports as being 10 times more sensitive than plating. Both the DNA probe and the HGMF may be most useful in a research laboratory where extended periods of time are available for lengthy identification of *E. coli* O157:H7 in foods.

Research methods to isolate *E. coli* O157:H7 are often cumbersome, costly and time and labor intensive. In an effort to rapidly and inexpensively identify *E. coli* O157:H7 several detection methods have been developed. Latex agglutination tests are a rapid method used to identify *E. coli* O157:H7. Non-sorbitol-fermenting colonies are selected from sorbitol MacConkey agar plates and identified using commercially available latex-conjugated O157 antisera (Chapman, 1989). March and Ratnam (1986) used a commercially available latex agglutination test kit to test clinical isolates of *E. coli* O157:H7. The test kit rapidly detected *E. coli* O157:H7 in each of the 100 known positive fecal samples and was negative for the bacteria in each of the known negative fecal samples. However, Borczyk et al. (1990) proved that some sorbitol negative *Escherichia* bacteria such as *E. hermanii*, *E. coli* O148:NM, and *E. coli* O117:H27 can cause false positives when using this latex agglutination test kit because of shared antigens.

In one method, synthetic oligonucleotide probes that were derived from VT1 and VT2 genes were used in a polymerase chain reaction (PCR) procedure to detect these genes in some enteric pathogens (Pollard et al., 1989). The probes demonstrated a high specificity in detecting verotoxin *E. coli* including O157:H7 (Samadpour et al., 1990). Researchers have examined the use of a dipstick immunoassay to detect *E. coli* O157:H7 in ground beef within 16 hours (Kim and Doyle, 1992). Pure cultures of *E. coli* were readily detectable at 1 x 10^5 and 1 X 10^6 organisms per ml. The test was less sensitive when the pure culture was 1 X 10^3 organisms per ml. The dipstick immunoassay detected 0.1 to 1.3 cells per gram in artificially inoculated ground beef; however, the method has a false positive rate of 2.0%. More recently Park et al. (1994), used a direct immunofluorescence (DIF) antibody stain to detect *E. coli* O157:H7 directly from human fecal specimens with 2 hours. Twelve *E. coli* O157 were identified by the DIF method and confirmed by culture for 100% specificity. In an alternative method, Abbott et al. (1994) used the MicroScan dried conventional gram-negative identification panel from Baxter Diagnostics to presumptively identify *E. coli* O157:H7. Eighty five percent of the O157:H7 strains tested had a unique biochemical profile number that was not observed among non-O157:H7 strains tested. Abbott et al. concluded that the dried MicroScan identification panels could be used to presumptively identify potential strains of O157:H7.

Choosing a rapid method for the identification of *E. coli* O157:H7 will depend largely on the requirements of the purchasing laboratory. Buying decisions may depend on test availability, reliability, specificity, price, and the speed in which results can be obtained.

Outbreaks Associated with Food

Infections due to *E. coli* O157:H7 are not a reportable disease in many states. As of October of 1992 only 11 states require reporting of the isolation of this bacteria.

Therefore, it is difficult to determine the true incidence of *E. coli* O157:H7 infections in the United States. Since 1982, 16 deaths in the United States due to *E. coli* O157:H7, have been reported to the Center for Disease Control (National Live Stock & Meat Board, 1993). No other strains of enterohemorrhagic *E. coli* serotypes have caused an outbreak. Outbreaks have taken place in schools, day-care centers, nursing homes, restaurants, and the community at large. Table 2.1 summarizes the reported outbreaks of *E. coli* O157:H7 in the United States. Outbreaks have also been documented in Canada and Great Britain (Johnson et al., 1983; Pai et al., 1984; Riley et al., 1983; Wells et al., 1983). Outbreaks are detected because of an unusually large number of people hospitalized with severe diarrhea, hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP).

The first documented case of food infection attributed to *E. coli* O157:H7 was in 1982. That year there were two outbreaks of *E. coli* O157:H7 resulting in gastrointestinal illness. The outbreaks affected at least 47 people in Oregon and Michigan who had eaten at the same restaurant chain. The outbreak was characterized by severe abdominal pain, watery diarrhea followed by grossly bloody diarrhea, and little to no fever. In both outbreaks, three ingredients were eaten together - reconstituted dehydrated onions, hamburger meat, and pickles. No single ingredient could be independently associated with the disease because they had all been served together. However, in the Michigan outbreak, *E. coli* O157:H7 was isolated from a frozen hamburger patty from a suspected lot (Riley et al., 1983; Wells et al. 1983).

E. coli O157:H7 is particularly pathogenic to special population groups such as infants, the elderly, and the immunocompromized. The lethality of *E. coli* O157:H7 to the elderly was demonstrated in Canada in 1985. A deadly outbreak of *E. coli* O157:H7 affected 55 of 169 residents and 18 of 137 staff members in a Canadian nursing home. Carter et al. (1987) characterized the outbreak into two phases: the first phase of the primary infection was attributed to a contaminated ham, turkey, and cheese sandwich

(none of the food was available for microbiological examination) and the second phase was due to person-to-person transmission. HUS developed in 12 affected residents, 11 of which ultimately died. Overall, 19 of the affected residents died. There were no deaths among the staff members. Two years after the *E. coli* O157:H7 serotype was first identified another outbreak occurred in a Nebraska nursing home which implicated ground beef. Ryan et al. (1986) reported that 34 people out of 101 residents in a nursing home became ill and 4 ultimately died. Epidemiological evidence strongly implicated ground beef that was served. However, Ryan et al. was unable to isolate *E. coli* O157:H7 from the suspected lot. It should be noted that at this time an enrichment step using modified *E. coli* broth (MEC) was not being utilized in laboratory identification which could have limited the success of isolation in the suspected ground beef. It should also be noted that there were only 6 frozen, raw hamburger patties left for researchers to test for *E. coli* O157:H7.

Ground beef was again associated with an outbreak of *E. coli* O157:H7 in 1986 when Washington State experienced a statewide outbreak of *E. coli* O157:H7 (Ostroff et al., 1990). In Walla Walla, Washington, 37 people were infected, 14 of which had *E. coli* O157:H7 confirmed. Seventy-seven percent of the primary cases ate at the same Mexican restaurant which included 9 of the 14 cultured confirmed cases. Two infected people eventually died. The same strain of *E. coli* O157:H7 was isolated from 3 nursing home residents in Dayton, Washington. In both of these outbreaks, ground beef was implicated and had been purchased from the same supplier. *E. coli* O157:H7 was isolated from ground beef at the restaurant but not at the time of the outbreak.

Twenty mentally retarded persons in Utah that were housed in two different institutions became ill with *E. coli* O157:H7 infections concurrently. Thirteen of these were culture confirmed, 2 were probable, and 5 were possible. Eight residents developed HUS and 4 ultimately died. Three employees were cultured confirmed for *E. coli* O157:H7. Both facilities had one food source in common; both had received ground beef

from a single lot distributed by a government commodities distribution program (Pavia et al., 1990). In 1988, precooked ground beef patties were implicated in an outbreak of *E. coli* O157:H7. The Minnesota Department of Health was informed of 4 junior high children that were hospitalized with abdominal cramps and bloody diarrhea. Thirty two students out of the 1562 student body became ill with *E. coli* O157:H7. Four of the children were hospitalized (no HUS was present) and there were no fatalities. Precooked ground beef patties were implicated although no *E. coli* O157:H7 was isolated from precooked patties from the implicated manufacturing dates (Belongia et al., 1991).

E. coli O157:H7 gained national attention, again in 1993, when over 400 people became sick in Washington, California, Idaho, and Nevada after eating at a fast food chain. Four children died, from suspected person-to-person contact and from contaminated hamburger meat. Dr. Phillip Tarr of the Children's Hospital in Seattle reported on the unusual virulence of this strain of *E. coli* O157:H7. He reported that no more than 12 bacteria, in an undercooked ground beef patty, were enough to cause illness and death (Spencer, 1993). O'Brien et al. (1993) analyzed stool specimens from 5 of the patients who had bloody diarrhea and were infected during this outbreak. The objective of the research was to determine if the infecting strain was unique in its ability to cause such a large outbreak. O'Brien et al. did not find an increase in virulence of the *E. coli* O157:H7 serotype responsible for the outbreak. Her conclusion was that the level of contamination and/or the temperature at which the hamburgers were cooked are responsible for the size of the outbreak. O'Brien et al. results show that proper cooking of ground beef patties is essential to consumer safety.

Further implicating beef as a reservoir of *E. coli* O157:H7 (Morbidity and Mortality Weekly Report, 1991) was an outbreak attributed to the consumption of rare roast beef eaten at an agricultural threshing show in North Dakota in July of 1990. Seventy attendees were infected and food preparation and serving techniques were

implicated in the outbreak. However, food samples were not available for microbiological analyses.

Although *E. coli* O157:H7 has often been associated with ground beef, in 1986 it was implicated in an outbreak in Ontario where several kindergarten children suffered from the illness after visiting a dairy farm and sampling raw milk. (Borczyk et al., 1987; Vasavada et al., 1988). The isolation of *E. coli* O157:H7 from raw milk provided further evidence of a bovine reservoir.

The first documented outbreak of *E. coli* O157:H7 that showed the bacteria could be transmitted by water occurred in 1989 in Missouri. The outbreak was associated with the towns water supply and infected 243 people resulting in 4 deaths. Although no *E. coli* Ol57:H7 was isolated from city water, water was the only common exposure in the community. The source of the *E. coli* O157:H7 was never discovered. The bacteria may have originated from a backflow during repairs of 45 broken water meters or at the time of two water main breaks were repaired. Cattle were not considered a source of the contaminate however, the local deer population might have been. (Swerdlow et al., 1992).

The ability of *E. coli* O157:H7 to be spread by person-to-person contact was documented by Spika et al. (1986). Children in a North Carolina day care center became ill with the bacteria and then transmitted the disease to family members. Food was ruled out as a vehicle due to the movement of the illness between different age classes at the day care center and because almost all the children ate the same meals. An interesting feature of this outbreak was the absence of bloody diarrhea in many of the ill children.

