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

By

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THERMAL INACTIVATION OF *ESCHERICHIA COLI*

O157:H7 IN GROUND BEEF PATTIES

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WE COMPLETED THE COURSE TOGETHER.

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CHAPTER I

INTRODUCTION

Ground beef patties are a basic component of the American diet and are served in homes, fast food and white table cloth restaurants as well as schools, hospitals, nursing homes and prisons. A 1991 report by the American Meat Institute revealed that ground beef constituted approximately 42% of the total beef consumed in the U.S. (AMI, 1991). Undercooked ground beef patties have been implicated as a carrier for *Escherichia coli* O157:H7; an enteropathogenic microorganism linked to human illness having symptoms ranging from diarrhea, to hemorrhagic colitis, to hemolytic uremic syndrome and sometimes death. This is a foodborne pathogen which could strike anywhere in the country geographically, as well as, any segment of the population, whether they be grouped by age or economics. The first objective of this review is to trace its relatively recent emergence as a foodborne pathogen and its impact on ground beef manufacturing.

In order to determine the effect *E coli* O157:H7 has on the manufacture of ground beef patties, 1) it must be known how often it occurs in ground beef and 2) what temperature and holding time relationship will inactivate it. To determine *E. coli* O157:H7 occurrence in ground beef, a feasible method for isolation and detection must be found. To determine thermal inactivation, D values and thermal death times must be established and subsequently tested for accuracy under industry like conditions and on a large scale.

Public and government concerns for microbiological safety of precooked beef patties led the Food Safety Inspection Service (FSIS) to consider a required endpoint

temperature of 71.1°C. Of equal importance are the factors which contribute to the manufacturing of ground beef patties and the influence those factors have on the palatability or the tenderness, juiciness and flavor perceived by consumers. Therefore, the second objective is to determine what influence endpoint temperature, fat level and method of cooking have on the palatability of the final product.

Fulfillment of these two objectives will lead to the establishment of time and temperature relationships for the production of microbiologically safe precooked beef patties (at different fat formulations) without sacrificing the quality characteristics which consumers associate with ground beef.

CHAPTER II

REVIEW OF LITERATURE

Escherichia coli

Background

E. coli is a predominant gram-negative rod isolated from the intestinal tract of warm-blooded animals and is widely distributed in nature, primarily found in feces. Since 1892, it has been used as an indicator of fecal pollution in water and food. One of the "coliform group", the genus is divided into many biotypes and serotypes, some of which can be pathogenic to humans (Frazier and Westhoff, 1988). *E. coli* is only one of several agents which can cause bacterial gastroenteritis. Two mechanisms have been described by which *E. coli* can mediate diarrhea. Enterotoxigenic strains colonize the mucosal surface of the small bowel and produce heat-labile (LT) or heat-stable (ST) enterotoxins which cause fluid secretion and diarrhea. Enteroinvasive *E. coli* strains penetrate and multiply within colonic epithelial cells. These were the only reported methods by which *E. coli* caused illness until 1977 when Konowalchuk et al. reported nontoxigenic *E. coli* strains inducing a distinctive cytotoxic effect on vero cells different from LT. They termed this previously unknown toxin VT (toxic to Vero cells). In the study, ten *E. coli* strains induced a cytotoxic response in Vero cells and seven were associated with diarrhea in human infants. Levine et al. (1978) also confirmed the presence of this cytotoxin induced by various *E. coli* strains. Therefore, another group of

E. coli, enteropathogenic *E. coli*, were identified which were not invasive, nor did they produce LT or ST enterotoxins.

Verotoxin Associated with Human Disease

As stated earlier, Konowalchuk et al. (1977) were the first to identify *E. coli* VT and their study involved strains which had produced diarrhea in human infants. Several studies since that time have linked VT^+ *E. coli* to symptoms of Hemolytic Uremic Syndrome (HUS, commonly referred to as kidney failure). Karmali et al. (1983) conducted a study from September, 1980 to October, 1982 in Canada. Eleven of 15 (73%) HUS cases had evidence of infection by VT^+ *E. coli*. This was confirmed in 1985 by Karmali et al. in a study of 40 pediatric patients with HUS in which 30 (75%) patients had evidence of Verotoxin-producing *Escherichia coli* (VTEC) infection. O157 was one of six different serogroups implicated.

Not only diarrhea and HUS but other symptoms such as hemorrhagic colitis (HC, commonly referred to as bloody diarrhea) have been associated with VT+ *E. coli* infection as well. Johnson et al. (1983) reported an outbreak of VT infection in a patient with HC and were the first to link directly to the *E. coli* O157:H7 strain specifically. They also reported that 2 of the 6 sporadic O157:H7 strains isolated in 1979 and 1980 were associated with hemorrhagic colitis. Again, none of the isolates produced ST or LT entertoxins or were invasive. Subsequent studies have linked HC and HUS to Verotoxin-producing *E. coli* O157:H7 (Gransden et al. 1986; Spika et al., 1986)

Outbreaks Associated with Food

The first documented outbreaks of *E. coli* O157:H7 in the United States were reported by Riley et al. (1983). This outbreak was in Oregon (February, 1982) and the

second was in Michigan (May, 1982). The illness was characterized by severe abdominal cramps, initially watery diarrhea followed by grossly bloody diarrhea, and little or no fever. It was associated with eating at restaurants belonging to one chain and consuming any one of three sandwiches containing a beef patty, rehydrated onions and pickles. Assays on stool cultures did not yield previously recognized pathogens but 9 of 12 stools did contain *E. coli* O157:H7. The same pathogen was present at a level of 50 organisms per gram in a ground beef patty from a suspected lot used at the Michigan restaurant during the outbreak period (Wells et al., 1983).

After these outbreaks, a surveillance system was established to identify and study sporadic cases of this illness in the United States (Remis et al., 1984). Between August 1982 and April 1984, the cases of 103 persons whose illnesses were compatible to hemorrhagic colitis were reported in 28 states. Stool specimens were obtained from 74% of the cases and *E. coli* O157:H7 was identified in 36% of those specimens. Most common symptoms were severe abdominal cramps followed by watery diarrhea then bright-red bloody diarrhea (some experienced nausea and vomiting, two developed HUS). No work was done to trace the vehicle of transmission.

The next outbreak of *E. coli* O157:H7 infection in the U. S. occurred in September, 1984 in a nursing home and was reported by Ryan et al. (1986). Thirty-four residents of the 101 bed facility developed diarrheal illness. Fourteen people were hospitalized with symptoms of crampy abdominal pain and grossly bloody diarrhea, four died. When it was determined that 17 of the 19 residents with bloody diarrhea had eaten ground beef before becoming ill, ground beef was implicated as the vehicle of transmission. *E. coli* O157:H7 was the only bacterial pathogen identified in the laboratory investigation of stool specimens from residents. However, no *E. coli* O157:H7 was recovered from the leftover frozen patties from the meal served.

Outbreaks were reported in Canada by Pai et al. (1984). They conducted a study in 1983 over a 6 month time period in Calgary where *E. coli* O157:H7 was isolated from

19 of 125 patients with grossly bloody diarrhea. The diarrheal illness was usually self-limited, and 3 children developed HUS. The illness followed consumption of hamburgers for 15 patients.

A later outbreak of *E. coli* O157:H7 enteritis in Canada of September 1985 affected 55 of 169 residents and 18 of 137 staff members at a nursing home (Carter et al., 1987). This outbreak came in two phases, a primary wave associated with previously prepared and unrefrigerated ham, turkey and cheese sandwiches made by a foodhandler who had recently recovered from diarrhea and a secondary wave associated with person-to-person transmission of infection. Hemolytic uremic syndrome developed in 12 affected residents (22%) and 11 of those died. Overall, 19 (35%) of the affected residents died, 17 (31%) from causes attributable to their infection. Laboratory investigations of stool samples identified *E. coli* O157:H7 as the only pathogen present in all specimens but one. Carter et al. summarized by saying elderly persons have an increased susceptibility to *E. coli* O157:H7 infection and high mortality rates when the infection occurs.

No outbreaks of *Escherichia coli* O157:H7 illness were reported in the United Kingdom until 1988 (Morgan et al., 1988). This outbreak affected 24 persons over a 2 week period and was characterized by severe abdominal pain and bloody diarrhea of short duration. Eleven patients were hospitalized and there was one death. Ground beef was not the vehicle of transmission, but a case-control study suggested that handling potatoes (possibly contaminated with manure) was the risk factor.

In addition to ground beef, meat sandwiches and potatoes, raw milk has also been implicated in two cases and one outbreak where children developed HC and HUS after drinking raw milk from dairy herds where *E. coli* O157:H7 was isolated from healthy heifers (Martin et al., 1986; Borczyk et al., 1987).

Isolation and Detection

Since *E. coli* was an emerging food borne pathogen, little was known of its survival characteristics (response to high or low temperatures) or its growth response to culture medium. Research to determine *E. coli* O157:H7's characteristics was conducted by Doyle and Schoeni (1984). Survival studies were done in ground beef to determine the rates of thermal inactivation (D values) as well as stability at frozen temperatures over several months. Results indicated that O157:H7 inoculated in 1 gram of ground beef and thermally processed in a water bath was more sensitive to heat than *Salmonella*, but O157:H7 could survive in ground beef patties for 9 months at -20°C with little change in number. The optimum temperature for growth in culture medium (trypticase soy broth) was 37°C for it grew poorly at 44-45.5°C, the temperature used for recovery of *E. coli* from foods. This makes it unlikely to be detected using established methods for detection of other strains of *E. coli*.

It became equally important to determine means of isolating *E. coli* O157:H7 from food, this being difficult because the microorganism is present in low numbers and other competiting microflora (including different strains of *E. coli*) are present as well. Therefore, research efforts have concentrated on detection through one or more O157:H7 unique biochemical reactions such as the inability to ferment sorbitol, lack of fluorescence in the presence of 4-methylumbelliferyl-B-D-glucuronide (MUG) and production of indole.

One of the first methods of isolation reported, utilized O157:H7's inability to ferment sorbitol as a method of detection. Farmer and Davis (1985) devised a H7 antiserum-sorbitol fermentation medium. Inoculated O157:H7 colonies did not ferment sorbitol and were immobilized in the semisolid medium, while almost all other *E. coli* strains gave a different pattern. The same characteristic (inability to ferment sorbitol) was utilized again to isolate O157:H7 colonies when plating. MacConkey-sorbitol agar

(MSA) was used as a means of identifying the sorbitol negative O157:H7 colonies which were white as opposed to the sorbitol positive, red colonies.

Szabo et al. (1986) used a direct plating medium incorporating tryptophan, sorbitol, an indicator dye and a fluorogenic substrate. Several type of bacteria grew on the medium, but only sorbitol negative, MUG negative, indole positive colonies are blue, non-fluorescent under Ultra-violet light and red upon exposure to the indole reagent (after overnight incubation at 44.5°C). *E. coli* O157:H7 recovery from artificially contaminated ground beef using this method was ≥ 90%. Todd et al. (1988) developed a rapid detection method using hydrophobic grid membrane filters and a highly specific monoclonal antibody (MAb) which binds the somatic antigen. This method gives presumptive identification of *E. coli* O157:H7 in 24 hours within a range of 10 colony forming units (CFU)/g to 10^3 CFU/g inoculum level and ≤ 95% recovery.

Because O157:H7 is present in such low numbers and the possibility of missing the strain in a sample is high, enrichment procedures were added to direct plating to increase the possibility of detection. Szabo et al. (1990) developed a procedure utilizing modified tryptic soy broth and a hydrophobic grid membrane filter with an enzyme-labeled antibody (HGMF-ELA). The lower limit of sensitivity was reduced to ≤ 1 CFU/g, an improvement over 10 CFU/g detected by direct count methodology. This was important since samples containing less than 10 CFU/g have been implicated in food-borne outbreaks of hemorrhagic colitis (Szabo et al., 1990).

A different enrichment method for the isolation of *E. coli* O157:H7 from ground beef was developed by Okrend et al. (1990a). They utilized modified EC broth to which novobiocin was added which limited growth of gram positive organisms. After 24 hours of static incubation or 6 hours of shaking at 35°C, dilutions were plated on MSA. Sorbitol negative colonies were spread on Levine eosin methylene blue agar (EMB) and stabbed on phenol red sorbitol agar with 4-methylumbelliferyl B-D-glucuronide agar (PRS-MUG). Those cultures that were typical on EMB, sorbitol and MUG negative on

PRS-MUG were biochemically and serologically confirmed. At an inoculum level of 0.4 - 0.6 CFU/g, the 6 hr method isolated 9 of 13 samples while the 24 hr method isolated 13 of 13.

When direct plating, selecting colonies from selective media for further testing is difficult because O157:H7 colonies are not the only white CFU on MSA agar. A method which reduced the number of false suspect colonies picked from primary plating was developed by Okrend et al. (1990c). The addition of 5-bromo-4-chloro-3-indoxyl B-D-glucuronide (BCIG) allowed the differentiation of B-glucuronidase positive from B-glucuronidase negative colonies. *E. coli* O157:H7 colonies are sorbital negative, B-glucuronidase negative and remain white while sorbitol negative, B-glucuronidase positive colonies turn green /blue. *E. coli* O157:H7 was isolated from 11 of 12 inoculated meat samples (0.7 CFU/g) using MSA-BCIG compared to 8 of 12 samples using MSA without BCIG.

Some scientists examined methods of isolation which would be more sensitive and specific than direct plating. One method of isolation made use of bacteriophages which have extreme specificity for their host organisms and have been used to identify several kinds of bacteria. Coliphages are bacteriophages that specifically infect *E. coli.* Ronner and Cliver (1990) have identified a coliphage (AR1) that plaqued on 14 strains of O157:H7 but not on other *E. coli.* Of all enterobacteria tested, only *Shigella dysenteriae* plaqued, suggesting a relationship between production of this toxin and susceptibility to coliphage AR1. A second method utilized DNA probes prepared by Levine et al. (1987) for the detection of plasmids which mediate epithelial cell attachment of verocytotoxin-producing strains.

Methods utilizing phages and DNA probes are more sensitive than direct plating techniques; however, they are expensive and labor intensive. Okrend et al. (1990b) tested a screening method using the commercially available 3M Petrifilm test kit-HEC-for hemorrhagic *E. coli* O157:H7 and found the test kit to be a reliable negative screen

for O157 antigen. The method incorporated an enrichment procedure and reactive disc blot enzyme linked imminoabsorbent assay (ELISA). The test kit made possible the identification of negative samples in 26-28 hours, leaving a small number of presumptive positive samples for confirmation testing.

