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R-PHYCOERYTHRIN AS A TIME-TEMPERATURE INTEGRATOR, AND TRIOSE PHOSPHATE ISOMERASE AS A TEMPERATURE INDICATOR OF THERMAL PROCESSING IN BEEF PATTIES

By

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ABSTRACT

R-PHYCOERYTHRIN AS A TIME-TEMPERATURE INTEGRATOR AND TRIOSE PHOSPHATE ISOMERASE AS A TEMPERATURE INDICATOR OF THERMAL PROCESSING IN GROUND BEEF PATTIES

By

Sarah E. Smith

The D values for an 8-strain *Salmonella* cocktail in log phase were 16.34, 2.72, 0.44, and 0.15 min at 55, 58, 61, and 63°C, respectively, with a z value of 3.90°C in ground beef. Frozen storage of inoculated beef and starvation conditions in media prior to inoculation decreased the thermal resistance of the *Salmonella* cocktail. The D values of the thermally injured *Salmonella* cocktail were not different ($P<0.05$) from the *Salmonella* cocktail in log phase. Similar results were seen with the heat shocked *Salmonella* cocktail except at 58°C where it had a lower ($P<0.05$) thermal resistance.

Encapsulated R-Phycoerythrin (PE) was inserted into the center of a beef patty that had been inoculated with a *Salmonella* cocktail. In 113g patties, a normalized PE fluorescence less than 0.29 was correlated to a 5 $\log_{10}$ decrease in *Salmonella*. At this cut off value, PE was able to correctly categorize 93% of the high and low fat beef patties as undercooked or properly cooked according to current USDA regulations.

Five 1g samples within each patty were assayed for triose phosphate isomerase (TPI) enzyme activity after cooking. Large variability in activity was seen within each patty and internal temperatures were estimated to differ by as much as 25.7°C. TPI activity was affected by the heterogeneous nature of ground beef, but was able to identify temperature variability within patties.

To Mom and Dad

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LIST OF SYMBOLS AND UNITS

E_A	activation energy of a process	kJ mole^{-1}
F	processing value	min
F_{Tref}	processing value at the reference temperature	min
k	reaction rate constant	min^{-1}
ko	pre-exponential factor	min^{-1}
n	reaction order	
R	universal gas constant	J mole K^{-1}
t	time	min
T	temperature	°C
x	concentration of a target attribute at time t	
x_o	concentration of a target attribute at time zero	

CHAPTER 1

INTRODUCTION

Inadequate cooking or survival of pathogens during cooking is a common cause of foodborne disease outbreaks associated with meat products (Bean and Griffin, 1990). Between 1988 and 1992 a total of 2,423 foodborne outbreaks were reported, with bacterial pathogens causing 79% of all outbreaks (Bean et al., 1997). This group of pathogens consists mainly of *Salmonella*, *Campylobacter*, and *Escherichia coli* O157:H7, all of which have been found in ground beef. It is well known that ground beef is one of the most common vectors for bacterial pathogens and that thermal processing is the main solution to inactivation of these microbes. One of the best known outbreaks involving *E. coli* O157:H7 resulted from the consumption of undercooked ground beef.

The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) published meat and poultry regulations in Title 9 of the Code of Federal Regulations. Processors are required to follow published time-temperature cooking schedules for ground beef. Recently, lethality performance standards have been added to allow flexibility in processing procedures (USDA-FSIS, 1999). In cooked beef, roast beef, and cooked corned beef the regulations state that any processing schedule may be used, as long as it is equivalent to a 6.5-$\log_{10}$ reduction in *Salmonella*. A 5-$\log_{10}$ reduction in *Salmonella* has been proposed for cooked, uncured, meat patties. These processing standards are based on microbial thermal death time studies performed in a laboratory and have not been verified using actual meat products.

Processing compliance should be documented with challenge studies using a cocktail of *Salmonella* serotypes. Particular serotypes are not specified by the USDA. However, the cocktail should contain *Salmonella* strains that exhibit relatively high heat resistance and have been previously implicated in foodborne outbreaks (USDA-FSIS, 1999).

The emergence of pathogens with the ability to resist antibiotics and traditional heat treatments has prompted research in the area of microbial thermal inactivation. Recent research has investigated the effects of sub-lethal heat treatment prior to thermal inactivation. Studies have been completed with single strains of *Salmonella* and *Listeria* and a cocktail of *E. coli* O157:H7, but not with a *Salmonella* cocktail. Many different environmental conditions alter the thermal inactivation parameters of pathogens. The effect of fat concentration of meat, starvation conditions, and freezing of inoculated ground beef on the thermal inactivation parameters of a *Salmonella* cocktail are unknown.

Even though current USDA regulations are based on the thermal destruction of bacterial pathogens, current methodologies for monitoring adequacy of heat treatments are not. Time-temperature integrators (TTIs) are devices capable of quantifying a time-temperature impact on a quality parameter without acquiring the actual thermal history. A TTI that correlates to a 5 or greater $\log_{10}$ decrease in *Salmonella* could assist processors in proving process lethality. Processors are limited by the inability to take pathogens into production facilities to conduct challenge studies on actual processing equipment. An extrinsic TTI could be brought into a plant environment to assess the thermal process, while an endogenous TTI would already be present for monitoring.

Thermal inactivation parameters were determined for phycoerythrin (PE) in a buffer system. PE with a z value of 5.99 was able to distinguish between undercooked, properly cooked, and overcooked thermal processing schedules for ground beef (Orta-Ramirez, 1999). The inactivation of PE did not follow a first order reaction, yet the lethalities calculated for PE and *Salmonella,* assuming non-linear kinetics, were very predictable. A mathematical model could be developed to correlate the thermal destruction of PE fluorescence to the destruction of *Salmonella* in meat products. More work is needed to evaluate this extrinsic fluorescence based TTI in beef patties.

Triose phosphate isomerase (TPI) is an endogenous enzyme in ground beef that has shown potential as a time-temperature integrator for thermal processing. The z value of TPI was reported as 5.56°C, within the range given for *Salmonella* in ground beef (Orta-Ramirez et al., 1997). A difference was detected in TPI activity between ground beef patties cooked to 65.6 and 71.1°C. More research is needed to evaluate the potential of TPI as a temperature indicator in ground beef patties during cooking.

The objectives of this project were (1) to investigate the effect of fat content of meat, growth phase of microorganism, starvation conditions, and sub-lethal heat treatment on thermal inactivation parameters of *S.* Typhimurium DT104 and a *Salmonella* cocktail, (2) to verify the ability of PE to indicate proper thermal inactivation of *Salmonella* in a ground beef patty, and (3) investigate the potential of TPI to indicate temperatures within ground beef patties.

CHAPTER 2

LITERATURE REVIEW

2.1. Evolution of thermal inactivation regulations

Title 9 of the Code of Federal Regulations has traditionally been used to outline the USDA meat processing requirements needed for the destruction of pathogens that cause foodborne disease (USDA-FSIS, 2000). In 1990, the USDA-FSIS finalized regulations for cooked beef, roast beef, and corned beef. These regulations consisted of 16 time/temperature schedules (USDA-FSIS, 1990) to ensure a 7-log reduction in *Salmonella*. There were no federal regulations concerning fully cooked, uncured meat patties until 1996.

The regulations were reviewed after the 1993 outbreak of *Escherichia coli* O157:H7 caused by the consumption of undercooked ground beef patties. Schedules for beef patties were established based on the thermal destruction of *Salmonella* and *E. coli* O157:H7 (Goodfellow and Brown, 1978; Doyle and Shoeni, 1984). Meat processors, subjected to federal inspection, were required to use one of the following time/temperature protocols for fully cooked, uncured meat patties: 66.1°C (151°F)/41 sec, 66.7°C (152°F)/32 sec, 67.2°C (153°F)/26 sec, 67.8°C (154°F)/20 sec, 68.3°C (155°F)/16 sec, 68.9°C (156°F)/13 sec, or 69.4°C (157°F) (and up)/10 sec (USDA-FSIS 1996). The time-temperature regulations for cooked beef, roast beef, and corned beef were also altered to reflect a 6.5-log decrease in *Salmonella*, a reduction from previous regulations. Thirty different schedules are allowed for these products ranging from a minimum internal temperature of 54.4°C (130°F) with a holding time of 112 min to 71.1°C (160°F) for 10s.

Guidelines for cooking meat products in foodservice establishments are published in The Food Code of the Food and Drug Administration (FDA, 1999). The Food Code recommends that roast beef and corned beef be cooked to one of the following time/temperature combinations: 54°C (130°F)/121 min, 56°C (132°F)/77 min, 57°C (134°F)/47 min, 58 (136°F)/32 min, 59°C (138°F)/19 min, 60°C (140°F)/12 min, 61°C (142°F)/8 min, 62°C (144°F)/5 min, or 63°C (145°F)/3 min. Whereas, hamburger patties are recommended to be cooked to 70°C (158°F) for < 1s, 68°C (155°F) for 15s, 66°C (150°F) for 1 min, or 63°C (145°F) for 3 min.

Recently the USDA-FSIS, in addition to new time/temperature schedules, has allowed processors to establish processes based on lethality standards (USDA-FSIS, 1999). Lethality performance standards are based on the destruction of a specific number of *Salmonella* microorganisms.

Lethality performance standards are outlined in terms of *decimal reductions* or $\log_{10}$ reductions in *Salmonella*. A D value is defined as the time (min) required to reduce a target population by one log at a given temperature. The larger the D value, the greater the heat resistance of a culture. A z value is defined as the temperature increase necessary to reduce a D value by one log. The z value is an indication of temperature sensitivity. The lower the z value the more temperature sensitive the organism. Therefore, for accurate process control, thermal inactivation parameters for *Salmonella* and other pathogens must be established in the meat product of interest.

In addition to lethality performance standards, ready-to-eat products also must meet stabilization and handling standards. Stabilization standards are designed to prevent growth of spore-forming bacteria that can cause food borne illness. It was anticipated that most processors would meet this standard by rapidly cooling products following cooking. The third standard, handling, was introduced to prevent infectious pathogens from being introduced into the product following processing, stabilization, or final packaging.

Performance standards tell establishments what degree of effectiveness their HACCP plans will be expected to achieve while allowing processors the flexibility of incorporating individualized processing procedures. If an establishment chooses to use an alternative treatment or intervention, such as organic rinses or curing, in combination with heat treatment, it is the responsibility of the establishment to demonstrate a cumulative equivalency in microbial reduction. The process should not only show specific reductions in *Salmonella*, but also the reduction of all other foodborne pathogens of concern.

Although *E. coli* O157:H7 has been identified in beef products with greater frequency, it has been shown to be less heat resistant than *Salmonella* (Doyle and Shoeni, 1984; Line et al., 1991). *Listeria monocytogenes*, another pathogen of concern in beef products, has shown to exhibit a higher heat resistance than *Salmonella*. Yet its incidence is much lower and often is the result of post-cooking contamination. Therefore monitoring the thermal reduction of *Salmonella* in cooked products would indicate destruction of most other pathogens.

Compliance to lethality performance standards must be verified using challenge studies. Challenge studies are laboratory studies designed to mimic commercial processes to verify the destruction of pathogens. The USDA-FSIS is recommending the use of multiple serotypes of *Salmonella* for challenge studies. The use of a combination of serotypes is referred to as a cocktail. The pathogenic strains comprising the cocktail should be relatively heat resistant and also should be among those historically implicated in food borne outbreaks. Yet the USDA-FSIS does not specify each strain that should be included in a cocktail to be used in challenge studies. Thus, different cocktails will render varying kinetic parameters, with different processors using different standards to verify processing schedules. These variations could be eliminated if one universal cocktail was defined for use.

A proposed lethality performance standard for fully cooked, uncured meat patties includes a 5 $\log_{10}$ reduction in *Salmonella* (USDA-FSIS, 1996). However, in review, the FSIS has concluded that a higher lethality standard may be needed to produce safe ground beef patties. It appears that the lethality performance standard for meat patties will be increased to 6.5 $\log_{10}$ (Hasiak, 2000). While this is under further investigation, the time/temperature schedules published in 1996 remain in effect. Although these thermal processing guidelines are available, there is still a need to verify adequate cooking once a product has been processed.

2.2. Thermal inactivation of *Salmonella* strains and *Escherichia coli* O157:H7 in meat

In 1999 the USDA-FSIS finalized the approved use of lethality performance standards for beef processing. Quantitative information on lethality of pathogens is required to establish effective schedules. Thermal processing has evolved into the most common means of preventing food borne disease. Variables affecting heat resistance of pathogens include species, muscle type, pH, and fat content (Murphy et al., 2000). Thermal inactivation parameters can also be influenced by initial population of pathogens, age of the cultures, and heat shock injury (Murphy et al., 1999)

2.2.1. *Salmonella*

The first regulations for roast beef processing were based upon results obtained by Goodfellow and Brown in 1978. Thermal death time studies were performed on a *Salmonella* cocktail comprised of *S.* Typhimurium, *S.* Newport, *S.* Agona, *S.* Bovis-Morbificans, and *S.* Muenchen in ground meat. The resulting D values were 61-62 min at 51.6°C (125°F), 3.8-4.2 min at 57.2°C (135°F), and 0.6-0.7 min at 62.7°C (145°F), depending on the recovery method used. The z value of *Salmonella* was found to be 5.56°C. From these D values, processing time/temperature requirements were established to achieve a 7 $\log_{10}$ reduction in *Salmonella* in ground beef ranging from 195 min at 128°F (53.3°C) to 5 min at 144°F (62.2°C) (USDA-FSIS, 1990).

More recently, in response to new recommendations by the USDA-FSIS, thermal death time studies have been completed utilizing an 8-strain *Salmonella* cocktail in ground beef (Juneja et al., 2000). Juneja et al. (2000) attempted to identify the most representative and appropriate strains of *Salmonella* to include in a cocktail used to quantify heat resistance in meat products. No correlation between the heat resistance and the origin of the *Salmonella* spp. could be established due to significant variation in the heat resistance among strains. The D values reported in meat were higher at 58 and 60°C than those calculated in chicken broth. The D values of the *Salmonella* cocktail in ground beef are presented in Table 2.1. The D values found by Juneja et al. (2000) were lower than those reported by Goodfellow and Brown (1978) and Orta Ramirez et al. (1997) in ground beef. The z values were higher, thus indicating the 8-strain *Salmonella* cocktail was less temperature sensitive. These differences could be explained by the choice of strains used.

Thermal death time studies using another *Salmonella* cocktail were performed in ground chicken (Murphy et al., 2000). Six *Salmonella* strains were inoculated into ground chicken breast patties prior to thermal processing. The D values were higher in ground chicken than those in peptone-agar solution at all temperatures tested. Juneja et al. (2000) also found higher D values in beef as compared to chicken broth (Table 2.2). This cocktail was less heat resistant than *S.* Senftenberg in ground turkey (Veeramuthu et al., 1998). The D values reported here were higher than Juneja et al. at all common temperatures. The higher D values observed might be due to the inclusion of *S.* Senftenberg in the cocktail. Early studies have demonstrated the unusual heat resistance of *S.* Senftenberg in buffer ($D_{57°C}$ = 31 min) (Ng et al., 1969) and poultry ($D_{66°C}$ = 3.5 min) (Milone and Watson, 1970).

Orta-Ramirez et al. (1997) tested the thermal resistance of *S.* Senftenberg in ground beef using 10 by 75 mm thermal-death-time tubes. D values were 53.00, 15.17, 2.08, and 0.22 min at 53, 58, 63, and 68°C, respectively, with a z value of 6.24°C. These results are in good accordance with those reported by Murphy et al. (2000), which also included *S.* Senftenberg.

Thermal resistance of pathogens is influenced not only by the strain(s) chosen, but also the microbial growth phase of the organism during testing. Heddleson et al. (1991)

Table 2.1 D values[a] of *Salmonella* cocktail in ground beef (Juneja et al., 2000)

Temperature (°C)	D value (min)
58	8.65 ± 0.03
60	5.48 ± 0.04
62.5	1.50 ± 0.01
65	0.67 ± 0.04

[a]D values are expressed as the mean of two replicate experiments ± standard deviation.

Table 2.2. D values[a] for *Salmonella* in chicken breast meat (Murphy et al., 1999)

Temperature (°C)	D value (min)
55.0	30.1
57.5	12.9
60.0	5.88
62.5	2.51
65.0	1.16
67.5	0.287
70.0	0.175

[a]D values are expressed as the mean of three triplicate thermal death time studies.

reported that maximum heat resistance occurred during stationary phase of *Salmonella* spp. in phosphate buffer. Freezing of *Salmonella* cultures in liquid media also can lower thermal resistance (Smith, 1995). Enumeration of *S.* Typhimurium strain 55 cultures that were frozen for 24h at -30°C during log phase exhibited a 2 $\log_{10}$ decrease in comparison to cultures that were chilled for 24h at 0°C. Greater recovery of *S.* Typhiumurium strain 55 was observed using cells frozen in stationary rather than log phase.

Environmental stresses and their effects on pathogen heat resistance have received a lot of attention in recent years. Heat shock is defined as the exposure of cultures to sub-lethal heat treatments. The stress associated with sub-lethal heat shock induces the rapid synthesis of specific proteins, known as heat-shock proteins (Juneja et al., 1998). Increased thermal tolerance to a second heat challenge, which would normally be lethal, has been observed in microbial cells that have synthesized these heat-shock proteins.

Bunning et al. (1990) reported that heat shocking or exposing *Salmonella* cultures to temperatures just slightly above their normal growth temperature added a degree of protection against lethal effects. Heat shocking *S.* Typhimurium at 48°C for 30 min increased the D value from 21.3 min to 96.1 min at 52°C in broth. Yet, when the cultures were heat shocked at 42°C and 52°C, no significant difference was seen in D values at an inactivation temperature of 57.8°C. Mackey and Derrick (1987) heat shocked *S.* Thompson cultures at 48°C for 30 min before performing thermal inactivation studies in minced beef. The D values at 54°C and 60°C were increased by factors of 2.4 and 2.7, respectively.

Juneja et al. (1998) reported that heat shocking a four-strain cocktail of *E. coli* O157:H7 at 46°C for 15 to 30 minutes increased the heat resistance in ground beef. In inoculated ground beef, the non-heat-shocked cells decreased by 2.17 logs colony forming units (CFU)/g within 4 min and by 7.8 logs CFU/g after 15 min at 60°C. Whereas the heat shocked cocktail decreased 0.35 and 6.38 $\log_{10}$ CFU/g, respectively under the same inactivation conditions. Thermal death time studies were performed on *E. coli* O157:H7 in tryptic soy broth (TSB) and a ground beef slurry after heating at a sub-lethal temperature of 45°C for 30 min (Williams and Ingham, 1997). The heat shock increased the D values by 37, 68, and 50%, respectively, at 54, 58, and 62°C in tempered TSB. Yet, the D values at 58°C in a ground beef slurry did not differ from those of the non-heat-shocked cultures.

These results are similar to those from a heat shock study with *L. monocytogenes*. Farber and Brown (1990) reported that after heat shocking *L. monocytogenes* in a sausage mix at 48°C for 30 min before heating at the final test temperature, D values at 62 and 64°C were not significantly different from non-heat-shocked values. No differences in D values were seen until after 120 min of heat shocking at 48°C, which produced a culture 2.4 times as heat resistant at 64°C.

In addition to heat-shocked cultures, thermally injured cells have also been studied. Thermally injured cultures are defined as microbial cells that are recoverable on non-selective media but not detected on selective media. Therefore, thermally injured *Salmonella* would grow on a tryptic soy agar, but not on bismuth sulfite. The difference between survivor counts on the two media would give an indication of the percentage of cells that have been injured. Ahmed et al. (1995) heated *E. coli* O157:H7 at 50, 55, and 60°C for differing times to produce injured cells. *L. monocytogenes* cells were thermally injured after being heated at 52°C for 1h (Smith and Archer, 1988). The degree of thermal injury was determined by plating the survivors on tryptose phosphate agar with and without sodium chloride. Thermally injured cells are of concern if present in a food product, but remain undetected with normal enumeration procedures. Therefore, thermal inactivation of *Salmonella* has been shown to be influenced by the freezing history of the meat product, growth phase and previous thermal treatment of the cultures.

2.2.2 *Escherichia coli* O157:H7

Thermal inactivation of a four-strain cocktail of *E. coli* O157:H7 was determined in ground beef (Table 2.3) (Juneja et al., 1997). The z value was reported as 6.0°C. Thermal death time studies of *E. coli* O157:H7 in ground beef were also conducted in thermal death time tubes (10 by 75 mm) by Orta-Ramirez et al. (1997). The D values ranged from 46.10 min at 53°C to 0.12 min at 68°C. The z value was reported as 5.60°C.

At comparable temperatures, Juneja et al. (1997) reported that *E. coli* O157:H7 was less heat resistant at lower temperatures (58°C), yet more resistant at higher temperatures (63°C). Differences in D values could be attributed to the use of different *E. coli* O157:H7 strains. Juneja et al. (1997) performed the thermal inactivation studies on a 4-strain mixture of *E. coli* O157:H7, whereas Orta-Ramirez et al. (1997) used a single strain.

Thermal resistance of *E. coli* O157:H7 in ground beef is influenced by the fat content in meat. D values were higher in high fat as compared to low fat ground beef (Table 2.4). D values reported by Line et al. (1991) for *E. coli* O157:H7 in ground beef at 57.5°C were in good agreement with Juneja et al. (1997). Juneja et al. (1997) reported a D value of 4.95 min at 57.5°C in ground beef containing 10% fat. Line et al. (1991)

Table 2.3. D values[a] for *E. coli* O157:H7 in ground beef (Juneja et al., 1997)

Temperature (°C)	D value (min)
55	21.13 ± 0.25
57.5	4.95 ± 0.16
60	3.17 ± 0.18
62.5	0.93 ± 0.01
65	0.39 ± 0.00

[a]D values are expressed as the mean of two replicate experiments ± standard deviation.