Once *E. coli* O157:H7 became a recognized pathogen several informal monitoring surveillance systems were set up in the United States and Canada. In 1982, the Center for Disease Control (CDC) reported that between August 1982 and April 1984, *E. coli* O157:H7 was isolated from the stools of 28 people from 11 states. During a 6 month period in 1983, researchers (Pai et al., 1984) in Calgary, Canada selectively examined stool specimens from patients at three Calgary hospitals. *E. coli* O157:H7 was isolated

from 19 of 125 patients with grossly bloody diarrhea and one sibling with non-bloody diarrhea. The illness appeared to be associated with consumption of hamburgers by 15 of the patients. However, consumption of hamburger could not positively be identified as the source of the infection. Gransden et al. (1986) conducted a 2 year study of *E. coli* O157:H7 beginning in September of 1983 at the British of Columbia's Children's Hospital. *E. coli* O157:H7 was isolated from 9 patients with HUS and 25 patients with diarrhea. Of the 34 positive *E. coli* O157:H7 patients 25 had bloody diarrhea. Two of the infected patients died.

E. coli O157:H7 is a pathogen that is found world wide. The first community outbreak of *E. coli* O157:H7 in the United Kingdom was reported by Morgan et al. (1988). Unlike previously reported outbreaks, in the United States that implicated ground beef, the outbreak in the UK implicated raw vegetables, and particularly potatoes. Food handlers became infected with *E. coli* O157:H7 after handling raw vegetables that may have been fertilized with manure. It is interesting to note that the infected food handlers did not transmit the infection to people for whom which they prepared food.

E. coli O157:H7 has infected and has the potential to infect people in a wide age range. People of all ages can be susceptible to an infection from this bacteria however, immunocompromised people are at the greatest risk. *E. coli* O157:H7 has been identified in over 16 countries on 6 continents around the world. Only the United States, Canada, and Great Britain have reported outbreaks (Anonymous, 1994a).

Table 2.1 Reported outbreaks of *E. coli* O157:H7 in the United States

Month	Year	State	Number affected	Likely vehicle	Reference	
Feb.	1982	OR	26	Ground beef	Riley et l.	(1983)
May	1982	MI	21	Ground beef	Riley et al.	(1983)
Sept.	1984	NE	34	Ground beef	Ryan et al.	(1986)
Sept.	1984	NC	36	Person-to-person	Spika et al.	(1986)
Oct.	1986	WA	37	Ground beef	Ostroff et al.	(1990)
June	1987	UT	51	Ground beef	Pavia et al.	(1990)
Oct.	1988	MN	54	Precooked beef	Belongia et al.	(1991)
Dec.	1989	MO	243	Water	MMWR	(1993)
July	1990	ND	65	Roast beef	MMWR	(1991)
Nov.	1991	MA	32	Apple cider	Besser et al.	(1993)
Jan.	1993	WA, ID, NV CA	400	Ground beef	Spencer et al.	(1993)

Animal Sources

Dairy cattle have been suspected reservoirs of *E. coli* O157:H7 because ground beef and raw milk have both been implicated in outbreaks. Martin et al. (1986) screened both dairy and beef cattle for *E. coli* O157:H7. The microorganism was isolated from healthy dairy heifers but not from beef cattle. Therefore, Martin et al. concluded that the organism may be more common in dairy cattle than other cattle. Other researchers (Borcsyk et al., 1987; Ostroff et al., 1990) have been able to trace *E. coli* O157:H7 involved in outbreaks back to the implicated farms. However, *E. coli* O157:H7 has been isolated from cattle that were not associated with a community outbreak (Szabo et al., 1990; Wells et al., 1991; Samadpour et al., 1990). McNamara (1994) conducted a surveillance program to detect *E. coli* O157:H7 in bob veal kidneys. Five samples or .4% of 1,408 samples collected were positive for *E. coli* O157:H7. Out of 5,542 fancy veal kidney samples only 3 samples or 0.1% were positive for *E. coli* O157:H7. The largest study to date involved a nationwide (28 states) on farm study to determine the prevalence of *E. coli* O157:H7 in preweened dairy heifers. Only 25 of the 7,000 or >1.0% of the animals tested were found to harbor *E. coli* O157:H7 (Spencer, 1993). Table 2.2 summarizes the results of studies conducted to determine the prevalence of *E. coli* O157:H7 on farms in the United States. This data generally reflects one-time sampling. Additional research needs to be conducted into the carrier status of individual infected animals. Identifying infected animals and culling them from the herd has the potential to decrease the incidence of *E. coli* O157:H7 in ground beef products.

There is presently no data that show any animals normally become diseased from *E. coli* O157:H7 (Menning, 1993). Because animals do not show signs of *E. coli* O157:H7 infections, it is impossible to cull suspected animals from beef and dairy herds. Asymptomatic animals increase the potential of contaminating meat and milk used to manufacture food products.

In an effort to identify other animal hosts of *E. coli* O157:H7, Beery et al. (1985) and Stavric et al. (1993) conducted studies to determine if chickens could serve as hosts for *E. coli* 0157:H7. They concluded that *E. coli* O157:H7 can colonize in the cecae of chickens and chicks and is excreted in the feces. These findings indicate that chickens may serve as reservoirs of the bacteria and may contaminate meat at the time of slaughter or cross contaminate non-contaminated meats during handling.

The animal sources of *E. coli* O157:H7 indicate that beef, chicken, and dairy products are susceptible to contamination by the bacteria. The ecology of *E. coli* O157:H7 is not known. Studies need to be conducted to determine how this bacteria infects animals so preventative steps may be taken to limit its colonization of farm livestock. The animal sources combined with *E. coli* O157:H7's ability to survive in a wide variety of environmental conditions makes it a serious foodborne pathogen that can contaminate a wide variety of foods.

Table 2.2 Summary of *E. coli* O157:H7 in cattle with farm origins.

Location Year	No. tested	O157:H7 identified	Description of cattle	Reference
WI 1986	226	5	Dairy heifers	Martin et al.(1986)
WA 1987	539	7	Dairy heifers, calves, and adults	Wells et al. (1990)
Canada 1986	67	2	Dairy heifers and cows	Borczyk et al. 1987)
WA 1987	27	0	Dairy heifers calves	Wells et al. (1990)
Unkn.1988	1,408	5	Bob veal kidneys	McNamara (1994)
Unkn. 1989	5,542	3	Fancy veal kidneys	McNamara (1994)
Canada 1990	22	8	Dairy cattle	Szabo et al. (1990)
WA 1990	539	6	Dairy cattle	Ostroff et al. (1990)
WA 1990	28	9	Dairy calves	Samadpour et al. (1990)
WA/WI 1991	1,266	18	Dairy cattle	Wells et al. (1991)
Nat.wide 1993	2,000	25	Dairy heifers	Spencer et al. (1993)

Food Sources

Once suspected animal sources were identified researchers attempted to determine the prevalence of *E. coli* O157:H7 in various foods. Doyle and Schoeni (1987) isolated *E. coli* O157:H7 from 3.7% ground beef, 1.5% pork, 1.5% poultry, and 2.0% lamb in retail samples collected. This is the first report of *E. coli* O157:H7 isolated from food other than ground beef. Therefore, Doyle and Schoeni concluded that his results indicate *E. coli* O157:H7 is not a rare contaminant of fresh meats and poultry. However, this paper does not address the potential of cross contamination of the poultry, pork and lamb retail samples by the retail beef samples. The ability of *E. coli* O157:H7 to grow in a variety of foods was further researched by Szabo et al. (1990). *E. coli* O157:H7 was artificially inoculated with 100 to 200 colony forming units (CFU) into vegetables, dairy products, and ground beef. *E. coli* O157:H7 was recovered from each of the vegetables, dairy products and meat that was inoculated. In the same research paper Samadpour et al. (1990) obtained food samples from a local Seattle grocery store and also tested them for *E. coli* O157:H7 using a DNA probe. Four of the food samples tested positive for the bacteria. They included ground beef, raw goat milk, blueberries, and surimi-based delicatessen salad (possible cross contamination). McNamara (1994) began a surveillance program of raw beef and poultry products to determine the prevalence of *E. coli* O157:H7. Two samples or 0.1% of 1,647 beef brisket and ground beef samples collected were positive for the bacteria. McNamara also analyzed 6,133 samples of broiler backs and necks for *E. coli* O157:H7. No O157:H7 was isolated from the samples.

E. coli O157:H7 has an uncharacteristic ability to survive acid environments when compared to other microorganisms. *E. coli* O157:H7 was implicated in an outbreak involving apple cider in 1991 which demonstrated the microorganisms ability to grow in an acidic environment (Besser et al. 1993). Apple cider is distinguished from apple juice by its lack of pasteurization. Fresh unwashed pressed apples were used to make apple

cider in which no preservatives used. *E. coli* O157:H7 was able to survive in the cider for 20 days at refrigerated temperatures. The bacteria is believed to have originated from cow manure used to fertilize the trees and then contaminated the apples when they fell to the ground. Because of this outbreak, Zhao et al. (1993) examined the ability of *E. coli* O157:H7, at concentrations of 1×10^2 CFU or 1×10^5 CFU, to grow in apple cider with and without preservatives. Zhao et al. found that .1% sodium benzoate was an effective preservative while .1% potassium sorbate was not. The highest rate of inactivation occurred when the two preservatives were used in combination with one another. More recent research characterizing the acid tolerance and survival of two different strains of *E. coli* O157:H7 in apple cider was conducted by Miller and Kaspar (1994). They found that both of the strains used in their research were significantly more acid-tolerant than a control stain of non-enterohemorrhagic *E. coli.* They also found that acid tolerance differed between the two strains and lowering the storage temperature (4°C) enhanced the survival of *E. coli* O157:H7 in a 2.0-4.0 pH range.

Abdul-Raouf et al. (1993a) demonstrated that *E. coli* O157:H7 would grow at pH 4.7-5.4 when incubated (21-30°C) in a beef slurry mixed with citric or lactic acid. Interestingly, acetic acid under the same conditions inhibited the growth of *E. coli* O157:H7.