Pathogenicity

Initial studies to determine pathogenicity centered around the previously noted findings of Konowalchuk et al. (1977) and were conducted on several different strains of *E. coli*. Scotland et al. (1980) confirmed these findings when 25 strains of *E. coli* were isolated from infants with diarrhea which were found to be VT+ and also failed to produce ST or LT interotoxins. Wade et al. (1979) also confirmed Konowalchuk's findings by reporting of toxins which were not LT, ST or invasive and were undetectable in standard assays. They used *E. coli* strain O26:H19 and noted that 3 patients excreting cytotoxic O26 had bloody diarrhea.

E. coli O157:H7 was never mentioned in association with VT production until the study of Scotland et al. (1987) in which 54 strains *of E. coli* were assayed for production of Vero cytotoxin VT1 and VT2. Twenty-six strains from cases of diarrhea, HC and HUS produced VT. Of the 26 strains, 21 were the H type 7 (non-motile) except for 2 other non-motile strains. All hybridized with at least one of two DNA probes specific for VT genes.

The characterization of the *E. coli* toxin was conducted by O'Brien et al. (1983) who reported that the Vero cytotoxin produced by 11 *E. coli* strains had the same subunit structure, isoelectric point, comparable biological activities and the same relative heat stability (up to 65^{o}C for 30 min) as the Shiga toxin 60R. They concluded a family of Shiga-like toxins exists and proposed that it be named "*E. coli* Shiga-like toxin" (SLT).

Questions as to how the VT toxin was mediated naturally arose and Scotland et al. (1983) determined that the genes for VT production derived from the *E. coli* H19 strain are carried on a bacteriophage and not on a plasmid as in most *E. coli* or chromosomally located as with *Shigella*. Later it was determined that the *E. coli* O157:H7 strain 933 has two toxin converting phages, 933J and 933W (Strockbine et al. 1986). These two toxins are genetically related but have antigenically distinct cytotoxins with similar activities referred to as Shiga-like toxin I and II (SLT I & II). SLT I and II have been associated with diarrhea, HC and HUS. Willshaw et al. (1987) confirmed that the genes controlling production of VT in *E. coli* O157:H7 strain 933 were phage encoded and they developed DNA probes to identify verocytotoxin producing *E. coli* (VTEC) to detect their presence in fecal specimens. DNA probes were used by Smith et al. (1987) during a one year study in England and Wales. VTEC were identified in 39% of fecal samples of patients with hemorrhagic colitis. Thirty of 32 VTEC strains were O157 serotype.

Heat Shock Proteins

One phenomenon found in nature among different types of cells is the induced synthesis of several specific proteins when the environmental temperature shifts upward (Yamamori and Yura, 1980). Temperature induced proteins were later referred to as heat shock proteins (hsp) and Kusukawa and Yura (1988) reported that their transcription at high temperatures was mediated by sigma 32 (s^{32}, a RNA polymerase that makes all other mRNA). The report stated that maintenance of a constant level of hsp was considered necessary for normal cell growth as well as survival at extreme temperatures. One hsp among 20 was singled out (GroE) as playing a key protective role for *E. coli* survival between 20 and 40°C.

How the heat shock proteins were mediated was addressed by Squires et al. (1991) who reported that one gene (Clp B) specifies for two hsp, F84.1 and F86.5. Kitagawa et al. (1991) indicated that Clp B expression is under direct control of sigma 32, as well as Clp P (hsp) and suggested the possibility of an ATP-dependent protease, Clp B - Clp P complex playing an important role against thermal stress in *E. coli*.

The effect of heat shock on the recovery and/or survival of *E. coli* O157:H7 has been studied by Murano and Pierson (1992). They reported that the heat shock of aerobically grown cells resulted in an increase in the mean D value after a 55°C heat treatment by a factor of 2.1 over nonheat-shocked controls.

Animal Sources

The reservoir for *E. coli* O157:H7 is unknown; however, epidemiological studies have shown an association between infection and consumption of under cooked ground beef and raw milk (Borczyk et al. 1987; Martin et al., 1986; Pai et al., 1984; Riley et al., 1983; Ryan et al., 1986). Since dairy cows are sources of both, they are suspected as reservoirs for *E. coli* O157:H7 and independent studies were conducted to determine if they could be the vehicle of transmission.

Martin et al. (1986) investigated two separate cases of children who developed HC and HUS after consuming raw milk. *E. coli* O157:H7 was isolated from healthy heifers from both herds. Dairy cattle were again considered the reservoir after a large outbreak of VTEC O157:H7 infection among 60 kindergarten children in Ontario. A trip to a dairy farm where they consumed raw milk, resulted in 48 children with abdominal cramps, most had watery diarrhea and 3 developed HUS. *E. coli* O157:H7 was found in 43 children's stools and 7 fecal samples from the dairy herd (Borczyk et al., 1987). Calves have also been implicated as reservoirs. Orskov et al. (1987) isolated *E. coli* O157:H7 from 13 calves with coli bacillosis in Argentina.

Unlike dairy cattle, beef cattle tested to date have not been reservoirs. Stool samples from 531 healthy beef cattle in western U.S. and 68 cattle in the Northwestern U.S. did not yield O157:H7 (Martin et al., 1986).

Because other serotypes of VTEC can produce human illness besides O157:H7, two studies were conducted in Germany for the detection of VT+ *E. coli* in healthy cattle by Montenegro et al. (1990). Among 259 animals investigated, 10.8% were found to carry VT+ *E. coli* strains, 40% of the strains belonged to serogroups known to be pathogenic for humans. In a second, separate study, fecal samples were taken from dairy cattle which were analyzed by colony hybridization with VT1 and VT2 specific gene probes. Thirty-eight percent of the colonies isolated by the VT2+ gene probe belonged to *E. coli* serogroups that are pathogenic. In Canada where they have the most documented foodborne infections due to VTEC, 19.5 % of dairy cows and 11.6% of the total cattle are VT+. *E. coli* O157:H7 was found in 4 of the 600 animals. These accumulated findings have led some regulatory authorities to warn that the increased slaughtering and processing of dairy cattle may increase the potential of *E. coli* O157:H7 contamination (Vasavada, 1988).

Chicken may also serve as reservoirs, a conclusion from the study conducted by Beery et al. (1985). They determined that *E. coli* O157:H7 can colonize in chicken in the cecae for up to 90 days after inoculation at day 1 (without effecting the appearance of the chickens) and be passed through the colon with fecal excrement. This finding is important because chickens which were reservoirs for *E. coli* O157:H7 could become externally contaminated during slaughter and contaminate other chickens during processing (ex: chill tank). *E. coli* O157:H7 could then cause food borne illness either through undercooking of chicken or cross contamination with other foods.

Occurrence in Meat

The prevalence of Verotoxin-producing *E. coli* (VTEC) capable of causing human illness by transmission through contaminated meat has been addressed by different scientists. Read et al. (1989) studied samples selected randomly from meat processing plants in the Toronto, Ontario area. After running assays on 225 samples of ground beef, 235 samples of pork and 200 samples of chicken, they considered ground beef and pork but not chicken significant sources of VTEC. A study conducted in Britain by Smith et al (1991) examined samples from chicken and pork sausages for VTEC using DNA probes for VT genes. Hybridization was detected in 25% of 184 sausage samples but none of the 112 chicken samples. Three of the VTEC serotypes identified in pork sausages have been isolated from cases of human infection.

Only one study has been conducted to isolate the *E. coli* O157:H7 strain from meat purchased in the United States as well as Canada. Doyle and Schoeni (1987) assayed 896 samples of fresh meats and poultry with a hydrophobic grid membrane filter-immunoblot procedure developed to isolate the organism from food. O157:H7 was isolated from 3.7% of beef, 1.5% of pork, 1.5% of poultry and 2.0% of lamb samples. Thiry-one percent of the beef sampled and 7.1 % of the pork from Calgary, Canada contained O157:H7. They concluded that the organism is not a rare contaminant of fresh meat and poultry.

Ground Beef Manufacture

Effect of Fat Level

Fat level plays a significant role in ground beef manufacture from the stand point of palatability, in particular those traits of tenderness which are associated with juiciness.

Cole et al. (1960) found a consumer panel of husbands scored a 25% fat formulation most desirable among 15, 25, 35 and 45% fat levels in ground beef patties, while a trained taste panel gave highest scores of tenderness and juiciness to a 35% fat formulation. Cross et al. (1980) prepared ground beef patties of 16, 20, 24 and 28% fat. Trained sensory panelists evaluated each treatment for differences in tenderness, juiciness, connective tissue amount, mouth coating and beef flavor intensity. As the fat level increased in the patties, they gave higher tenderness and juiciness scores and ratings of lower connective tissue amount. It was also concluded that the difference between low and high fat ground beef narrows considerably during cooking because as the fat content increases in a raw pattie, the total loss of fat increases in the cooked pattie.

Instrumental shear values (force necessary to separate a ground beef pattie) have been correlated to tenderness as perceived by a trained sensory panel (Beilken et al., 1991; Brady et al, 1985; Burrill et al., 1962; Cross et al., 1978; Segars et al., 1975) and are also affected by fat level. Huffman and Powell (1970) compared 15, 25, and 35% fat formulations in ground beef patties and reported that the shear value for patties with 35% fat was significantly lower than for patties containing 15 or 25% fat.

Similar results were found by Berry and Leddy (1984) when the effects of fat level (14, 19, 24%) in ground beef patties were tested using electric broiling, charbroiling, conventional oven roasting, convection oven roasting, electric grill frying, and microwave cooking. As expected, higher tenderness and juiciness values were associated with higher fat levels in patties. Microwave cooking produced low sensory panel ratings regardless of fat level, while patties cooked by frying had the highest ground beef flavor intensity scores. It was concluded that properties of cohesiveness, hardness and density were influenced by fat level in a similar manner for all cooking methods.

Two recent studies (Berry, 1992; Troutt et al., 1992a) also studied the effect of fat level on sensory characteristics of cooked patties. Both stated that as fat level increased,

the shear force decreased. Troutt et al. declared low fat (5-10%) patties to be firmer in texture, more crumbly at end of chewing, less juicy and flavorful with less oily mouth coating than 20-30% fat patties. It was also stated that increases in temperature from 71° to 77°C accentuated differences in palatability between low and high fat patties. Berry (1992) stated that patties processed with 0% fat were rated lower in juiciness and flavor compared to all other fat levels and suggested that alterations in processing and cooking would probably be necessary to achieve acceptance of extremely low-fat beef patties.

Because of diet and health concerns, consumers have been urged to reduce dietary fat and consume more complex carbohydrates. With this in mind, Troutt et al. (1992b) conducted a study to determine the effect of incorporating texture-modifying ingredients into low fat (5-10%) ground beef. They used six combinations of dietary fibers, starches and polydextrose. Treatments containing these ingredients had cooking losses of 20-40% less than controls. Patties containing three way combinations of ingredients more closely resembled the 20% fat controls for texture traits. Patties with texture-modifying ingredients had less oily mouthfeel, but were also less juicy and had slightly lower beef flavor intensity scores.

Juiciness and tenderness is not only affected by fat level but also by moisture. Swift et al. (1954) reported that increasing proportions of fat or moisture are related to increases in juiciness and tenderness in bologna as preceived by a trained taste panel. It was also stated that for bologna, juiciness and tenderness varied more noticeably with changes in moisture content than fat content.

Since added moisture has been reported to contribute as much or more than fat in emulsified products, it has been used to replace fat in sausage products. Ahmed et al. (1990) conducted a study to determine the feasibility of replacing fat with added water in low fat pork sausage. Six sausage blends consisting of three fat levels (15, 25, & 35%) and two levels of added water (3 & 13%) were packaged in chubs or mechanically formed patties. Results showed increased amounts of added water in low fat sausage

resulted in cooking losses, color and textural characteristics similar to control sausage. Sensory panel ratings did not differ between sausage formulated to contain 35% fat (3% added water) and 15% fat. It was concluded that acceptable pork sausage may be produced at 15% fat with added water.

Since the American Heart Association has advised the general public to reduce dietary cholesterol and saturated fat in an effort to reduce the risk of heart disease, a study was conducted by Hoelscher et al. (1987) to determine the relationship between initial fat content of raw ground beef patties and the cholesterol and caloric content of cooked patties. It was determined that the cholesterol content was not related to initial fat content and the caloric content of cooked patties was curvilinearly related to initial fat content of the raw patty. This was due to an increased loss of fat during cooking as the initial fat content increased.

Comminution

Comminution, the reduction of particle size, is necessary to formulate ground beef patties from beef trim and several different methods can be used. Berry et al. (1987) manufactured flaked and formed beef and pork steaks using eleven different Comitrol® cutting heads to give a wide range of flake sizes to determine the effect of particle size on textural and cooking properties. Data from the trained panel, Instron and cooking indicated that as flake size increased, detectable fiber, amount of connective tissue and shear force all increased. Textural properties of beef and pork steaks were affected the same by flake size. It was also concluded that thickness of the flake particle was as important as width.

Grinding is a traditional comminution method and often used as a basis of comparison to determine the acceptability of other comminution methods such as flake cutting. Randall and Larmond (1977) found that patties processed by both grinding and

flake cutting methods were acceptable with a significantly higher score being given to the ground patty by a consumer panel. In the same study, trained sensory panelists described ground patties as having a finer grind or smaller particle size, being more tender, less rubbery, more juicy and greasy than flake-cut patties.

Chopping was compared to grinding by Berry (1980) where trained sensory panelists gave chopped product higher scores for juiciness, initial and final tenderness and initial and final connective tissue. He concluded that when considering palatability, chopping was a suitable substitute for grinding as the initial means of comminuting trimmings.

Effect of Temperature on Tenderness

The final internal temperature to which meat is cooked also influences many of the palatability attributes. Bouton and Harris (1972) and Draudt (1972, referenced by Berry, 1975) each reported three similar effects of heating on samples of muscle.

1. 50°C is the temperature of maximum shear value (area of meat or protein denaturation)

2. 60°C yields a decline in shear value (associated with collagen shrinkage) (reported also by Machlik and Draudt, 1963)

3. 75°C produces appreciably greater shear values (associated with the hardening reaction.)

Davey and Gilbert (1974) also identified two separate phases of toughening developing during cooking at increasing temperature. A three to four fold increase in toughening occurring in the first phase between 40 and 50°C with a further doubling of toughness occurring in the second phase between 65 and 75°C. They associated the first with myosin solubility indicating denaturation while the second phase was associated with collagen shrinkage which forces out meat juice. Martens et al. (1982) defined the

temperature that differentiated between thermal denaturation of myosin, collagen and actin. They concluded that firmness increased with thermal denaturation of myofibrillar proteins (myosin 40-60°C; actin 66-73°C) and that fiber cohesivity decreased with collagen denaturation (56-62°C). It was further stated that reduction in juiciness was associated with actin denaturation while cooking loss increased over the whole temperature range.