Table 2.4. D values (min)[a] of *E. coli* O157:H7 in ground beef as affected by fat content

Line et al., 1991			Ahmed et al., 1995			
	Fat (%)			Fat (%)		
Temp (°C)	2.0	30.5	Temp (°C)	7	10	20
51.6	78.2	115.5	50	55.34	80.66	92.67
57.2	4.1	5.3	55	11.40	15.30	19.26
62.7	0.30	0.47	60	0.45	0.46	0.47

[a]D values are expressed as mean value from three experiments

performed thermal inactivation studies in ground beef containing 2.0% and 30.5% fat and found D values at 57.2°C to be lower and higher, respectively, than Juneja et al. (1997). These results further confirm higher thermal resistance in higher fat ground beef. The D values reported by Ahmed et al. (1995) were 1.4 and 6.9 magnitudes lower at 55 and 60°C, respectively, than those reported by Juneja et al. (1997) in 10% fat ground beef. Differences in results could be attributed to Juneja et al. (1997) using a 4-strain mixture of *E. coli* O157:H7, whereas Ahmed et al. (1995) performed studies with a single strain. Differences could also be explained by recovery methods used in each of the studies. Ahmed et al. (1995) also found that higher fat content decreased the z value of *E. coli* O157:H7 in beef products.

Juneja et al. (1998) assessed the influence of storage conditions on thermal resistance of *E. coli* O157:H7 in ground beef. After 48h of frozen storage, numbers of *E. coli* O157:H7 decreased 5.05 logs after 16 min at 60°C in comparison to *E. coli* O157:H7 in fresh ground beef that decreased 6.7 logs under the same conditions. Thus thermal inactivation of *E. coli* O157:H7 is dependent on the strain chosen, the fat content and the storage conditions of the beef product.

2.3. Time-temperature integrators

Thermal processing is one of the major methods of food preservation. The variability in factors contributing to the effectiveness of a thermal process often require the specific determination of appropriate heat treatments. Variables that affect thermal processing of meat products include species, muscle type, pH, fat content, and storage conditions. Even with reliable heat treatment recommendations it is still essential to monitor the cooking process to assure that the desired effects are achieved.

Consumers and food handlers have accepted the fact that visible signs, such as color changes in food, are indicators of safe processing. However, recent research has shown that color and texture indicators are not reliable. Sufficient numbers of ground beef patties were found to turn brown well before reaching the internal target temperature of 160°F (Hunt et al., 1995). The USDA-FSIS continues to advise consumers to use food thermometers when cooking meat and poultry.

It is very difficult to use a thermometer and thermocouple in commercial processing plants to verify that the time-temperature requirements have been met for a ground beef patty. Berry et al. (1999) found that the internal temperature of beef patties varied as much as 18°C when the center temperature was 71°C. Current regulations state that commercial establishments must verify the time and temperature within ± 1°F on one beef patty on a production line each hour. Yet with the large temperature gradients in a patty, these regulations may not be adequate.

The enforcement of Hazard Analysis Critical Control Point (HACCP) programs for meat processors has decreased the incidence of microbial contamination, yet methods to verify efficacy of thermal processes are still needed. Methods to evaluate the accuracy of thermal processes are classified into three different approaches: (1) *in situ* methods, (2) physical-mathematical methods, and (3) time-temperature integrators (TTIs) (Hendrickx et al., 1995; Van Loey et al., 1996).

2.3.1. *In situ* methods

In the *in situ* methods, changes in a food component are evaluated before and after thermal processing. Attributes frequently measured are microbial contaminants, sensory factors, or nutrients. In practice, however, monitoring often proves laborious, time-consuming, and expensive. In addition, the concentration of the attribute must be large enough to be detected. Therefore, other more convenient and successful methods have been and must be developed.

2.3.2. Physical-mathematical models

Physical-mathematical models combine previous knowledge from the kinetic parameters of a food quality attribute with a time-temperature profile to calculate the impact of the thermal treatment on the parameter of interest. Limitations of these methods are attributed to the need for time-temperature data. These temperature profiles can be collected by either direct physical measurement or from constructive computation. Problems arise when direct recording is not possible due to processing conditions or when inaccuracies occur with time-temperature histories that are reconstructed by mathematical modeling. Conservative estimates used in these models often result in over processing which in turn leads to significant nutrient loss and quality reduction of the product. Another limitation with physical-mathematical models is the lack of data for kinetic models for new technological advances in cooking, including ohmic and microwave heating.

2.3.3. Time-temperature integrators (TTIs)

TTIs can overcome the limitations created by the *in situ* and physical-mathematical modeling approaches. A TTI has been defined as a device that undergoes an irreversible and precisely measurable change in response to a time-temperature history that mimics the change in a target attribute exposed to the same thermal history (Hendrickx et al., 1995). A major advantage of a TTI is the quantification of the integrated time-temperature impact on a quality attribute without acquiring the actual time-temperature history of the product. Ideal TTIs would be simple and inexpensive to prepare, easily recoverable with an accurate and user-friendly read-out. TTIs should be incorporated into a food product without disrupting the heat transfer within the product while quantifying the thermal process on a target attribute.

To quantify the treatment that was performed on the target attribute, the TTI must show the same time-temperature dependent response as that of the target attribute when the temperature is the only rate determining factor. Mathematically, this can be expressed as:

$$(F_{Tref})_{target} = (F_{Tref})_{TTI}$$

Assuming an nth order TTI system, the rate equation can be expressed as:

$$dX/dT = - k_x X^n$$

where k_x is the rate constant and n the reaction order. For a first order reaction under isothermal conditions, the integrated equation is:

$$\ln (X_o/X) = k_x t$$

where X_o is the initial value of the target attribute. In many cases, according to the Arrhenius equation the rate constant can be expressed as:

$$k_x = k_o \exp (-E_A/RT)$$

where k_o is the rate constant at a reference temperature, E_A is the activation energy, R is the universal gas constant and T is the absolute temperature.

In a general case where the reaction order is different than unity, with the reaction rate constant and independent of the order of reaction, the F value can be written as (Hendrickx et al., 1995):

$$(F_{T_{ref}})_x = \int_0^t \exp\left[\frac{E_A}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] dt$$

which represents the equivalent heating time at a reference temperature resulting in the same lethality as the time-varying temperature profile. This equation is valid for the target attribute only when the activation energies of both the TTI and the target parameter are the same. In the simplest form of TTI, z values of the target attribute and TTI should be equal. An overestimation of the actual processing impact by the use of a TTI with a higher z value should be avoided, as this could lead to undercooking of food products, and a public health risk. A TTI with a lower z value than the target attribute would lead to overprocessing and decreased quality of the cooked product.

TTIs can be classified into biological, chemical or physical systems. In the biological TTI category, measuring devices detect a change in biological activity, such as numbers of microorganisms or level of enzyme activity after heating. Chemical and physical systems are based on a chemical or physical response, respectively, toward time and temperature (Hendrickx et al., 1995). A TTI can be extrinsic or intrinsic (Van Loey et al., 1996). An extrinsic TTI is incorporated into a food, whereas intrinsic TTIs are naturally present in the food. Three approaches can be distinguished with regard to the application of a TTI: dispersed, permeable, or isolated. In a dispersed system, the TTI is homogeneously distributed throughout the food. Permeable systems allow for some diffusion of a TTI through a food product, whereas isolated systems allow for well defined positioning of a TTI within a food product.

Most biological TTIs are based on microbiological or enzymatic assays. Microbiological TTIs can be used to monitor thermal processing through the use of count-reduction systems and survivor-kill systems (Van Loey et al., 1996). Utilizing count-reduction systems, the impact of the thermal process can be quantified from the survivor counts. The survivor-kill system indicates the presence or absence of microbial growth (an indication of sufficient thermal impact).

For low-acid foods, *Bacillus stearothermophilis, B. subtilis* 5230, *B. coagulans*, and *Clostridium sporogenes* are used for monitoring wet heat sterilization (Hendrickx et al., 1995). Pflug (1980) used a spore solution of *B. stearothermophilis* in a plastic rod to verify the sterilization of green beans, corn, and peas. Upon comparison of the results with values determined using the physical-mathematical model, it was concluded that the plastic rods could be used effectively to validate sterility. An overview of other applications with microbial TTIs has been published by Hendrickx et al. (1995). The main disadvantages of microbiological TTIs include the length of incubation, low level of analytical precision, the need to properly calibrate the spores to determine their thermal resistance and the possibility of contamination.

Protein-based systems, particularly enzyme systems, have some advantages as TTIs over microbial systems. The relative ease of assaying remaining enzyme activity make protein-based systems very applicable. In addition, enzymatic TTIs are less expensive to prepare, while being rapid and accurate. α-Amylase of *Bacillus licheniformis* immobilized on glass beads and *Bacillus amyloliquefaciens* in the presence of polyolic alcohols or carbohydrates have been studied as possible TTIs (De Cordt et al., 1992; 1994). Application of these TTIs is limited by the temperature of enzyme inactivation.

Very few chemical or physical indicators are currently developed. Initially compounds within food products, for which analytical methods of detection were already established, were studied as potential TTIs. These compounds included thiamin, pantothenic acid, vitamin C, and methylmethionine sulfonium salt (Kim and Taub, 1993). The limitations of these methods included lengthy procedures for recovery and post treatment assay. Kim and Taub (1993) also studied compounds that form during thermal processing of foods. Difficulties arose when they could not find a compound that was easily assayed and whose concentration after processing could be used to verify sterility.

2.4. R-Phycoerythin

R-Phycoerythin (PE), phycocyanin, (PC) and allophycocyanin (APC) are three major proteins that comprise a class of fluorescent compounds named phycobiliproteins (PBP) (MacColl and Guard-Friar, 1987). Phycobiliprotiens are photosynthetic antenna complexes that provide characteristic colors to cyanobacteria, red algae, and cryptomonads. They are located on the cytoplasmic face of the thykaloid membrane of the chloroplasts (Glazer, 1994). These compounds harvest solar energy in regions of the visible spectrum that show low chlorophyll absorption and transfer the excitation energy to chlorophyll in the photosynthetic membrane.

The PBP structure is comprised of apoproteins and chromophores called *bilins,* which are linear tetrapyrrole groups covalently attached to the apoprotein. The *bilins* fluoresce in the visible range. The two major chromophores are phycoerythrobilin and phycocyanobilin, at least one of which will be present in all phycobiliproteins. Three minor bilins have been identified: phycourobilin, criptobviolin, and the 697-nm bilin.

PE, red in color, has absorption bands at 498-568 nm and fluorescent emission bands at 570-620 nm. The blue proteins, PC and APC, have absorption bands at 550-630 nm and 598-680 nm and fluorescent emission bands at 630-650 nm and 660-680 nm, respectively.

R-phycoerythin (R-PE) contains three polypeptide chains: α, β, and γ or γ'. These subunits are usually organized as $\alpha_6\beta_6\gamma$ or $\alpha_6\beta_6\gamma$'. The subunit structure has a diameter of 12.7 nm with a ratio of $\alpha + \beta : \gamma$ of 12:1. The α subunit contains two phycoerythrobilins, the β subunit contains three subunits, and the γ subunit contains two phycoerythrobilins and two phycourobilins for a total of thirty-four chromophores (Glazer, 1994). The molecular mass has been reported to range between 240 and 260 kDa. The entire amino acid sequence of the α and β subunits of PE from *Porphyridium cruentum* has been identified (Glazer and Hixon, 1977; Sidler et al., 1989). Heat or chemical denaturation can cause conformational changes in the native structure of PBP resulting in partial or complete loss of optical properties (Ogawa et al., 1991; Creighton, 1993).

2.4.1. R-Phycoerythin as a TTI to verify adequate processing of beef products

Orta-Ramirez (1999) investigated the effectiveness of PE as an extrinsic, single component, fluorescence-based TTI to monitor thermal processing in beef products. PE was easily extracted from edible algae, abundant (up to 20% dry weight) and had a wide range of thermal tolerance under different chemical conditions. In addition, after thermal processing, the fluorescent response of PE was readily detectable without intermediate recovery steps.

The effect of pH on the spectral properties of R-PE was investigated (Orta-Ramirez, 1999). Fluorescence intensity was similar at pH values from 5.0-9.0. At pHs below 4.0 or above 10.0 R-PE did not fluoresce. In addition, it was shown that a solution of R-PE exhibited a bright pink-red color at neutral pH values, and shifted to purple at pHs above and below neutrality.

It was shown by Ogawa et al. (1991) that at pH near 4.0, R-PE exists in the form of aggregates. At pH 7.0, PE has a compact, native conformation with the chromophores tightly packed in the interior. The protein is partially unfolded at pH 10.0. The addition of sucrose, SDS, NaCl, or ME at pH 4.0 resulted in a complete loss of R-PE fluorescence even before heating. Similarly, at pH 10.0, the protein structure was very sensitive to the addition of solvents due to being partially unfolded.

The effects of pH on thermal inactivation of R-PE were also studied (Orta-Ramirez, 1999). D values were calculated at temperatures from 60-90°C for pH values from 4.0-10.0. The R-PE was most thermally resistant at pH 6.0 and 7.0 (pH 6.0: D_{70} = 12258.72 min, D_{80} = 239.74 min, D_{90} = 1.43 min; pH 7.0: D_{70} = 2204.75, D_{80} = 461.87 min, D_{90} = 1.21 min). The z values increased from pH 4.0 (z = 4.59°C) to pH 9.0 (z = 9.15°C) and then decreased once again at pH 10.0 (z = 4.44°C).

A large range of z values was obtained by manipulating the environment of R-PE, thus prompting the study of combined effects of pH and additives. Overall, sucrose and β-mercaptoethanol (ME) had stabilizing effects, while the addition of sodium dodecyl sulfate (SDS), sodium chloride (NaCl), and urea caused protein denaturation of PE.

It was concluded from these preliminary experiments that by modifying the environment, the z value of PE can be adjusted to that of a target pathogen in meat products. The D values of R-PE in 0.012 M borate buffer, pH 9.0 ranged from 512.82 min at 60°C to 10.57 min at 70°C. The z value of 5.99°C fell within the range given for z values of *Salmonella* of 5.6°C to 6.2°C (Goodfellow and Brown, 1978; Orta-Ramirez et al., 1999). This range of z values was chosen for comparison due to the fact that *Salmonella* is the target microorganism in thermal processing of beef products (USDA-FSIS, 2000).

Non-isothermal experiments were also completed to verify the adequacy of R-PE as a TTI for thermal processing of meat products (Orta-Ramirez, 1999). Results indicated that R-PE could be used to distinguish between undercooked, properly cooked, and overcooked samples. Thus this protein could be used to verify proper processing of meat products once a cut-off value of residual fluorescence was determined for thermal processing. Though the R-PE was able to differentiate between adequate and inadequate heat processes, the PE did not respond accurately to the time temperature combinations tested. The General Method (Pflug, 1997) was used to calculate predicted fluorescence values corresponding to the heating protocols. A weak correspondence ($R^2 < 0.80$) was seen between the predicted and observed values, indicating that non-isothermal inactivation of PE did not follow first-order kinetics.

When normalized fluorescence (fluorescence at time t/ initial fluorescence) was plotted against calculated lethalities for each process, an exponential relationship was found. Thus it was concluded that even though the thermal inactivation of PE did not follow first order kinetics, it could be used as a TTI to predict the thermal destruction of *Salmonella* during thermal processing of beef products. Therefore, even though the z value of PE will not be equal to the z value of *Salmonella*, results show that the PE destruction is very predictable. PE will be able to distinguish between undercooked and properly cooked beef products.

2.5. Triose phosphate isomerase

Triose phosphate isomerase (TPI) is an enzyme that catalyzes the interconversion of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde 3-phosphate (GAP) (Webb and Knowles, 1975). TPI has been isolated and crystallized from many different sources including calf skeletal muscle (Beisenhertz, 1955), rabbit skeletal muscle (Czok and Buecher, 1960; Norton et al., 1970), chicken breast muscle (Bonner et al., 1976) and human skeletal muscle (Dabrowska et al., 1978).

TPI activity is determined through the following coupled reactions:

$$\text{Glyceraldehyde-3-phosphate} \xrightarrow{\text{Triose phosphate isomerase}} \text{dihydroxyacetone phosphate (DHAP)}$$

$$\text{DHAP + NADH} \xrightarrow{\text{glycerol-3-phosphate dehydrogenase}} \text{glycerol-phosphate + NAD}$$

Glyceraldehyde-3-phosphate is the substrate of the reaction and the glycerol-3-phosphate dehydrogenase (GDH) acts as the coupling enzyme (Beisenhertz, 1955). It has been shown that TPI activity is affected by choice of buffer and pH during the assay procedures. In a neutral pH range (7 to 8) there is little variation in activity, but at pH 6.3, the activity is reduced by half (Beisenherz, 1955). It has also been shown that the activity of TPI is doubled in triethanolamine-HCl buffer in comparison to bicarbonate-CO_2 at pH 7.5 (Beisenherz, 1955). Oesper and Meyerhof (1950) reported that a buffer containing 0.05 M phosphate reduced TPI activity by 75%.

2.5.1 Triose phosphate isomerase as a TTI for thermal processing of ground beef

Endogenous enzymes have been suggested as potential indicators of proper thermal processing in beef products. Orta-Ramirez et al. (1997) conducted thermal inactivation studies on several enzymes, *E. coli* O157:H7 and *S.* Senftenberg. For an endogenous protein to be an ideal indicator of thermal processing, it should exhibit the same temperature sensitivity (z value) of the target microorganism. The z value reported for TPI in ground beef of 5.56°C, (Orta-Ramirez et al., 1997) was close to the value for *Salmonella* (z = 5.64°C) (Goodfellow and Brown, 1976), the pathogen used to establish processing guidelines for ground beef patties.

Sair et al. (1999) investigated the relationship between TPI activity and cooking endpoint temperature in ground beef patties. TPI activity decreased with increasing cooking temperatures in ground beef patties (24.4% fat) from 60.0 to 71.1°C. Yet no differences in TPI activity could be distinguished when patties were cooked to internal temperatures of 71.1 to 82.2°C. Enzyme activity in beef patties decreased from a raw value of 2,303U/g meat to 6.3 U/g meat or less at temperatures greater than or equal to 71.1°C.

Larger standard deviations were seen in TPI activity at lower processing temperatures suggesting that large temperature variations were present within a ground beef patty during processing (Sair et al., 1999). This hypothesis was supported by Berry et al. (1999) who reported temperature differences up to 18°C using infrared thermography on cross sections of patties cooked to a final internal temperature of 71.1°C.

The use of TPI to verify that roast beef has been properly processed was investinged by Hsu et al. (2000). Ground semimembranous muscle was cooked to achieve a 7 $\log_{10}$ reduction using either low, medium, or high temperature roast beef processing schedules (USDA, 1996). TPI activities were similar ($P<0.05$) in ground meat cooked using three equivalent time-temperature regulations. The lowest TPI activities in adequately processed roast beef model systems and pilot plant studies were 2.1 and 1.56 U/g, respectively. It was suggested that a residual TPI value less than or equal to about 2 U/g could be used to indicate proper thermal processing of roast beef cooked under any of the time-temperature regulations.

CHAPTER 3

THERMAL INACTIVATION OF *SALMONELLA* TYPHIMURIUM DT104 AND AN 8-STRAIN *SALMONELLA* COCKTAIL UNDER DIFFERENT CONDITIONS

3.1. ABSTRACT

Thermal death time studies using three strains of *Salmonella* Typhimurium DT104 in ground beef (4.8% fat) revealed that the D values of each strain were not different (P>0.05) at 55 or 58 °C. At 61 °C, the D value of strain 10127 (0.57 min) was higher (P<0.05) than those of strains 01071 (0.43 min) and 10601 (0.41 min). The D values for strain 10127 in ground beef were similar at 58, 61, and 64 °C, regardless of fat content. However, at 55 °C the D value in high fat ground beef (21.98 min) was more than two fold greater than that in the low fat ground beef (9.05 min).

The heat resistance of the *Salmonella* cocktail in stationary phase decreased after inoculation and frozen storage of ground beef at 55, 58 and 61°C. Thermal resistance of the cocktail decreased (P<0.05) at 55, 61, and 63°C after 14d of starvation in 0.1% peptone water at 4°C. The D values for the thermally injured (56°C for 30 min) and heat shocked (48°C for 30 min) cocktails were not different (P<0.05) from one another at any of the test temperatures. The D values of the thermally injured cocktail were also not different (P<0.05) from the *Salmonella* cocktail in log phase. The heat shocked cocktail was less heat resistant than the non-heat shocked cocktail at 58°C. The z value of the thermally injured cocktail was the highest recorded, thus these cultures were the least temperature dependent.

3.2. INTRODUCTION

Current command and control regulations require that beef patties be cooked to a certain temperature for a specified amount of time to eliminate pathogens. Proposed regulations (USDA-FSIS, 1999) would supplement specific time temperature requirements with lethality performance standards. A 5 $\log_{10}$ reduction in *Salmonella* has been proposed as the lethality performance standard for fully cooked, uncured meat patties (USDA-FSIS, 1999). The FSIS has not finalized this standard due to concerns that a process equivalent to a 5 $\log_{10}$ reduction in *Salmonella* may not be sufficient to protect public health. The lethality performance standard for roast beef requires the use of a combination of thermal and non-thermal processes sufficient to achieve a 6.5 log reduction in *Salmonella* (USDA-FSIS, 1999).

The USDA-FSIS (2000) recommends the use of a cocktail or a combination of *Salmonella* serotypes to verify compliance to the performance standard. This cocktail should consist of *Salmonella* strains that exhibit relatively high heat resistance and have been previously implicated in foodborne outbreaks (UDSA-FSIS, 2000). The D and z values for different *Salmonella* serotypes were determined in chicken broth and meat products in order to identify the most appropriate strains to use for defining heat resistance in meat products (Juneja et al., 2000). A comparison of these strains to *E. coli* O157:H7 and other *Salmonella* strains would be useful to verify that the recommendations are appropriate.