To test the efficiency of organic acids in controlling *E. coli* O157:H7, Cutter et al. (1993) used 0, 1, 3, 5% acetic, lactic, or citric acids at 24°C were sprayed on beef carcasses that had been artificially inoculated with O157:H7. In results that differed from Abdul-Raouf et al. (1993a), Cutter et al. found that acid type was not a significant treatment factor ($P>0.1$). However, 5% lactic acid was the most effective for decreasing *E. coli* O157:H7 numbers and greater reductions were seen in adipose verses lean tissue. Differences in the research results between Abdul-Raouf et al. and Cutter et al. could be due to differences in the strains of *E. coli* O157:H7 used to perform the research. Cutter et al. did report that the resistance of *E. coli* O157:H7 to acids varied between strains.

Cutter's observation that O157:H7 survived acid carcass washing better in lean tissue may be of concern to manufactures of low fat ground beef patties. Further research needs to be conducted to determine why acetic acid inhibits the bacteria. Understanding this mechanism may help researchers develop an effective carcass wash system. The above information demonstrates *E. coli* O157:H7's ability to survive freezing and grow in diverse environments. These environments include modified atmosphere packaging (MAP), low pH (4.7-5.4), and at a temperature range of 12-40°C. A low infective dose coupled with *E. coli* O157:H7's ability to grow in a multitude of environments makes it a foodborne pathogen of great significance.

E. coli O157:H7's ability to grow on vegetables was demonstrated by Abdul-Raouf et al. (1993b). He conducted research to determine the survival and growth of *E. coli* O157:H7 on iceberg lettuce, shredded cucumbers, and shredded carrots in modified atmosphere packaging (3% oxygen and 97% nitrogen). The bacteria grew on inoculated vegetables stored at 12-21°C but populations decreased at 5°C. Overall, this research demonstrated the ability of *E. coli* O157:H7 to grow on raw salad vegetables at 12-21°C under modified atmosphere packaging. Further research performed by Abdul-Raouf et al. (1993a) examined the survival of *E. coli* O157:H7 in a salad made with beef and up to 40% mayonnaise. The beef salads had a pH range of 5.4-6.07. Again *E. coli* O157:H7 exhibited a significant increase in population when the inoculated salad was held at 21-30°C and a decrease in population at 5°C. Abdul-Raouf et al. concluded that *E. coli* O157:H7 is capable of growing at pH levels associated with beef salads.

Glass et al. (1992) determined that fate of *E. coli* O157:H7 during the production and subsequent storage of fermented dry sausage. She found that O157:H7 can survive the fermentation and drying steps in the production of fermented sausages and subsequent storage.

Because dairy cattle are suspected reservoirs of *E. coli* O157:H7, Arocha et al., (1992) examined the ability of *E. coli* O157:H7 to survive during the manufacture of

cottage cheese. Pasteurized skim milk was artificially inoculated with *E. coli* 0157:H7 before it was used to make cottage cheese. *E. coli* O157:H7 was able to multiply in the low pH and high acidity environment (5.0-6.5) of lactic acid producing bacteria during the manufacture of cottage cheese. Therefore, if processing time and temperature standards are not met or postpasteurization contamination occurs *E. coli* O157:H7 may jeopardize the safety of the cottage cheese products.

The association of *E. coli* O157:H7 with undercooked ground beef has lead the Food Safety and Inspection Service (Federal Register, 1993) to issue temperatures and holding times for fully-cooked uncured meat patties (beef, veal, and pork). Heat treatments include, but are not limited to, frying, broiling, baking, or roasting. Fully cooked patties must be cooled to an internal temperature of 40°C or below within 2 hours after heat processing. The temperatures and holding times are as follows: 66.1°C/41 sec, 66.7°C/32 sec, 67.2°C/26 sec, 67.8°C/20 sec, 68.3°C/16 sec, 68.9°C and up/10 seconds. (Code of Federal Regulations, 1993).

In an effort to determine the survival of *E. coli* O157:H7 in ground beef, Shipp (1992) artificially inoculated *E. coli* O157:H7 ($1x10^3$) into ground beef patties formulated to either 10, 20, or 30% fat. Subsequent isolation and identification found that *E. coli* O157:H7 survived in ground beef patties made with 10 and 20% fat when cooked to 66 and 71°C. No *E. coli* O157:H7 was detected in 30% fat formulated patties at internal temperatures of 60, 66, and 71°C. Shipp et al. concluded that the survival of the bacteria may have been due to the development of heat shock proteins or the difference in how lean meat transfers heat when compared to fat in ground beef.

Various researchers (Doyle and Schoeni, 1984; Line et al., 1991; and Shipp 1992) have performed research to determine the decimal reduction time D values (decimal reduction time-the time required to destroy 90% of the organism) and Z values (°F required for the thermal destruction curve to traverse one log cycle) in thermally processed ground beef. Doyle and Schoeni reported D-values and Z values in 1.0 grams

of ground beef samples containing 17-20% fat. Line et al. also used 1.0 gram samples but used 2.0% or 30.0% fat. Both research groups found that *E. coli* O157:H7 was more heat sensitive than *Salmonella* at 135° and 145°C. Line et al. results indicate that D values (minutes) were longer for fatty (30%) ground beef samples. Therefore, *E. coli* O157:H7 exhibits a low resistance to heat and should be killed by proper cooking.

Shipp (1992) conducted research to specifically confirm the effectiveness of Doyle and Schoeni's values in an actual 112.5 ground beef patties containing 12-17% fat. Shipp found that the D values of patties thermally processed at 57°, 60°C, and 63°C were approximately one-half of those previously reported by Doyle and Schoeni. Differences in results may have been due to 1.0 gram verses 112.5 gram sample sizes used to cook, different strains of *E. coli* O157:H7 were used, and Doyle and Schoeni used the stationary phase of growth while Shipp use a mid-log phase.

E. coli O157:H7 has been shown to survive in a wide variety of growing conditions that encompass an even wider variety of foods. The bacteria will survive modified atmosphere packaging (MAP), low pH, freezing, and temperatures ranging from 5°C to 40°C. Researchers have also that *E. coli* O157:H7 will produce heat shock proteins when exposed to heat. Methods used to report and therefore trace the bacteria must improve so an accurate determination of the magnitude of this bacteria's occurrence can be established. New products and packaging conditions that are developed need to be accessed for their effect on the survival of *E. coli* O157:H7. Lastly, research must continue to focus on developing laboratory methods that are timely, sensitive, and inexpensive so that ingredients and finished products may be screened as part of food safety programs.

Ground Beef Manufacture

It has been estimated that over 1.36 million metric tons of ground beef are consumed each year. Ground beef is consumed in fast food restaurants, schools, the military, day care, hospitals, health care facilities and in households. (A.M.I., 1992) Ground beef is produced directly in some slaughter plants from in-house trimmings. Ground beef can also be produced by retailers and grinders who buy boxed beef, bulk trimmings, or carcasses. There are currently 2,905 ground beef operations in the United States and less than 900 of these slaughter cattle. Any given patty or pound of ground beef can contain any combination of domestic cow meat, domestically fed beef or imported meat. In 1992, fast food hamburgers accounted for about 47% of fast food sales and 15% of hotel, restaurant and institution sales. (USDA-APHIS, 1994). A growing area of the food industry, which includes ground beef, is in the development of low-fat products. Bruhn et al. (1992) reported that three out of four consumers indicated that partial fat replacement would be of interest to them in the food that they eat. Another interesting finding of Bruhn et al. is that products prepared with fat substitutes were general considered, by the consumer, to be more healthy than traditional products. In October of 1990 the United States Department of Agriculture (USDA) announced plans to purchase new low-fat ground beef patties for use in the National School Lunch Program (Pinkerton, 1990). In response to this demand food scientist have put forth a major effort to develop fat replacers with attributes such as mouthfeel, tenderness, juiciness and flavor.

Effect of Fat Level

Consumers are increasingly aware of the amount of fat in their diet. Bruhn et al. (1992) conducted a survey to assess consumer nutritional concerns and behavior. He found that 84% of the respondents considered taste the most important factor when

shopping for food, 71% chose product safety, and 69% chose nutrition. Today's beef is 27% leaner than the USDA handbook values of the mid 1980's. Fat is now routinely trimmed from meat . The challenge to researchers and food companies is to formulate and manufacture ground beef that is lower in fat and still maintains palatability and yields.

Researchers have responded by examining the effects of fat level on a variety of sensory characteristics such as: palatability, tenderness, juiciness, texture, flavor, and cooking properties. In 1980, Cross et al. used a trained taste panel to study the effect of fat level (16, 20, 24, and 28%) on ground beef that was cooked on a Farberware broiler. The panel judged the cooked ground beef on tenderness, juiciness, flavor, connective tissue amount, and mouth coating effect. Berry et al. (1985) found that increasing fat levels in formulations resulted in higher tenderness and juiciness scores. Ground beef flavor was not significantly affected by fat level. This is not particularly surprising considering that the lowest fat level tested was at 16%. Berry and Leddy (1984) again found that higher tenderness and juiciness values were associated with increased fat levels of 24%. However, patties formulated to 24% fat did not differ in flavor intensity from the 14 and 19% fat formulations, but the 24% fat patties did show a decline in flavor intensity.

Hoelscher et al. (1987) formulated ground beef patties to contain 5, 10, 15, 20, 25, and 30% fat. Patties were either pan-fried or broiled, frozen then thawed and reheated by a microwave oven and refrozen. Hoelscher analyzed the patties for proximate analysis, cholesterol and total calories. At the lower fat levels 0, 5, and 10%, there was no significant difference ($P>0.05$) between the fat content of the raw and cooked products. However, as fat level increased from 10 to 30%, the difference in the final fat content of the raw verses cooked ground beef patties was apparent. Therefore, Hoelscher concluded that regardless of the difference in the initial fat level (0-30%) the cholesterol content of the final cooked product may not be significantly different. A 25% reduction of calories was noted with 10% lean ground beef and a 50% reduction in calories was noted with a 2% low-fat ground beef patty as compared to a regular 30% fat ground beef patty.