Another view was presented by Purchas (1973) who reported that cold shortened beef increased in toughness from 50° to 60°C and continued to rise at higher temperatures of 70 and 80°C. Along the same line, Bouton et al. (1981) reported that both peak shear yield and initial force values for cold-shortened beef increased with temperature up to about 70°C.

Conversely, significantly higher shear values at 55°C than at 77°C or greater were found by Goodwin et al. (1962) when cooking turkey breast and thigh meat. They also reported that temperatures of 88 and 94°C produced a drier product which tended to crumble. Rate of cooking also influences tenderness in beef muscle shear force values. Locker and Daines (1974) concluded that slow cooking gave higher cooking losses than fast, but lower shear force values. Bramblett and Vail (1964) when investigating the qualities of less tender meat as affected by cooking at very low oven temperatures for long periods observed that the muscles cooked at 68°F required 2-4 times as long to cook as did muscles cooked at 93°F. However, Warner-Bratzler and Kramer shear shear force values indicated that meat cooked at 68°F was more tender than meat cooked at 93°F. Meat cooked at 68°F also had slightly better appearance and flavor but had greater cooking losses and was less juicy. Conversely, rate of cooking had no significant effect on shear values for turkey breast or thigh (Goodwin et al., 1962).

Reheating also affects tenderness by affecting sarcomere length. Bouton and Harris (1981) reported that sarcomere length values tended to decrease with increasing temperature and time and that recooking at 80°C significantly reduced sarcomere lengths.

Methods of Cooking

Studies have been conducted to determine the effect of different cooking methods on the physical, chemical and sensory characteristics of meat. One approach is to determine what happens to meat during different cooking methods. Reid and Harrison (1971) chose four heat methods, deep fat frying (DF), oven braising (OB), pressure braising (PB) and oven roasting (OR) to observe the effects of these cooking methods on certain histological characteristics of beef muscle cooked to 70°C. Measurements of muscle fiber width were taken and observations for color, type of connective tissue, distribution of fat and estimated quantity of connective tissue were made on 475 sections of beef muscle. They concluded that the effects of heat on the selected histological characteristics of beef muscle did not vary significantly among the four heat treatments.

A more applied approach was taken by Schock et al. (1970). The same four cooking methods (DF,OB,PB,OR) were used to cook beef muscle to 70°C. For every heat treatment they observed a relationship between rate of heat penetration and cooking time. Heat penetration was fastest in PB pieces of muscle followed by DF, OB and OR with cooking time following the same order. Cooking losses however were greatest for PB and the least for OR. No differences attributable to heat treatment were found for flavor, tenderness or overall acceptability.

The effect of temperature and air circulation on weight and volume yields for beef roasts was studied by Schoman and Ball (1961) using a specially designed oven. They found yield to be a function of evaporation loss and yield decreased as temperature and air circulation increased. When cooking at low temperature with forced air circulation and at the pressure of saturated steam, yield increased and roasting time decreased; however, saturated steam gave the product a moist heat aroma and texture.

Comparisons were made between oven roasting and deep fat cooking by Visser et al. (1960). Since heat conductivity of liquid fat is about 6 times that of air, it was observed that the fat transferred heat to the meat more rapidly than the air in the oven, although the temperature of the fat was less than that of the oven. After cooking was terminated, the roasts stood at room temperature until the maximum internal temperature was noted. The internal temperature of oven roasts did not rise, however, the internal temperature of roasts cooked in deep fat to 45° and 55°C rose approximately 10°C, roasts cooked to 65° and 70°C rose 5°C and roasts cooked to 85°C rose very little. They concluded, that in general, as the internal temperature of the meat increased, the average cooking time and cooking losses increased significantly.

Dry Heat Cookery

Different thermal processing methods affect the tenderness of meat differently. Berry (1975) compared microwave, roasting, oven broiling and deep fat frying as reported in Table 2.1. Shear values are given for whole beef muscle cooked using the four methods. Whole muscles cooked in the microwave had higher shear force values than whole muscles cooked by oven-broiling or deep fat frying (Carpenter, 1968; Ream, 1971).

Table 2.2 lists cooking losses using the same four methods of thermal processing. Cooking losses were significantly greater when using microwave cooking vs roasting (Kylen et al., 1964; Ream, 1971); oven-broiling (Carpenter et al., 1968; McCrae, 1973), and deep fat frying (Carpenter et al., 1968). When comparing microwave cooking to a convection oven, Cremer (1982) conducted a study in which trained sensory panelists scored beef patties heated in the convection oven higher for appearance, flavor and acceptability than patties cooked in a microwave.

Table 2.1. Shear force values (kN) as affected by method of cookery

Cooking method					
Microwave	Roasting	Oven-broiling	Deep fat frying	Muscle	Citation
3.8	3.2			beef longissimus	Berry (1974)[d]
4.4[a]		3.9[b]	3.5[c]	beef longissimus	Carpenter et al. (1968)[d]
4.2[a]	4.5[a]	4.4[a]		beef semi-tendinosus	McCrae (1973)[d]
6.8[a]	6.1[b]			beef longissimus	Ream (1971)[d]
7.8[a]	6.9[a]			beef longissimus	Taylor (1969)[d]

[abc] Means in the same row bearing different superscripts are significantly different ($P < 0.05$).
d as referenced by Berry (1975)

Table 2.2. Total cooking losses as affected by method of cookery

Cooking method					
Microwave	Roasting	Oven-broiling	Deef fat frying	Muscle	Citation
27.5	23.0			beef longissimus	Berry (1974)[c]
35.0[a]		30.4[b]	29.1[b]	beef longissimus	Carpenter et al. (1968)[c]
28.3[a]	28.2[a]	23.7[b]		beef semi-tendinosus	McCrae (1973)[c]
31.7[a]	17.5[b]			beef longissimus	Ream (1971)[c]
39.4	27.6			beef longissimus	Taylor (1969)[c]
38.8[a]	17.5[b]			beef longissimus	Kylen et al. (1964)[c]

[ab] Means in the same row bearing different superscripts are significantly different ($P < 0.05$).
[c] as referenced by Berry (1975)

Precooking Effects on Ground Beef Patties

The meat industry produces precooked ground beef patties which are heated and served by institutions such as schools. Some advantages which have been claimed are reduced cooking time prior to serving along with lower cooking losses, and greater versatility. Cross et al. (1979) tested this theory in their study which produced 7 formulations (some with soy) of ground beef patties commercially processed. Results revealed that frozen patties whether precooked or raw required approximately the same amount of time to reach an internal temperature of 55°C. Also, patties which were precooked, frozen and reheated were tougher and drier and lost more weight during processing than those not precooked.

Joseph et al. (1980) evaluated precooked ground beef patties using two kinds of commercial precooking broilers (one dry heat, one moist heat) to thermal process ground beef patties and compare the impact on physical, chemical and sensory attributes of each method. Patties were evaluated both by a trained and consumer panel. Precooked patties were lighter in frozen weight and smaller in diameter than raw patties and could be transported at less cost. When served, precooked/frozen/reheated patties sustained greater total weight loss. It was concluded that precooking of ground beef patties resulted in increased moisture retention but increased fat loss during final heating of product. When cooked in a moisture-controlled oven broiler, there was decreased flavor desirability. There was also increased tenderness of heated patties (unlike Cross et al, 1979) but no apparent effect on amount of detectable connective tissue, flavor intensity or ready-to-serve appearance.

Tests have also been conducted by Cross and Berry (1980) to determine if the addition of frozen lean to patty formulations or subsequent surface perforation affected the palatability or the cooking properties of ground beef patties. Results indicated that

the addition of frozen lean at 40% level had no important effects on ground beef palatability and surface treatment of the patty added no advantage.

Texture : Definition

Texture has been defined in numerous ways depending on the object being described. Even when speaking specifically of food, the meaning of texture varies when speaking of vegetables, candy or meat. One approach more specific than others, was referred to by Szczesniak (1963) who quoted Ball et al. (1957). Ball et al. divided texture of meat into "sight" and "feel" definitions. The "sight" definition reads "Texture of meat is the microscopic appearance of muscle from the standpoint of smoothness or firmness of grain..." The "feel" definition is worded: "The texture of cooked meat is the feel of smoothness or fineness of the muscle tissue in the mouth.'". Szczsniak (1963) referred to the mechanical characteristic of texture as the reaction of food to stress.

Measurement of mechanical characteristics have been accomplished by several methods including the Warner-Bratzler shear and Kramer shear which enjoy more popularity than other methods. Studies have been conducted to determine how accurately mechanical shear force measurements relate to tenderness as preceived by a trained sensory panel.

A study was conducted by Burrill et al. (1962) using five methods of measuring tenderness in beef muscle, Warner-Bratzler shear, Kramer shear maximum force, Kramer shear total work, taste panel scores and panel chews. Highly significant correlation coefficients were found in every case for all the various combinations used to test tenderness. The correlation coefficient between taste-panel scores and Warner-Bratzler shear was -0.83; between taste panel and Kramer maximum force was -0.72. (the difference not being significant). Cross et al. (1978) also reported shear force readings

which were highly correlated with sensory evaluations; however, they were evaluating ground beef patties with a Instron Universal testing machine.

Segars et al. (1975) developed a punch and die test cell for the Instron Universal Testing machine which applied shearing deformation to the meat sample which the Instron force-measuring system recorded. Six whole muscles from U.S. Choice and U.S. Commercial grade animals were used in the evaluations which were conducted by a trained sensory panel and the new test cell. Correlation coefficients of the sensory attributes and instrumental properties ranged from 0.92 to 0.98.

In a recent study conducted by Beilken et al. (1991) compression, shear tensile and punch tests were used to assay a ground meat product and determine the effect of various treatments. These measurements, along with chemical and moisture retention were related to textural attributes. Most of the sensory attributes (80%) were described by a combination of Warner-Bratzler shear and compression measurements.

Conclusion

Ground beef patties are an integral part of the every day diet in the United States. Research must be conducted to ensure the microbiological safety of ground beef patties from recent food borne pathogens such as *E. coli* O157:H7. Rather than allow a public scare to decrease consumption of this mainstay of the American diet, proper research must be conducted which takes into consideration the biological characteristics of *E. coli* O157:H7, and considers the changes these characteristics might under go in inoculated ground beef patties of varying fat formulations. In time, a relationship may be determined which will insure the thermal inactivation of *E. coli* O157:H7 without sacrificing the quality characteristics consumers expect in ground beef patties.

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CHAPTER III

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

Abstract

Ground beef patties (112g) were inoculated with *Escherichia coli* O157:H7, placed in plastic bags and cooked in water to determine the D value at 57°C, 60°C and 63°C. Using this model system, the D value was found to be 168 sec at 57°C, 26 sec at 60°C and 11.6 sec at 63°C. Thermal death times were subsequently determined at 60°C, 63°C and 66°C for two inoculum levels (10^3 CFU/g, 10^5 CFU/g) in low fat (2-5%) and high fat (24-32%) beef patties. Holding times equivalent to an 8D cook at 60°C, 12D cook at 63°C and 16D cook at 66°C were necessary to inactivate a 10^3 CFU/g inoculum of *E. coli* O157:H7 in high fat patties. At an inoculum level of 10^5 CFU/g, holding times equivalent to a 35D cook at 60°C, 44D cook at 63°C and 65D cook at 66°C were necessary to inactivate *E. coli* O157:H7 in high fat patties. Low fat patties required longer holding times than high fat patties. These findings indicated an increased survival of *E. coli* O157:H7 over extended holding times. Ground beef was eliminated as the basis for the increased survival when similar results were obtained using growth medium. Cells treated under identical thermal processing conditions resulted in the synthesis of heat-shock proteins. Therefore, the survival of *E. coli* O157:H7 over holding times significantly longer than the D values would have predicted was due to an increase in heat resistance. These results must be taken into consideration when predicting the

microbiological safety of precooked beef patties.

Introduction

Escherichia coli O157:H7 has been implicated in several foodborne outbreaks of hemorrhagic colitis associated with the consumption of ground beef *(12,15,16)*. Public and government concerns for the microbiological safety of precooked beef patties led the Food Safety and Inspection Service to consider a required endpoint temperature for precooked beef patties of 71.1°C. The effects of a mandatory endpoint temperature considerably higher than the one commonly used by industry (60° to 66°C) would be decreases in palatability and yield caused by decreases in product moisture and increased toughness and dryness *(1,2,8,13)*.

Little information is available on the distribution and survival of *E. coli* O157:H7 or other enterohemorrahagic serotypes in fresh and processed retail beef products. Doyle and Schoeni examined the distribution of this organism in fresh ground beef and reported that 6 (3.7%) of 164 beef samples assayed contained *E. coli* O157:H7 *(4)* . More recently, Read et al. reported that 11% (25 of 225 samples) of ground beef samples contained the organism *(14)*.

Research has been conducted to determine the survival of *E. coli* O157:H7 at various temperatures in thermally processed ground beef. Work to date involved determining the decimal reduction time (D value), the time required to destroy 90% of the *E. coli* cells in ground beef. Doyle and Schoeni used 1 gram samples of inoculated ground beef and determined the D value at six different temperatures *(3)*. They found *E. coli* O157:H7 to be more heat sensitive than *Salmonella.* However, the samples examined in this study were very small and do not represent an equivalent thermal transfer process for the typical industry ground beef pattie (112 gram). To date, no reports have been published

to confirm that these D values would inactivate a known level of *E. coli* O157:H7 in ground beef.

The objectives of this study were to establish D values for *E. coli* O157:H7 in ground beef patties and confirm that the D values generated would actually inactivate a known level of *E. coli* O157:H7 inoculated into ground beef patties. These results will be used to determine the holding times necessary to inactivate E. coli O157:H7 in ground beef patties at endpoint temperatures less than 71.1°C.

Materials and Methods

Bacterial strains and culture conditions.

E. coli O157:H7 ATCC #43895 was obtained from the American Type Culture Collection (Atlanta, GA). This strain was isolated from raw ground beef implicated in a hemorrhagic colitis outbreak. The culture was stored at -75°C (Queve Cryostar freezer) and was routinely grown in Luria broth (LB)*(7)*. Inocula for experiments consisted of cells grown to mid-log phase at 37°C with continuous shaking @ 100 rpm (Lab-Line® Orbit Environ-shaker, Melrose Park, IL.). Cells were harvested by centrifugation (Beckman J2-MC centrifuge; Beckman Instruments Inc., Palo Alto, CA) at 13,000 x g for 10 minutes, washed, and resuspended in fresh LB to the desired cell density (10^5, 10^7 or 10^9 colony forming units (CFU)/ml, determined by plating on LB agar) prior to inoculation of ground beef.