Salmonella Typhimurium DT104 has emerged as a particularly dangerous *Salmonella* phage type that is resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (Hollingsworth et al., 1997). In 1996, 32% of the *S.*

Typhimurium isolates tested at the Centers for Disease Control and Prevention demonstrated the ACSSuT resistant pattern associated with Definitive Type 104 (Morbidity and Mortality Weekly Review, 1997). D values for a clinical isolate of *Salmonella* Typhimurium DT104 in chicken broth ranged from 4.16 min at 55°C to 0.27 min at 62°C (Juneja et al., 2000).

Several factors influence the thermal resistance of *Salmonella*, including age of the culture, nutrient content of the growth medium, heat treatment history, and initial population. According to Heddleson et al., (1991) maximum heat resistance of *Salmonella* spp. occurred in cells that had reached stationary phase in tryptic soy broth after 24h of incubation. The numbers of viable pathogens also can be affected by the nutrients available in the growth medium. Dykes and Withers (1999) examined sub-lethal damage in *Listeria monocytogenes* cells starved at 4°C vs. fresh cultures. Growth of fresh cells was minimally affected by selective agar. In contrast, numbers of starved cells were greatly reduced after direct plating on a selective agar. Potential public health risks could arise if starved cultures within raw food products remain undetected on selective media.

The history of heat treatment and its effect on thermal resistance of pathogens has been studied intensely in recent years. Mackey and Derrick (1987) reported that the D values of *S.* Thompson in minced beef increased from 10.5 to 25.0 min at 54°C and from 0.46 to 1.26 min at 60°C after a previous heat treatment at 48°C for 30 min. Thermal resistance of *E. coli* O157:H7 in ground beef at 60°C increased following thermal treatment at 46°C for 15 to 30 min. Muscle type, pH, fat and moisture content of the heating menstruum also influence thermal resistance of microorganisms. Therefore, thermal inactivation parameters of a microorganism must be established in the food of interest with controlled environmental conditions for accurate process control.

The objectives of this study were to determine (1) the thermal resistance of *S.* Typhimurium DT104 in ground beef of two fat contents and (2) to assess the effects of microbial growth phase, freezing, starvation conditions and non-lethal heat treatment on thermal resistance of a *Salmonella* cocktail.

3.3. MATERIALS AND METHODS

3.3.1. Bacterial strains

Three human isolates of *Salmonella* Typhimurium DT104 (10601, 10127, 01071) were obtained from B. Swaminathan (Centers for Disease Control and Prevention, Atlanta, GA). The following eight *Salmonella* strains were obtained from V.K. Juneja (Agricultural Research Service, Eastern Regional Research Center, USDA-ARS, Philadelphia, PA): *S.* Thompson FSIS 120 (chicken isolate), *S.* Enteriditis H3527 and H3502 (clinical isolates phage type 13A and 4, respectively), *S.* Typhimurium H3380 (human isolate DT104), *S.* Hadar MF60404 (turkey), *S.* Copenhagen 8457 (pork), *S.* Montevideo FSIS 051 (beef), and *S.* Heidelberg F5038BG1 (human isolate). All strains were preserved in vials containing tryptic soy broth (TSB) (Difco, Detroit, MI) with 10% glycerol at –80 °C.

3.3.2. Culture preparation

To propagate the cultures, one loopful of frozen culture was transferred to 9 mL of TSB in 20 mL culture tubes. Cultures were maintained by daily transfers in TSB (37°C, 18-24h), with a minimum of two consecutive transfers before use. Each inoculum was prepared from either an 18-24h (log phase) or 48h (stationary phase) culture containing approximately 10^9 CFU/ml. The eight *Salmonella* strains received from Juneja were grown in separate culture tubes and then combined in equal volumes just before centrifugation to produce a cocktail for thermal death time studies.

On the day of each experiment, cultures were pelleted by centrifugation at 6,000 × g for 10 min at 4 °C and resuspended in sterile 0.1% buffered peptone water to a concentration of about 10^{10} CFU/ml. Following centrifugation, the starved *Salmonella* cocktail was resuspended in 5 mL of 0.1% peptone water and stored at 4 °C for 14 days. The cultures were enumerated by serially diluting in 0.1% peptone water and plating on Petrifilm® aerobic count plates (3M, St. Paul, MN) in duplicate.

To produce thermally injured and heat shocked *Salmonella* cocktails, 4×10^{12} CFU/mL were added to 200 mL of tempered TSB in a 3L Erlenmeyer flask and heated for 30 min at 56 or 48°C, respectively (Bunning et al., 1990; Mackey and Derrick, 1987). Preliminary studies showed that a 1-log decrease in growth was observed on bismuth sulfite agar (selective agar) when compared to tryptic soy agar with 0.9% yeast extract (non-selective agar), thus producing a 90% thermally injured culture after heating at 56°C for 30 min.

3.3.3. Ground beef preparation

Low fat beef was obtained from the Michigan State University Meat Laboratory. High fat beef was received from a local processor. The meat was ground twice through a 4 mm diameter plate in a Kitchen Aid grinder (Model K5-A, Hobart, Troy, OH), vacuum packaged in 60g portions, and frozen at -12 °C. The frozen meat was transported on dry ice to Iowa State University and irradiated (10 kGy) to eliminate indigenous microflora.

The irradiated beef was tested for sterility by plating a 1:10 dilution onto Petrifilm® Aerobic count plates.

Moisture, fat, and protein contents were determined by AOAC (1996) methods 950.46B, 991.36, and 981.1, respectively. For determination of pH, 10g of ground beef were homogenized with 90 mL of distilled water using a Polytron homogenizer (Model PT 10/35, Brinkman Instruments, Westbury, NJ) at speed setting 3 for 30s. The pH of the homogenized mixture was measured using a combination electrode (Model 145, Corning, Medfield, MA). Analyses were done in triplicate.

3.3.4. Inoculation of ground beef

Microorganisms were added dropwise, using aseptic procedures, to obtain a target concentration of 10^8 CFU/g ground beef. The meat was mixed in a sterile bowl using sterile gloves to ensure even distribution. Inoculated meat was transferred into a 60 mL syringe (Becton Dickinson and Co., Franklin Lakes, NJ) after which 1g aliquots were extruded into 5 × 25.5 cm sterile polyethylene laminated nylon bags (Butcher and Packer, Detroit, MI). Sterility of the bags was confirmed by mixing 9 mL of 0.1% peptone water in random bags and plating the resultant liquid on Petrifilm® Aerobic count plates. Bags containing the meat were rolled to a thickness of 1 mm using a template and then heat sealed.

For the freezing studies, the inoculated meat was held at –9 °C for 7 days. Meat was tempered to 4 °C under cold running water prior to thermal death time studies.

3.3.5. Thermal inactivation procedure

The bags containing meat were placed in a rack and completely submerged in a Polystat circulating water bath (Model 1268-52, Cole-Palmer Instrument Co., Chicago, IL) set at 55, 58, 61, 64 or 66 °C. The temperature was monitored using a thermocouple (Type T, length 8.6 cm, width 1.0 mm, accuracy ± 0.1 °C, Omega Engineering, Stamford, CT) connected to a datalogger system (Daqbook 100, Omega) inserted and sealed in the pouches. The heating lag time was 8s. Time zero was defined as the time when the meat reached the target temperature. Preliminary studies were conducted to determine appropriate time-temperature combinations to achieve ≥ a 5 $\log_{10}$ reduction in pathogens. Meat was removed at specific time intervals, held on ice at 4 °C and used within 4h. Three replicate batches of ground beef were heated at each temperature.

3.3.6. Enumeration of *Salmonella*

Cooked ground beef was transferred to sterile Whirlpak™ bags (18 oz, Nasco, Ft. Atkinson, WI) and manually homogenized for 1 min with 9 mL of 0.1% sterile peptone water. Bacterial counts were determined by decimal dilution of the ground beef in sterile 0.1% peptone water and enumerated using Aerobic Petrifilm Count Plates (3M, St. Paul, MN). All plates were incubated at 37 °C for at least 24 hr.

3.3.7. Calculation of D values and z values

Bacterial counts were converted to logarithms. D values were estimated assuming linear inactivation kinetics. D values (in minutes) were calculated by linear regression of surviving bacteria vs. time from at least 4 data points with a correlation coefficient >0.80 using Microsoft Excel Version 5.0a (Microsoft Corp., Redmond, WA). Thermal resistance curves were determined by plotting log D values vs. temperature. The z value was determined as the negative reciprocal of the slope of the thermal resistance curve. Significant differences of means were determined using paired t-tests ($P<0.05$) (JMP, SAS Institute, 1998)

3.4. RESULTS AND DISCUSSION

3.4.1. Composition

Low fat ground beef (pH 5.65 ± 0.01) contained 4.8% ± 1.1% fat, 72.4% ± 0.2% moisture and 15.8% ± 2.6% protein. High fat ground beef (pH 5.72 ± 0.01) contained 19.1% ± 0.7% fat, 63.4 % ± 0.6% moisture, and 14.4% ± 1.9% protein. No microorganisms were detected in the irradiated meat.

3.4.2. Heat resistance of *Salmonella* Typhimurium DT104

Thermal death time studies were performed using three strains of *S.* Typhimurium DT104 in ground beef (4.8% fat) (Table 3.1). The D values of the three strains were not different ($P>0.05$) at 55 or 58 °C. At 61 °C, the D value of strain 10127 (0.57 min) was higher ($P<0.05$) than those of strains 01071 (0.43 min) and 10601 (0.41 min). At 64 °C the D value for strain 10601 (0.07 min) was about half that of the other two strains ($P<0.05$). The D values calculated for the three strains at 58 °C were similar to those reported by Humphrey et al., (1997) for three wild strains of *S.* Typhimurium DT104 on pork muscle ($D_{58\ °C}$ = 2.61 min).

Maurer (2000) reported D values of 16.34 min, 2.72 min, 0.44 min and 0.15 min at 55, 58, 61, and 63°C, respectively, for the log phase 8-strain *Salmonella* cocktail in 19.1% ground beef. Thus, the *S.* Typhimurium DT104 strain 10127 was more thermally resistant at 55 and 61°C. If beef was cooked according to the processes established for this cocktail, a 5D reduction in *S.* Typhimurium DT104 might not occur. Thus, this cocktail would underestimate thermal processing schedules needed to accomplish a 5D reduction if more heat resistant strains of *Salmonella* were present.

Table 3.1 D values[a] and regression parameters of three strains of *Salmonella* Typhimurium DT104 in high (19.1%) and low (4.8%) ground beef.

Bacterium	Fat Content	Temp (°C)	y-intercept	Slope	R^2	D value (min)
S. Typhimurium DT104-10127	High	64	6.30	-6.20	0.97	0.16 ± 0.01
		61	6.32	-1.54	0.97	0.65 ± 0.03
		58	6.57	-0.38	0.95	2.63 ± 0.19
		55	6.49	-0.05	0.95	21.98 ± 2.15
S. Typhimurium DT104-10127	Low	64	7.13	-6.70	0.98	0.15 ± 0.01
		61	6.97	-1.75	0.96	0.57 ± 0.01
		58	7.01	-0.44	0.95	2.26 ± 0.10
		55	6.97	-0.11	0.95	9.05 ± 0.13
S. Typhimurium DT104-10601	Low	64	7.03	-14.43	0.99	0.07 ± 0.01
		61	6.67	-2.47	0.98	0.41 ± 0.01
		58	7.87	-0.49	0.95	2.15 ± 0.07
		55	6.40	-0.10	0.93	10.55 ± 1.04
S. Typhimurium DT104-01071	Low	64	6.97	-7.20	0.95	0.14 ± 0.01
		61	7.49	-2.33	0.94	0.43 ± 0.02
		58	7.24	-0.49	0.97	2.06 ± 0.13
		55	7.09	-0.10	0.96	10.27 ± 0.78

[a]D value is expressed as the mean ± standard error of the mean of three replicate determinations.

D values for *Escherichia coli* O157:H7 in high fat (19.1%) ground beef were reported as 22.47 min, 2.05 min, 0.32 min, and 0.18 min at 55, 58, 61, and 63°C, respectively (Maurer, 2000). Thus, *E. coli* was more heat resistant at 55°C, than *S.* Typhimurium DT104 strain 10127.

The D values for strain 10127 in ground beef were similar at 58, 61, and 64°C, regardless of fat content. However, at 55°C the D value in high fat ground beef (21.98 min) was more than two fold greater than that in the low fat ground beef (9.05 min). Similar effects due to fat content were seen with *E. coli* O157:H7 in ground beef. (Line et al., 1991; Ahmed et al., 1995; Maurer, 2000).

3.4.3. *Salmonella* cocktail

The combination of the 8-strain *Salmonella* cocktail in stationary growth phase with freezing of inoculated meat was also investigated. D values for the 8-strain *Salmonella* cocktail in stationary phase without freezing were previously reported as 18.66 min, 3.39 min, 0.57 min, and 0.20 min at 55, 58, 61, and 63°C, respectively in high fat (19.1%) ground beef (Maurer, 2000). Thermal resistance of the *Salmonella* cocktail in stationary phase decreased ($P<0.05$) after inoculation and frozen storage of ground beef at 55, 58 and 61°C (Table 3.2). Formation of ice crystals during freezing which disrupt the cell membrane is likely responsible for the observed decrease in viability (Doyle and Cliver, 1990; Smith, 1995). Given these findings, greater total lethality may be achieved when cooking previously frozen raw products as compared products that have not been frozen.

Table 3.2 D values[a] and regression parameters of an 8-stain *Salmonella* cocktail in stationary phase after freezing inoculated ground beef (19.1% fat).

Temp (°C)	y-intercept	Slope	R^2	D value (min)
63	6.47	-5.13	0.90	0.20 ± 0.01
61	7.08	-3.54	0.94	0.28 ± 0.02
58	6.74	-0.42	0.85	2.36 ± 0.14
55	6.87	-0.08	0.85	12.52 ± 1.27

[a]D value is expressed as the mean ± standard error of the mean of three replicate determinations.

The thermal stability of *E. coli* O157:H7 was increased due to cold-shock protein synthesis after freezing (-18°C) in comparison to fresh (3°C) hamburger patty storage (Jackson et al., 1996). Heat resistance of the *Salmonella* cocktail decreased in ground beef after freezing. Juneja et al., (1997) reported that freezing of *E. coli* O157:H7 cultures (-18°C) in ground beef did not affect the heat resistance. Differences could be due to storage conditions including sample size, length of storage, and storage temperature. During studies completed here, 1g of ground beef was frozen at -9°C for 7 days. Juneja et al., (1997) and Jackson et al., (1996) held 3g samples for 48h and 113g samples for 9h at -18°C. Sample size could have an impact cooking rates. In addition, cold-shock protein induction and biochemical changes in the bacterial membrane might be affected by the time and temperature of storage.

Storing the *Salmonella* cocktail in 0.1% peptone water at 4°C for 14 days to produce starved cells resulted in D values of 5.85 min, 2.93 min, 0.22 min, and 0.11 min at 55, 58, 61, and 63°C, respectively, thus reducing the heat resistance at all four temperatures tested. Differences ($P<0.05$) were seen between the D values of the starved *Salmonella* cocktail and the cocktail in log phase at 55, 61, and 63°C.

No literature could be found on the effect of starvation on thermal inactivation, yet starvation at 4°C reportedly reduced the growth of *L. monocytogenes* and *E. coli* O157:H7 cultures on selective media. According to Dykes and Withers (1999) growth of fresh *L. monocytogenes* cultures showed a reduction in growth numbers of 8% or less when plated on a selective compared to a non-selective medium. However, populations of cells that had been stored in phosphate-buffered saline (pH 5.5) for 4 weeks were reduced by more than 2 log (99%) on selective medium.

In addition, when *E. coli* O157:H7 cultures were starved in phosphate buffered saline, Arnold and Kasper (1995) reported that viable counts decreased 100 fold after 48 hr. Although it appears that extended freezing may decrease thermal resistance, the presence sub-lethally damaged strains may constitute in a substantial health risk, since these pathogenic strains could remain undetected in foods.

D values for the thermally injured and heat shocked *Salmonella* cocktail were not different ($P< 0.05$) from one another at any of the temperatures tested (Table 3.3). Maurer (2000) reported similar D values for the *Salmonella* cocktail in log phase the same temperatures tested. The D values for the heat shocked cocktail were also not different from the non-heat shocked cocktail at 63, 61, or 55°C. However, at 58°C, the D value of the heat shocked cocktail was lower ($P< 0.05$).

Studies have shown that thermotolerance of bacteria is greatly affected by the heating menstuum during heat shock and subsequent thermal inactivation (Farber and Brown, 1990; Juneja et al., 1998). Thermal inactivation studies were performed with

Table 3.3. D values and regression parameters of an 8-strain *Salmonella* cocktail after thermally injury (56°C for 30 min) or heat shock (48°C for 30 min).

Treatment	Temp (°C)	y-intercept	Slope	R^2	D value (min)
56°C – 30min	63	5.69	-3.86	0.82	0.26 ± 0.01[a]
	61	7.58	-2.47	0.88	0.41 ± 0.05[b]
	58	5.68	-0.34	0.88	2.98 ± 0.22[c]
	55	7.13	-0.09	0.88	11.14 ± 1.75[d]
48°C – 30 min	63	7.71	-6.03	0.90	0.17 ± 0.02[a]
	61	8.20	-2.84	0.97	0.35 ± 0.01[b]
	58	7.73	-0.53	0.92	1.90 ± 0.12[c]
	55	7.53	-0.09	0.91	11.71 ± 2.62[d]

[abcd] Means ± the standard error of the mean followed by the same letter were not different (P<0.05).

E. coli O157:H7 in tryptic soy broth (TSB) and a ground beef slurry after heating at a sublethal temperature of 45°C for 30 min (Williams and Ingham, 1997). Heat shock increased the D values by 37, 68, and 50%, respectively, at 54, 58, and 62°C in tempered TSB. However, the D values at 58°C in a ground beef slurry were not different.

These results are in good accordance with a heat shock study involving *L. monocytogenes*. Farber and Brown (1990) reported that after heat shocking *L. monocytogenes* at 48°C for 30 min, D values in a sausage mix were not different (P < 0.05) at 62 and 64°C. D values did not change until the cultures were heat shocked for 120 min at 48°C.

Mackey and Derrick (1987) heat shocked *S.* Thompson cultures at 48°C for 30 min before performing thermal inactivation studies in minced beef. The D values at 54°C and 60°C increased by factors of 2.4 and 2.7, respectively. Xavier and Ingham (1997) reported that heat shocking at 42°C for 60 min produced in higher D values in casein peptone-soymeal peptone broth for *S.* Enteriditis at 52, 54, and 56°C, but not at 58°C. In addition, Bunning et al. (1990) reported that heat shocking *S.* Typhimurium at 48°C for 30 min increased the D value from 21.3 min to 96.1 min at 52°C in broth. However, when the cultures were heat shocked at 42°C and 52°C, no difference was seen in D values at an inactivation temperature of 57.8°C. These results suggested that 42°C is too low to initiate the synthesis of heat-shock proteins, while 52°C led to a progressive loss of bacterial viability.

Heat shocking a four-strain cocktail of *E. coli* O157:H7 at 46°C for 15 to 30 min increased thermal resistance in ground beef (Juneja et al., 1998). Using the untreated cocktail populations decreased by 2.17 and 7.8 log_{10} CFU/g, respectively, after 4 and 15 min of heating at 60°C. Populations in the heat shocked cocktail decreased 0.35 and 8.5 log_{10} CFU/g under the same inactivation conditions. Differences in the results reported here could be due to the use of a cocktail rather than a homogenous culture.

3.4.4. z Values

Thermal inactivation of all three *Salmonella* Typhimurium DT104 strains (z=4.13-5.07°C) was less temperature dependent than that of *E. coli* O157:H7 (z = 3.60) and the *Salmonella* cocktail in log phase (z = 3.90) in ground beef with 19.1% fat as indicated by the higher z values (Table 3.4) (Maurer, 2000). The z value for *S.* Typhimurium DT104 strain 10127 was higher in low as compared to high fat ground beef. Ahmed et al. (1995) reported similar findings in which the z value for *E. coli* O157:H7 increased from 4.35 to 4.78°C in ground beef as the fat content decreased from 20 to 7%.

The z value of the *Salmonella* cocktail in stationary phase after freezing was not different from the cocktail in stationary phase ($z = 4.08°C$) (Maurer, 2000) ($P<0.05$). There was no difference between the z values for the starved cocktail and the cocktail in log phase. In addition, the z values for the heat shocked and thermally resistant cocktail were not different ($P<0.05$) from the non-heat shocked cocktail in log phase.

Table 3.4 The z values[a] and regression parameters of *Salmonella* Typhimurium DT104 and an 8-strain *Salmonella* cocktail in stationary phase after freezing in high (19.1%) and low (4.8%) fat ground beef.

Bacterium	Fat content	y-intercept	Slope	R^2	z value (°C)
S. Typhimurium DT104-10127	High	14.11	-0.23	0.99	4.28 ± 0.05
S. Typhimurium DT104-10127	Low	11.80	-0.20	1.00	5.07 ± 0.02
S. Typhimurium DT104-10601	Low	14.38	-0.24	1.00	4.13 ± 0.07
S. Typhimurium DT104-01071	Low	12.50	-0.21	0.99	4.77 ± 0.06
Salmonella cocktail (48h frozen)	High	14.18	-0.24	0.97	4.20 ± 0.06
Salmonella cocktail - starved	High	13.75	-0.23	0.95	4.28 ± 0.01
Salmonella cocktail - (56°C for 30 min)	High	12.92	-0.22	0.98	4.64 ± 0.09
Salmonella cocktail - (48°C for 30 min)	High	13.86	-0.23	0.99	4.28 ± 0.27

[a]The z value is expressed as the mean ± standard error of the mean of three replicate determinations.