Additional research was conducted by Berry (1992) on the effects of low-fat levels (0, 4, 8, 12, 16, 20) on sensory, shear, cooking, chemical properties of ground beef patties. His research concluded that as fat levels decreased shear force levels increased and tenderness, juiciness and flavor decreased. Berry also concluded that shear force increases as fat levels decrease in ground beef patties. That same year Troutt et al. (1992a) utilized 5, 10, 15, 20, 25, and 30% fat levels. Trout et al. considered 5 and 10% fat formulations to be low fat. Five and 10% were less juicy and flavorful, firmer in texture, and more crumbly at the end-of-chewing and exhibited a darker raw red color. Shear values increased as fat level decreased. Berry (1994) cooked ground beef, patties at either 4 or 20% fat on an electric grill or in combination with an overhead broiler unit. As may be expected patties with a 20% fat level had higher flavor, juiciness and tenderness scores and lower shear and compression values, and cook yields than patties formulated to 4% fat. The overall conclusion from the research presented above is that as fat level decreases so does flavor, juiciness, and tenderness. Therefore, decreasing fat levels may lead to a ground beef patty that is unacceptable to consumers.

Shipp (1992) formulated ground beef patties to 10, 20, and 30% fat levels. Shipp found that as fat level increased from 10-30% the peak shear force decreased from 88 to 71 kN. As might be expected, Shipp found that as moisture decreased fat level increased.

Fat Replacers

Because the palatability of ground beef is directly related to fat content the formulation of low-fat ground beef by simply removing fat results in a product with a decrease in palatability, flavor intensity, juiciness and tenderness. In an attempt to lower fat levels, but maintain palatability and yields, non-meat ingredients and fat replacers have been used in ground beef formulations. Helm (1991) reported that the fat replacer market would be growing at an annual rate of 15-20% and is estimated to be about forty million

dollars. Helm also reports that in the United States alone, three out of four adults or aproximately 141 million Americans will be reaching for low-calorie and/or reduced-fat products. Keeton (1991) classified fat replacers into 4 categories:

1) Added water

2) Proteins (soy, milk, whey, wheat, oats, corn)

3) Carbohydrates (starches, gums, maltodextrins, dextrins, carrageenan)

4) Synthetic compounds (sucrose, polyester, polydextrose)

The first category is added water which can be used to replace a portion of the animal fat. In 1990, Ahmed et al. replaced fat with water in fresh pork sausage. Ahmend et al. used 3 fat levels (15, 25, and 35%) and 2 levels of added water (3 and 13%). He found that an acceptable pork sausage can be produced with 15% fat if water is added to replace fat. Miller et al. (1993) attempted to add water (10%) and/or low levels of phosphate (.25%) to low fat (10%) ground beef so that juiciness and tenderness could be increased. Ground beef (10%) without phosphate and water had lower juiciness, texture, flavor, overall palatability, and higher off-flavor when compared to ground beef made with added water or ground beef made with 22% fat. Miller et al. concluded that 10% low fat patties with added water and/or phosphate could have characteristics equal to 22% fat ground beef. This research did not address the effects of storage instability from increased water activity (A_w). Additional research should be conducted to determine the shelflife of the added water and phosphate formulation.

The second category of fat replacers are proteins which now include ingredients such as soy, milk, whey, wheat, oats, and corn. Brown and Zayas (1990) incorporated corn germ protein flour (CGPF) in ground beef patties at levels of 2.5, 5.0, and 7.5%. CGPF increased the retention of both fat and water and therefore increased the yields of the patties in which it was used. Analysis of data from a trained taste panel found that as concentrations of CGPF increased the flavor and meat aroma decreased. However, no objectionable flavor and aroma were found in the CGPF extended patties. ConAgra Co.

has introduced a retail low fat ground beef that get 28% of its calories from fat (Anonymous, 1992b). The formula includes lean trimmed meat, oat flour, beef stock and salt. Oat derived fat replacers simulate the mouthfeel and moisture retention properties of fat .

Soy proteins have been widely used for extending ground beef patties, formed meats, and pizza toppings. When consumers were asked why they buy foods that contain soy proteins, they responded that the reason was a perceived health benefit (McMindes, 1991). Soy proteins can be divided into 3 categories 1) soy flour (50% protein), 2) soy concentrate (70% protein), 3) and isolated soy protein (90% protein). Huffman and Powell (1970) looked at varying fat levels (15, 25, 35%) and 2% added soy in ground beef patties. Patties with added soy had higher tenderness scores than patties without added soy. When this study was conducted it did not evaluate the palatability of the product. Investigation of the use of textured soy protein (TSP) in ground beef by Drake et al. (1975) revealed that TSP level had a highly significant effect on flavor and fat level had no effect when evaluated by a taste panel. The taste panel scored patties without TSP significantly higher than patties containing 15 and 20% TSP. Patties with 25% TSP had the lowest scores for flavor. Cross et al. (1979) found that all beef patties were significantly ($P<0.05$) tougher than patties containing a combination of soy flour and soy concentrate. The addition of soy protein to the formulation did not significantly affect sensory panel ratings for juiciness.

Further research by Ziprin et al. (1981) used defatted soy flour, soy concentrate, soy isolate, and textured soy flour to make 20% fat ground beef patties. There was no difference ($P<0.05$) in flavor juiciness, texture and satisfaction between the all beef patties and the soy extended patties. However, the extended patties had significantly higher yields than the all-beef patties.

Twenty percent added soy was evaluated by Berry et al. (1985) and Brewer et al. (1992). Soy extended patties had higher yields when compared to all beef patties. Berry

et al. found that ground beef flavor intensity scores were unaffected by patty formulation. However, Brewer et al. results did indicate that soy extended patties had lower beef flavor scores. Both researchers found that the taste panel could detected a noticeable soy off flavor. In a more recent study Berry (1990) studied the influence of freezing rate, frozen storage temperature, and storage time on the quality of all beef patties and patties extended with 20% soy protein concentrate. Berry's results indicate that there was not extensive changes in patty quality due to freezing rate and the addition of 20% soy reduced the rate of quality deterioration (color, flavor, odor, and increased thiobartituric acid volumes) during storage. Although Berry was looking for the effect of freezing rate etc., his data indicates that soy extended patties had lower numerical sensory values than did the all beef patties.

McMindes (1991) compared ground beef patties made with either 8.1% or 12.4% isolated soy proteins (10% fat) to ground beef patties made with 20% fat. There was no difference in appearance or texture when the soy patties were compared to the all beef patties. However, both soy formulations (10% fat) scored higher for flavor than did the 20% fat all beef patty. Despite some negative sensory attributes, soy protein will continue offer food manufactures an ingredient that is a nutritionally complete high quality protein that is essentially fat free.

Carbohydrates are the third category which include starches, gums, maltodextrins, dextrins and carrageenan. The term "functional blend" has been used to refer to various ingredient blends which are formulated to achieve certain goals, one of which is fat reduction. Taki (1991) investigated various ingredients in an effort to develop a functional blend that would produce juicy, tender, and flavorful low-fat meat. The result was a blend of modified food starch, rice flour, salt, emulsifier, flavor, and gums (control moisture release). Ten percent low-fat ground beef patties manufactured with this blend, 90% lean trim, and 7.0% water performed better in tenderness, juiciness and yield than

patties (8.1-12.8% fat) made with formulations of all beef, prehydrated soy isolate, hydrolyzed vegetable protein, salt, and water.

Carrageenan is included in the carbohydrate category and is a high molecular weight polysaccharide, derived from various red seaweeds, that are used as both a binder and extender in meat products. There are three different types of carrageenan: iota-kappa- and lambda-carrageenan. Iota- and kappa-carrageenans act as gelling agents. Iota-carrageenan gels strongly with calcium ions to form a clear, elastic, syneresis-free gel that resets after shear which results in moisture retention. Just like not all carrageenans are alike neither are all iota-carrageenans therefore, carrageenans must be selected carefully (Giese, 1992).

The development of a 10% fat ground beef patty formulation that utilizes carrageenan took place at Auburn University in Alabama. (Huffman, et al., 1991). Based on a consumer sensory panel, Huffman defined an optimal control as being a 20% fat ground beef patty. They also concluded that overall acceptability of a ground beef patty was highly correlated with beef flavor intensity. To enhance flavor a 2:1 ratio of salt to hydrolyzed vegetable protein (HVP) was used at levels of 0.25% and 0.125% respectively. Iota-carrageenan was chosen for the formula to enhance juiciness and flavor. Sensory panelists rated carrageenan formulated patties higher for flavor intensity, tenderness, and juicier than patties with 8 or 20% fat. The carrageenan formulation had lower fat and therefore, calorie content than did 8% fat patties. Huffman concluded that many of the negative attributes of low-fat fresh meat products may be eliminated through the use of combining iota carrageenan and water.

To determine consumer acceptability of Huffman's carrageenan extended ground beef patty formulation, Dunkelberger (1991) home delivered ground beef patties to 91 randomly selected households. The household were sent 1) less than 10% fat plus quality enhancers (carrageenan) ground beef, 2) less than 10% fat ground beef without enhancers, 3) 20% fat ground beef. Consumers gave more favorable ratings to both the 10% fat

ground beef with quality enhancers and the 10% fat ground beef without enhancers for preparing and cooking stages. Consumer ratings at the eating stage were similar between the 10% fat ground beef with quality enhancers and the 20% fat ground beef. These results indicate favorable consumer acceptance of low-fat ground beef with added carrageenan as a quality enhancer. However, this research did not address consumer willingness to pay higher price at the supermarket for low-fat ground beef products with carrageenan fat replacers.