Inoculation methods for ground beef patties.

In initial experiments, a small volume of the *E. coli* inocula was added to ground beef trim (12 to 17% fat) to obtain the desired inoculum level. The inoculum was dispersed by adding the culture a drop at a time over the ground beef trim. Following the addition of the inoculum, the beef was mixed (Leland ribbon-paddle mixer), then ground twice through a 0.32cm plate (grinder model 5424852, Biro Mfg. Co, Marblehead, OH)

in an attempt to ensure even distribution of the inoculum. Patties of 112 grams were then formed (Hollymatic Super pattie machine, Chicago, IL). This method did not distribute the inoculum evenly in the patties (preliminary study) so a spray method was developed and subsequently used in which twenty eight (112g) ground beef patties (12 to 17% fat) were inoculated with *E. coli* O157:H7 by spraying each side twice with the *E. coli* inocula (.75 ml/spray). Following inoculation, patties were reground and reformed into patties (112g). Final inoculum levels were confirmed by sampling a raw patty and plating appropriate dilutions on MacConkey Sorbitol Agar [(MSA) Oxoid, Unipath Limited, Hampshire, England].

D values of E. coli O157:H7 in ground beef patties.

Inoculated patties were placed in plastic bags (15 x 21cm), (KOCH, Kansas City, MO) and heated in a shaker water bath at 40 rpm (model BKS-350, Gallenkamp & Co., Sussex, England) to internal temperatures of 57°C, 60°C and 63°C. Internal temperatures were monitored using copper constantan thermocouples (model OM 302 Temperature logger; Omega Engineering Inc., Stanford, CT). Nine patties were thermally processed per temperature. Once the initial temperature was reached, one patty was removed to determine the inactivation at the come-up-time, two more were removed for time 0 and immediately frozen in liquid nitrogen to represent industry's Individually Quick Frozen (IQF) technique. Duplicate patties were removed at appropriate intervals for 57°C (135 sec), 60°C (23 sec) or 63°C (12 sec) *(3)*. All patties were quick frozen and stored frozen for subsequent microbiological analyses (Frigidaire deluxe freezer, Sears, Chicago, IL).

Surviving *E. coli* were determined by serially diluting three samples (11g) per pattie in 0.1% peptone (Difco Laboratories, Detroit, MI) and plating appropriate dilutions on MSA. Following incubation at 37°C for 24 hrs, typical colonies (white, opaque, raised) were counted and serologically confirmed as *E. coli* O157:H7 using an Oxoid latex agglutination kit (Unipath Limited, Hampshire, England). Duplicate tests were

conducted for each holding time and temperature treatment. These results were plotted to determine the thermal inactivation curve for each temperature from which a D value was determined.

A resuscitation or recovery procedure *(3)* and a hydrophobic grid membrane filter (HGMF) technique reported by Todd et al. *(19)* were initially compared to the direct plating procedure described above to determine the most reliable method for enumeration of *E. coli* O157:H7 from precooked ground beef patties. All typical colonies were confirmed as *E. coli* O157:H7 using an Oxoid latex agglutination kit specific for the O157:H7 serotype.

Thermal inactivation of E. coli O157:H7 in ground beef patties.

In experiments to determine the thermal death times, patties were formulated for two fat levels (high 29% ±5 and low 3% ±2), inoculated at two different inoculum levels (10^3 CFU/g and 10^5 CFU/g) and cooked to three different endpoint temperatures of 60°C, 63°C and 66°C. Each endpoint temperature by fat combination was subjected to a series of holding times to inactivate a known level of *E. coli* O157:H7. The thermal inactivation procedure consisted of placing patties (n=12) in plastic bags and heating in a shaker water bath set at 40 rpm to the desired internal temperature (determined by thermocouples in each pattie). The time necessary to reach the desired internal temperature was 10 min (±5 min.). Two patties were removed at each of the six different holding times; the purge was removed and the patties were placed in an ice slurry. The thermal process was duplicated for each fat level, inoculum level and endpoint temperature. Patties were not IQF frozen as before because microbiological analyses were conducted immediately. No difference in survival of *E. coli* O157:H7 between patties frozen and those chilled in an ice slurry was detected. Thermal inactivation procedures were repeated 16 times (increasing holding times each repetition) before thermal death times were achieved that inactivated the inoculum level. The data from two replications of each fat level, inoculum level and temperature were reported.

The presence of any surviving *E. coli* cells was determined by macerating a sample (12.5g) from each chilled pattie with 112.5 ml of modified *E. coli* (mEC) broth *(11)* in a Seward stomacher (Tekmar Company, Cincinnati, Ohio). After stomaching, the sample was transferred to a 250ml Erlenmeyer flask and incubated 24 hours at 37°C (VWR Scientific Inc. Model 3020, Sugar Land, TX). Duplicate samples (12.5g) were analyzed for each pattie. Following enrichment, appropriate dilutions were plated in duplicate on MSA. Plates were incubated at 37°C for 24 hours. Typical colonies on MSA plates were streaked on Eosin-methylene blue agar [(EMB) Oxoid, Unipath Limited, Hampshire, England] and incubated for 24 hours. Typical *E. coli* colonies were serologically tested for the O157:H7 serotype using an Oxoid latex agglutination kit. Duplicate tests were conducted for each temperature and holding time combination.

^{35}S labeling of cells and SDS-Page of proteins.

E. coli O157:H7 was grown to mid-log phase cells in LB broth and harvested by centrifugation at 13,000 x *g* for 10 minutes. To prepare the inoculum for labeling studies, cells were washed and resuspended in 100 ml of M9 broth (+ amino acids and Vitamin B1)*(9)*. Three tubes containing 10 ml of M9 broth containing ^{35}S methionine (20 mCi/ml) were inoculated with 1 ml of the *E. coli* O157:H7 inoculum to achieve 10^8 CFU/ml and immediately thermal processed at 37°C for 25 min; 60° and 66°C for 10 min (plus a 9 min Come-Up-Time) in a shaker water bath (40 rpm). This procedure mimicked the thermal processing conditions previously used. Following heat treatment, cells were harvested by centrifugation and frozen (-20°C).

Frozen cells labeled with ^{35}S were thawed, mixed with an equal volume of 10% trichloroacetic acid. The precipitates were collected by centrifugation and washed twice with cold 5% trichloroacetic acid and once with acetone. The precipitates were dissolved in 200ml of sample buffer (0.0625 M Tris [pH 6.8], 2% sodium dodecyl sulfate, 10% glycerol, 10mM dithiotreitol, 0.001% bromophenol blue) and heated at 100°C for 3 min *(20)*. Proteins were separated using a Bio-Rad Mini-Protean II SDS polyacrylamide gel

electrophoresis (Bio-Rad Laboratories, Richmond CA) with a discontinuous buffer system described by Laemmli *(6)*. Slab gels were used, 10% acrylamide as the separation gel and 5% acrylamide as the stacking gel. SDS-Page molecular weight standards of 14,400 to 97,400 daltons were used. Gels were stained with Coomassie Blue stain (Mini-Protean II Dual Slab Cell, Bio-Rad Laboratories, Richmond, CA). After staining, gels were rinsed under running water for one hr and fixed by rocking in a solution consisting of 32.02g sodium salicylate, 1ml glycerol and 200ml dH_2O for 30 min (Rocker Platform, Bellco Biotechnology, Vineland, NJ). After drying in an Easy Breeze™ SE 1200 drying frame (Hoefer Scientific Instruments, San Francisco, CA) for 2 hours at lowest heat setting, gels were exposed on Kodak XAR film for 2 days at -75°C.

Results

Inoculation and enumeration methods for ground beef patties.

Initial results comparing inoculation techniques indicated that the method of inoculation affects the distribution of the cells. Ground beef inoculated a drop at a time to a level of 10^6 CFU/g resulted in a even distribution of the cells. However when ground beef was inoculated to 10^8 CFU/g using this same technique, an uneven distribution of cells resulted. Additional grinding of the ground beef did not thoroughly disperse the cells and improve the uniform distribution of the inoculum. Larger volumes of inocula would have dispersed the *E. coli* more uniformly, but the integrity of the patties would have suffered. The spray technique was devised as a means of inoculating pre-formed patties to reduce the uneven distribution of the inoculum. The spray technique was found to distribute the inoculum more evenly at low and high inoculum levels as determined from plate counts of inoculated, raw patties as shown in Table 3.1.

Initial experiments were also conducted to determine the most reliable method for the enumeration of *E. coli* O157:H7 from precooked ground beef patties. The HGMF

technique was effective for enumerating *E. coli* when high counts were present. However, when lower numbers of cells were present and low dilutions had to be plated, the prefilter and filter were often blocked with meat fibers. This impeded the efficient and rapid plating of samples and therefore preventing us from using this procedure. Comparison of a direct plating procedure on MSA to a resuscitation plating procedure indicated that plate counts of precooked beef patties did not increase with the resuscitation plating on Trypticase Soy Agar. In fact, higher counts were obtained with direct plating on MSA as shown in Table 3.2. This procedure was chosen as the enumeration technique for recovery of *E. coli* from precooked beef patties.

D values in ground beef patties.

The thermal inactivation of *E. coli* O157:H7 at 63°C in ground beef patties (12 to 17% fat) is shown in Figure 3.1. Thermal inactivation curves indicated at all three temperatures (57°C, 60°C, 63°C) that the D values were approximately one-half those previously reported by Doyle and Schoeni *(3)*. At 57°C, the D value was found to be 168 sec compared to 270 sec reported previously. At 60°C, the D value was found to be 26 sec compared to 45 sec reported previously and at 63°C the D value was found to be 11.6 sec compared to 24 sec reported previously.

Thermal death times (seconds necessary to kill a given number of organisms at a specified temperature) were established for 10^3 CFU/g and 10^5 CFU/g inoculum levels in low and high fat ground beef patties and are shown in Table 3.3. The thermal death times were much longer than expected using the D values determined in this study. At a 10^3 inoculum level, high fat patties required 3.67 min at 60°C, 2.34 min at 63°C and 1.34 min at 66°C. At a 10^5 inoculum level, high fat patties required 15 min at 60°C, 8.58 min at 63°C and 5.42 min at 66°C. Low fat patties required longer holding times than high fat patties at both inoculum levels. At 10^5 CFU/g, in low fat patties the holding times became impractical (25 min at 60°C, 22 min at 63°C and 14.42 min at 66°C) for

industry conditions and were not conclusively determined. Lower temperatures (60°C) required longer holding times than higher temperatures (63°, 66°C).

To determine the predicted level of heat inactivation of *E. coli* O157:H7, the actual time necessary to inactivate the inoculum level (10^3 or 10^5 CFU/g) was divided by the D values to obtain a D cook value or D kill. The results obtained using this approach are shown in Table 3.4. At a 10^3 inoculum level in high fat patties an 8D cook was required at 60°C, a 12D cook at 63°C and a 16D cook at 66°C. High inoculum levels (10^5) in high fat patties required 35D cook at 60°C, 44D cook at 63°C and 65D cook at 66°C. Low fat patties required longer D cooks than high fat patties.

Temperature-induced protein synthesis.

Gel electrophoretic patterns of heat shock proteins synthesized in *E. coli* O157:H7 cells which were heat treated are presented in Figure 3.2. Similar protein bands of 90, 66 and 30kDa were shown at 60°and 66°C with a heavier concentration of heat shock proteins being observed at 60° than 66°C. Eight different proteins induced by the heat treatment were observed at 60° and 66°C and range in size from 95 to 30kDA.

Discussion

The spray technique for inoculating patties was an improvement over the drop by drop inoculation of ground beef trim; however, the distribution of the inoculum in the ground beef patties was still not completely homogeneous. This inability to distribute inoculum evenly in a 112g ground beef pattie can present a problem. When inoculating small batches of ground beef (1g) the entire batch may be used to form a sample for microbiological analysis; however, a 112g pattie is too large to form a single sample. Therefore, consideration must be given to sample size, number and location to obtain a true indication of the numbers of *E. coli* O157:H7 present throughout the pattie.

The HGMF technique was effective for enumerating *E. coli* O157:H7 from precooked meat samples when plating high dilutions; however, at low dilutions, meat fibers blocked the prefilter causing the HGMF technique to be a labor intensive, time consuming means of enumeration. Due to the time restraints imposed by the volume of samples, this procedure was not used. Resuscitation or recovery plating was also considered because it allows injured cells in a sample time to repair before selective medium is added. This may allow for the enumeration of injured cells that may survive in a food sample. However, no benefits were observed with resuscitation plating on TSA. This may be due to oxidative stress on the heat treated *E. coli* O157:H7 cells which were incubated aerobically.

Differences in D values between this study and Doyle and Schoeni *(3)* may be a reflection of the differences in sample size (1g vs 112g). Larger samples take longer to reach the internal end point temperature than smaller samples. This would result in an increase in the latent thermal inactivation and a decrease in the number of survivors. Also Doyle and Schoeni *(3)* used stationary phase *E. coli* O157:H7 while this study used mid-log phase. Mid-log cells may be more susceptible to stress than stationary phase cells. Also Doyle and Schoeni used a different strain of *E. coli* O157:H7 which may have been more heat resistant than the one used in this study *(3)*.

The thermal death times for *E. coli* O157:H7 are presented in Table 3.3. and indicate that as the level of inoculum increased the time necessary for inactivation increased. Also the lower the fat content, the longer the holding time necessary to inactivate the *E. coli* O157:H7. This may be explained by the decreased rate of heat transfer through lean as opposed to fat tissue.

When predicting the level of inactivation, the results indicated the D values determined in the first study were not useful in determining the length of time necessary to inactivate 10^3 CFU/g or 10^5 CFU/g inoculum levels of *E. coli* O157:H7 in ground beef patties. In practice, the thermal death time predicted for a 10^3 CFU/g inoculum

level would be four times the D value, a thermal process adequate to kill 999.9 cells. This can be referred to as a 4D cook. When referring to Table 3.4., a predicted 4D cook (10^3 inoculum level) in high fat patties actually was a 8D cook at 60°C, a 12D cook at 63°C and a 16D cook at 66°C. At a 10^5 inoculum level in high fat patties, a predicted 6D cook was a 35D at 60°C, a 44D cook at 63°C and 65D cook at 66°C. At both inoculum levels, low fat patties required longer D cooks than high fat patties. As shown in Table 3.4., an increase in temperature meant a decrease in the ability of the D value to predict the thermal death time. These findings indicate an increased resistance or survival ability on the part of *E. coli* O157:H7 with increasing temperatures.