3.5. CONCLUSIONS

The new performance standards require meat processors to validate the efficacy of their processes to reduce microbial contamination if cooking procedures vary from published compliance and safe harbor guidelines. Results stated here indicate that the particular pathogen, the state of the meat (fresh or frozen), and the fat content must be considered when designing thermal processes. Based on our findings, this 8-strain *Salmonella* cocktail may not be able to adequately predict a 5-log reduction for some pathogens including more heat resistant strains of *S.* Typhimurium DT104 at low temperatures (≤55°C). In addition, great care should be taken during thermal processing to reduce the lag time of heating meat products, so as not to produce thermotolerant cells that may require greater process lethalities to ensure a safe meat product.

CHAPTER 4

EVALUATION OF PHYCOERYTHRIN AS A TIME-TEMPERATURE INTEGRATOR IN BEEF PATTIES

4.1. ABSTRACT

R-phycoerythrin encapsulated in glass capillary tubes was inserted into ground beef patties of varying fat content and size to evaluate its applicability as a time-temperature integrator. The normalized fluorescence of PE was 0.31 or below when 113g high fat (19.1%) patties were processed to meet USDA-FSIS time-temperature regulations or lethality performance standards. All of the patties exhibited at least a 5-log decrease in a *Salmonella* cocktail or met one of the safe harbor guidelines when the PE fluorescence value was lower than 0.31.

Similar results were seen in 113g low fat (4.8%) ground beef patties. Analysis of the normalized fluorescence of PE revealed that a value of 0.29 was indicative of a properly cooked patty. Of the 41 patties tested, only one sample which met the USDA-FSIS thermal processing guidelines had a PE value greater than 0.29, meaning that the PE did not correctly identify this patty as being properly cooked.

A normalized PE fluorescence of 0.21 or below was found when 60g ground beef patties were properly cooked according to the USDA-FSIS safe harbor guidelines. This endpoint fluorescence value was lower than in the 113g patties. Improper application of methodologies designed for larger patties limited the collection of accurate results. Further studies will be needed to validate phycoerythrin as a time-temperature integrator for proper thermal processing of ground beef patties.

4.2. INTRODUCTION

Title 9 of the Code of Federal Regulations has traditionally been used to outline the USDA processing requirements to destroy food borne pathogens in meat products (USDA-FSIS, 1999). Recently the USDA-FSIS, in addition to time/temperature schedules, has allowed processors to establish thermal processes based on lethality standards (USDA-FSIS, 1999). Lethality performance standards are based on the destruction of a specific, significant number of *Salmonella* microorganisms.

One proposed lethality performance standard for fully cooked, uncured meat patties allows the use of any thermal process to achieve a 5 $\log_{10}$ reduction in *Salmonella* (USDA-FSIS, 1999). Although these thermal processing guidelines are available, there is still a need to verify adequate cooking.

A time temperature integrator (TTI) is a device that undergoes an irreversible and precisely measurable change in response to time-temperature history that mimics the change in a target attribute exposed to the same thermal history (Hendrickx et al., 1995). A major advantage of a TTI is the quantification of the integrated time-temperature impact on microbial destruction without acquiring the actual time-temperature history of the product. Ideal TTIs would be simple, inexpensive to prepare and easily recoverable with an accurate and user-friendly read-out. TTIs should be incorporated into a beef patty without disrupting heat transfer while quantifying the thermal process on microbial destruction.

Phycoerythrin (PE), a phototosynthetic antenna complex protein in cyanobacteria, red algae, and cryptomonads, was investigated as an extrinsic, single component, fluorescence-based TTI to monitor thermal processing in beef products (Orta-Ramirez, 1999). PE, red in color, has absorption bands at 498-568 nm and fluorescent emission bands at 570-620 nm. In earlier experiments, the z values of PE increased from pH 4.0 (z = 4.59°C) to pH 9.0 (z = 9.15°C) and then decreased at pH 10.0 (z = 4.44°C). A large range of z values was obtained by manipulating the environment of R-PE. It was concluded from these experiments that the z value of PE could be adjusted to that of a target pathogen in meat products by modifying the content, pH, and concentration of the buffering solution. The z value of PE in 0.012 M borate buffer, pH 9.0, of 5.99°C fell within the range given for z values of *Salmonella* of 5.6°C to 6.62°C (Goodfellow and Brown, 1978; Orta-Ramirez et al., 1997, Juneja et al., 2000). This range of z values was chosen for comparison since *Salmonella* is the target microorganism in thermal processing of beef products based on the new lethality standards (USDA-FSIS, 2000).

In non-isothermal studies, residual PE fluorescence in a buffer system could distinguish between undercoooked, properly cooked, or overcooked thermal processes for ground beef. It was concluded that inactivation of PE did not follow first order kinetics. A non-linear model was developed to fit the thermal inactivation of *S.* Senftenberg and fluorescence loss of PE. In both cases, lethality was very predictable, suggesting that a mathematical relationship could be established between the lethality of *S.* Senftenberg and the fluorescence loss of R-PE.

The objectives of this study were to (1) verify the ability of PE to distinguish between cooking schedules of varying time and temperature combinations within a beef patty and (2) to identify a minimum fluorescence reduction to indicate proper cooking of a ground beef patty.

4.3. MATERIALS AND METHODS

4.3.1. Ground beef patties

Low fat ground beef was obtained from the Michigan State University Meat Laboratory. High fat ground beef was received from a local processor. The meat was ground through a 3.175 mm diameter plate in a grinder (Tor Rey, Model M-32-5, Monterrey, Nuevo Leon, Mexico), vacuum packaged in 1135g portions, and frozen at -12°C. The frozen meat was transported on dry ice to Iowa State University and irradiated (10 kGy) to eliminate indigenous microflora. The irradiated beef was tested for sterility by plating a 1:10 dilution on Petrifilm® Aerobic count plates. Ground beef was thawed at 4°C for 24h prior to use.

Moisture, fat and protein contents were determined by AOAC (1996) methods 950.46B, 991.36, and 981.1, respectively. For determination of pH, 10g of ground beef plus 90 mL of distilled water were homogenized using a Polytron homogenizer (Model PT 10/35, Brinkman Instruments, Westbury, NJ) at speed setting 3 for 30s. A combination electrode (Model 145, Corning, Medfield, MA) was used to measure the pH of the ground beef slurry. Analyses were done in triplicate.

4.3.2. Bacterial strains

Eight *Salmonella* strains were obtained from V.K. Juneja at the Agricultural Research Service, Eastern Regional Research Center of the USDA. The strains were *S.* Thompson FSIS 120 (chicken isolate), *S.* Enteriditis H3527 and H3502 (clinical isolates phage type 13A and 4, respectively), *S.* Typhimurium H3380 (human isolate DT104), *S.* Hadar MF60404 (turkey), *S.* Copenhagen 8457 (pork), *S.* Montevideo FSIS 051 (beef), and *S.* Heidelberg F5038BG1 (human isolate). The strains were preserved at –80 °C in tryptic soy broth (TSB) (Difco, Detroit, MI) containing 20% glycerol.

4.3.3. Culture preparation

To propagate the cultures, one loopful of frozen culture was transferred to 100 mL of TSB in a 200 mL culture bottle. Cultures were maintained by daily transfers in TSB, with a minimum of two consecutive 24h transfers before use. Each inoculum was prepared from a 18-24h (log phase) culture containing approximately 10^9 CFU/ml. A preliminary growth curve study was conducted to determine the times of incubation needed to achieve log phase (Maurer, 2000). The eight cultures used in the *Salmonella* cocktail were each grown in separate culture tubes and combined in equal volume before centrifugation just prior to thermal death time studies.

On the day of experimentation, cultures were pelleted by centrifugation at 6,000 × g for 15 min at 4 °C and resuspended in sterile 0.1% buffered peptone water to a concentration of about 10^{10} CFU/ml. The inoculums were serially diluted in 0.1% peptone water and plated in duplicate on tryptic soy agar (TSA) (Difco, Detroit, MI) containing 0.6% yeast extract to determine initial cell numbers.

4.3.4. Inoculation of ground beef

The 10 mL inoculum was added dropwise to thawed ground beef to obtain a target concentration of 10^8 CFU/g meat. The meat was mixed using a sterilized Kitchen Aid mixer (Model K5-4, Hobart, Troy, OH) at speed setting 3 for 6 minutes. Inoculated meat (113 or 60g) was transferred to a 100 × 15 mm sterile polystyrene Petri dish (VWR Scientific Products, West Chester, PA) using a sterile spatula and pressed into beef patties. The inoculated patties were kept at 4°C and used within 6h.

4.3.5. Preparation of phycoerythrin

Phycoerythrin (PE) was previously isolated from fresh cultures of *Porphyra yezoenis* (Orta-Ramirez 1999). The protein was stored in 60% ammonium sulfate in a light impermeable glass bottle at 4°C. Each sample of PE was dialyzed overnight against distilled deionized water. The protein was then diluted in 0.025 M borate buffer, pH 9.0, to achieve an absorbance of 0.1 at 565 nm.

Capillary tubes (Accu Fill 90 Micropet, Cat. No. 4624, Becton-Dickenson, and Co., Parsippany, NJ) were filled with PE solution (200μL) by capillary action. To allow for even headspace at both ends, 50 μL were removed using a micropipet. One end was sealed using a gas/oxygen flame and the other with Teflon tape.

4.3.6. Ground beef cooking preparations and procedures

A thermocouple (Type T, length 8.6 cm, width 1.0 mm, accuracy ± 0.1°C, Omega Engineering, Stamford, CT) was attached in parallel to the capillary tube with dental floss near the tip and tape at the other end (Figure 4.1A). Dental floss was used to introduce the least disruption to heat transfer within the patty while binding the thermocouple and capillary tube together. The thermocouple and capillary tube were inserted horizontally into the beef patty (Figure 4.1B). The thermocouple was connected to a datalogger system (Daqbook 100, Omega Technologies Inc.) to record the temperature at the center of the patty every second throughout the cooking process.

Ground beef patties were cooked on a griddle (Model 07032, Presto Jumbo griddle, National Presto Industries, Inc., Eau Claire, WI) set to 177°C (350°F). Preliminary studies revealed the griddle cycled ± 7°C around the target surface temperature. The griddle was preheated for a minimum of 30 min prior to use. During the cooking process, patties were heated for 2 min, flipped, and heated for another 2 minutes, then flipped every minute until the process was completed based on the procedures of Berry and Bigner-George (1999). Each beef patty was cooked in the same location on the griddle.

Cooking times for the 113g patties ranged from 6 to 13 min producing maximum internal temperatures of 54.2 to 75.9°C. The 60g patties were cooked for 4 to 8 min producing internal temperatures of 60.7 to 75.9°C. The patties were immediately transferred to sterile Whirlpak™ bags (18 oz, Nasco, Ft. Atkinson, WI) and placed in an ice water bath for quick cooling. Ten 113g patties, either high or low fat, were cooked each day for 10 days for a total of 50 low fat and 50 high fat patties. The high fat and low fat patties were grilled over 43 and 9 day periods, respectively. Eighteen high fat 60g patties were cooked on consecutive days for a total of 54 patties.

Following the cooling period, patties were sliced open vertically with a sterile knife and measurements were taken to determine the vertical placement of the thermocouple and capillary tube containing PE. Patties were accepted if the thermocouple and capillary tube were located within ±5% from the center point of the cooked patty thickness. The capillary tubes were removed, sterilized, kept on ice, and analyzed within 6h. Each heated patty was weighed following the cooling period to calculate the percentage of cook yield (Equation 1).

$$\%\text{ Cook yield} = (\text{Cooked weight} / \text{Raw weight}) \times 100 \qquad (1)$$

A 25g sample of the ground beef was removed from the center of the patty using a sterile 50 mL beaker as a template and placed into a 7 by 12 cm sterile stomacher bag (Seward Medical, London, UK). The beef samples were kept at 4°C and analyzed within 6h.

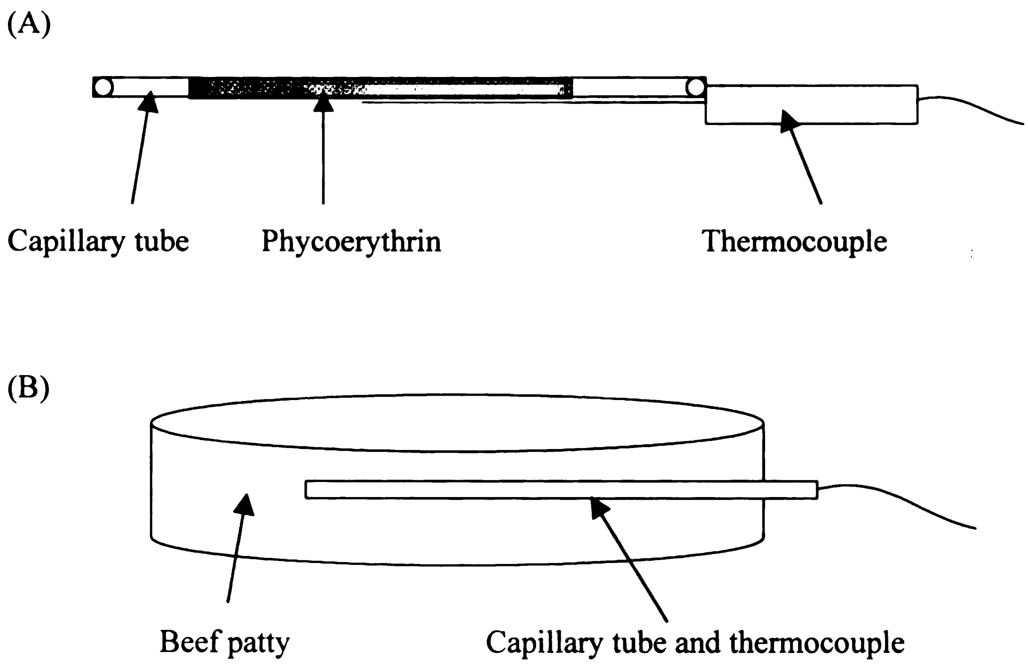


Figure 4.1. (A) Attachment of the capillary tube containing phycoerythrin to a thermocouple, (B) Insertion position of the capillary tube and thermocouple into a beef patty

4.3.7. PE fluorescence measurements

PE was measured at maximum emission wavelength using a CytoFluor II Microwell Fluorescence reader equipped with CytoFluor II software, Version 2.0c (Biosearch Incorporated, Bedford, MA). Heated and unheated PE solutions were transferred from capillary tubes into a 96-well fluorescence plate reader (Cat. no. DG5515, Life Science Products Inc., Denver, CO). The CytoFluor lamp was allowed to warm-up for a minimum of 10 min prior to use. The excitation setting used was 485/20 nm and emission setting 590/35 nm. These settings allowed for excitation readings at 485 ± 20nm and emission readings at 590 ± 35 nm. After a mixing time of 20s, 10 readings were taken on each plate. PE fluorescence measurements were expressed as normalized values calculated by dividing the fluorescence at time t (F) by the initial fluorescence before heating (F_o).

4.3.8. Enumeration of surviving bacteria

Cooked ground beef (25g) was diluted in 225 mL of lactose broth (Difco, Detroit, MI) and stomached for 90s in a masticator (Model 0410, IUL Instruments USA, Inc. Cincinnati, OH). Bacterial counts were determined by decimal dilution of the ground beef in sterile 0.1% peptone water and plated on TSA containing 0.6% yeast extract. All plates were counted after 48h of incubation at 37°C for. Destruction of the 8-strain *Salmonella* cocktail was expressed as the log of the ratio between the count at time t (N) and the initial count (N_o). This was calculated by subtracting the log of the final count after heating from the log of the initial count before cooking (log N_o). The resulting values gave the log numbers of *Salmonella* colonies per gram of beef destroyed by the heat treatment.

4.3.9. Calculation of process lethality

Lethality values were calculated using the General Method (Pflug, 1997) with a reference temperature of 65°C (Equation 2). The z value used for R-PE was 5.99°C (Orta-Ramirez, 1999).

$$\text{Lethality} = \frac{1}{10^{((65-T)/5.99)}} \qquad (2)$$

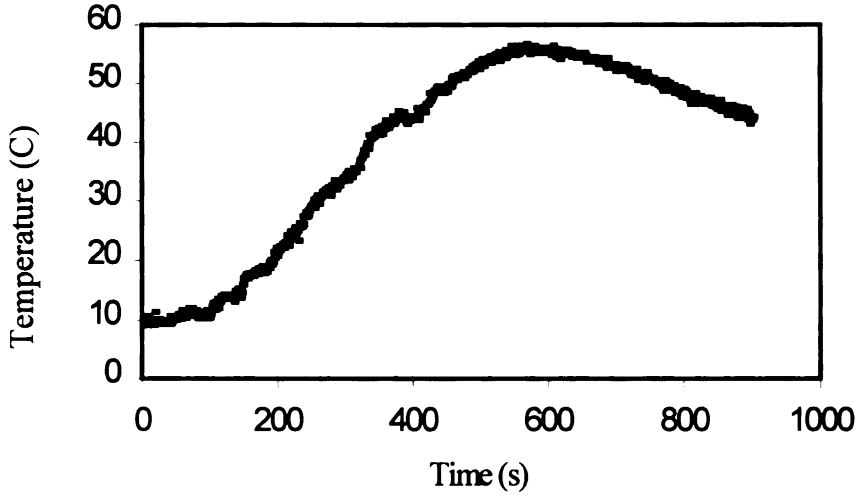
where T = temperature (°C) at time t. Each temperature recorded over time by the datalogger system was converted into an equivalent lethality. Individual lethalities were then summed to give the total lethality of the process.

4.4. RESULTS AND DISCUSSION

4.4.1. Effect of maximum center temperatures on *Salmonella* in high fat ground beef patties.

High fat ground beef (pH 5.72 ± 0.01) contained 63.4 % ± 0.6% moisture, 19.1% ± 0.7% fat, and 14.4% ± 1.9% protein. No microorganisms were detected in the irradiated meat. Typical center temperature histories during cooking for ground beef patties (19.1% fat) are given in Figure 4.2. Inserting a thermocouple into the center of a

(A)



(B)

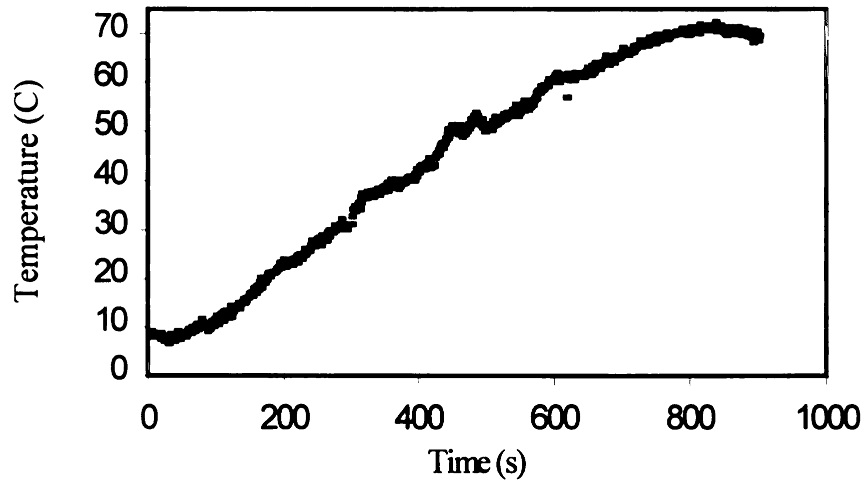


Figure 4.2. Representative time-temperature cooking profiles of a 113g high fat (19.1%) ground beef patty. (A) Illustrates a patty cooked to 56.4°C and total lethality of 5.25s; (B) Illustrates a patty cooked to 71.9°C and total lethality of 1420s.

beef patty allowed for measurement of the maximum temperature achieved at the center of each patty. The maximum center temperature of the 41 high fat patties ranged from 56.8 to 75.9°C. Regulations provided by the USDA-FSIS (1996) offer processors time-temperature guidelines for proper thermal processing of ground beef patties. These regulations vary from an endpoint temperature of 66.1°C (151°F) for 41s to 69.4°C (157°F) (and up) for 10s. These safe harbor guidelines were designed to produce a 5 $\log_{10}$ reduction in *Salmonella* in ground beef patties.

As center temperature of the beef patties was increased, the number of surviving *Salmonella* ($R^2 = 0.83$) decreased (Figure 4.3). From the time-temperature data recorded during each cooking process, it was possible to categorize each patty as undercooked or properly cooked according to the safe harbor guidelines. Out of 41 patties with acceptable thermocouple placement, 19 patties did not meet the required time-temperature regulations provided by the USDA. Therefore 22 patties met or exceeded the time-temperature safe harbor guidelines.

Four patties met at least one of the time-temperature guidelines provided by the USDA, but the *Salmonella* cocktail did not decrease by 5 $\log_{10}$. This same trend was seen by Orta-Ramirez (2000) in non-isothermal experiments with the *Salmonella* cocktail in ground beef and could have serious implications on the safety of meat products. Ground beef patties cooked according to safe harbor guidelines may not provide a 5 $\log_{10}$ decrease in the number of surviving *Salmonella*.