In 1992a, Egbert et al. performed two additional research projects on the carrageenan formulation developed at Auburn University. The first research project was designed to determine the aerobic refrigerated storage stability of carrageenan formulated patties during simulated retail display. In the first project no differences ($P<0.05$) were found between low-fat all beef patties and carrageenan-based patties for populations of psychrotrophs or coliforms. In the first research project Egbert et al. added 2 or 3% potassium lactate to the carrageenan formulation without negative effects on sensory properties. The patties with 2 or 3% potassium lactate had lower aerobic and psychrotrophic populations than either all beef or carrageenan-based patties without potassium lactate. The second research project conducted that same year by Egbert et al. (1992b) compared the same carrageenan formulation and low-fat all beef patties, in a study to determine the effect of simulated retail in both oxygen-permeable and oxygen-impermeable packaging on microbial populations and color values. Again no differences ($P<0.05$) were found in aerobic, phsychrotrophic, mold, or yeasts between the two products. Both formulations of low-fat patties in oxygen-permeable packaging had greater product discoloration than patties in oxygen-impermeable packaging. Patties made with the carrageenan formulation were more red ($P<0.05$) than the low-fat formulated patties. In both of Egbert et al. studies (1992a, 1992b), the presence of pathogenic bacteria such as *E. coli* O157:H7 was not evaluated. The survival of pathogenic bacteria must be part of the evaluation of new products and new product

packaging. As reported earlier, *E. coli* O157:H7 will not grow at 41°C which is the temperature used to identify coliforms in these research projects.

Further research conducted by Brewer et al. (1992) formulated a mix of sodium tripolyphosphate (0.2%), modified food starch (0.5%), carrageenan (0.5%), and water (6.0%). Patties formulated with this mixture had higher beef flavor and juiciness scores than did patties formulated with soy. The carrageenan mixture also had lower off-flavor, and rubbery texture scores than patties formulated with soy. These results indicate that greater consumer acceptance may be obtained with ground beef patties utilizing carrageenan verses soy fat extenders.

More recently Bullock et al. (1994), used the carrageenan formulation developed by Huffman, et al. (1991) but, formulated the carrageenan with lower value beef cuts to manufacture low-fat ground beef patties. The carrageenan formulation was made with either 90% lean cow trimmings, 50/50 Choice trimmings, 95% lean Choice trimmings, defatted clods and rounds, cow knuckles, and cow chucks. These five treatments were compared to a 20% fat control. After a 24 weeks of frozen storage patties were grilled on a McDonald's grill with a 165°C surface temperature to an internal temperature of 74°C. The 20% fat control patties had greater ($P<0.05$) shear force values and cooking losses. Bullock found that all the low-fat patty treatments were rated higher ($P<0.05$) in flavor intensity than control patties. Bullock's research reaffirms that carrageenan has the potential to be an acceptable fat replacer in low-fat ground beef patties.

Lowering the fat content or using fat replacers in ground beef often changes the perception of mouthfeel characteristics by the consumer. Research has been conducted to utilize lipid-based fat substitutes to achieve a reduce calorie product. These particular fat substitutes are resistant to digestive lipases, which make the substitutes unavailable for absorption. Liu et al. (1991) used either corn, cotton seed, palm, peanut, or soybean plant oils (partially hydrogenated) to achieve 10% fat ground beef patties. Sensory panelists found ground beef patties formulated with corn or palm oil were not different ($P<0.05$)

than 10% fat all beef patties. No differences were found in overall acceptability of ground beef patties manufactured with partially hydrogenated plant oils. Lui et al. used an untrained consumer-type panel that rated for overall acceptability only. Further research should be conducted using a trained sensory panel and mechanical evaluation before acceptability is determined.

Effect of Temperature on Tenderness

Consumers may initially purchase a product that has fat replacers because of a perception of health. However, once purchased, consumers regard tenderness as the principle attribute associated with texture. This is evident by consumer preferences for tender cuts of meat. The methods by which meat is cooked and endpoint temperatures can greatly impact tenderness. Davey and Gilbert (1974) found that toughening took place in two distinct temperature ranges of 40-50°C and 65-75°C. Toughening was associated with the loss of myosin, collagen shrinkage, cooking losses, and protein coagulation. A decrease in toughness was seen in the temperature range between 50 and 65°C. In an effort to explain this temperature texture interaction Bouton and Harris (1981) used a Warner-Bratzler shear device to measure the changes in shear force of meat cooked between 50-65°C. Bouton and Harris research results confirmed that increasing cooking temperatures from 50-60°C or 65°C produced a decrease in shear force values. They attributed this change in tenderness to changes in connective tissue and collagen denaturation.

Researchers may recommend optimal cooking temperatures for a tender beef product, but it is the Federal Government's (Federal Register, 1993) guidelines that control how precooked patties used for food service institutions are to be prepared. The FSIS guidelines require patties to reach an internal temperature of 71°C. To determine the effect of this guideline, Troutt et al. (1992a) cooked ground beef patties at various fat

levels 5, 10, 15, 20, 25, and 30% to either 71° or 77°C. Patties cooked to 77°C had greater cooking losses, longer cooking times, less beef flavor than patties cooked to 71°C. Patties cooked to 77°C had higher ($P<0.05$) Instron Warner-Bratzler compression values than patties cooked to 71°C. Cooking to 77°C vs. 71°C made the differences in low fat patties (firmer texture, more crumbly, less juicy and less flavorful) more apparent than cooking to 71°C. Berry (1994) cooked ground beef patties (4 or 20% fat), on an electric grill, to either medium (70°C) or well-done (80°C) using photographic scales developed by American Meat Science Association. Patties cooked to medium verses well-done resulted in higher initial tenderness scores and lower Instron peak shear values. Subsequent studies by Shipp (1992) found that Instron peak shear values increased significantly from 0.60 to 0.88 kN as the endpoint cooking temperature, of ground beef patties, increased from 60° to 71°C.

Methods of Cooking

Cooking method markedly affects the palatability of meat and therefore many methods of cooking have been used by researchers to determine the effects of cooking on food. The American Meat Science Association (1978) in cooperation with the National Live Stock & Meat Board publishes guidelines for cooking meat. They describe roasting as a method by which heat is transmitted to the meat by convection, either by normal or forced air in a closed preheated oven. Broiling is defined as a method by which meat is cooked by direct radiant heat. The meat is placed above or below the heat source. The heat in broiling usually radiates from one direction, so the meat must be turned during cooking. Braising is a method in which meat is cooked slowly, in a moist (added water) atmosphere within a closed system such as a covered pan.

Early research conducted in 1960 by Visser et al., cooked roasts obtained from the psoas major, adductor, rectus femoris, and vastis lateralus muscles by either oven roasting

or cooking in deep fat at 100° or 110°C. Oven roasts required 2-3 times longer cooking times than those roasts cooked in fat. A difference in cooking times can be attributed to the fact that air does not transfer heat as well as fat. Oven cooked roasts and roasts cooked in deep fat at 110°C had higher tenderness scores than roasts cooked in deep fat at 100°C. They concluded that as the internal temperature of the meat increased, the cooking times and cooking losses increased significantly.

Additional research was conducted by Schoman and Ball (1961) on the effects of temperature and air circulation on weight and yields of beef roasts. Their results revealed that yield is a function of evaporation loss and decreased as temperature and air circulation increased. However, low temperatures combined with forced air circulation and at the pressure of saturated steam resulted in increased yields and decreased roasting times.

Multiple methods of dry and moist heat cooking and their effects on beef *semimembranosous* were evaluated by Schock et al. (1974). The cooking methods used were deep-fat frying (DF), oven-roasted (OR), oven-braised (OB), and pressure- braised (PB) at 10 psi to an internal temperature of 70°C. Schock concluded that Warner-Bratzler shear values, color-difference, flavor, tenderness, and overall acceptability were not affected significantly by treatments. Schock et al. results concurred with Visser's findings, in that oven roasted samples had the lowest rate of heat penetration and therefore the longest cooking times.

Cross and Berry (1980) took a different approach in that they examined production practices affecting the palatability and cooking properties of ground beef patties. The effects of patty size, surface perforation, and source of frozen lean (40% domestic, Mexican, or Australian) beef was examined. Patties were broiled on electric Faberware broilers and doneness was determined by a photographic scale. Source of the frozen lean did not have a significant effect on sensory, cooking, and physical properties. No advantage was seen in perforating the uncooked patties. However, 102g patties

received significantly lower ratings for tenderness, juiciness, and flavor intensity when compared to 114g and 227g patties.

Holdt et al. (1986) used a flat top electric grill to cook 18% fat ground beef patties. Cooking temperatures of 121°, 148°, and 177°C were used to cook to endpoint temperatures of 10°, 25°, 40°, 55°, and 70°C. Holdt's investigation revealed that the lower cooking temperatures of 121° and 148°C required significantly ($P<0.05$) more time to reach internal temperatures of 70°C than did using the 177°C cook temperature. Holdt concluded that grilled patties are more desirable when cooked at lower grill temperatures. She based her conclusion on decreased Kramer shear values, increased yields, increased patty diameter, and increased patty moisture levels when patties were cooked to the lower temperature treatments of 121° and 148°C.

Methods of cooking were investigated by Hoelscher et al. (1987) who formulated ground beef patties to 0, 5, 10, 15, 20, and 25% fat levels. After thawing, patties from each formulation were either broiled or pan-fried. Patties that were broiled tended to have more fat loss during cooking. At the 30% fat level ground beef patties that were pan-fried had higher fat content than broiled. Cooked patties were subjected to reheating in a microwave oven. Reheating caused a further loss of fat for both pan-fried and broiled. Unfortunately no sensory evaluation was done on this project's patties.

Ground beef patties at either 4 or 20% fat formulations were cooked by an electric grill alone or in combination with an overhead broiler unit to either medium or well done (photographic scale) (Berry, 1994). Cooking method did not influence ($P>0.05$) cooked patty temperature or tenderness. Broiler grilling reduced the fat retention in 20% patties when compared to grilling alone while no differences were seen at the 4% fat level. The use of high temperature cooking with low-fat patties to well-done apparently produced a high retention of fat compared to the use of similar procedures for patties of high fat content. However, cooking low-fat ground beef patties to well-done by either method

lessened tenderness, juiciness, and flavor quality. Therefore, the two methods of cooking did not influence tenderness measurements whereas cooking times and temperature did.