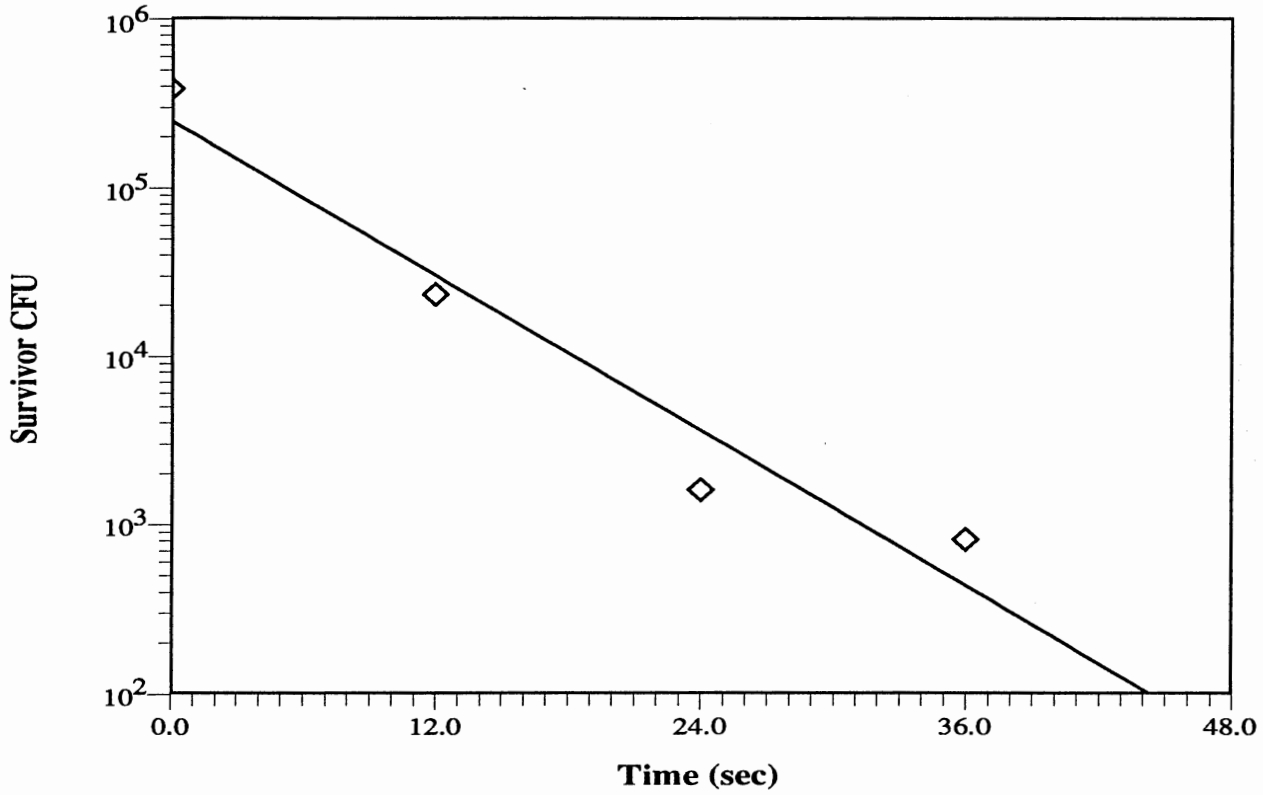
On June 5, 1990, the Food Safety Inspection Service (FSIS) of the US Department of Agriculture proposed a 5D process be required in cooking uncured beef patties, based on a study by Goodfellow and Brown on *Salmonella (10)*. In this proposal, the FSIS recommends that a minimum temperature of 66°C for 41 sec be required to achieve the 5D kill process *(5)*. Since *E. coli* O157:H7 implicated in outbreaks has been recovered from ground beef at 10^2 CFU/g and from baby beef at levels ranging from 10^1 to 10^3 CFU/g *(19)*, a 5D process would be considered appropriate. Also, Doyle and Schoeni *(3)* reported *E. coli* O157:H7 to be more heat sensitive than *Salmonella* under the same conditions. However; our study indicated (Table 3.4.) a 16D cook was necessary to inactivate a 10^3 inoculum level in high fat patties at 66°C. A longer D cook (20D) was required for low fat patties. Therefore, the 5D kill process proposed by the FSIS is not adequate to inactivate the levels of *E. coli* O157:H7 reported as recovered from ground beef and baby beef.

This study has shown that *E. coli* O157:H7 can survive at high temperatures over extended holding times. This phenomenon may have been attributed to an insulating effect created by the ground beef which protected the inoculum from the effects of the heat treatment. However that theory was eliminated as the basis for the heat resistance when similar results were obtained using growth medium and identical thermal

processing conditions. Many bacterial strains have been reported to survive at high temperatures due to the production of heat shock proteins *(17)*. These heat shock proteins play a key protective role in supporting normal cell growth and survival at extreme temperatures. Murano and Pierson *(10)* reported a heat shock protein (71kDa) immunologically similar to sigma^{32} subunit of RNA polymerase which was produced by *E. coli* O157:H7 in response to temperatures up to 55°C. Yamamori and Yura *(20)* also reported coordinate induction of three heat-shock proteins (76, 73, and 64kDa) (probably RNA polymerase, sigma^{32}). Sherman and Goldberg reported a major protein of 60kDA and minor components of 100, 90, 40 and 30kDa *(18)*. They identified the 90kDA protein as protease La, a known heat shock protein. Protein bands of similar size (90, 66 and 30kDa) were found at both heat treatments (60°and 66°C) in this study.

In conclusion, D values were established for *E. coli* O157:H7 in ground beef patties. From the D values, holding times were established which inactivated *E. coli* O157:H7 at temperatures lower than 71.1°C. These holding times were longer than the D values would have predicted and were influenced by the level of fat in the beef pattie. This study indicates that the practice of predicting the thermal death time for a microorganism by multiplying the D value by the inoculum level is not a true indicator of the temperature and holding time necessary to inactivate *E. coli* O157:H7. Since consumption of low fat ground beef is rising, the microbiological safety of the product must be addressed. Further research is currently underway to determine if the thermal death times established in the laboratory on a small scale using one method of thermal processing (water bath) will transfer to an industry setting where other methods of thermal processing are used and patties are processed on a large scale.

Figure 3.1 Thermal inactivation curve of E. coli O157:H7 at 63 C in cooked ground beef patties



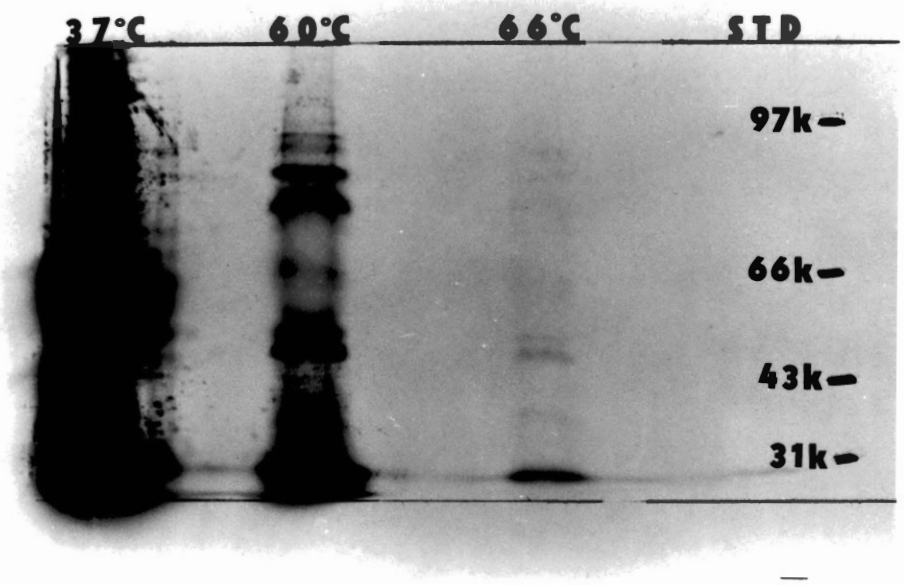


Figure 3.2. Audiogradiogram of SDS Polyacrimide Gel Electrophoresic separation of *E. coli* O157:H7 heat shock proteins labeled with ^{35}S methionine.
(labels indicate molecular wt markers in kDaltons)

Table 3.1. Percent *E. Coli* O157:H7 recovered from ground beef patties using two inoculation methods.[a]

	Inoculation level	
Method	10^6	10^8
Spray[b]	43.3%	33.3%
Drop[c]	10.3%	4.0%

[a] appropriate dilutions plated on MSA agar.
[b] Six patties (12 to 17% fat) were sprayed with *E. coli* O157:H7; three with a 10^6 CFU/ml inoculum and three with 10^8 CFU/ml inoculum (1.5ml/patty).
[c] Two 336 g samples of ground beef were inoculated with 4.5 ml of *E. coli* O157:H7 each. One with 10^6 CFU/ml inoculum and the other 10^8 CFU/ml inoculum. The samples were formed into patties.

Table 3.2. Recovery of *E. coli* O157:H7 (CFU/g) from precooked beef patties.

	Resuscitation (TSA/MSA[a])	Direct Plating (MSA[b])
Trial 1		
patty 1	$1.0x10^1$	$<1x10^1$
Trial 2		
patty 1	$<1.0x10^1$	$1.2x10^2$
patty 2	$<1.0x10^1$	$1.0x10^2$

[a] Overlay of MSA (MacConkey Sorbitol Agar) on TSA (Trypticase Soy Agar).

[b] MSA direct plating procedure

Table 3.3.Thermal death times (min) necessary to inactivate a given inoculum level of *E. coli* O157:H7 in ground beef patties.[a]

Inoculum Level	Fat Level[b]	Temperature (°C) 60	63	66
10^3	high	3.67	2.34	1.34
(CFU/g)	low	5.0	2.83	1.67
10^5	high	15.0	8.58	5.42
(CFU/g)	low	25.0	22.0	14.42

[a] times reflect maximum values of two replications
[b] high fat (24-32%); low fat (2-5%)

Table 3.4. Calculated D value cook[a] times necessary for thermal inactivation of *E. coli 0157:H7* at two inoculum and fat levels.

Fat level[b]	Inoculum level[c]	Endpoint Temperature (°C) 60	63	66[d]
High	10^3	8D	12D	16D
High	10^5	35D	44D	65D
Low	10^3	12D	15D	20D

[a] thermal death time (sec)/ D value (sec) = D cook
[b] high fat (24-32%); low fat (2-5%)
[c] CFU/g
[d] D value extrapolated (Doyle and Schoeni, 1984)

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CHAPTER IV

FATE OF *ESCHERICHIA COLI* O157:H7 IN THERMALLY PROCESSED GROUND BEEF PATTIES

Abstract

Ground beef patties were formulated to 10, 20, and 30% fat and inoculated (10^3 CFU/g) with *Escherichia coli* O157:H7. Patties were cooked to 60°, 66° and 71°C with appropriate holding times and frozen. No *E. coli* survived in the 66°C or 71°C thermally processed patties regardless of fat level. Yields and moisture significantly decreased and peak shear values increased as temperature increased. *E. coli* O157:H7 survived in 10 and 20% fat patties cooked to 60°C. Cooking to 66°C (+ holding time) resulted in a safe product with greater yields, moisture and tenderness than products cooked to 71°C.

Introduction

Escherichia coli O157:H7 has been implicated in several foodborne outbreaks of hemorrhagic colitis associated with the consumption of ground beef (Pai et al., 1984; Riley et al., 1983; Ryan et al., 1986). Public and government concerns for the microbiological safety of precooked beef patties has led the Food Safety and Inspection Service to consider a required endpoint temperature of 71°C for precooked beef patties. This temperature, higher than industry practice (60 to 66°C), would decrease palatability and yield (Bouton et al., 1981; Davey and Gilbert, 1974; Martens et al., 1982) in order to

control *E. coli* O157:H7. The importance and relevance of the D values (time required to destroy 90% of the bacteria) has been addressed by only Doyle and Schoeni (1984). However, only one gram of ground beef was used to estimate the survival/time relationship at six different temperatures, while the typical ground beef pattie weighs 112 g. In addition, samples were processed in a laboratory setting, not using industrial like practices. To date, no reports have been published using the D values generated by Doyle and Schoeni to establish thermal death times (holding time necessary to inactivate a known levels of *E. coli* O157:H7 inoculated into ground beef).

In a previous study (Shipp et al., 1992a), D values for *E. coli* O157:H7 were determined in ground beef patties and subsequently tested on inoculated patties to determine the thermal death times. The objectives of this study were to 1) determine the accuracy of the thermal death times previously generated in the laboratory and 2) establish the time and temperature relationships necessary to produce a microbiologically safe, palatable beef pattie under industry like conditions.

Materials and Methods

Bacterial strains and culture conditions

E. coli O157:H7 #43895 was obtained from the American Type Culture collection (Atlanta, GA). This strain was isolated from raw ground beef implicated in a hemorrhagic colitis outbreak. The culture was stored at -75°C (Queve Cryostar freezer) and was routinely grown in Luria broth [(LB) Difco Manual, Detroit, MI] at 37°C. To ensure the use of a consistent, synchronous culture the growth curve of *E. coli* O157:H7 was determined in LB broth incubated at 37°C with continuous shaking @ 100 rpm (Lab-Line Orbit Environ-shaker, Melrose Park, IL). Inocula for experiments consisted of mid-log phase cells harvested by centrifugation at 13,000 x *g* for 10 min (J2-MC

Beckman, Palo Alto, CA). Cells were resuspended in fresh LB to 10^5 Colony Forming Units (CFU)/ml prior to inoculation into ground beef.

Formulation of patties

Bull carcass trim (frozen 3 mo) and commercial trimmings (50% fat, frozen 3 mo) were used to achieve three 11.4 kg batches at fat levels of 10, 20 and 30% and a batch size of /fat level. Each formulation was ground (.32cm plate, Biro Mfg. Co, Marblehead, OH) and patties (112g) were formed (Hollymatic Super pattie machine, Chicago, IL) and inoculated by spraying with *E. coli* O157:H7 (.75 ml/spray) to achieve 10^3 CFU/g. Patties were subsequently reground and reformed into patties.

Cooking procedure

Three replications of ground beef patties were cooked to three different endpoint temperatures of 60°, 66° and 71°C. The thermal process consisted of cooking the patties using an impingement oven (model 1022, Lincoln Food Service, Ft. Wayne, IN) at 218°C varying conveyor speeds to achieve the desired endpoint temperature of 60°, 66° or 71°C (conveyor speed/endpoint temperature determined in a preliminary study). Patties were removed aseptically from the oven, placed on trays and held in a convection holding oven (Jero Thermaflo Cook & Hold, Tulsa, OK) for the appropriate time and temperature. Table 4.1 presents the holding times previously determined by Shipp et al., 1992b. After freezing in a liquid nitrogen bath (separate bath per fat and temperature treatment), the patties were packaged in plastic bags and stored (-18°C) for microbiological, proximate and texture analyses. Cooking yields were calculated by dividing the weight of the cooked patties by the raw patties.