There was also a patty that did not meet any of the safe harbor guidelines but *Salmonella* decreased by greater than 5 $\log_{10}$. The lowest temperature stated in the processing regulations is 66.1°C. A patty may be cooked at a temperature below 66.1°C

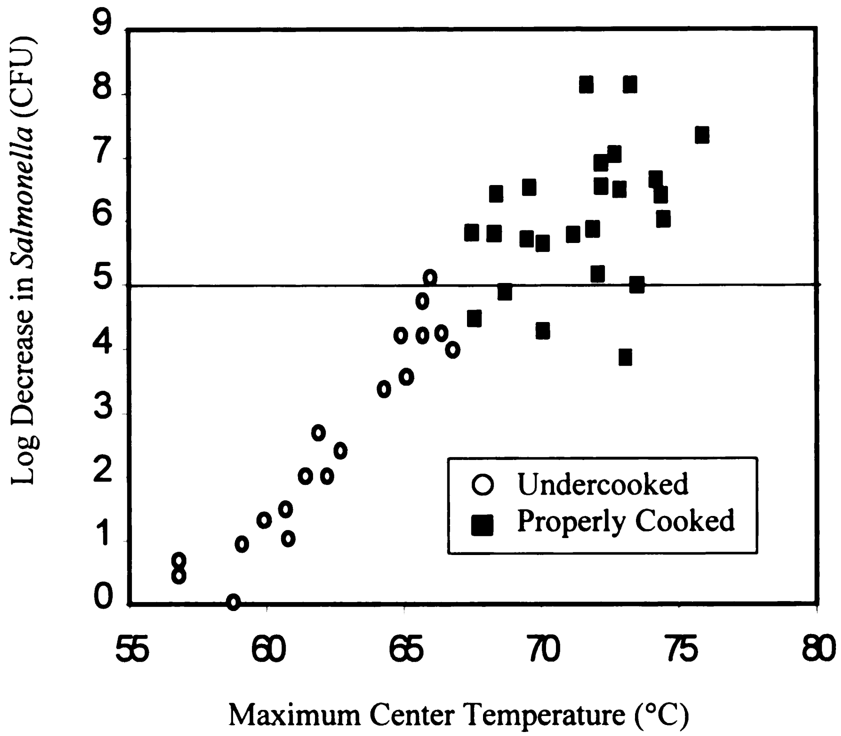


Figure 4.3. Effect of maximum internal center temperature on the log decrease in *Salmonella* cocktail of ground beef patties (19.1% fat). Patties were categorized as undercooked or properly cooked according to the time-temperature regulations of the USDA (2000).

for a longer time to achieve a 5 $\log_{10}$ decrease in *Salmonella*. Yet according to the safe harbor guidelines this patty would be classified as undercooked. The advantage of the proposed lethality performance standard is that a beef patty may be cooked according to any time-temperature combination as long as a 5 $\log_{10}$ decrease in *Salmonella* is verified.

4.4.2. Effect of lethality on *Salmonella* in high fat ground beef patties.

In addition to time-temperature guidelines, the USDA is in the process of establishing lethality performance standards for ready-to-eat ground beef patties. Lethality standards allow processors to cook meat products according to any time-temperature combination if validation of a significant decrease in *Salmonella* is proven. A proposed lethality performance standard for fully cooked, uncured meat patties includes a 5 $\log_{10}$ reduction in *Salmonella* (USDA, 1999). Lethality values were calculated from the thermal process each patty received.

Lethalities of the 41 patties ranged from 5.25 to 4980s at 65°C. The lethality equivalent to the time-temperature combinations given by the USDA-FSIS for ground beef patties processed using safe harbor guidelines was calculated using the General Methods (Pflug, 1997). The lethality for all time-temperature combinations was 66s at 65°C. Any patty with a lethality greater than 66s at 65°C could be considered properly processed by USDA definitions. The range of processing lethality used in this study resulted in undercooked and properly cooked beef patties. An increase in process lethality resulted in a decrease in cooking yield in beef patties ($R^2 = 0.86$). This suggests that the time and temperature data used to calculate process lethality were accurately collected. Accurate process lethalities offer a balance between undercooked patties that could lead to a public health risk and overcooked patties with decreased quality.

A correlation was seen between the log decrease in *Salmonella* and lethality of the cooking process ($R^2 = 0.86$) (Figure 4.4). This was a higher correlation than between *Salmonella* and maximum center temperature, suggesting that using process lethalities to predict destruction of *Salmonella* in beef patties may be more accurate than using center temperature. Twenty-two patties had lethalities greater than 66s at 65°C and met one of the time-temperature regulations of the USDA. There were 7 patties with lethality greater than 66s at 65°C that did not meet either the time-temperature regulations or the 5-$\log_{10}$ decrease in *Salmonella*. Lethality is calculated using the entire time-temperature history. A total lethality may be greater than 66s at 65°C if a beef patty were held at a temperature below 66.1°C for an extended length of time. Time-temperature regulations do not extend below 66.1°C, therefore patties with lower maximum endpoint temperatures would be considered undercooked. A 5 $\log_{10}$ decrease was consistently seen when process lethality was greater than 300s at 65°C.

4.4.3. Relationship between normalized PE fluorescence and *Salmonella* in high fat ground beef patties.

Normalized PE fluorescence in cooked patties ranged from 0.05 to 0.86. Normalized fluorescence was compared to the log decrease in the *Salmonella* cocktail in forty-one 113g high fat ground beef patties. A linear trend was seen between normalized fluorescence and the number of surviving *Salmonella* ($R^2 = 0.74$) in cooked beef patties. Using linear regression, it was possible to calculate the normalized PE fluorescence that corresponded to a 5 $\log_{10}$ decrease in *Salmonella* (Figure 4.5). Normalized fluorescence

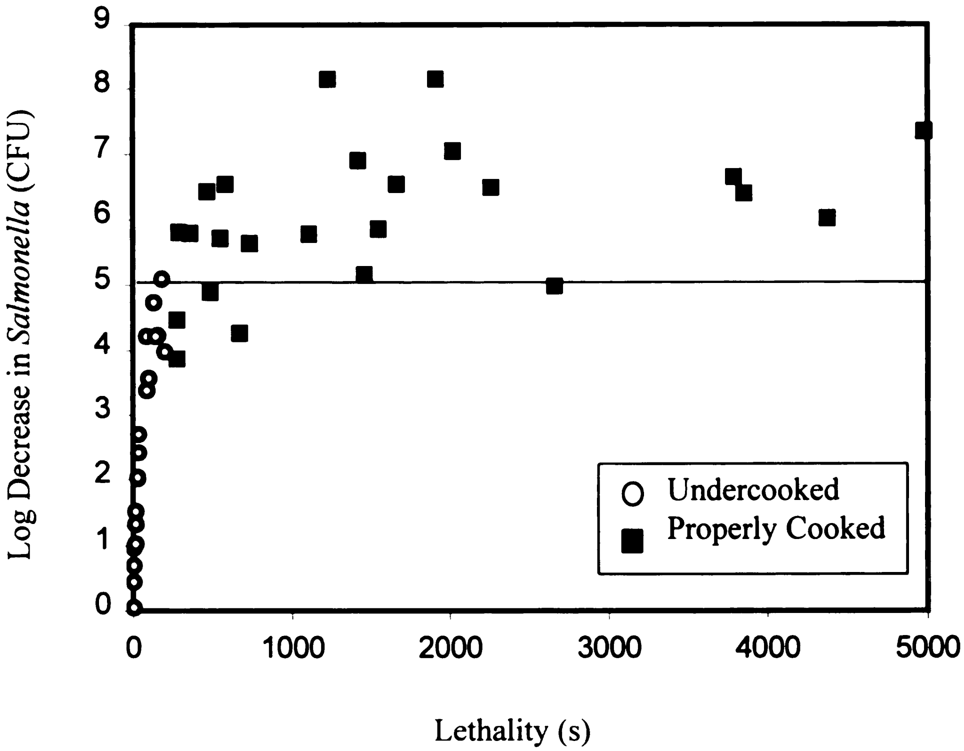


Figure 4.4. Effect of process lethality on the log decrease in *Salmonella* in ground beef patties (19.1% fat). Patties were categorized as undercooked or properly cooked according to the time-temperature regulations of the USDA (2000).

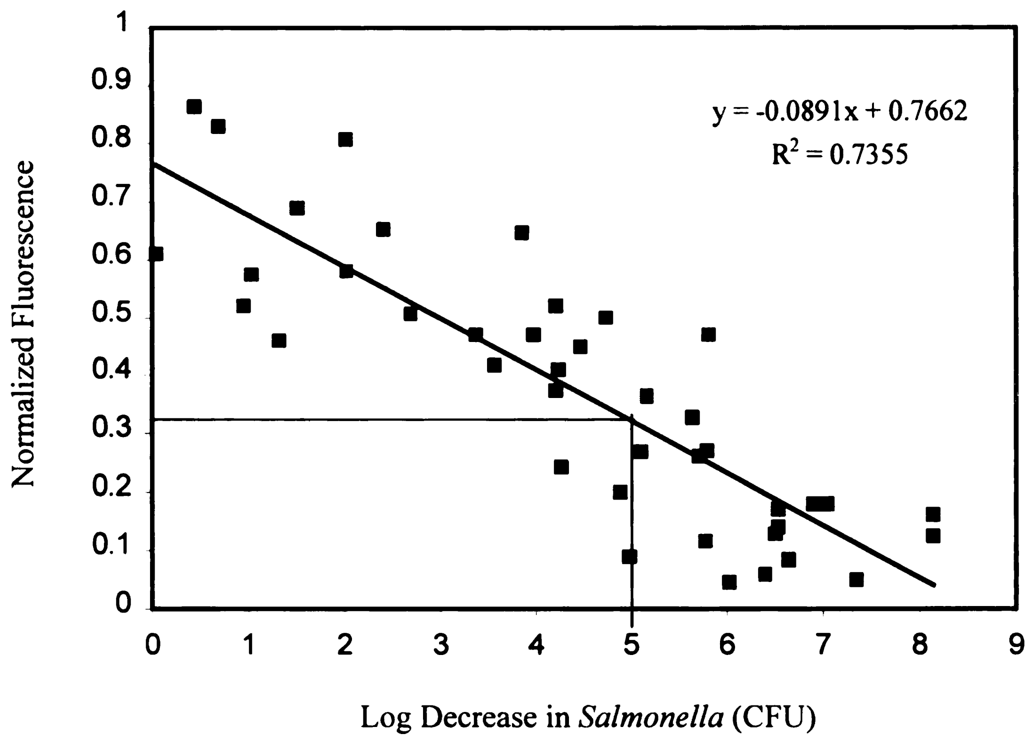


Figure 4.5. Correlation between normalized fluorescence and log decrease in *Salmonella* cocktail in ground beef patties (19.1% fat).

of PE was reduced to 0.31 (CI 99%) or below inside patties with a 5-$\log_{10}$ decrease in *Salmonella*. A normalized PE fluorescence of 0.31 or below may be used to verify proper thermal processing of ground beef.

The relationship between normalized fluorescence and log decrease in *Salmonella* was used to evaluate this endpoint fluorescence value (Figure 4.6). A line was drawn horizontally across the graph to indicate a 5 $\log_{10}$ decrease in *Salmonella*. Cooked patties above this line indicated a 5 $\log_{10}$ decrease in *Salmonella*, patties below indicated less than a 5 $\log_{10}$ decrease. A vertical line indicates a normalized PE fluorescence of 0.31. Patties plotted to the left of this line indicated a normalized fluorescence less than 0.31 and to the right of the line greater than 0.31. With the addition of these lines the graph was divided into 4 quadrants.

Patties in the upper left quadrant had a 5 or greater $\log_{10}$ decrease in *Salmonella* and were correctly categorized by PE as being properly cooked with a normalized fluorescence value below 0.31. Sixteen patties were observed in the upper left quadrant. Patties in the top right quadrant had a 5 or greater $\log_{10}$ decrease in *Salmonella*, yet the PE incorrectly categorized the patties as being undercooked with a normalized value above 0.31. Three patties were categorized in the upper right quadrant. Beef patties in this quadrant were processed correctly, yet PE indicated otherwise, therefore safe product would be disposed of thus incurring unnecessary financial losses.

Patties categorized in the lower right quadrant had less than a 5 $\log_{10}$ decrease in *Salmonella* and were properly distinguished by the PE, with a normalized fluorescence value greater than 0.32. Twenty patties were observed in the lower right quadrant.

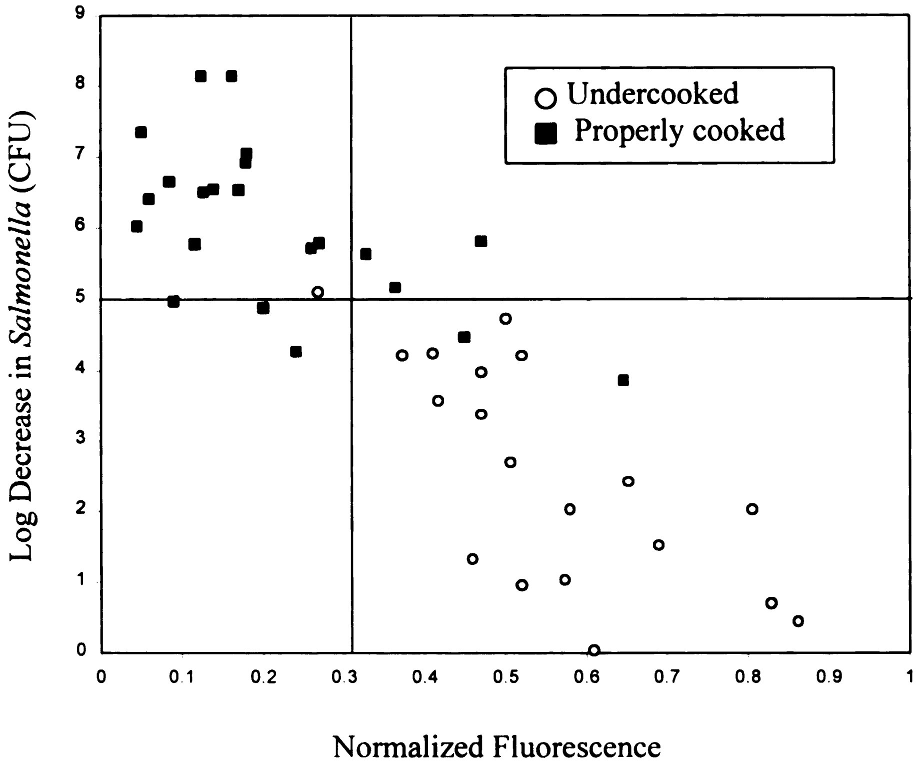


Figure 4.6. Relationship between normalized phycoerythrin fluorescence and log decrease in *Salmonella* in ground beef patties (19.1% fat). Patties were categorized as undercooked or properly cooked according to the time-temperature regulations of the USDA (2000).

Eighteen of these did not meet any of the USDA time-temperature guidelines. Two patties met the guidelines but without a 5 $\log_{10}$ decrease in *Salmonella*. PE correctly classified the patties as undercooked following lethality standard guidelines, but the patties were improperly categorized according to the time-temperature regulations.

Finally, patties in the lower left quadrant had less than a 5 $\log_{10}$ decrease in *Salmonella*, but the PE indicated a safe product with a normalized value less than 0.32. These two patties suggest a problem with PE as a TTI. Both of these patties met one of the time-temperature safe harbor guidelines of the USDA without a 5 $\log_{10}$ decrease. PE correctly categorized the patties according to either time-temperature guidelines or lethality performance standards. Overall, 88% of the cooked patties were correctly identified by a normalized PE fluorescence of 0.31.

4.4.4. Effect of maximum center temperature on *Salmonella* in low fat ground beef patties.

Low fat ground beef (pH 5.65 ± 0.01) contained 4.8% ± 1.1% fat, 72.4% ± 0.2% moisture and 15.8% ± 2.6% protein. No microorganisms were detected in the irradiated meat. Typical center temperature histories during cooking of low fat (4.8%) ground beef patties were very similar to that given in Figure 4.2.

The maximum center temperature inside the 41 low fat patties ranged from 54.2 to 75.6°C. As the center temperature of the beef patties increased, the number of surviving *Salmonella* ($R^2 = 0.87$) decreased (Figure 4.7). As with the high fat patties, the time-temperature schedules recorded during each cooking process were used to categorize each patty as undercooked or properly cooked according to the time

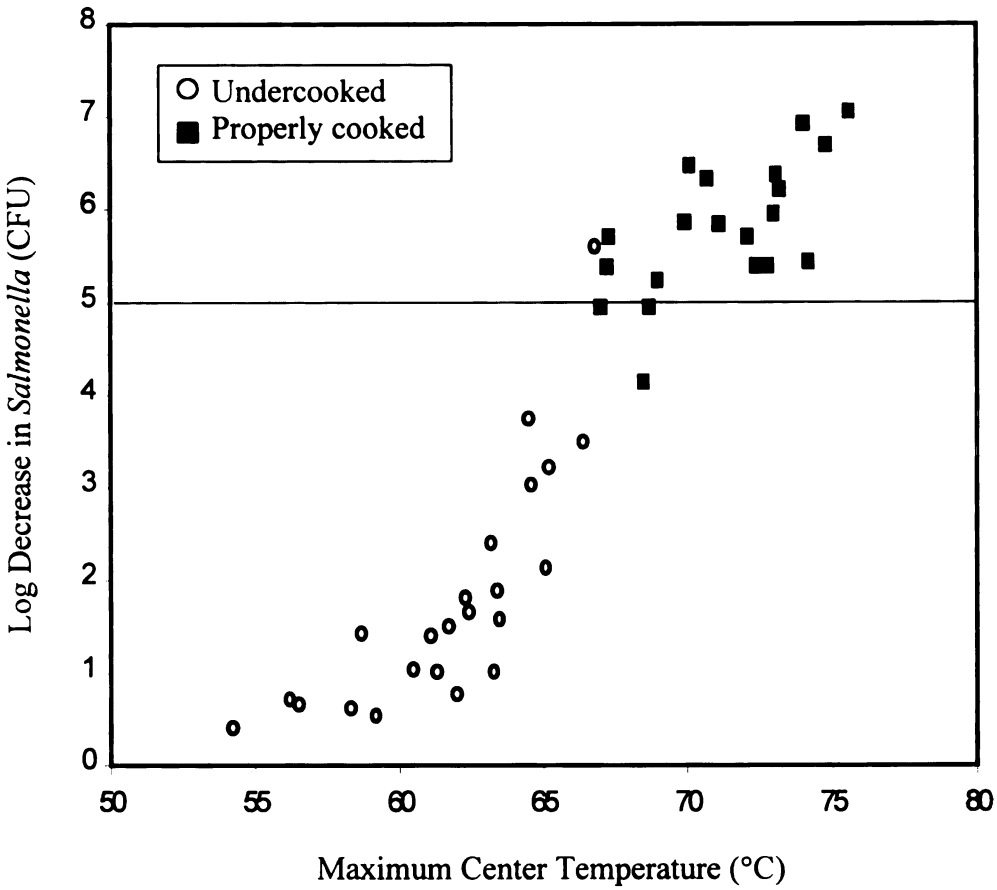


Figure 4.7. Effect of maximum internal center temperature on log decrease in *Salmonella* in ground beef patties (4.8% fat). Patties were categorized as undercooked or properly cooked according to the time-temperature regulations of the USDA (2000).

temperature regulations for ready-to-eat ground beef patties.Out of 41 patties with acceptable thermocouple locations, 22 patties did not meet the required time-temperature regulations provided by the USDA. Thus 19 patties met or exceeded the time-temperature safe harbor guidelines. Three patties met at least one of the time-temperature guidelines provided by the USDA but without a 5 $\log_{10}$ decrease in the *Salmonella* cocktail. This suggests that the number of *Salmonella* survivors in beef patties cooked according to the time-temperature regulations from the USDA-FSIS may not decrease by 5 $\log_{10}$ in all cases. One patty did not meet any of the safe harbor guidelines and resulted in greater than 5 $\log_{10}$ decrease in *Salmonella* cocktail. This patty may have been cooked at a temperature below 66.1°C for a longer time to achieve a 5 $\log_{10}$ decrease in *Salmonella*, but it would not be classified as properly cooked under the USDA regulations.

4.4.5. Effect of lethality on *Salmonella* in low fat ground beef patties.

The lethalities of the 41 patties ranged from 1.51 to 3980s at 65°C. The range of processing lethality used in this study resulted in undercooked and properly cooked patties. An increase in lethality resulted in a decreased cooking yield for the 113g beef patties ($R^2 = 0.70$).

A correlation was seen between the log decrease in *Salmonella* and lethality of the cooking process ($R^2 = 0.88$) (Figure 4.8). There was a higher correlation coefficient with lethality and log decrease in *Salmonella* than with maximum center temperature. Lethality was able to successfully predict appropriate reductions in the number of *Salmonella* survivors better than maximum center temperature. Twenty-four patties had lethalities greater than 65s at 65°C and met one of the time-temperature regulations. Four

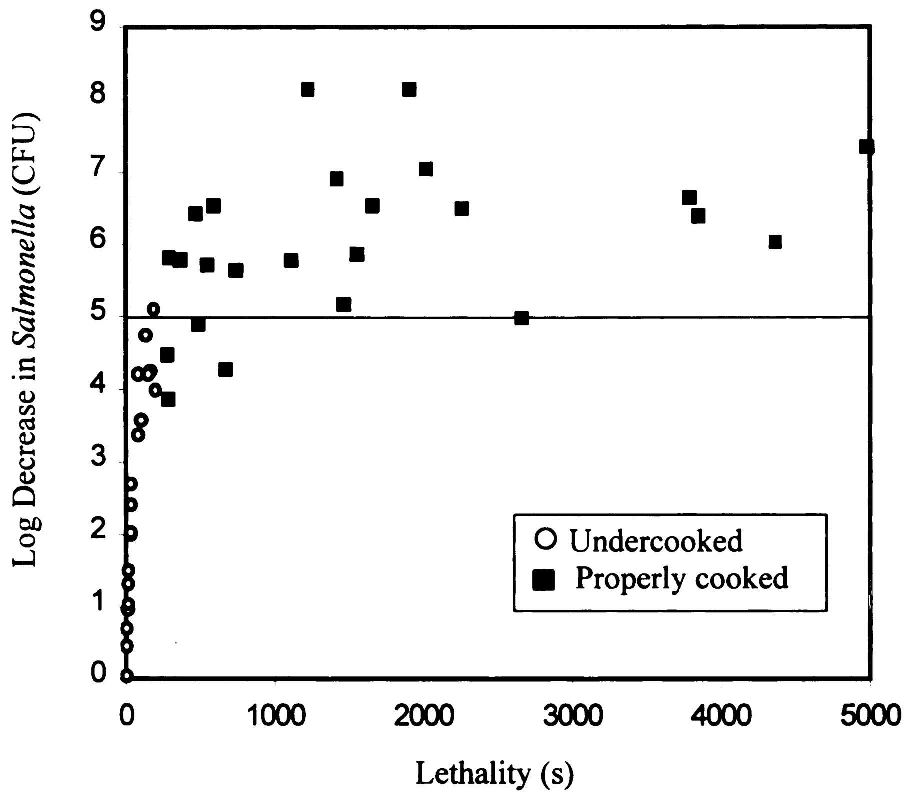


Figure 4.8. Effect of process lethality on log decrease in *Salmonella* ground beef patties (4.8% fat). Patties were categorized as undercooked or properly cooked according to the time-temperature regulations of the USDA (2000).

patties with lethalities greater than 66s did not meet either the safe harbor guidelines or lethality performance standards. A patty may have been cooked for an extended period of time at a temperature below those listed in the safe harbor guidelines. These patties would be considered properly cooked when performance lethality standards are finalized for ground beef patties. Lethality greater than 105s at 65°C consistently reduced the number of *Salmonella* survivors by 5 $\log_{10}$.