Impingement cooking is a new form of technology that is becoming increasingly popular (Henke, 1985). Air impingement technology uses high-velocity forced columns of heated air to strike perpendicularly on a product being heated. The forced air removes the cold boundary surrounding the product. Shipp (1992) cooked ground beef patties with 10, 20, and 30% fat levels to three endpoint temperatures of 60°, 66°, and 71°C using an impingement oven. Shipp's research revealed that yields decreased from 74.4% to 65.28% as end point temperature increased from 60°C to 71°C and as might be expected, moisture decreased significantly from 60.34 to 58.32% as endpoint temperature and fat level increased. As the impingement method of cooking becomes increasingly popular, further research needs to be conducted into the effects of impingement cooking on yields, proximate analysis, and sensory evaluation.

Microwave and convection ovens are two additional methods of cooking that have become popular in the past 15 years; especially with ready to prepare foods. Cremer (1982) heated 96 partially thawed precooked beef patties (25% fat) in an institutional microwave and convection ovens. Beef patties heated in the convection oven had significantly higher ($P<0.01$) values for appearance, flavor, and general acceptability than patties heated in the microwave oven. However, Cremer et al. found that the microwave used less energy to function than did the convection oven. Cremer's research did not address potential inconsistencies with "hot" and "cold" spots within the two cooking devices and the impact these spots might have on the internal temperatures of the ground beef patties.

Additional research performed in 1992a by Troutt et al. used a preheated electric skillet to cook ground beef made of various fat levels and ground beef with texture-modifying low-fat formulations. Troutt et al. found that longer cooking times

were required with lower fat ground beef patties to reach the same degree of doneness as higher in fat patties.

Consumer demand for precooked frozen ground beef patties lead Cross et al. (1979) to investigate the effects of precooking and freezing ground beef patties verses raw frozen ground beef patties. Cross et al. heated the patties either by roasting or microwaving. Precooked patties were significantly ($P<0.05$) tougher, drier and lost more weight during cooking than patties cooked from the raw state. Precooked patties that were roasted for reheating were more tender as judged by sensory panel but had higher maximum shear force values when compare to precooked and microwave reheated patties.

As heat is transported through a ground beef patty, changes will occur depending on the ingredients, temperature raise and on the time involved. Many studies have been conducted into the effect of temperature on tenderness and the effect of cooking on meat. Cooking methods are most oven evaluated by their effect on sensory attributes, yields, and proximate analysis of the meat cooked. However, very little research has been conducted on the various methods of cooking and their effect on pathogenic bacteria associated with the meat As heat is transported through a ground beef patty changes will occur depending on the ingredients, temperature raise and on the time involve.

Conclusion

This literature review has established that 1) ground beef is a large part of the American diet 2) the *E. coli* O157:H7 bacteria is a pathogen closely associated with ground beef and 3) *E. coli* O157:H7 has the potential to kill infected patients, especially if they are very young or very old. What the literature reviewed has not revealed is previous research conducted to determine the survival of *E. coli* O157:H7 in low-fat ground beef formulated with fat replacers. There is also no research that has examined the survival of

E. coli O157:H7 in low-fat ground beef made with fat replacers , that has been cooked in various institutional ovens, such as microwave, convection and impingement. In order to better control this microorganism food industry facilities, food service establishments, and consumers must learn basic information about *E. coli* O157:H7's growth, sources of potential infection, cross-contamination, hand washing, general sanitation, and cooking temperature and times. Lack of knowledge in any of these areas may result in additional deaths. The beef industry cannot afford to have the consumption of beef decline due to the association of beef and pathogenic bacteria such as *E. coli* O157:H7.

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CHAPTER III

THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157H:7 IN LOW-FAT GROUND BEEF PATTIES

ABSTRACT

Four low-fat (10%) formulations (control, salt, carrageenan, soy) of ground beef patties, were manufactured and inoculated (10^3 CFU/g) with *Escherichia coli* O157:H7. Thermal death times were established by water cooking at four temperatures (60°, 66°, 68°, 72°C) and holding at these temperatures. Holding times were not required for patties cooked to 72°C or for control, salt and carrageenan patties cooked to 68° and 66°C. A 10 second holding time was required for 68°C soy patties. At 60°C, patties required 240 seconds (control and carrageenan), 120 seconds (salt) and 300 seconds (soy). *E. coli* O157:H7 was not isolated from inoculated patties after cooking in an impingement oven to 66, 68°C or 72°C and held. However, *E. coli* O157:H7 was isolated from control patties cooked to 60°C. Cooking to an internal temperature of 66°C resulted in a microbiologically safe low fat ground beef patty with greater yields, moisture and tenderness than patties cooked to 72°C or 68°C.

Introduction

The recent outbreak of *Escherichia coli* O157:H7 in undercooked ground beef in the Pacific Northwest has focused national attention on this microorganism and the proper cooking of ground beef (National Live Stock & Meat Board, 1993). The majority of outbreaks of *E. coli* O157:H7 have been attributed to the consumption of undercooked ground beef patties (Pai et al., 1984; Riley et al., 1983; Wells et al., 1983; Ryan et al.,

1986; Pavia et al., 1990; O'Brien et al., 1993). Due to the association of *E. coli* O157:H7 with undercooked ground beef, the USDA Food Safety and Inspection Service has issued heat processing temperature and time combinations for fully cooked meat patties. The temperature and time combinations are as follows: 66.1°C/41 seconds, 66.7°C/32 seconds, 153°C/26 seconds, 67.8°C/20 seconds, 68.3°C/16 seconds, 68.9 °C/13 seconds, and 69.4°C/ 10 seconds. It has been estimated that over 1.36 metric tons of ground beef is consumed each year in the United States. (A.M.I., 1992). Retail ground beef and ground beef served in fast food restaurants has an average of 20-30% fat (Hoogenkamp, 1991). However, health conscious consumers are requiring a reduced fat ground beef (approximately 10%) that still exhibits all the organoleptic properties of 20-30% ground beef. Consumer awareness of dietary fat intake has led to the development of low-fat ground beef formulations containing non-meat ingredients. Although a significant amount of research has been conducted into the sensory aspects and yields of low fat ground beef formulations, very little research has been conducted into the survival of *E. coli* O157:H7 on these low-fat formulations. Shipp (1992) found that *E. coli* O157:H7 survived in 10 and 20% fat ground beef patties and not in 30% fat formulations when ground beef patties were cooked to 60°C and held for 300, 260 and 220 seconds respectively. Additional research performed by Troutt et al. (1992a, 1992b) found, that low-fat (5 and 10%) ground beef patties took longer to cook as compared to higher fat beef patties (20-30%) when cooked using an electric skillet. Findings by both Shipp and Troutt et al. may indicate that Food Safety Inspection Service's temperature and holding time guidelines for fully cooked patties may need to be modified for low-fat ground beef formulations. Food manufactures can not afford to risk associating new low-fat ground beef products with a pathogen such as *E. coli* O157:H7. The objectives of this study were to 1) develop the temperatures and holding times (thermal death times) necessary to inactivate *E. coli* O157:H7 in low fat ground beef patties under laboratory conditions and 2) examine these thermal death times under industry like conditions.

MATERIALS AND METHODS

Bacterial strain and culture conditions

Escherichia coli O157:H7 (ATCC #43895) which was implicated in a hemorrhagic colitis outbreak due to the consumption of ground beef, was obtained from the American Type Culture Collection (Atlanta, GA). The culture was maintained at -75°C (Queve Crostar Freezer, Parkersburg, WV) until it was propagated statically, for 24 hours at 37°C, in Luria broth (LB). To obtain a consistent synchronous growth curve, two transfers were performed. The first transfer was performed by taking 1.0 ml of the originally inoculated LB and placing it into 99 ml of fresh LB. The newly inoculated LB was placed on a continuous shaker (Lab-Line Orbit Environ-shaker, Melrose Park, IL) at 120 rpm and 37°C for approximately 8.0 hours. The inoculation procedure was repeated for the second transfer but with approximately a 24 hour incubation period. The *E. coli* O157:H7 inocula was grown to mid-log phase to a density of 1 x 10^5 colony forming units (CFU/g). The initial density of inocula was confirmed by using a spectophotometer and obtaining an O.D. reading of approximately 0.45. The designated concentration of *E. coli* concentration was ascertained by plating directly on MacConkey sorbital agar.

Patty formulation

Fresh (2-5 days postmortem) lean bull meat and 50% fat beef trim were obtained from a local purveyor. Trimmings were individually ground through a 3/8" plate (grinder model 5424852, Biro Mfg. Co., Marblehead, OH) and mixed for 1.0 minute in a ribbon-paddle mixer (Leland, Detroit, MI) for sample homogeneity. Immediately following mixing, random samples from the lean and fat were taken for moisture and fat analysis. For each of the three replications, four treatments were formulated to 10% fat with the

following ingredients: 1) control treatment - no added ingredients; 2) salt treatment - .35% NaCl; 3) carrageenan treatment - 1.0 % carrageenan and flavorings (A. C. Legg Co., Birmingham, AL), .35% NaCl and 9% water; 4) the soy treatment - 5.15% soy protein concentrate (Response 4402, Central Soya Company, Inc. Fort Wayne, IN), .35% NaCl and 19.3% added water. All formulated treatments were mixed for one minute in a ribbon-paddle mixer. Patties (112g) were formed (Hollymatic super patty machine, Chicago, IL) and subsequently frozen at -20°C in stacks of 10 for approximately 3 - 4 weeks. Patties were later thawed at 50°C for 24 hours then at 40°C for 24 hours before inoculation.

Inoculation

After thawing, patties were separated and sprayed with *E. coli* O157:H7 inocula (1.5 ml of inocula per side) to achieve approximately a $1x10^3$ CFU/g concentration. Purity of the inocula was monitored by plating a sample on Brain heart infusion (BHI) agar for 24 hours at 37°C then gram staining to examine for possible gram positive organisms. *E. coli* O157:H7 was confirmed directly from the inoculated LB by using an Oxoid latex agglutination kit (Unipath Limited, Hampshire, England).