Isolation and Detection

The presence of any surviving *E. coli* cells was determined by macerating a 12.5g sample from each pattie (thawed, 4 hrs, 21°C) with 112.5 ml of modified E. coli (mEC) broth (Okrend et al., 1990) in a Seward stomacher (model 400, Tekmar Company, Cincinnati, OH). After blending, samples were transferred to flasks and incubated (static,

24 hrs, 37°C)(model 3020 VWR Scientific incubator, Sugar Land, TX). Duplicate samples (12.5g) were analyzed for each pattie. Following enrichment, appropriate dilutions were plated (in duplicate) on MacConkey Sorbitol Agar [(MSA) Oxoid, Unipath Limited, Hampshire, England]. MSA plates were incubated at 37°C (24 hrs). Typical colonies on MSA plates (white, opaque, raised) were streaked on Eosin-methylene blue agar [(EMB) Oxoid, Unipath Limited, Hampshire, England] and incubated (24 hrs). Each typical *E. coli* response on EMB (green metallic sheen) was serologically tested for the O157:H7 serotype using an Oxoid latex agglutination test (Unipath Limited, Hampshire, England). Four patties were analyzed from each fat x temperature treatment combination group (180 total patties, 36 patties for microbiological analysis).

Proximate analysis

Fat determinations were made on bull carcass trim and commercial trimmings prior to formulation of patties using the Babcock procedure. Moisture, fat and protein determinations were determined using six cooked patties per fat x temperature treatment according to AOAC (1984) procedures.

Instron Shear Measurements

Ten thawed (4 hrs, 21°C) patties for each fat x temperature treatment were analyzed. A 4 cm^2 section was removed from each pattie and peak shear force was recorded as it was sheared by a L.E.E.-Kramer (Voisey, 1977) attachment to an Instron Universal Testing Machine (Model 4502, Canton, Mass.).

Statistical analyses

Data from the three replications of the three cook temperatures by three fat formulation treatments (3x3 factorial) were analyzed using analysis of variance and multiple regression (Steel and Torrie, 1980) with the Statistical Analysis system (SAS, 1988).

Results and Discussion

Cooked pattie yields as affected by temperature are shown in Figure 4.1. Yields decreased from 74.7% to 65.28% as endpoint temperature increased from 60°C to 71°C ($P<0.05$). Troutt et al. (1992) also showed increased cooking losses with an increase in endpoint temperature in ground beef patties. Cooked pattie yields as affected by fat level are presented in Figure 4.2. Yields also decreased significantly as the fat level increased from 10 to 30% fat. Cross et al. (1980) reported an increase in fat loss during cooking associated with an increase in the percent fat in the raw pattie. Troutt et al. (1992) also reported highest cooking losses for 30% fat patties and lowest for 5 to 20% fat patties.

Shear force values for cooked patties as affected by cook temperature are shown in Figure 4.3. The peak shear values increased significantly (0.60 to 0.88 kN), in a curvilinear fashion, as the endpoint temperature increased from 60° to 71°C. This same trend was observed by Troutt et al. (1992) who reported that patties cooked to 77°C had higher shear force and total energy values than did those cooked to 71°C. Shear force values for cooked patties as affected by fat level are presented in Figure 4.4. The peak shear force decreased significantly (0.88 to 0.71 kN) as the fat level increased from 10 to 30% fat. Cross et al. (1980) reported that shear force and shear energy values decreased as fat level increased from 16 to 28% fat. Shear force values correspond to sensory traits such as tenderness and Cross et al. (1980) revealed that patties formulated to 16% fat were significantly tougher than patties containing 24 to 28% fat. Berry and Leddy (1984) also reported the same trend when their texture profile evaluation of ground beef patties indicated higher tenderness values associated with higher fat levels.

Figure 4.5 indicates the effect of endpoint temperature on the percent moisture in ground beef patties. Figure 4.6 indicates the effect of fat level on the percent moisture in cooked ground beef patties. As expected, moisture decreased significantly (60.34 to 58.32%) as endpoint temperature and fat level increased (62.83 to 57.27%). Troutt et al.

(1992) also showed a decrease in moisture as fat increased (5 to 30%) and temperature increased (71° to 77°C). Overall, these findings are in agreement with Berry (1992) who reported that shear force increased as fat levels decreased in ground beef patties. He also observed decreases in tenderness and juiciness as well.

The interation between cook temperature and fat level on the percent fat in the cooked pattie is shown in Figure 4.7. Patties with 10 and 20% fat formulations were not affected ($P<0.05$) but patties with 30% fat show a curvilinear relationship which peaks at 60°C and then decreases as the temperature increases. These findings could be attributed to an increase in fat loss during cooking associated with an increase in the percent fat in the raw pattie (Cross et al., 1980)

The interation between cook temperature and fat level on the percent protein in the cooked patties is shown in Figure 4.8. All three fat levels show a linear response which increases as the temperature increases, with 30% fat patties showing the sharpest increase. These changes in pattie composition are the inverse of the trends shown in the yield data. Yields decreased as cook temperature and fat level increased indicating losses in the pattie are associated with moisture and fat, not protein, and are in agreement with the reported findings of Troutt et al. (1992) and Cross et al. (1980).

Survival of E. coli O157:H7

As shown in Table 4.2., no *E. coli* O157:H7 was detected in the 30% fat formulation at internal endpoint temperatures of 60, 66 or 71°C. Furthermore, *E. coli* O157:H7 was not detected in the 10 or 20% fat formulations at internal endpoint temperatures of 66 and 71°C. However, *E. coli* O157:H7 was detected in two of three replications of the 10% fat formulation and one replication of the 20% fat patties cooked to an internal endpoint temperature of 60°C with appropriate holding times. This failure of the thermal death times to inactivate the *E. coli* O157:H7 in 10 and 20% fat formulations cooked to internal temperatures of 60°C suggests possible problems in transferring laboratory results directly to industry conditions. Three possible reasons for

this are: 1) heating method, 2) endpoint temperature and 3) heat shock proteins. Differences in the heating method (water bath vs impingement oven) and resulting differences in the rate of heat transfer may be a possible explanation. Individual internal temperatures were monitored in the water bath in the laboratory method (Shipp et al. 1992b) while individual temperatures were not monitored in the impingement oven. This possible variability of endpoint temperature in patties processed under industry conditions needs to be taken into consideration when applying thermal death times established in a laboratory.

Another possible explanation for survival is the biological characteristics of the *E. coli* microorganism. It has been shown to survive at high temperatures due to the production of heat shock proteins (Kusukawa and Yura, 1988). Murano and Pierson (1990) verified the production of heat shock proteins by the O157:H7 strain in response to heat shock at 55°C. A previous study (Shipp et al, 1992b) showed O157:H7 did produce heat shock proteins similar to proteins observed in other strains of *E. coli*. Highest concentrations of heat shock proteins were observed at the lowest temperatures of thermal processing, therefore offering explanation for increased survival at 60° over 66° and 71°C.

Conclusions

Thermal death times for *E. coli* O157:H7 established in a laboratory setting were not completely effective under industry conditions. Low fat ground beef patties cooked to lower endpoint temperatures (60°C) were found to be unsafe. This is thought to be a result of thermal processing under industry like conditions as well as higher levels of heat shock proteins being produced by *E. coli* O157:H7 at lower temperatures; therefore, increased resistance to thermal processing and holding times at lower temperatures. However, cooking to an endpoint temperature of 66°C for the appropriate holding time

resulted in a microbiologically safe product with greater yields, higher moisture levels and less peak shear force than products cooked to 71°C, regardless of fat level.

Figure 4.1 Effect of cook temperature on cooking yields for cooked ground beef patties inoculated with E. coli O157:H7

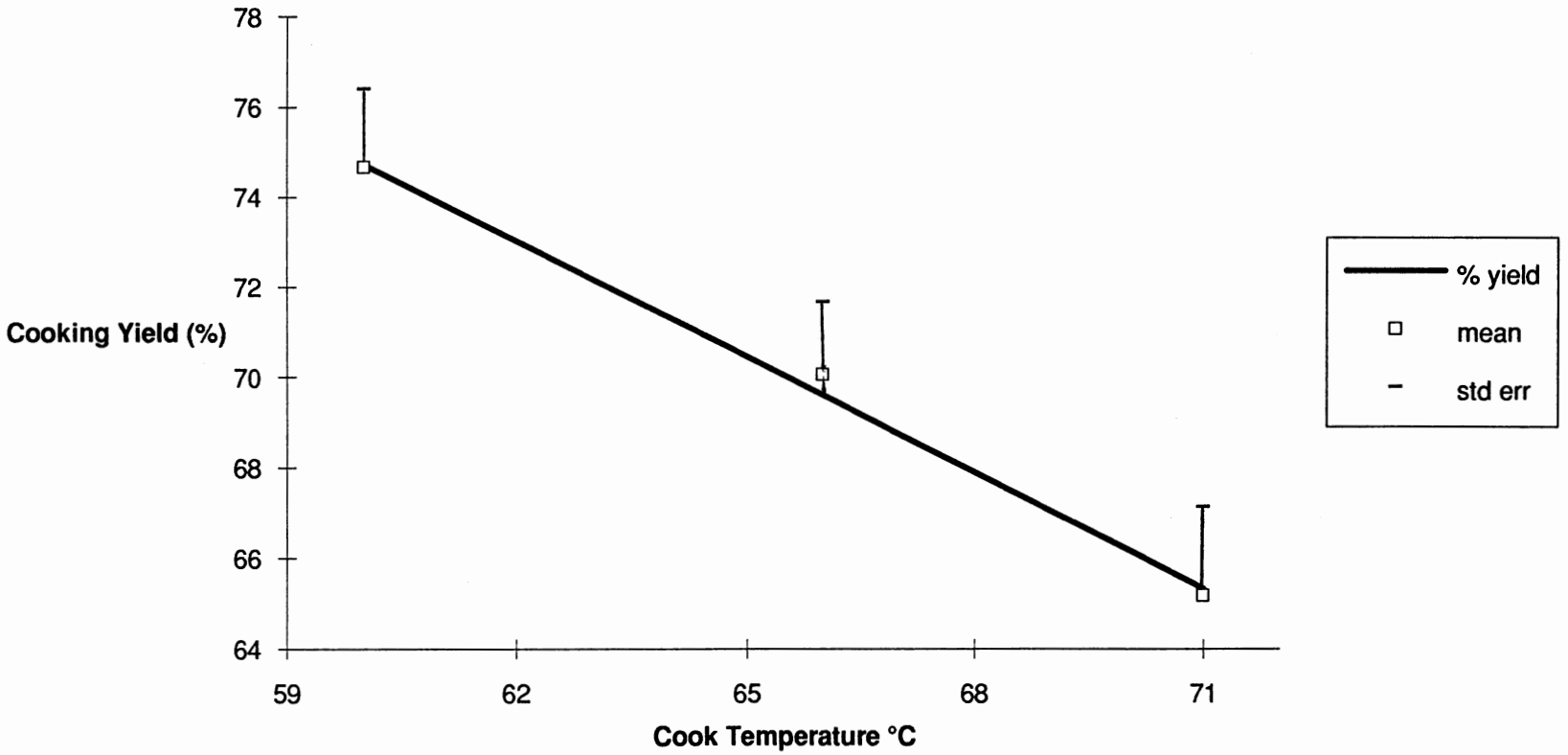

Figure 4.2. Effect of fat level on cooking yields for cooked ground beef patties inoculated with E. coli O157:H7

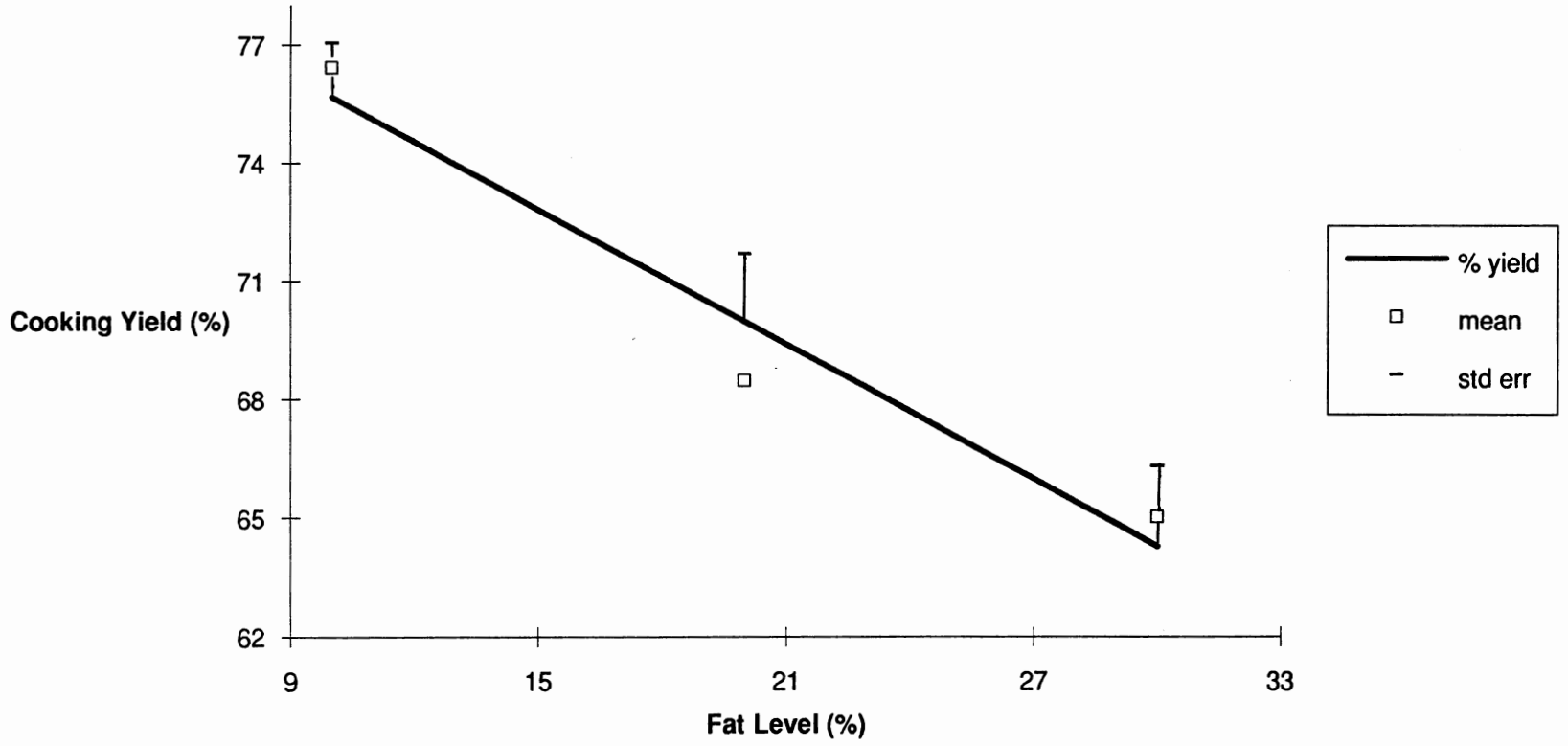

Figure 4.3. Effect of cook temperature on force required to shear cooked ground beef patties inoculated with E. coli O157:H7.