4.4.6. Relationship between normalized PE fluorescence and *Salmonella* in low fat ground beef patties.

Normalized PE fluorescence in cooked patties ranged from 0.03 to 0.90. Normalized fluorescence was compared to the log decrease in the *Salmonella* cocktail within forty-one 113g low fat ground beef patties. A linear relationship was seen between the normalized fluorescence and the number of surviving *Salmonella* (R^2 = 0.82) in cooked beef patties. This was a higher correlation than seen with the high fat patties. A normalized fluorescence of 0.29 (CI 99%) was calculated from linear regression to correspond with a 5 $\log_{10}$ reduction in the number of surviving *Salmonella* (Figure 4.9) in cooked patties. This value was very similar to the normalized PE fluorescence that indicated 5 $\log_{10}$ reduction in *Salmonella* in high fat ground beef patties. The relationship between normalized PE fluorescence and log decrease in *Salmonella* was used to evaluate the endpoint fluorescence value (Figure 4.10).

The sixteen patties in the upper left quadrant had a 5 or greater $\log_{10}$ decrease in *Salmonella* and were properly identified by PE with a normalized fluorescence below 0.29. Fifteen of these patties also met at least one of the time-temperature regulations. One patty was classified into the upper right quadrant which indicated greater than a 5

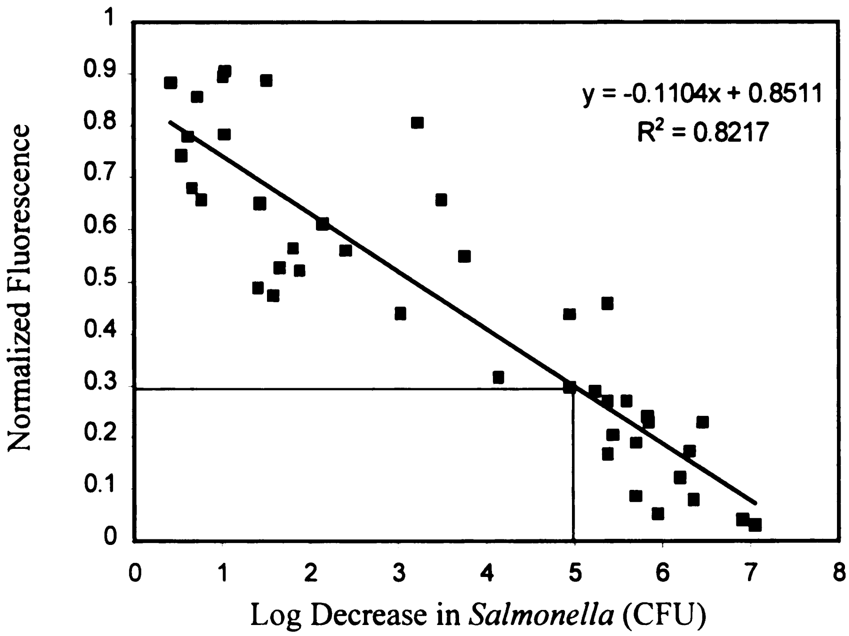


Figure 4.9. Correlation between normalized fluorescence and log decrease in *Salmonella* cocktail in ground beef patties (4.8% fat).

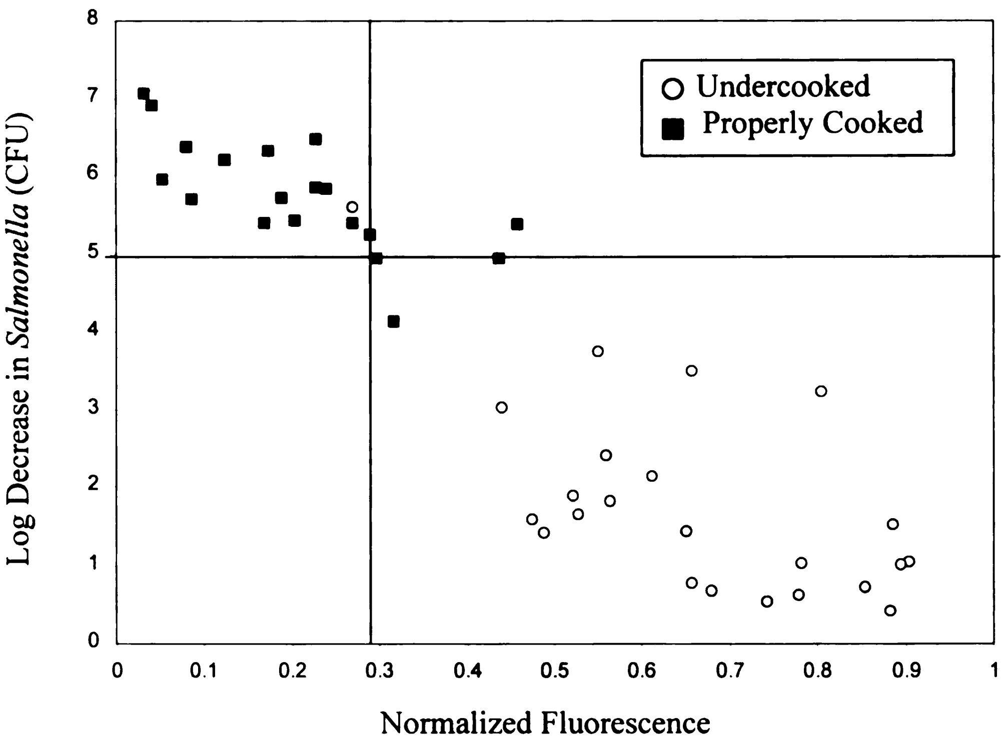


Figure 4.10. Relationship between normalized phycoerythrin fluorescence and log decrease in *Salmonella* in ground beef patties (4.8% fat). Patties were categorized as undercooked or properly cooked according to the time-temperature regulations of the USDA (2000).

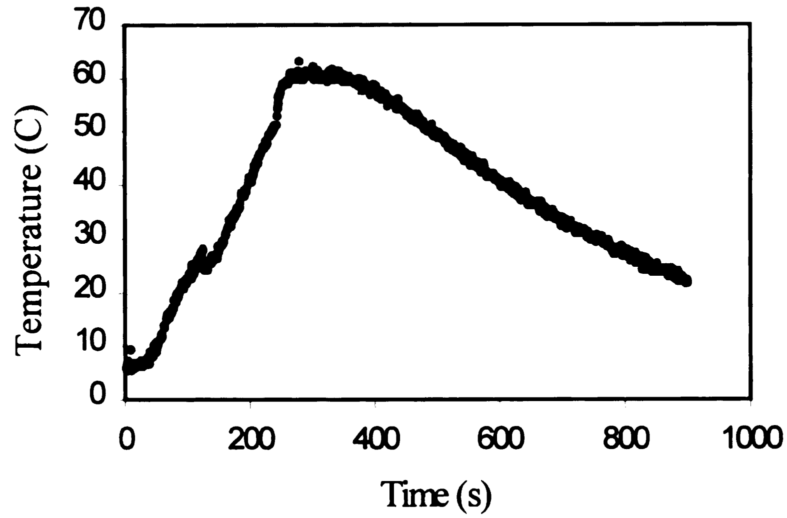
$\log_{10}$ reduction in *Salmonella*. However, PE incorrectly identified this patty as undercooked with a normalized fluorescence greater than 0.29. The lower right quadrant contained 24 cooked patties. Twenty-two patties did not meet any of the time-temperature regulations and were properly categorized by a normalized PE fluorescence of greater than 0.29. The remaining patty did meet one of the time-temperature regulations without a 5 $\log_{10}$ decrease in *Salmonella.* This patty was properly cooked according to current regulations, but improperly categorized by PE. There were no patties categorized into the lower left quadrant indicating that normalized PE fluorescence of 0.29 was able to correctly categorize undercooked patties. Overall 98% of the cooked patties were correctly categorized.

Smith et al. (2000) reported that *S.* Typhimurium DT104 cultures were less heat resistant in ground beef of lower fat content. Maurer (2000) found that *E. coli* O157: H7 had lower D values in 4.8% fat ground beef when compared to 19.1% fat. It was expected that normalized PE fluorescence values for low fat ground beef would have been greater than for high fat patties due to this decreased thermal resistance, but the two fluorescence cutoff points were very similar.

4.4.7. Effect of maximum center temperature on Salmonella in 60g high fat ground beef patties.

Grilling methodologies were applied to 60g ground beef patties to verify the applicability of PE as a time-temperature integrator in different size patties. The typical center temperature history during cooking of 60g ground beef patty is given in Figure 4.11. The maximum center temperature of the 42 patties ranged from 60.9 to 75.9°C. As the maximum center temperature of the cooked patty increased, the number of surviving

(A)



(B)

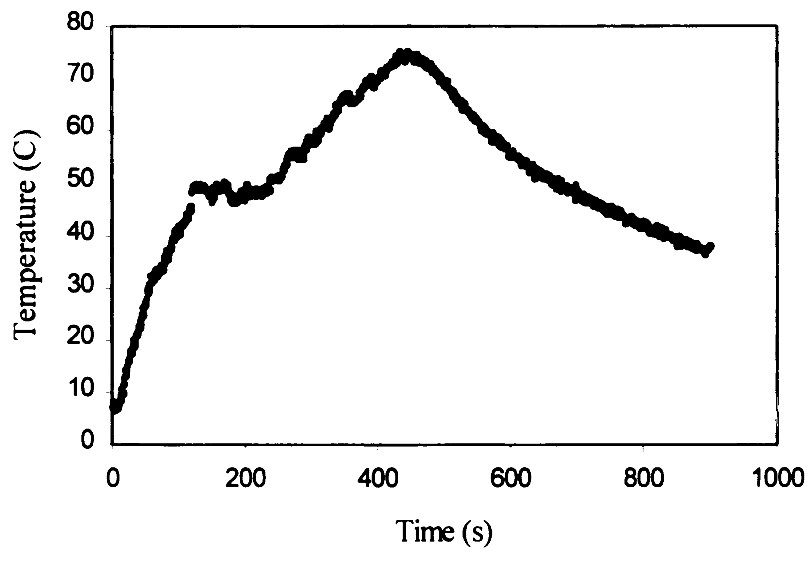


Figure 4.11. Representative time-temperature heating profile of a 60g high fat (19.1%) ground beef patty. (A) Illustrates a patty cooked to 63.1°C and a total lethality of 24.9s at 65°C; (B) Illustrates a patty cooked to 75.1°C and a total lethality of 2330s at 65°C.

Salmonella ($R^2 = 0.50$) decreased. In addition to differences in cooking time at each temperature of the cooking process, the low correlation could be due to the difficulty encountered with methodologies. When attempting to insert the thermocouple and capillary tube into a lower weight patties the thermocouple was frequently found to be closer to one surface or the other instead of in the center of the patty. Given that large decreases in the width of the patty were observed during cooking, the changing shape of the patty during cooking had a major impact on the final position of the thermocouple after cooking. Thus even though the thermocouple's final location was within the ± 5% of the center point, it may not have been in the proper position for the entire cooking time.

Twenty patties did not meet the required time-temperature regulations provided by the USDA. Therefore 20 patties met or exceeded the time-temperature regulations of the USDA. Although four patties met at least one of the time-temperature guidelines provided by the USDA, *Salmonella* decreased less than 5 $\log_{10}$ (Figure 4.12). This trend was seen in both the high and low fat 113g patties. Three patties did not meet the time-temperature safe harbors and yet the *Salmonella* cocktail was reduced by greater than 5 $\log_{10}$. The time-temperature history of these patties revealed extended cooking at temperatures below 61.1°C, the lowest endpoint temperature contained in the USDA time-temperature regulations.

4.4.8. Effect of lethality on *Salmonella* in 60g high fat ground beef patties.

Lethalities for the forty 60g cooked beef patties ranged from 11.4 to 2780s at 65°C. Therefore our range of lethality included processes for undercooked as well as

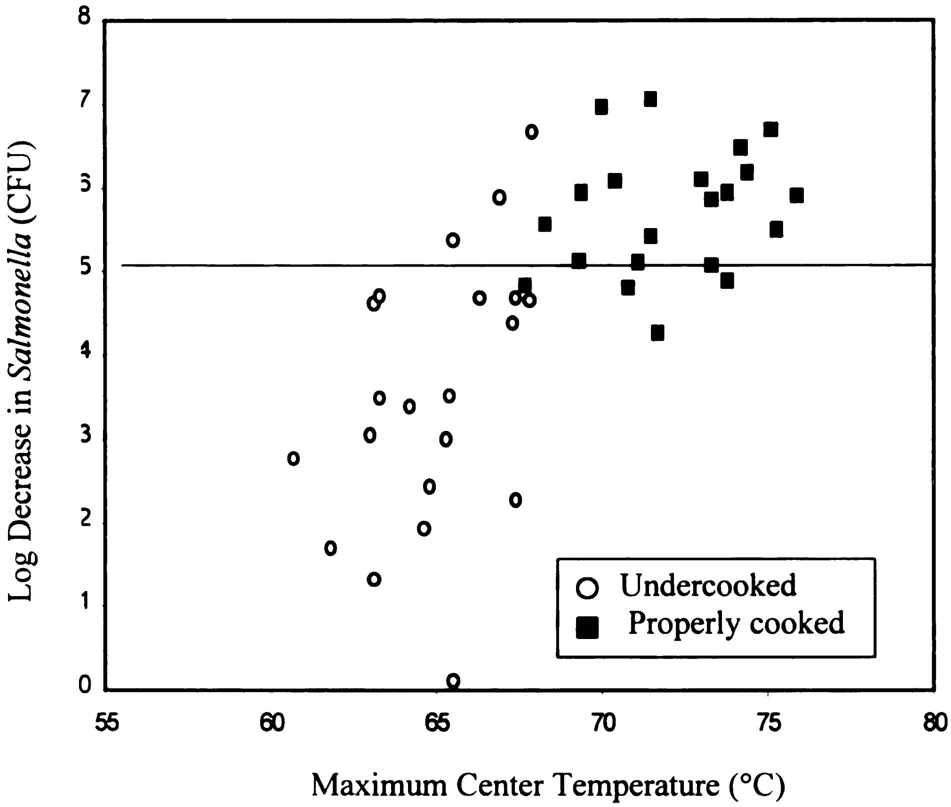


Figure 4.12. Effect of maximum center temperature on log decrease in *Salmonella* in 60g ground beef patties (19.1% fat). Patties were categorized as undercooked or properly cooked according to the time-temperature regulations of the USDA (2000).

properly cooked beef patties. There was a low correlation between the increase in lethality and the decrease in cooking yield of the 113g beef patties ($R^2 = 0.69$). Methodology weaknesses could again explain the low correlation seen.

A slight correlation was seen between log decrease in *Salmonella* and lethality of the cooking process ($R^2 = 0.58$) (Figure 4.13). This correlation was higher than the endpoint temperature correlation, suggesting that lethality measurement may be a more successful indicator of *Salmonella* destruction. The R^2 values for both relationships are low in comparison to the 113g patties suggesting that the placement of the thermocouple may not have been as accurate in patties of smaller size.

Eight patties with lethalities greater than 66s did not meet either time-temperature regulations or result in a 5-$\log_{10}$ decrease in *Salmonella*. When reviewing the time-temperature profiles of the cooking process, large time intervals at temperatures below 61.1°C were observed. The time at low temperatures contributes to the total lethality, but the temperatures are not included in the safe harbor guidelines and were not high enough to result in a 5 $\log_{10}$ reduction of *Salmonella*. A 5 $\log_{10}$ decrease was consistently observed in cooked ground beef patties with lethality greater than 135s at 65°C.

4.4.9. Relationship between normalized PE fluorescence and *Salmonella* in 60g high fat ground beef patties.

Normalized PE fluorescence in cooked patties ranged from 0.05 to 0.58. A linear trend was seen between the decrease in normalized fluorescence and the decrease in *Salmonella* survivors ($R^2 = 0.60$) in cooked beef patties. Using linear regression it was possible to calculate the normalized PE fluorescence that corresponded to a 5 $\log_{10}$

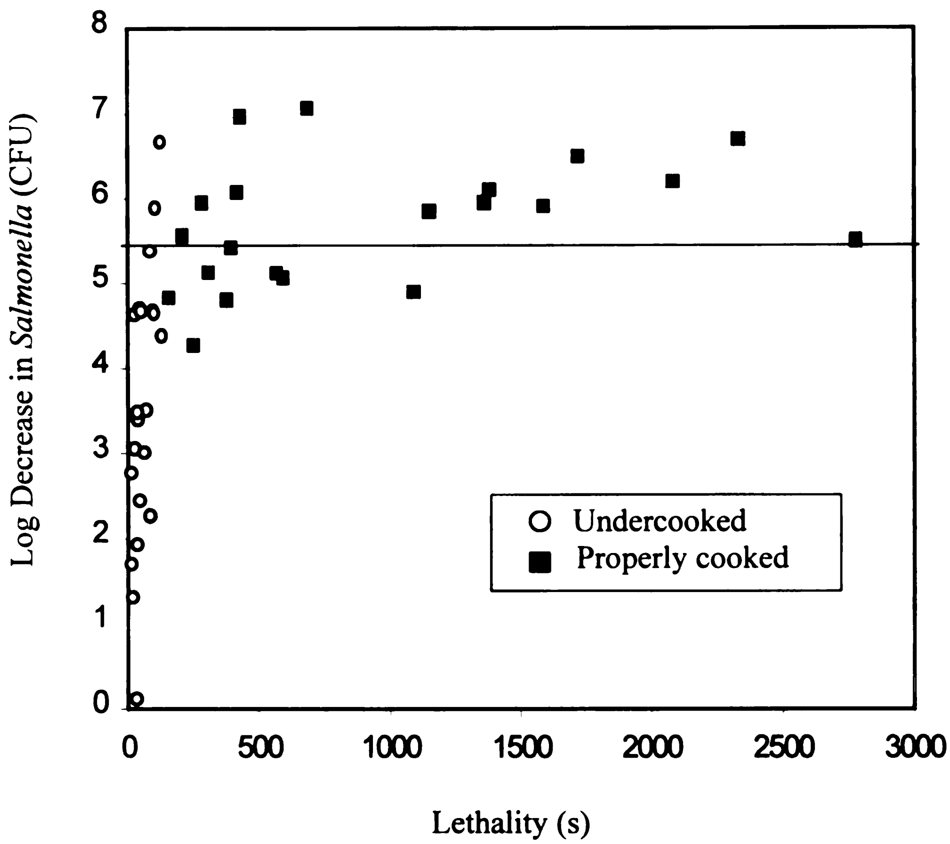


Figure 4.13. Effect of process lethality on the log decrease in *Salmonella* in 60g ground beef patties (19.1% fat). Patties were categorized as undercooked or properly cooked according to the time-temperature regulations of the USDA (2000).

decrease in *Salmonella* in the patties (Figure 4.14). The normalized fluorescence of the PE was 0.21 (CI 99%) or below inside patties with a 5 $\log_{10}$ or greater decrease in *Salmonella*. A normalized PE fluorescence of 0.21 or below may be used to verify proper thermal processing of 60g ground beef patties. This endpoint normalized PE fluorescence was lower than that reported for the high and low fat 113g patties.

Unusually low PE values were noted throughout the studies with 60g patties. It has been hypothesized that the PE was exposed directly to the heat of the griddle surface due to the narrow width of the beef patty. Therefore the lethality received by the PE was not representative of the lethality received by the patty.

A plot of log decrease in *Salmonella* against normalized fluorescence was used to evaluate the endpoint fluorescence value (Figure 4.15). Eighteen patties were classified into the upper left quadrant. These patties were correctly identified with a normalized PE fluorescence below 0.21 as being properly cooked. Two of the 18 patties did not meet at least one of the time-temperature guidelines, but *Salmonella* was reduced by 5 $\log_{10}$ or greater.

Two of the patties were incorrectly classified by the PE as properly cooked only because of the current time-temperature guidelines. Based on lethality performance standards these two patties were properly cooked. Two patty was observed in the upper right quadrant. The patty did not meet any of the time-temperature regulations, but the number of *Salmonella* survivors was reduced by at least 5 $\log_{10}$. Eighteen patties were classified into the lower right quadrant. One of the eighteen met time-temperature regulations, but did not exhibit a 5 $\log_{10}$ decrease in *Salmonella*.