Patties were then reground through a 0.32cm plate to distribute the inocula. Inoculated meat was then reformed into 112g patties. Inoculation level in the ground beef was verified by the isolation, detection, and confirmation of *E. coli* O157:H7 in raw patties.

Laboratory cooking procedure to determine holding times

Inoculated patties were placed in plastic bags (15 x 21cm), (KOCH, Kansas City, MO) for water bath cooking. Patties were cooked the same day of inoculation. All treatments were cooked together to internal temperatures of 60°, 66°, 68°, and 72°C using

a heated shaker water bath (model BKS-350, Gallenkamp & Co., Sussex, England). Copper constantan thermocouples were interfaced with a temperature recorder (Omega OM-5000 Data Logger, Omega Engineering, Inc., Stanford, CT), and placed in the geometric center of each patty to monitor internal temperatures. One water bath was used to cook the patties, and another water bath was used to establish holding times. The temperature of the first water bath was set 5°C higher than the target cook temperature in order to reach the target temperature within 10 min. (±3min). As the patties reached the treatment temperature they were immediately placed in the second water bath (holding) at the treatment temperature ±1°C to establish holding times. Each treatment temperature and formulation combination was subjected to a series of holding times to inactivate the known level of *E. coli* O157:H7. Once the initial target temperature was reached one patty was removed, from the water bath to determine the inactivation of *E. coli* O157:H7 at the target temperature without holding times. As each patty reached a predetermined holding time, the purge was removed and the bag and the patty were placed in liquid nitrogen to simulate the food industry's Individually Quick Frozen (IQF) procedures. Once frozen patties were stored in a -20°C freezer (Frigidaire deluxe freezer, Sears, Chicago, IL) until microbiological analyses could be performed. Specific holding times were established only after *E. coli* O157:H7 could not be detected in two replications of each temperature by holding time treatments.

Isolation and detection

Frozen patties were thawed (2 hr) at room temperature prior to microbiological analysis. Survival of *E. coli* O157:H7 in the cooked patties was determined by macerating a sample (12.5g) from each patty with 112.5 ml of modified *E. coli* (MEC) broth in a Seward stomacher (Tekmar Company, Cincinnati, OH). Samples were aseptically transferred to a 250 ml Erlenmeyer flask and incubated statically for 24 hours at 37°C

(VWR Scientific Inc. Model 3020, Sugar Land, TX). Duplicate samples (12.5g) were analyzed for each patty. Following enrichment, 0.1 ml of sample was spread on duplicate plates of MSA and plates were incubated at 37°C for 24 hours. Typical (white, opaque, raised) colonies were removed and streaked on Eosin-methylene blue agar (EMB-Oxoid, Unipath Limited, Hampshire, England) and incubated at 37°C for 24 hours. Typical *E. coli* colonies on EMB were serologically tested for the O157:H7 serotype using the Oxoid latex agglutination kit. Duplicate tests were conducted for each cooking temperature and formulation treatment combination.

Impingement cooking

Laboratory results were subjected to simulated manufacturing by using an impingement oven (model 1022, Lincoln Food Service, Ft. Wayne, IN). The bacterial strain, culture conditions and patty formulation was the same for the impingement cooking as the water bath cooking. Target internal temperatures of 60°, 66°, 68°, and 72°C were achieved by varying the belt speeds with the impingement oven at 218°C. Belt speeds were generated for each formulation treatment during a preliminary study. For each of the three replications, formulation treatment patties (n=9/duplicate) were randomized within each cooking temperature treatment. Treatment patties were cooked in descending order from 72°C to 60°C. After cooking, patties were aseptically removed from the oven, placed on trays and held in convection holding ovens (Jero Thermaflow Cook & Hold, Tulsa, OK) for the appropriate time and temperature, which was determined previously by the water bath cooking. Patties were subsequently frozen in individual containers of liquid nitrogen in order to eliminate cross contamination. Four patties from each formulation treatment by temperature were analyzed for *E. coli* O157:H7 in duplicate as previously described.

Textural analyses

Five thawed patties, in duplicate, were tested for each formulation treatment by temperature combination. A 4 cm^2 section was removed from the center of each patty and peak shear force was measured by using a L.E.E.-Kramer attachment on an Instron Universal Testing Machine (Model 4502, Canton, MA).

Proximate analyses

Two cooked patties from each formulation by temperature treatment combination were tested in duplicate for moisture, fat, and protein. Moisture was determined by oven drying, fat by ether extract, and protein by total combustion (AOAC, 1993).

Statistical analyses

Data collected from the 3 replications of four formulation treatments by four temperature treatments (4x4 factorial) were analyzed using analysis of variance ($P<0.05$), linear regression and least significant means procedure with the Statistical Analysis system (SAS, 1988).

RESULTS AND DISCUSSION

The objective of this study was to determine the temperature and holding times necessary to inactivate *Escherichia coli* O157:H7 in low-fat, precooked ground beef patties. Inoculated and cooked patties were also evaluated for texture, proximate analysis and yields. Figure 3.1 demonstrates the interaction of cooking temperatures and

formulation on the percent moisture in cooked low-fat ground beef patties. As might be expected, regardless of formulation, the moisture decreased as the cooking treatment temperature increased. The salt and carrageenan treatment experienced a curvilinear decrease in moisture, while the control and soy treatment formulations experienced a linear decrease in moisture. Carrageenan had higher moisture content ($P<0.05$) than the other formulation treatments, regardless of temperature. In results that are similar, Huffman et al. (1991) found that carrageenan formulated (7.2%) fat patties had a higher ($P<0.05$) moisture content than 8% and 20% fat all-beef patties. Bullock et al. (1994) found that patties formulated with carrageenan (7.5-8.1% fat) also had higher moisture values than 20% fat control patties.

There was no significant interaction between formulation treatment and cooking temperature ($P<0.05$) for shear force. Table 3.1 shows the main effect due to temperature on shear force values. More force was required to shear 72° and 68°C temperature treatments verses the 66° and 60°C temperature treatments ($P<0.05$). Troutt et al. (1992) and Shipp (1992) also showed that as temperature increased shear values increased.

The main effect due to formulation on shear force (Table 3.2) shows the control having the highest shear force and soy having the lowest shear force. Research performed by Huffman et al. (1991) revealed that carrageenan formulated patties with approximately 8% fat had ($P<0.05$) lower shear force values than did 8% and 20% fat.

The success of a manufacturing operation is often based on yields. This study found a formulation treatment by cook temperature interaction (Figure 3.2). Regardless of formulation, as temperature increased patty yields decreased. The carrageenan treatment had the highest yields ($P<0.05$) which may correlate to its ability to bind water. Similar results were obtained by Bullock et al. (1994) who found that carrageenan formulated patties (7.5-8.1% fat) had higher yields than 20% fat all beef patties. Brewer et al. (1992) also found that carrageenan formulated patties had less cook loss than did 8% and 20% fat all-beef patties.

Survival of *E. coli* O157:H7

Table 3.3 shows the holding time established by water cooking for each formulation and temperature treatment combination. Regardless of formulation, patties thermally processed in water to 72°C did not require holding times to produce a microbiologically safe product. Control, salt and carrageenan formulations did not require holding times when cooked to 68° and 66°C. However, the soy formulated patties required a 10 second holding time at both 68° and 66°C. The holding time required to inactivate *E. coli* O157:H7 in the control patties at 60°C is 60 seconds less (240 sec) than previously reported (300 sec) by Shipp (1992). The holding time required to inactivate *E. coli* O157:H7 at 66°C is 100 seconds less than previously reported (100 sec) by Shipp. The difference between holding time results may be due to different methods used to establish the holding times. Shipp used a single water bath to cook and hold inoculated patties. This research was conducted using two water baths. The temperature of the first water bath was set 5°C higher than the treatment temperature. Once patties reached the established treatment temperature they were removed and held in a second water bath the was set at the treatment temperature.

The survival of *E. coli* O157:H7 in low fat ground beef patties, cooked in the manufacturing simulation, is shown in Table 3.4. No *E. coli* O157H:7 was detected, regardless of formulation, when cooked in the impingement oven to 72°, 68°, and 66°C temperatures and held for the appropriate times. Shipp was also unable to detect *E. coli* O157:H7 in 10% fat formulations cooked to 66° and 71°C. No *E. coli* O157:H7 was detected at 60°C for the salt, carrageenan or soy formulations. However, positive samples for *E. coli* 0157:H7 were isolated from patties cooked to 60°C. These results are similar to Shipp who detected *E. coli* O157:H7 was detected in two of three replications of 10% fat all-beef formulated patties cooked to 60°C.

Microbiologically safe patties were produced in the water bath at 60°C; however, this was not duplicated under industry like conditions. The inability to reproduce laboratory results in industry like conditions may present future problems for cooking guideline development. Two possible explanations can be considered when addressing the inability of laboratory thermal death times to inactivate *E. coli* O157:H7. The first possible explanation is the difference when comparing the thermal conductivity of water bath cooking verses air impingement cooking. Differences in the way heat is transferred will affect patty temperatures and therefore, the survival of *E. coli* O157:H7. The second possible explanation for *E. coli* O157:H7 surviving at 60°C is the formation of heat shock proteins. Kusukawa and Yura (1988), Marano and Pierson (1993) and Shipp (1992) have all shown that *E. coli* O157:H7 has the ability to produce heat shock proteins. This biological characteristic of *E. coli* O157:H7 combined with extended holding times at 60°C temperatures may contribute to microbiologically unsafe low-fat ground beef patties.

CONCLUSIONS

Thermal death times were established in the laboratory to produce safe low-fat ground beef patties at 60°, 66°, 68°, and 72°C for the carrageenan, soy, salt, and control formulations. However, the lab results were not effective at 60°C with holding times when applied to industry like manufacturing conditions. Therefore, strictly using laboratory results without testing in industrial settings is not advised.

Table 3.1 - Shear force values for low-fat ground beef patties cooked to different endpoint temperatures and holding times.