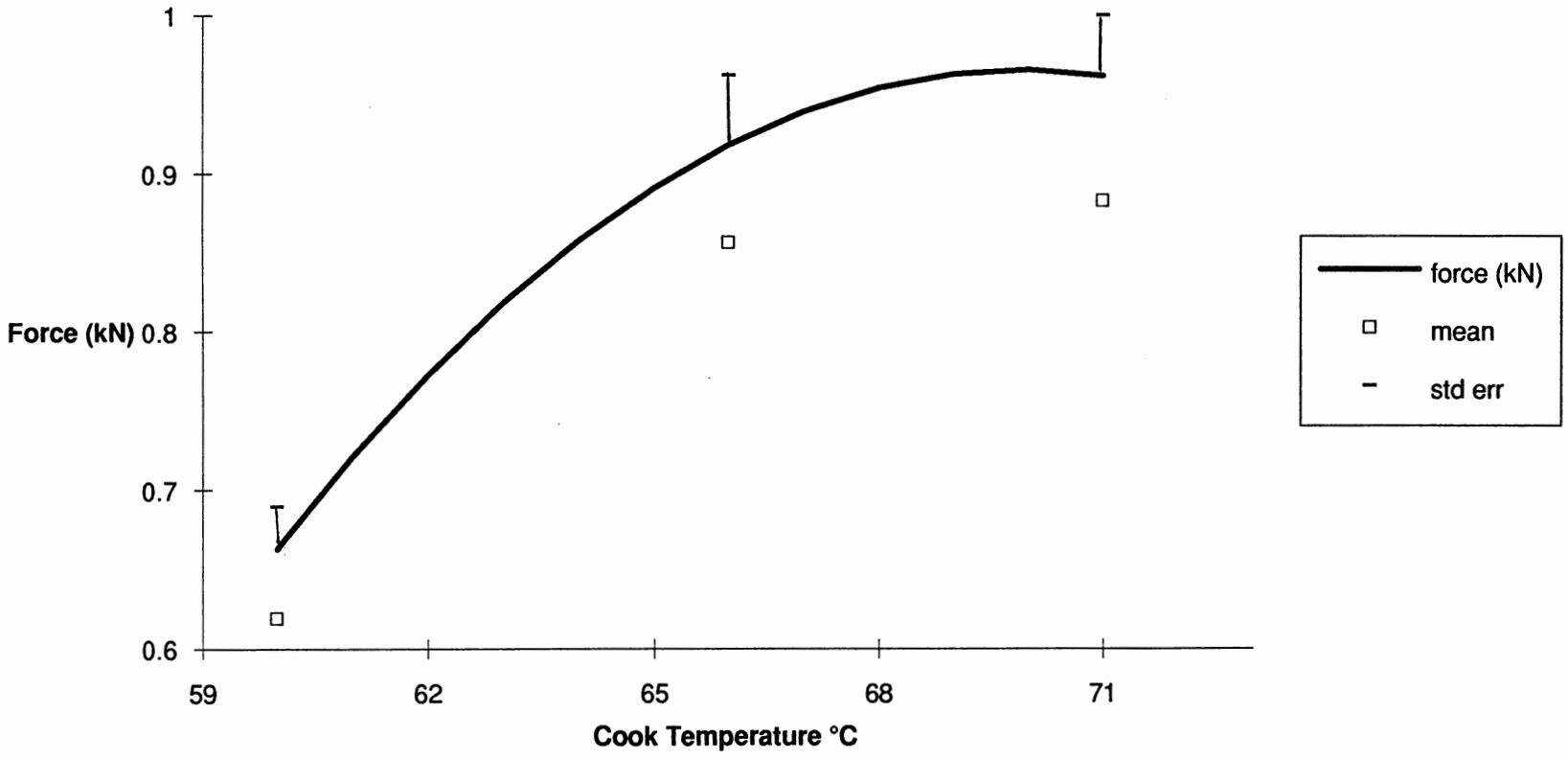

Figure 4.4. Effect of fat level on force required to shear cooked ground beef patties inoculated with E. coli O157:H7

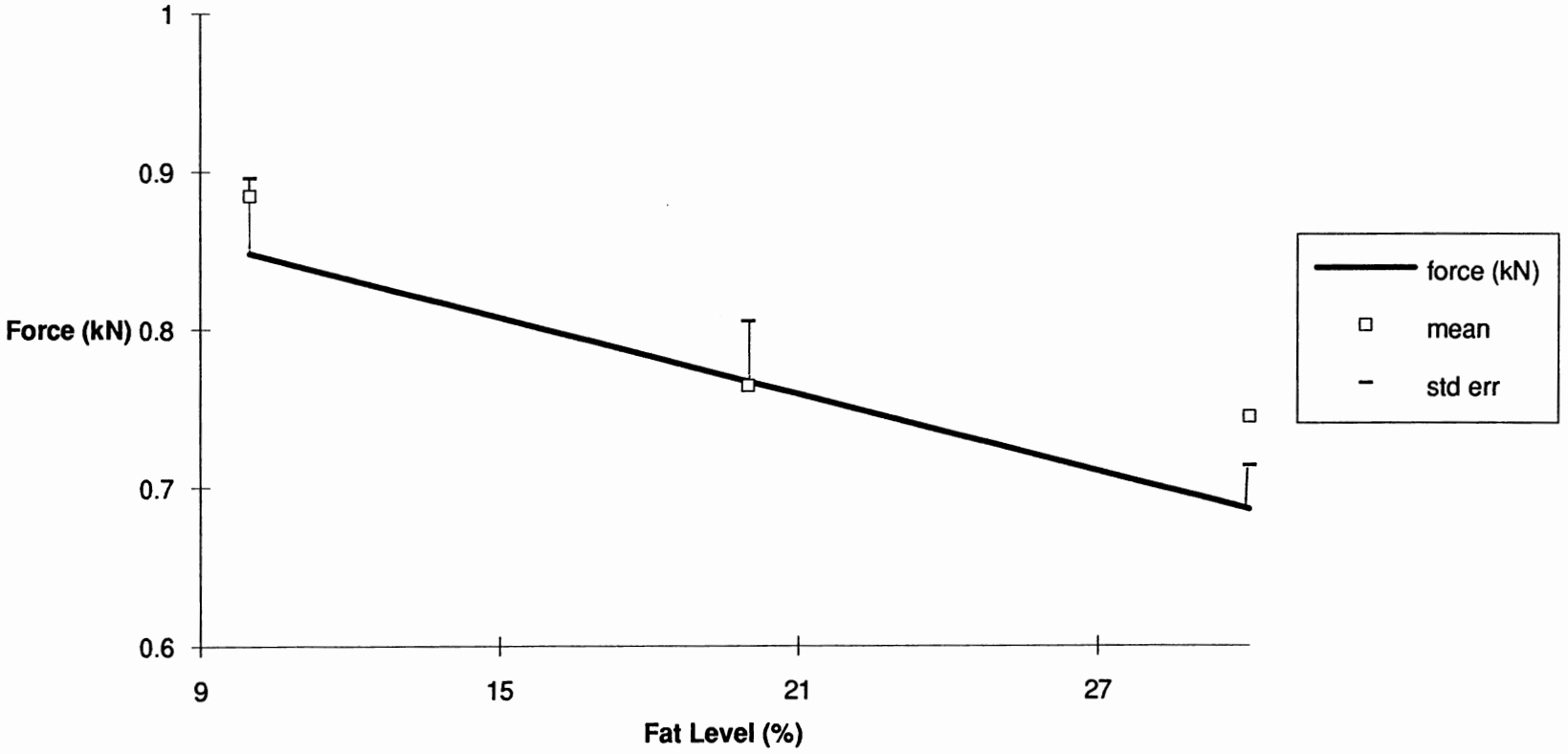

Figure 4.5. Effect of cook temperature on percent moisture for cooked ground beef patties inoculated with E. coli O157:H7

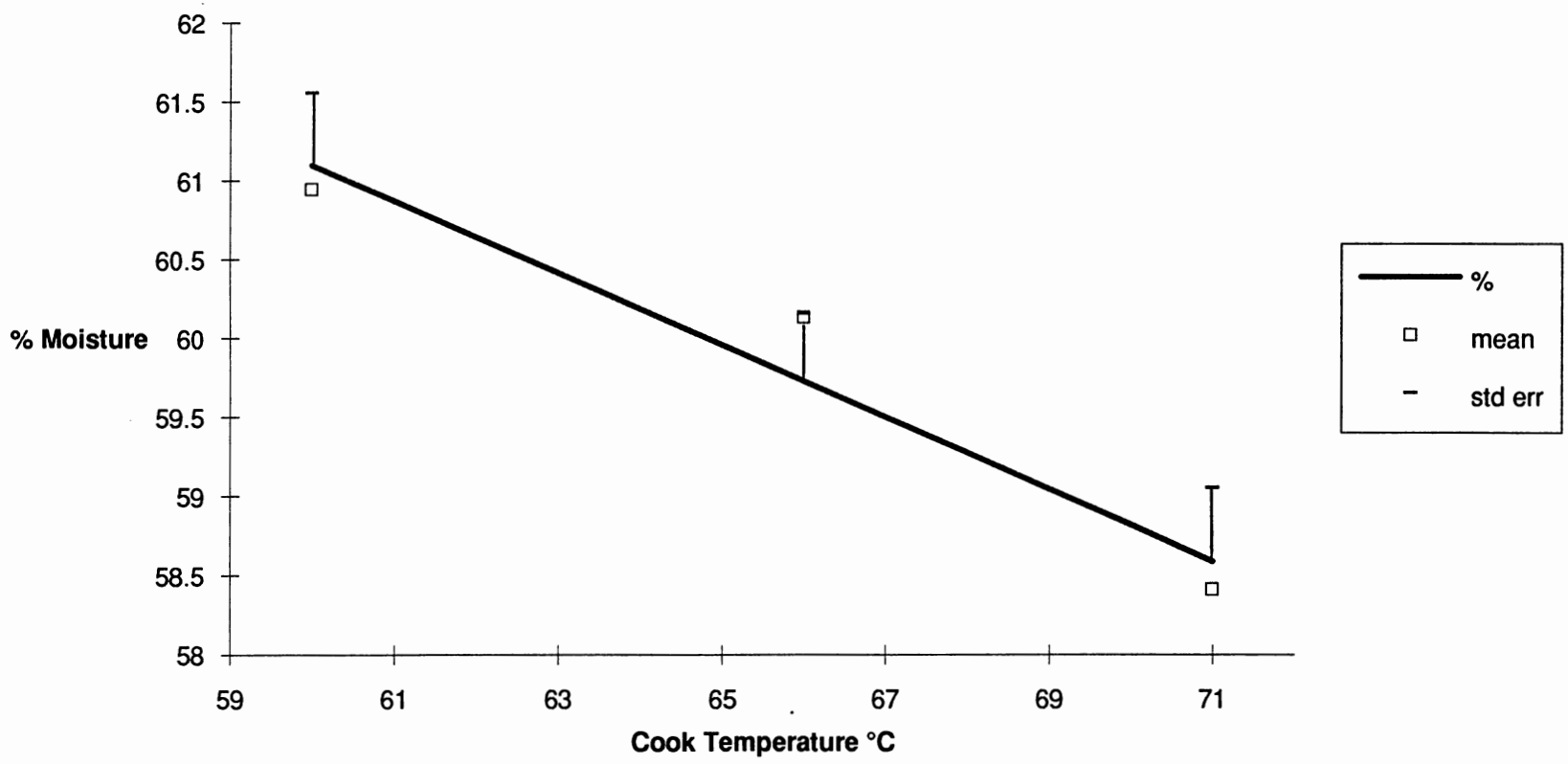

Figure 4.6. Effect of fat level on percent moisture for cooked ground beef patties inoculated with E. coli O157:H7

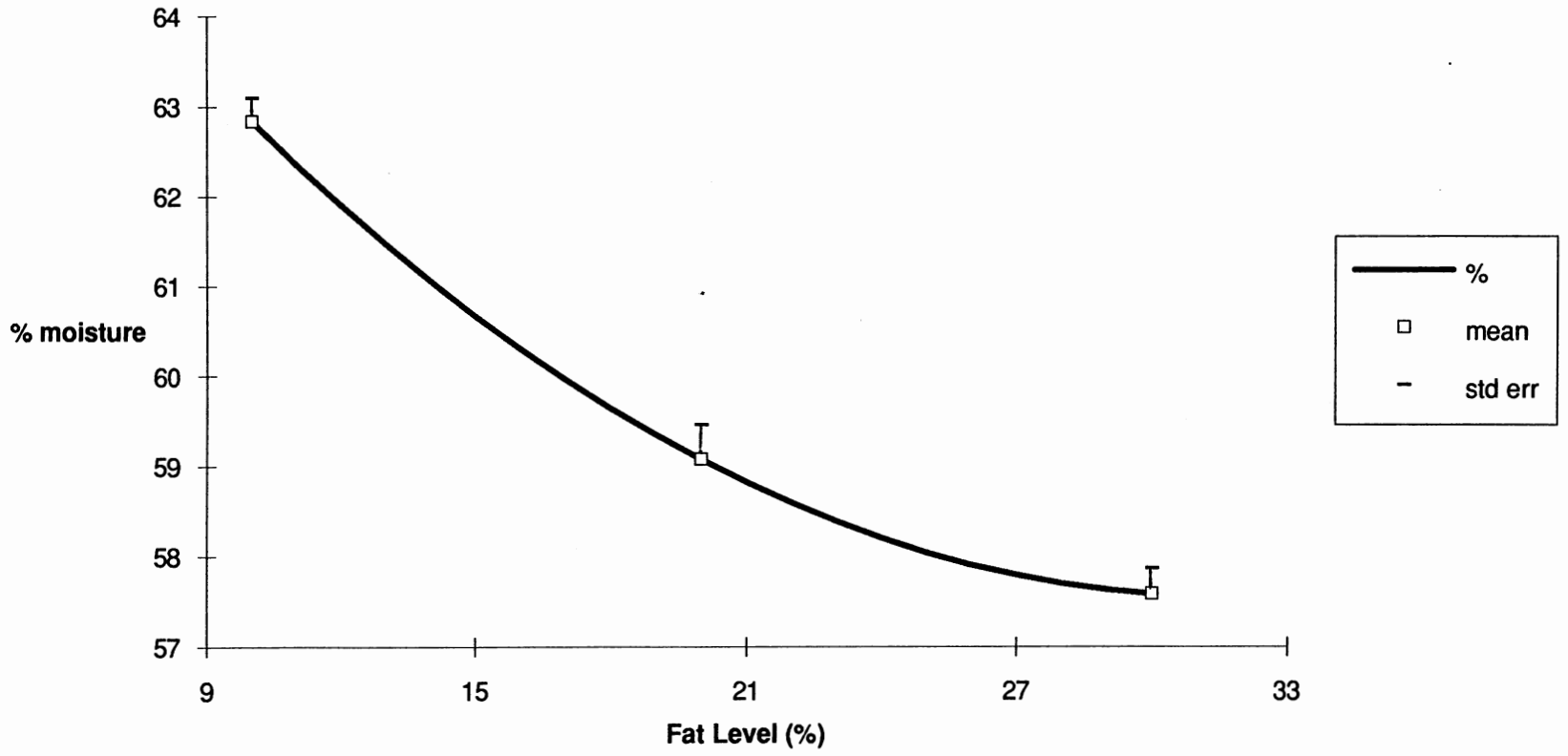

Figure 4.7 Effect of cook temperature and fat level on percent fat in cooked ground beef patties inoculated with E. coli O157:H7