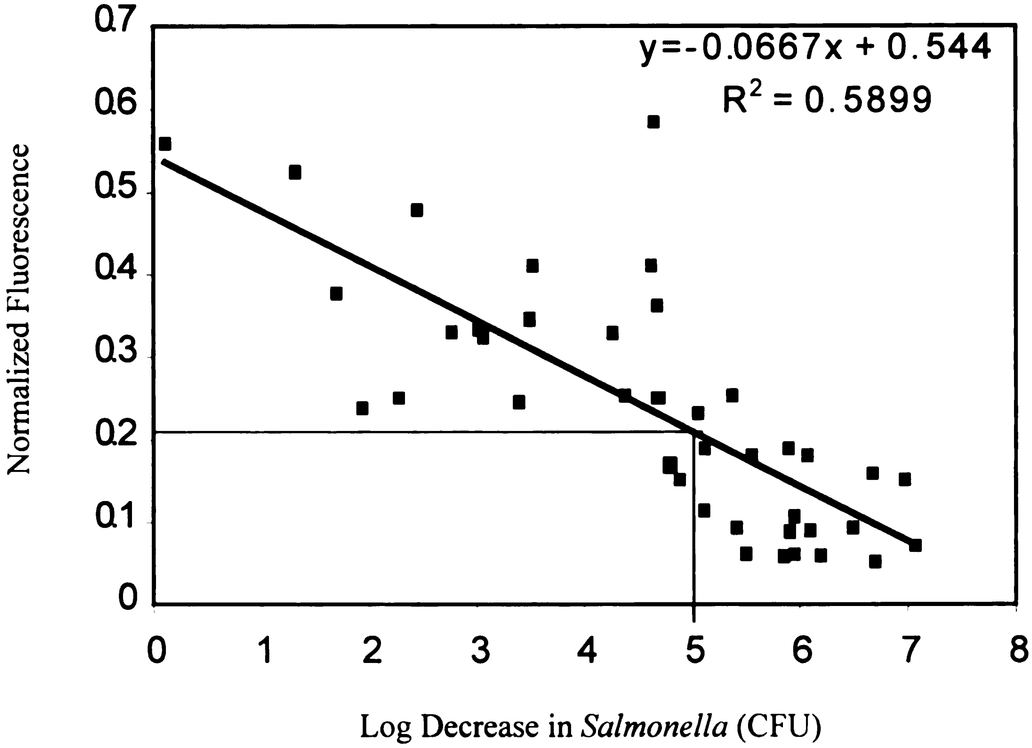


Figure 4.14. Correlation between normalized fluorescence and the log decrease in *Salmonella* in 60g ground beef patties (19.1% fat).

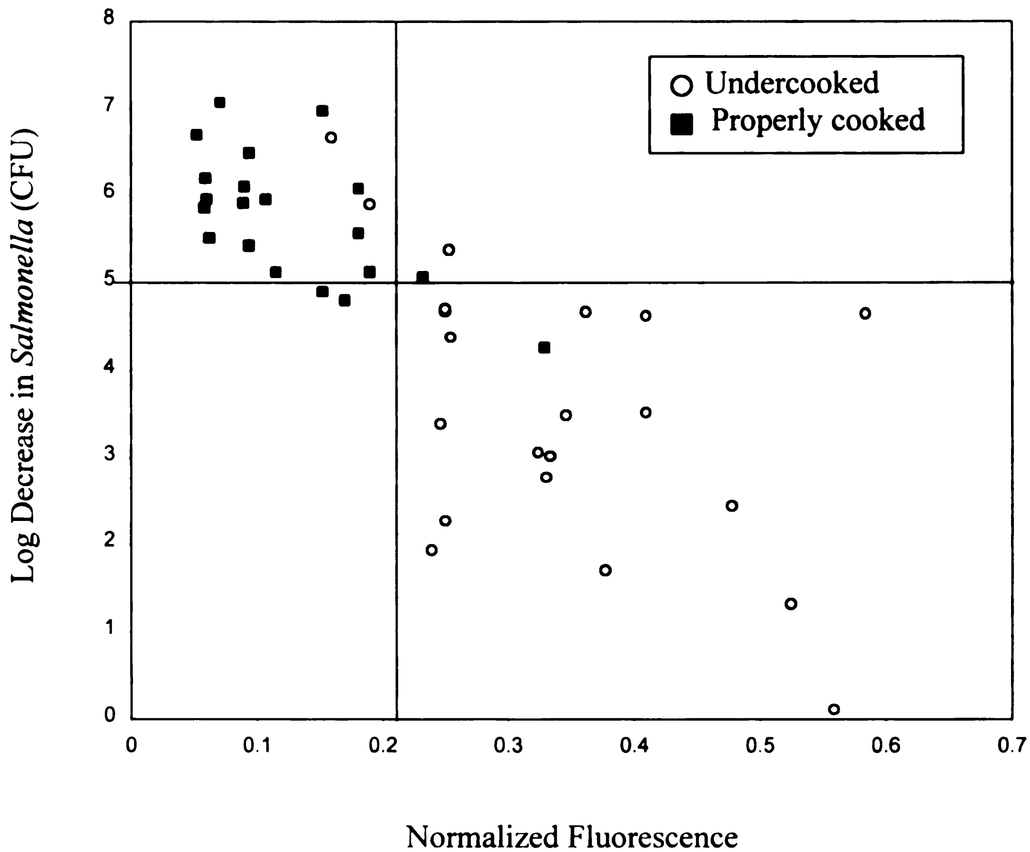


Figure 4.15. Relationship between log decrease in *Salmonella* and normalized phycoerythrin fluorescence in 60g ground beef patties (19.1% fat). Patties were categorized as undercooked or properly cooked according to the time-temperature regulations of the USDA (2000).

Two patties were categorized into the lower left quadrant. They both met at least one of the safe harbor guidelines but without a 5 $\log_{10}$ reduction in *Salmonella.* Overall a normalized PE fluorescence of 0.21 correctly identified 93% of the cooked beef patties. These results are positive, but due to the low correlation seen between normalized fluorescence and log decrease of *Salmonella,* new methodologies may need to be developed to avoid exposure of PE to the griddle surface.

4.5. CONCLUSIONS

Although thermal processing guidelines are available, there is still a need to verify adequate cooking of ground beef patties. Studies completed here reveal that PE has great potential as a TTI for verifying proper thermal processing of ground beef patties. In patties of two fat levels, PE was able to correctly categorize 93% of the ground beef patties as properly cooked or undercooked with a normalized fluorescence value less than 0.29. However, in 60g high fat beef patties, methodologies limited the applicability of PE. Future studies will need to address the encapsulation and delivery system for PE, as well as the varying size of beef patties and its affect on PE applicability.

CHAPTER 5

TRIOSE PHOSPHATE ISOMERASE AS AN INDICATOR OF THERMAL PROCESSING OF GROUND BEEF PATTIES

5.1. ABSTRACT

The maximum temperatures reached at the center point of 50 patties tested ranged from 62.5 to 72.7°C during cooking. Triose phosphate isomerase activities did not decrease with increasing maximum center temperature as expected. A large range in TPI activity was seen within each individual patty. The 18 patties that achieved lethality equal to or greater than 200s at 65°C exhibited a TPI activity of less than 20 U/g of meat. Maximum internal temperatures were calculated from TPI activity values for 5 locations within the patty. The estimated temperature differences within ground beef patties ranged from 3.11 to 25.68°C, but decreased with increasing process lethality. These results suggest that the lethality performance standard should be raised from the proposed 5.0 $\log_{10}$ to a 6.5 $\log_{10}$ reduction in *Salmonella* to ensure the safety of cooked ground beef patties.

5.2. INTRODUCTION

Recently the USDA-FSIS, in addition to new time/temperature schedules, has allowed processors to establish thermal processes for meat products based on lethality standards. A proposed lethality performance standard for fully cooked, uncured meat patties includes a 5 $\log_{10}$ reduction in *Salmonella* (USDA, 1999). However, in review, the FSIS has concluded that a higher lethality standard may be needed to produce safe ground beef patties. Although these thermal processing guidelines are available, there is still a need to verify adequate cooking.

Current regulations state that commercial establishments must verify the time and temperature within ± 1°F on one beef patty on a production line each hour. It is very difficult to use a thermometer or thermocouple in commercial processing plants to verify that the time-temperature requirements have been met in a ground beef patty. None of the established USDA methods to determine endpoint temperature are based on thermal inactivation of *Salmonella*, the microbial pathogen selected to define proposed lethality standards.

Berry et al. (1999) found that the internal temperature of beef patties varied as much as 18°C when the center temperature was 71°C. Liu and Berry (1996) reported that of 96 ground beef patties cooked for a predetermined time to a target temperature of 71°C, 16.7% did not reach 68°C. The lowest temperature measured within patties with 10% and 20% fat were 61 and 63°C, respectively. In addition, considerable variability in the mean time required for beef patties to reach a specific temperature was observed. The standard deviations from the means ranged from 0.22 to 0.59 min. Thus, while the average time for patties to cook to a certain internal temperature may be determined by initial studies, there is a high probability that some patties will be over- or undercooked. To overcome these difficulties in monitoring the thermal processing of ground beef, time-temperature integrators (TTIs) have been studied.

A TTI has been defined as a device that undergoes an irreversible and precisely measurable change in response to a time-temperature history that mimics the change in a target attribute exposed to the same thermal history (Hendrickx et al., 1995). A major advantage of a TTI is the quantification of the integrated time-temperature impact on microbial destruction within a beef patty can be determined without the actual time-temperature history of the product. Ideal TTIs would be simple, inexpensive to prepare, and easily recoverable with an accurate and user-friendly read-out. To be considered an ideal endogenous TTI, a protein should have a thermal inactivation rate constant (z value) that closely resembles the z value of the microorganism used to establish the processing schedules (Hendrickx et al., 1995; Van Loey et al., 1996).

The z value reported for TPI in ground beef, 5.56°C, (Orta-Ramirez et al., 1997) was very close to the value for *Salmonella* (z = 5.6°C) (Goodfellow and Brown, 1976), the pathogen used to establish processing guidelines for ground beef patties. Sair et al. (1999) reported that TPI activity decreased as cooking temperatures in ground beef patties (24.4% fat) increased from 60.0 to 71.1°C. No differences in TPI activity could be distinguished when patties were cooked to internal temperatures of 71.1 to 82.2°C. Larger standard deviations were seen in TPI activity at lower processing temperatures in ground beef patties, thus illustrating the large variations in internal temperatures that occur in beef patties during cooking.

The objectives of this study were to (1) investigate TPI activity within ground beef patties during cooking and (2) to evaluate TPI activity as an indicator of cooking temperature at discrete locations in ground beef patties.

5.3. MATERIALS AND METHODS

5.3.1. Ground beef patties

Frozen ground beef patties (113g) were acquired from J & B Meats (Lot # 1136G4, Coal Valley, IL) and held at -22°C until used. The frozen patties were stacked in layers separated by wax paper inside a plastic bag and then placed inside a cardboard box. The patties were thawed at 4°C for 24h prior to use. The raw patties were approximately 11 cm in diameter and 1 cm in height.

Moisture, fat, and protein contents were determined by AOAC (1996) methods 950.46B, 991.36, and 981.1, respectively. For determination of pH, 10g of ground beef were homogenized in 90 mL of distilled water using a Polytron homogenizer (Model PT 10/35, Brinkman Instruments, Westbury, NJ) at speed setting 3 for 30s. The pH of the homogenized mixture was measured using a combination electrode (Model 145, Corning, Medfield, MA). Analyses were done in triplicate.

5.3.2. Patty cooking procedures

A thermocouple (Type T, length 8.6 cm, width 1.0 mm, accuracy ± 0.1°C, Omega Engineering, Stamford, CT) connected to a datalogger system (Daqbook 100, Omega Technologies Inc.) was inserted horizontally into the beef patty. The temperature at the center of the patty was recorded every second during the cooking process.

Fifty ground beef patties were cooked during a 5 day period. The beef patties were cooked on a griddle (Model 07032, Presto Jumbo griddle, National Presto

Industries, Inc., Eau Claire, WI) set at 177°C. Preliminary studies showed that the griddle cycled ± 7°C. The griddle was preheated for a minimum of 30 min prior to use. During the cooking process, patties were heated for 2 min, flipped, and heated for another 2 minutes, then flipped every minute until the process was completed. Patties were removed from the griddle when an internal target temperature between 60 and 75°C was reached. Times of removal varied from 6 to 13 min. The patties were immediately transferred to Whirlpak™ bags (18 oz, Nasco, Ft. Atkinson, WI) and placed in an ice water bath for quick cooling.

Following the cooling period, patties were sliced vertically in half to reveal the cross section of the patty and measurements were taken to determine the placement of the thermocouple. Patties were accepted if the thermocouple and capillary tube were located within ±5% from the center point of the cooked patty thickness. A 50g sample of ground beef was removed from the center of each patty by coring with a plastic 100mL beaker and then vacuum packaged in plastic bags. The beef samples were frozen and held at -18°C until the enzyme assays were performed.

5.3.3. Protein extraction for TPI activity

A 1.0g sample of cooked ground beef was cored from the patty with a 16 × 150 mm disposable culture tube (VWR Scientific Products, West Chester, PA) in 5 locations. One sample was removed from the center (C) of thewith four additional samples removed from the top (T), bottom (B), left (L), and right (R) (Figure 5.1). The 1.0g samples were transferred to scintillation vials (Research Products Intl. Corp. Mount Prospect, IL),

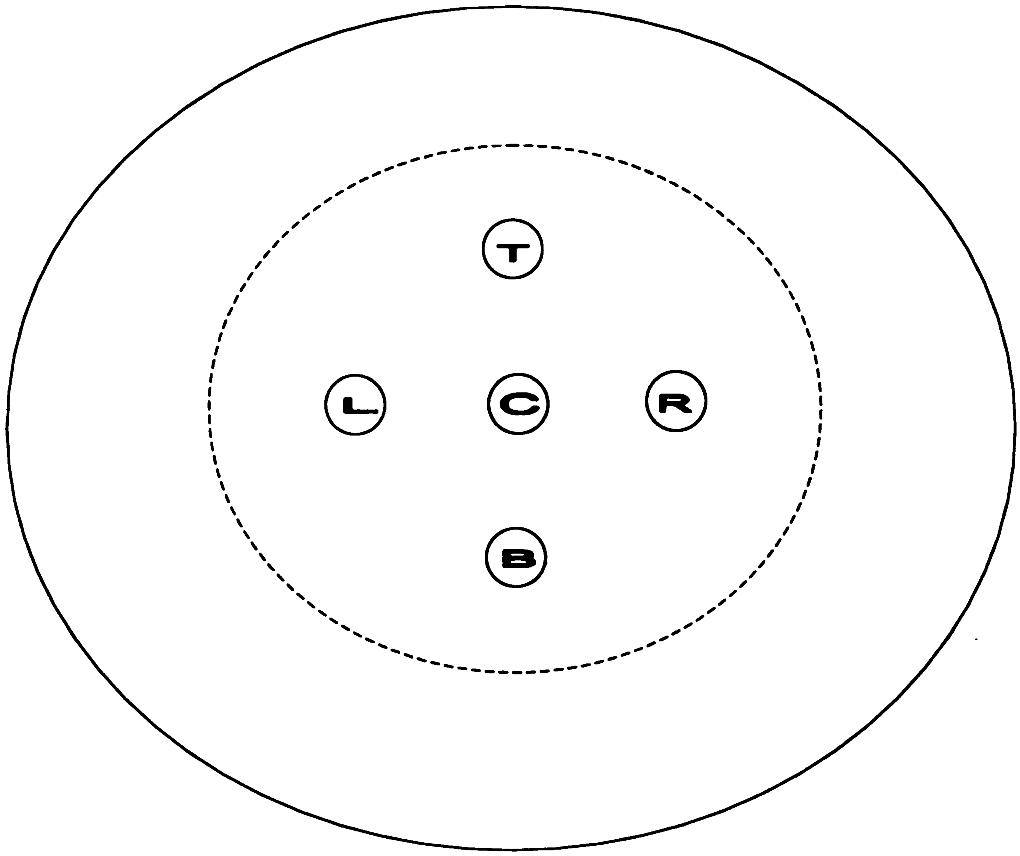


Figure 5.1. Location of 5 meat cores removed from each ground beef patty for triose phosphate isomerase enzyme activity analysis. Dashed line indicates the central 50g core.

and combined with 3 volumes of 0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 8.0 (PBS). The samples were mixed for 1 min using a Fisher Vortex Genie2™ (Scientific Industries, Inc., Bohemia, NY). The extracts were then stirred, without foaming, for 15 min at 4°C followed immediately by centrifugation at 6000 × g for 10 min at 4°C. The supernatents were filtered through Whatman no. 1 filter paper and then held on ice until assays were completed within 12h.

5.3.4. TPI activity determination

TPI activity was determined as described by Sair (1997). The change in absorbance was measured at 340 nm for 1 min at 25°C. The protein extract was diluted with PBS buffer as needed to achieve a change in absorbance of less than 0.2 per minute (Beisenherz, 1995; Norton et al., 1970). TPI activity was calculated using the following equation:

$$\text{Activity (U/L)} = \frac{1000 \times A \times V}{T \times \varepsilon \times d \times (\varphi/d)}$$

A = Absorbance

V = Assay volume (mL)

T = time (min)

ε = extinction coefficient (6.310 L × mmol^{-1} × cm)

d = distance of the light path (1 cm)

φ = sample volume (0.01 mL)

D = dilution of sample

Enzyme activities were expressed as units (U) per gram of meat.

5.3.5. Calculation of process lethality

Lethality was calculated using the General Method (Pflug, 1997) with a reference temperature of 65°C (Equation 2).

$$\text{Lethality} = \frac{1}{10^{((65-T)/5.44)}} \qquad (2)$$

where T = temperature (°C) at time t

The time-temperature data recorded by the datalogger system for each patty was converted into an equivalent lethality. Individual lethalities were then summed to give the total lethality of the process. Many different cooking schedules were used to achieve a range of lethalities. Utilizing the time-temperature safe-harbor guidelines for proper ground beef processing, an equivalent lethality was calculated to be 66s at 65°C using a z value of 5.44°C. This z value resulted in the smallest range of lethalities across all the time-temperature regulations. Thus any cooking process that resulted in a lethality of 66s at 65°C should be equivalent to the time-temperature regulations of the USDA.

5.4. RESULTS AND DISCUSSION

5.4.1. Effect of maximum center temperature on TPI activity in ground beef patties

Ground beef (pH 5.94 ± 0.04) contained 23.6 ± 5.2 % fat, 61.0 ± 0.3% moisture, and 15.9 ± 0.8% protein. The placement of a thermocouple in the center of each patty made it possible to collect data on the maximum temperature at this location during each cooking schedule. The maximum temperature at the center point of the 50 patties tested ranged from 62.5 to 72.7°C. USDA regulations currently in effect for proper thermal processing of ground beef patties include cooking schedules ranging from 66.1°C for 41s to 69.4°C or up for 10s. Under these regulations, thermal processes for each patty were classified as having met the regulations and being properly cooked, or not having met any of the time-temperature regulation and therefore were undercooked (Figure 5.2). Patties may have reached a temperature included in the regulations, but if they did not meet the time requirement, the patty was classified as undercooked.

Thirty-two patties were identified as being undercooked and 18 patties were properly cooked according to the USDA-FSIS time-temperature regulations. The maximum internal temperatures at the center point of patties that did not meet the USDA-FSIS regulations ranged from 62.5 to 68°C, with TPI activities ranging from 1.5 to 139.3 U/g of meat. The patties that met the time-temperature regulations achieved maximum center temperatures that ranged from 67.6 to 72.7°C. The TPI activities of the center point corresponding to these properly cooked patties ranged from 1.5 to 64.0 U/g of meat.

TPI activities did not decrease with increasing maximum center temperature (Figure 5.2). Sair et al. (1999) observed that TPI activity of beef patties decreased as the maximum internal temperature increased. Discrepancies could be seen due to the small sample size used to assay TPI activity. The enzyme assays reported here were completed with a 1g of ground beef, whereas Sair et al. (1999) used a much larger sample with a 7 cm diameter, that may have been more representative of an overall or average activity.