Cooking Treatment	Shear force (N)	SE[a]
60°C	.621[b]	.008
66°C	.619[b]	.008
68°C	.665[c]	.008
72°C	.679[c]	.008

[bc] Means followed by different superscripts are different ($P<0.05$)
[a] Standard error

Table 3.2 - Shear force values for cooked low-fat ground beef patty formulations

Formulation	Shear force (N)	SE[a]
Control	.747[b]	.008
Salt	.708[c]	.008
Carrageenan	.595[d]	.008
Soy	.534[e]	.008

[abcd] Means followed by different superscripts are different ($P<0.05$)
[a] Standard error

Table 3.3 - Holding times for low-fat ground beef patties inoculated with *E. coli* O157:H7[a]

	Temperature °C			
Formulation	60°C	66°C	68°C	72°C
Control	240 sec	0 sec	0 sec	0 sec
Salt	120 sec	0 sec	0 sec	0 sec
Carrageenan	240 sec	0 sec	0 sec	0 sec
Soy	300 sec	10 sec	10 sec	0 sec

[a]Holding times developed by water cooking.

Table 3.4 - Survival of *E. coli* O157:H7 in thermally processed low-fat ground beef patties[a]

	Temperature °C			
FORMULATION	60°C	66°C	68°C	72°C
Control	- + -	- - -	- - -	- - -
Salt	- - -	- - -	- - -	- - -
Carrageenan	- - -	- - -	- - -	- - -
Soy	- - -	- - -	- - -	- - -

[a]Three replications for each temperature x fat level subclass
+ = *E. coli* O157:H7 present in replication
- = No *E. coli* O157:H7 recovered in replication

Figure 3.1 Proximate moisture analysis of cooked low-fat ground beef as affected by formulation and cooking treatments

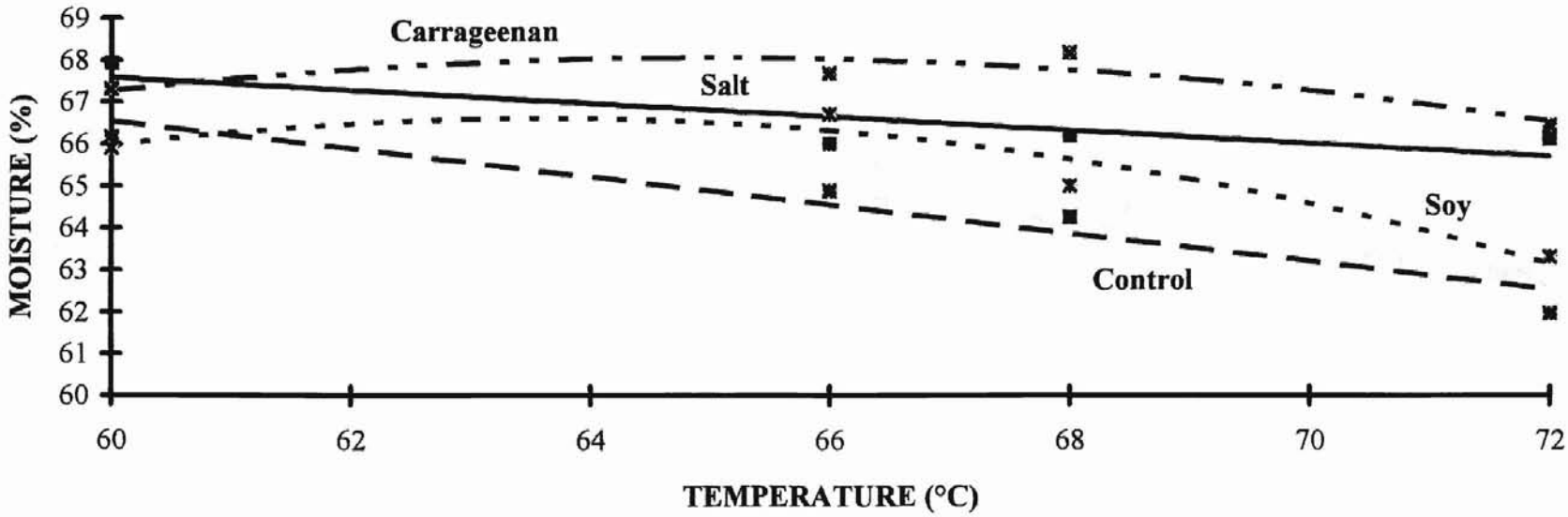
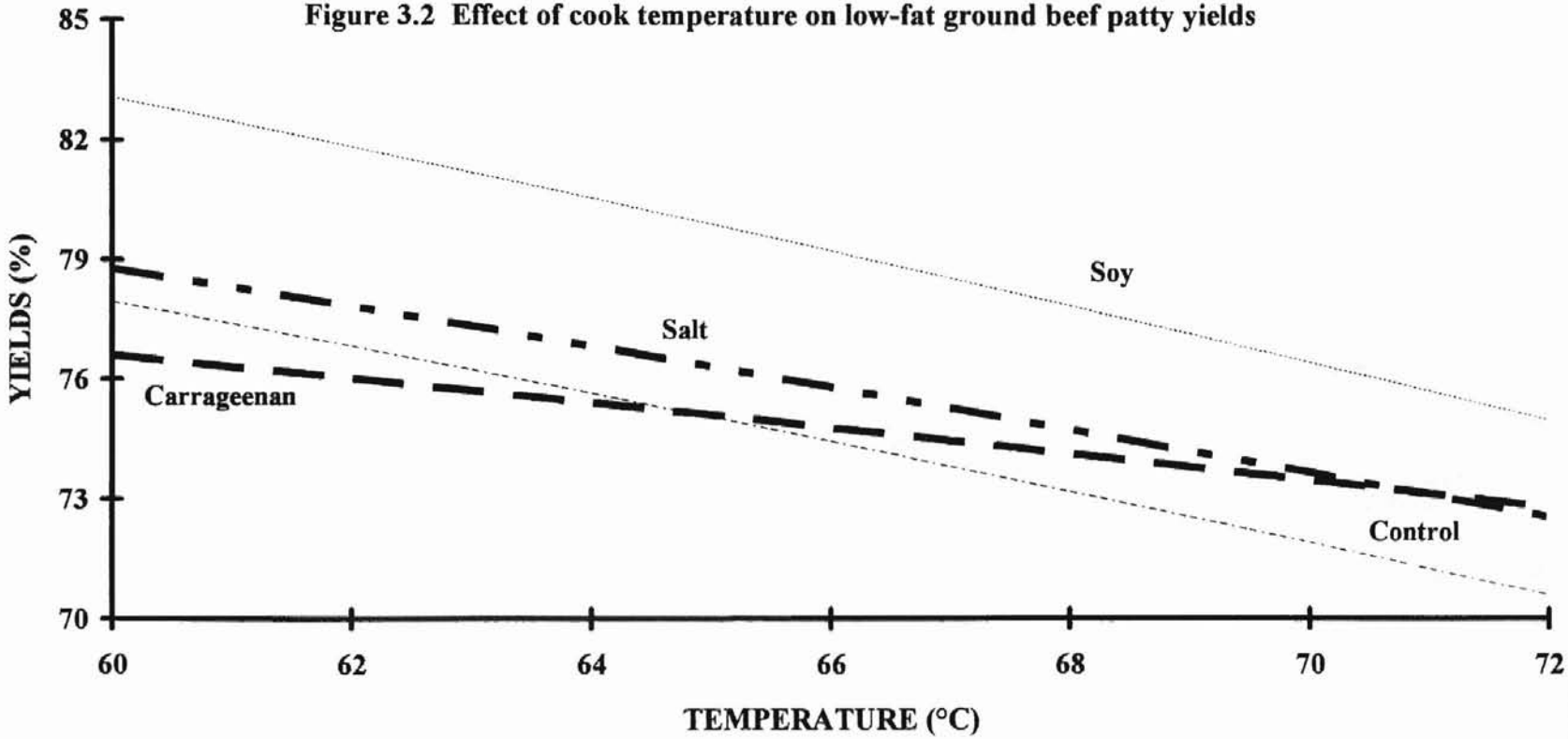


Figure 3.2 Effect of cook temperature on low-fat ground beef patty yields



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APPENDICES

APPENDIX A

MEDIA FORMULATIONS

Modified *E. coli* (MEC) broth

Bacto Tryptone	20.0 grams/liter
Lactose - alpha	5.00 grams/liter
Bacto bile salts #3	1.12 grams/liter
K_2HPO4	4.00 grams/liter
KH_2PO4	1.50 grams/liter
NaCl	5.00 grams/liter
Novobiocin	20.0 milligrams/liter

Luri Broth

Yeast extract	5.00 grams/liter
NaCl	10.0 grams/liter
Tryptone	10.0 grams/liter

MacKonkey Sorbitol Agar

Peptone	20.0 grams/liter
Sorbitol	10.0 grams/liter
Bile salts No. 3	1.5 grams/liter
NaCl	5.0 grams/liter
Neutral red	0.03 grams/liter
Crystal violet	0.001 grams liter
Agar	15.0 grams/liter

Eosin Methylene Blue Agar

Peptone	10.0 grams/liter
Lactose	10.0 grams/liter
Dipotassium hydrogen PO	2.0 grams/liter
Eosin Y	0.4 grams/liter
Methylene blue	0.065 grams/liter
Agar	15.0 grams/liter

APPENDIX B

INOCULUM GROWTH

1. Using a sterile pipette, scrape *E. coli* O157:H7 (ATCC #43895) from the frozen stock culture tube and swirl in 15 ml of 37°C Luri Broth (LB). Incubate statically for12 hours at 37°C.

2. Transfer .1 ml from the original LB tube into a second LB tube and incubate statically 8 hours at 37°C.

3. Transfer 1.0 ml from the second transfer tube and inoculate into 100 ml of LB and incubate 12 hours at 37°C using a shaker incubator set at 1,200 rpm.

4. Transfer 1.0 ml of the third transfer to 100 ml of LB and incubate for 2-3 hours at 1,200 rpm at 37°C.

5. After 2 hours take a spectophotometer reading every 15 minutes until an O. D. reading of .35 to .45 is obtained.

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