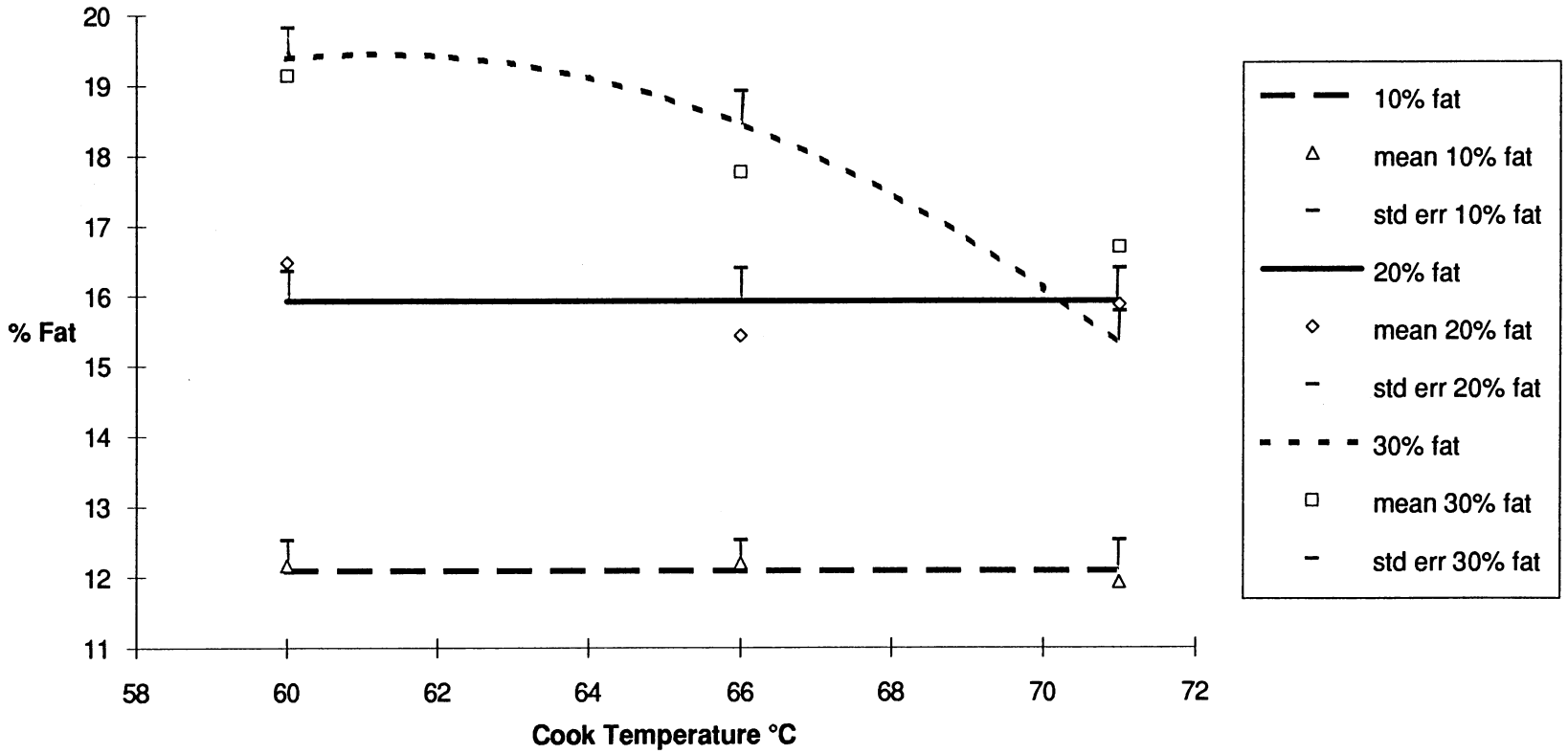

Figure 4.8 Effect of cook temperature and fat level on percent protein in cooked ground beef patties inoculated with E. coli O157:H7

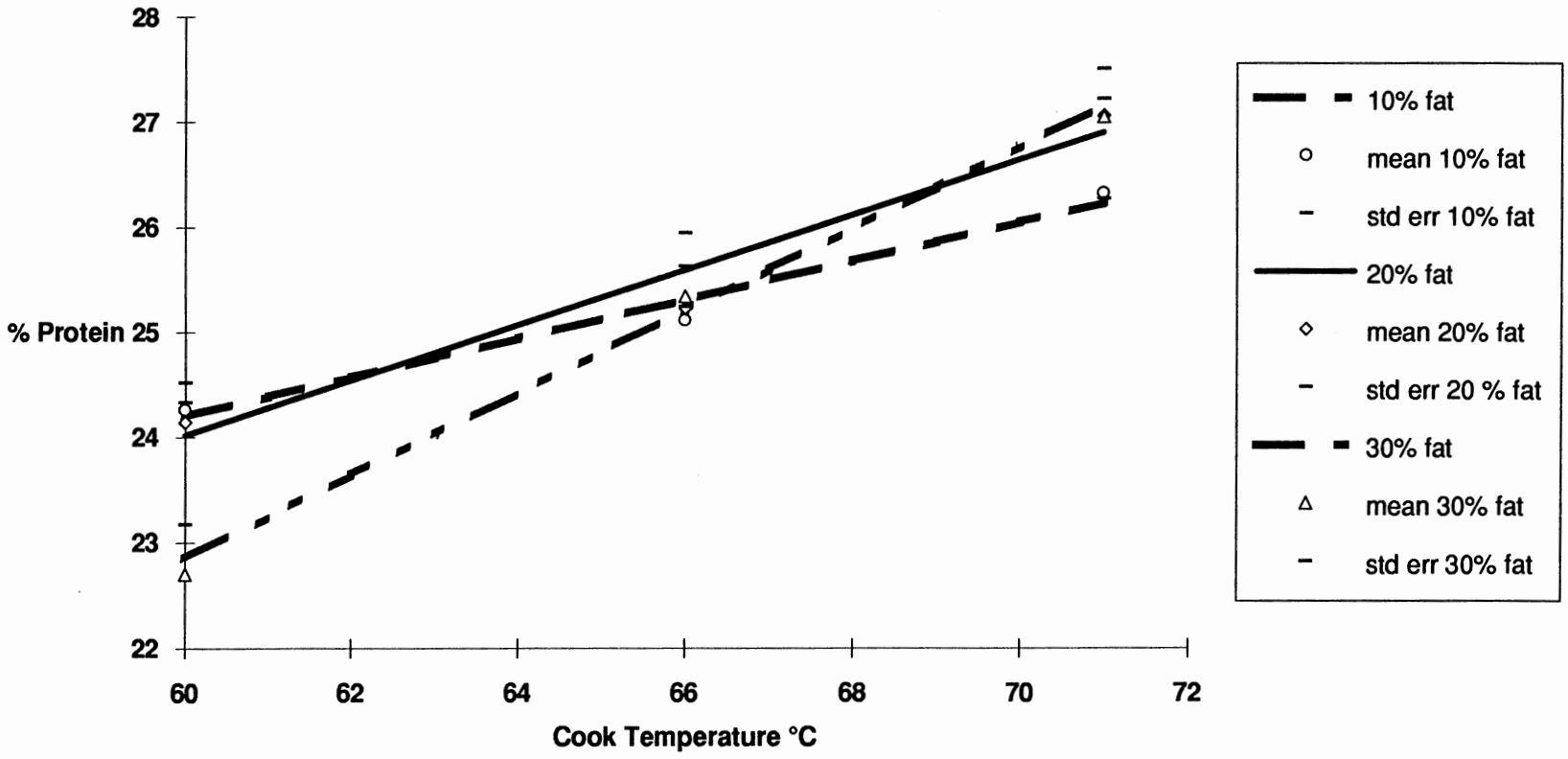

Table 4.1 - Holding times for ground beef patties inoculated with *E. coli* O157:H7[a]

	Temperature °C		
Fat Level	60	66	71
10%	300 sec	100 sec	0 sec
20%	260 sec	90 sec	0 sec
30%	220 sec	80 sec	0 sec

[a] established by Shipp et al. (1992b)

Table 4.2 - Survival of *E. coli* O157:H7 in thermally processed ground beef patties[a]

	Temperature °C		
Fat Level	60	66	71
10%	+ + -	- - -	- - -
20%	- - +	- - -	- - -
30%	- - -	- - -	- - -

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

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APPENDIXES

APPENDIX A

TABLE A.1

GEL ELECTROPHORESIS OF ^{35}S LABELED *E. COLI* O157:H7 PROTEIN

	Standard			60°C			66°C		
band	cm	%[a]	kDA	cm	%[a]	kDA	cm	%[a]	kDA
#1	2.5	17.8	97.4	2.8	20.1	95.0	3.4	23.9	90.5
#2	7.1	50.0	66.2	3.3	23.2	90.5	4.2	29.6	85.0
#3	10.4	73.6	42.7	4.0	28.2	87.0	4.9	34.5	80.0
#4	12.9	90.8	31.0	4.8	33.8	81.0	7.1	50.0	66.0
#5				7.1	50.0	66.0	9.1	64.1	49.5
#6				8.8	62.3	52.0	9.7	68.3	45.5
#7				9.5	66.9	46.5	11.0	81.0	35.8
#8				13.3	93.7	31.0	13.0	94.4	31.0

[a] relative migration

APPENDIX B

TABLE B.1

MEANS AND STANDARD ERRORS OF SIGNIFICANT VARIABLES IN CHAPTER IV

TEMP	YIELDS		MOISTURE		SHEAR FORCE	
(°C)	Mean	SE[a]	Mean	SE[a]	Mean	SE[a]
60	74.67	1.67	60.95	0.46	0.62	0.03
66	70.06	2.07	60.13	0.43	0.86	0.04
71	65.18	1.80	58.41	0.46	0.88	0.04

FAT	YIELDS		MOISTURE		SHEAR FORCE	
(%)	Mean	SE[a]	Mean	SE[a]	Mean	SE[a]
10	76.42	1.37	62.83	0.26	0.88	0.05
20	68.47	1.7	59.07	0.38	0.76	0.04
30	65.02	2.03	57.59	0.28	0.74	0.03

[a]Standard Error

TABLE B.2

MEANS AND STANDARD ERRORS OF SIGNIFICANT VARIABLES IN CHAPTER IV

PROTEIN

TEMP	10% FAT		20% FAT		30% FAT	
(°C)	MEAN	SE[a]	MEAN	SE[a]	MEAN	SE[a]
60	24.26	0.32	24.14	0.32	22.70	0.32
66	25.11	0.32	25.23	0.35	25.34	0.32
71	26.33	0.39	27.07	0.32	27.06	0.35

FAT

TEMP	10% FAT		20% FAT		30% FAT	
(°C)	MEAN	SE[a]	MEAN	SE[a]	MEAN	SE[a]
60	12.16	0.43	16.48	0.43	19.14	0.43
66	12.2	0.43	15.43	0.47	17.77	0.47
71	11.92	0.43	15.88	0.47	16.70	0.47

[a]Standard error.

TABLE B.3

REGRESSION EQUATIONS OF SIGNIFICANT VARIABLES IN CHAPTER IV

Variable	Parameter estimate		R^2
Yield	Intercept	141.142	0.204
	Cktemp	-0.474	
Yield	Intercept	81.373	0.295
	Fat	-0.570	
Shear Force	Intercept	-22.703	0.132
	Cktemp	0.300	
	$Cktemp^2$	-0.001	
Shear Force	Intercept	0.929	0.038
	Fat	-0.008	
Moisture	Intercept	78.857	0.130
	Cktemp	-0.127	
Moisture	Intercept	68.875	0.590
	Fat	-0.718	
	Fat^2	0.011	

TABLE B.4

REGRESSION EQUATIONS OF SIGNIFICANT VARIABLES IN CHAPTER IV

Fat (30%)	Intercept	-249.920	0.688
	Cktemp	3.790	
	$Cktemp^2$	-0.013	
Protein	Intercept	2.423	0.366
(10% Fat)	Cktemp	0.154	
(20% Fat)	Intercept	1.570	0.391
	Cktemp	0.159	
(30% Fat)	Intercept	-10.848	0.783
	Cktemp	0.240	

VITA

Deborah Kay Shipp

Candidate for the Degree of

Master of Science

Thesis: THERMAL INACTIVATION OF ESCHERICHIA COLI O157:H7 IN GROUND BEEF PATTIES

Major Field: Food Science

Biographical:

Personal Data: born in Oklahoma City, Oklahoma, April 9, 1951, the daughter of Orin and Evelyn Shipp.

Education: Graduated from Bethel High School, Shawnee, Oklahoma in 1969. Received Bachelor of Science Degree in Animal Science Food Industry option, Oklahoma State University, December 1989. Completed the requirements for the Master of Science degree in December, 1992.

Professional Experience: Internship at Wright Brand Foods, Summer, 1989. Instructor for Meat Technology, Spring 1991. Teaching and research assistant, Department of Animal Science, January, 1990 to May 1992.