All ground beef patties that reached a maximum center temperature of 71.1°C or higher had TPI activities below 20 U/g at the center point. Sair et al. (1999) reported that patties cooked to internal temperatures above 71.1°C had TPI activities below 6.3 U/g of meat. Lower TPI activities seen by Sair et al. (1999) could be due to the residual temperature increase after the patty reached the target endpoint temperature and was

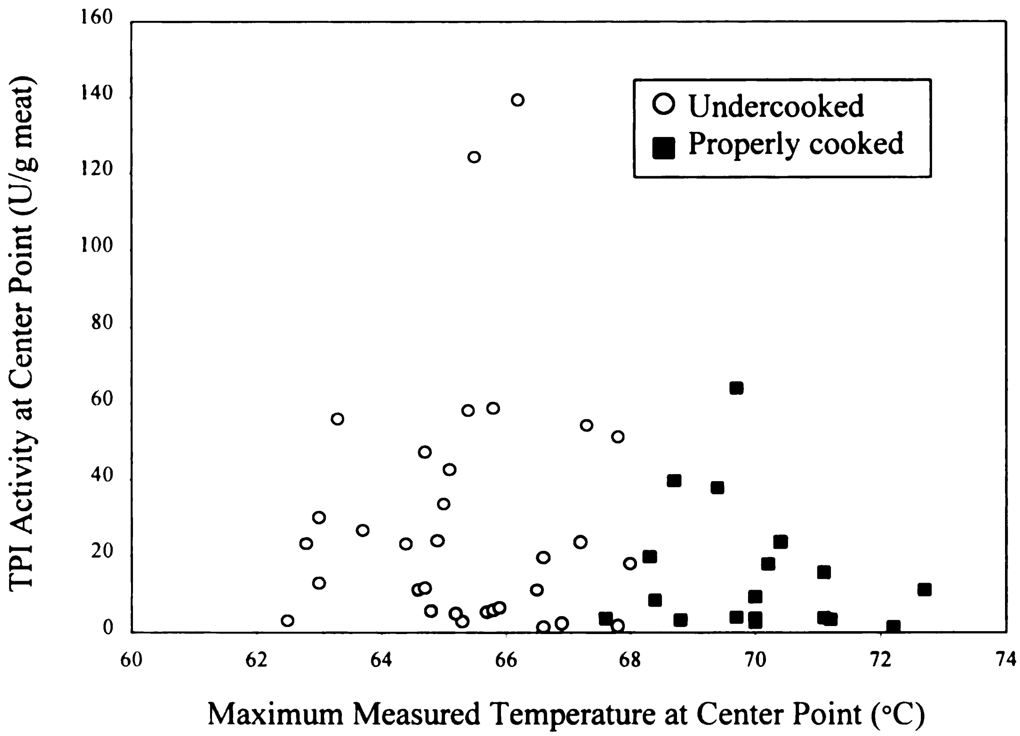


Figure 5.2. Effect of center point temperatures on the triose phosphate isomerase activity in the center of a beef patty. Patties were categorized as undercooked or properly cooked according to the USDA-FSIS time-temperature safe harbor guidelines (1996).

removed from the grill. Maximum temperatures reported in this study were the highest recorded within the patty during the cooking process. The lethality of different cooling processes used in the two studies could also be a factor affecting TPI activity. Sair et al. (1999) allowed patties to cool at room temperature, thus allowing the patties to remain at higher temperatures for a longer time than our study where patties were placed directly into an ice water bath.

When the TPI activities at all 5 locations were averaged, the values were different from the center point TPI activity within the same beef patty (Figure 5.3). This suggests that TPI activity of the center sample was not representative of the activity throughout the patty. Large variations in average TPI activity were seen in patties cooked to the same maximum center temperature. For example, patties undercooked to an internal temperature of 65-66°C exhibited average TPI activities from 2.4 to 63.2 U/g meat. Patties that were properly cooked to internal temperatures of 69-70°C had average TPI activities from 2.96 to 57.36 U/g meat. Variations in average TPI activity at the maximum center temperature did not decrease below 5 U/g meat until 72°C.

The heterogeneous nature of ground beef leads to large differences in heat and mass transfer and thus different heating rates within a patty (Pan and Singh, 1998). Troutt et al.(1992) reported that rendering of fat during cooking resulted in air pocket formation within a beef patty, which also has an effect on heat transfer. These effects on heat transfer throughout a beef patty can result in large temperature differences. Berry et al. (1999) reported internal temperature variations that exceeded 18°C on cut surfaces of a ground beef patty cooked to 71.1°C. TPI activity is very predictable, decreasing with increasing maximum internal temperature. The large variation in activity observed

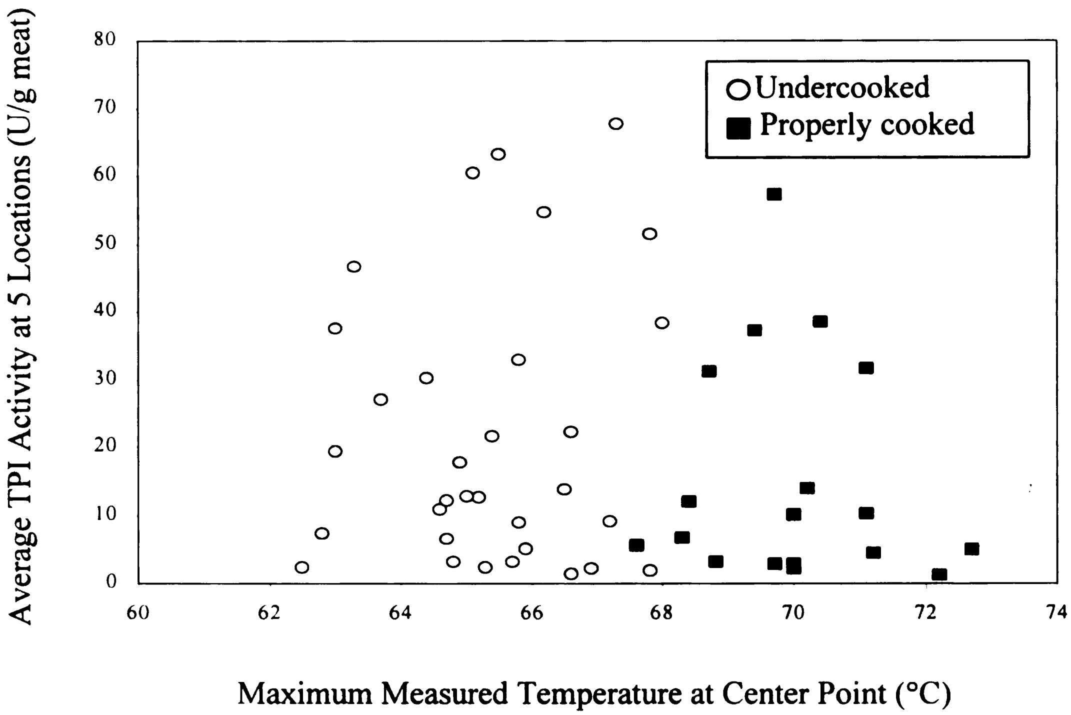


Figure 5.3. Effect of center point temperature on the average triose phosphate isomerase activity at five locations in the beef patty. Patties were categorized as undercooked or properly cooked according to the USDA-FSIS time-temperature safe harbor guidelines (2000).

suggests that there were large temperature variations within the beef patties during cooking.

5.4.2. Effect of lethality of the cooking processes on TPI activity in ground beef patties

Lethality was calculated to take into account the time-temperature history of the patty during cooking. Overall it was observed that as lethality of the cooking process increased, the TPI activity at the center point decreased. Beef patties with a lethality of 66s or greater at 65°C should be properly cooked according to the USDA safe harbor guidelines. There were 15 patties that had lethalities less than 66s at 65°C and had TPI activities greater than 20U/g meat, thus exhibiting the behavior expected of the TPI. Yet there were 13 patties that had lethality below 66s at 65°C and exhibited a TPI activity at the center point less than 20 U/g meat (Figure 5.4). For example, the lethality for one of the patties was calculated to be 22.3 s at 65°C and yet the TPI activity decreased from a raw value of approximately 2000 U/g to 7.3 U/g of meat. Thus if TPI was being utilized as an indicator of adequate thermal processing, this patty may be improperly identified as safe for consumption.

Two of the eighteen patties that achieved lethalities greater than 66s at 65°C failed to meet one of the time-temperature guidelines. The complete time-temperature history of the cooking process was used in lethality calculations, including the gradual increase from storage temperature to the maximum center temperature. Time-temperature regulations are based upon endpoint monitoring, which only accounts for the thermal processing time at the target temperature. The lethality of 66s at 65°C is equivalent to only the holding time at each temperature. Therefore, when the heating lag times are included, lethalities may be increased without an increase in maximum

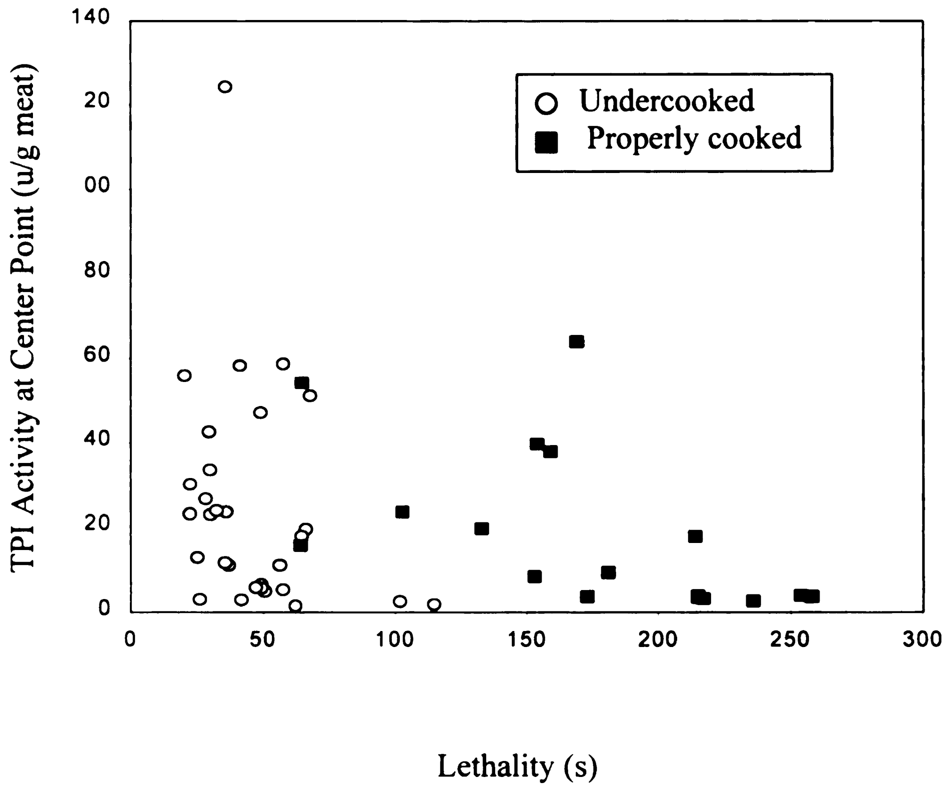


Figure 5.4. Effect of lethality of the cooking process plotted on the triose phosphate isomerase activity of the center point of a beef patty. Patties were categorized as undercooked or properly cooked according to the USDA-FSIS time-temperature safe harbor guidelines (2000).

center temperature, which explains why patties with lethalities greater than 66s at 65°C did not meet one of the safe harbor guidelines.

Three patties with lethalities greater than 66s at 65°C had TPI values above 20 U/g meat. These patties were properly cooked based on the time-temperature regulations, but TPI is indicating an undercooked patty. Once again, large temperature variations within the patties and small sample size may explain these discrepancies.

All of the patties that had a cooking schedule with lethality equal to or greater than 200s at 65°C exhibited a TPI activity of less than 20 U/g of meat. There was a better relationship between lethality and TPI activity than maximum internal temperature and TPI activity. Lethality, which offers an expression of the time-temperature history, may be a better indication of the magnitude of thermal processing than internal temperature alone.

The average TPI activity of the 5 sampling locations of each patty was calculated and compared to the TPI activities from Sair et al (1999) (Figure 5.5). Seventeen patties had lethalities less than 66s at 65°C and also exhibited average TPI activity of less than 20 U/g meat. Two patties had lethalities above 66s at 65°C, but did not meet any of the time-temperature regulations of the USDA. Three patties with lethalities greater than 66s at 65°C had TPI values above 20 U/g meat. One patty which was cooked with a lethality process less than 66s at 65°C met one of the time-temperature guidelines. Limitations in heterogeneity of ground beef and internal temperature differences mentioned above explain these variations.

5.4.3. Temperature variation within ground beef patties

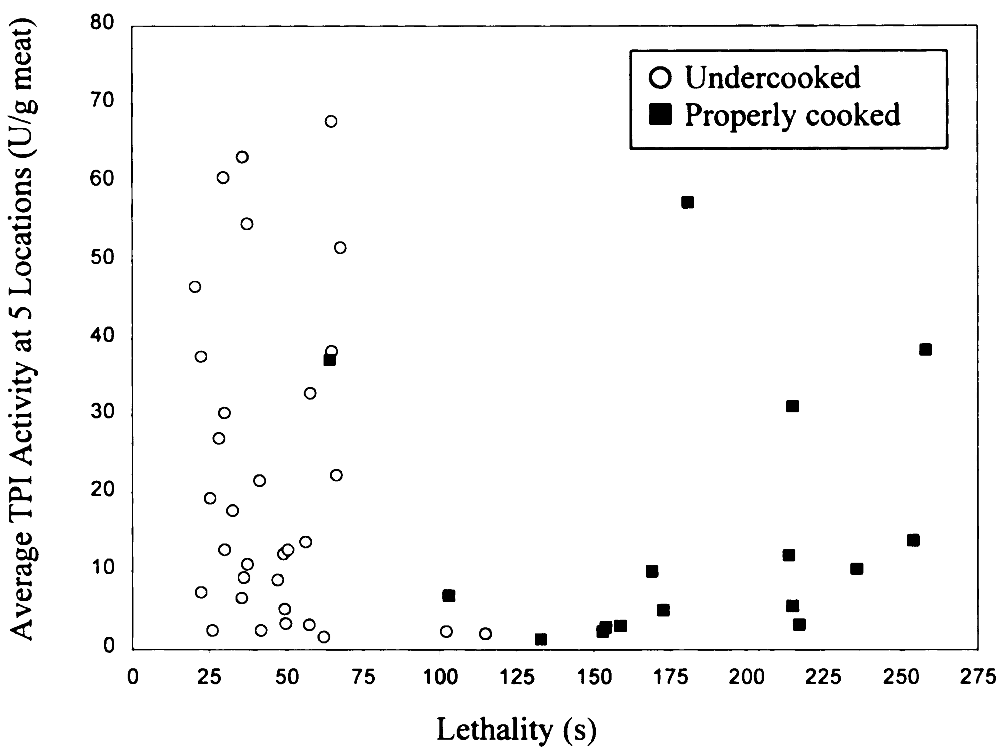


Figure 5.5. The effect of lethality of the cooking process on the average triose phosphate isomerase activity at five sampling points within a beef patty. Patties were categorized as undercooked or properly cooked according to the USDA-FSIS time-temperature safe harbor guidelines (2000).

Sair et al. (1999) reported TPI activities for beef patties cooked to four different endpoint temperatures in the range tested in this study. These values were used to create a standard curve to predict internal temperatures at the 5 locations within beef patties for which TPI activity was known (Figure 5.6). A very good correlation was seen between TPI activity and maximum internal temperature ($R^2 = 0.95$). From the equation describing this correlation, maximum internal temperatures were estimated from known activities.

From the calculated internal temperatures at each sampling point it was possible to estimate the temperature differences that occurred within the patties during cooking (Figure 5.7). At lethalities less than 66s, temperature differences within a patty ranged from a low of 3.11 to a high of 25.68°C. At lethalities greater than 66s, the temperature differences ranged from 7.0 to 22.2°C. Overall, as process lethalities increased temperature variations within beef patties decreased. This is in good agreement with Sair et al. (1999) who reported that the standard deviation of TPI activity decreased with increasing maximum internal temperature.

Temperatures calculated from TPI activities at the 5 different patty locations were averaged. A plot of the calculated average internal temperature ± standard deviations against the maximum measured internal temperature at the center of the patty is given in Figure 5.8. Large standard deviations were observed on this plot indicating that the range of temperatures within beef patties was very large. These studies indicate the need to determine mechanisms to accurately measure the cooking temperature in beef patties to verify proper thermal processing. Overall the standard deviations for the average calculated temperatures decreased as maximum internal temperatures increased.

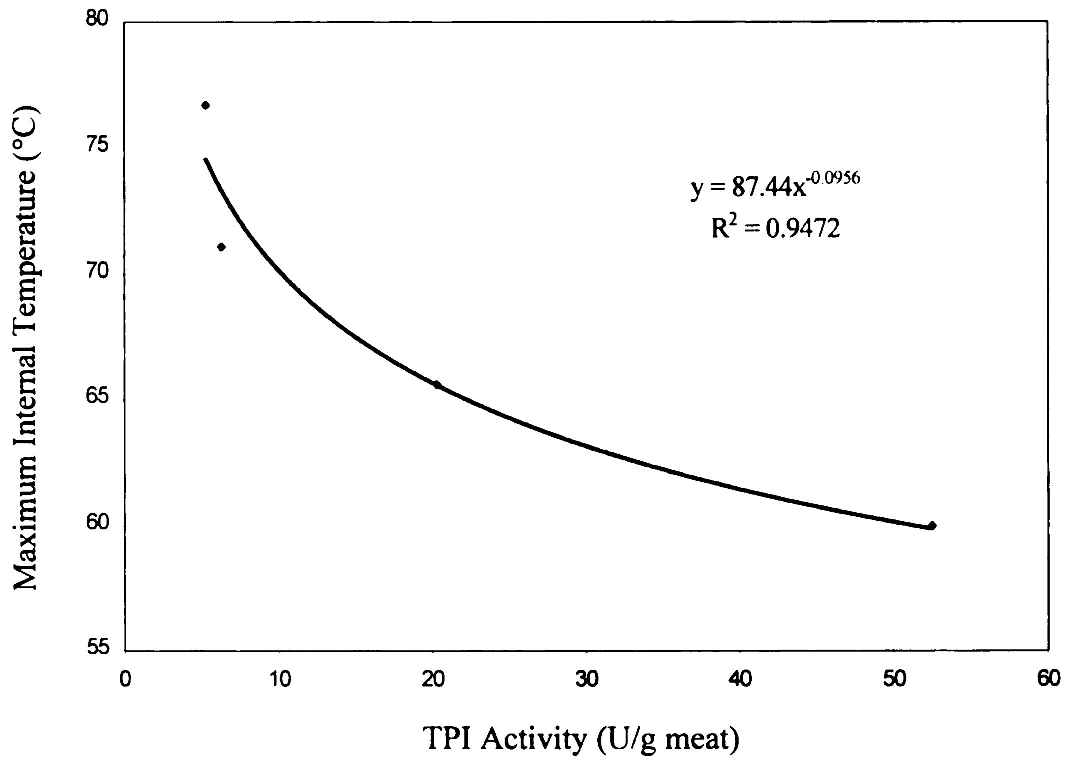


Figure 5.6. Standard curve showing the effects of maximum internal temperature in a ground beef patty on the of triose phosphate isomerase activity from Sair et al. (1999).

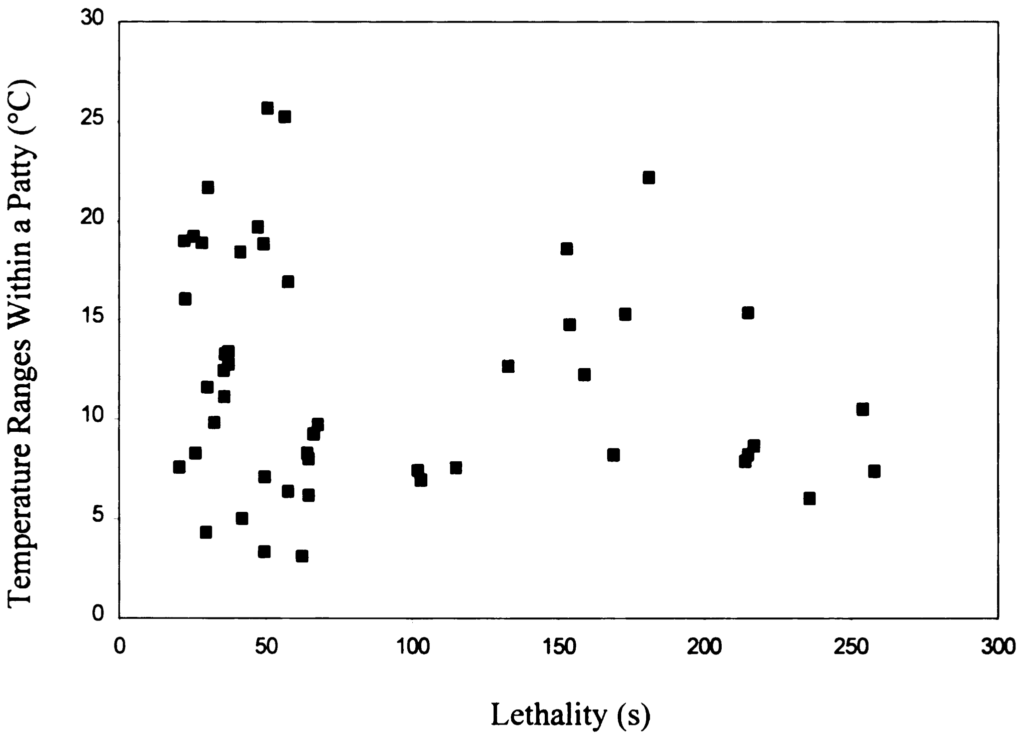


Figure 5.7. The effect of process lethality on the calculated temperature differences within ground beef patties.

No trend was seen between measured center temperature and the estimated average temperature throughout the patty suggesting that the center temperature may not be a good indicator of the thermal process employed on a patty (Figure 5.8). No correlation was observed between lethality and the calculated average temperature within beef patties suggesting that the small area measured by the thermocouple in the center of a patty may not be representative of the temperatures within the overall patty.

5.4.4. Effect of location on TPI activity of ground beef patties

The cold spot of a hamburger has always been assumed to be in the geometrical center of a patty. Therefore, it was predicted that the highest enzyme activity would be observed at the center point. Yet, only 26% of the 50 patties exhibited the highest activity at the center point. The majority (60%) of the patties tested had the highest TPI activities to the left or right of the center point, while the remaining 14% had the highest activity at the top or bottom point. The construction of the griddle used in this study could offer some explanation for these findings. Placement of the heating coil on the underside of the cooking surface can be seen from the diagram in Figure 5.9.

All patties were cooked individually in the center of the griddle. According to Figure 5.9, the top and bottom points of the beef patty were in closer proximity to the heating coils than the other three points. This may explain the lower TPI activities at points T and B in comparison to the other three points in a majority of the patties tested. Preliminary studies were performed to measure the temperature variations of the griddle surface during cycling. Higher temperatures were recorded in the middle of the griddle in comparison to the left and right sides. Again, explaining why the large number of patties had the highest enzyme activity on either the left or right side of center. Thus it is

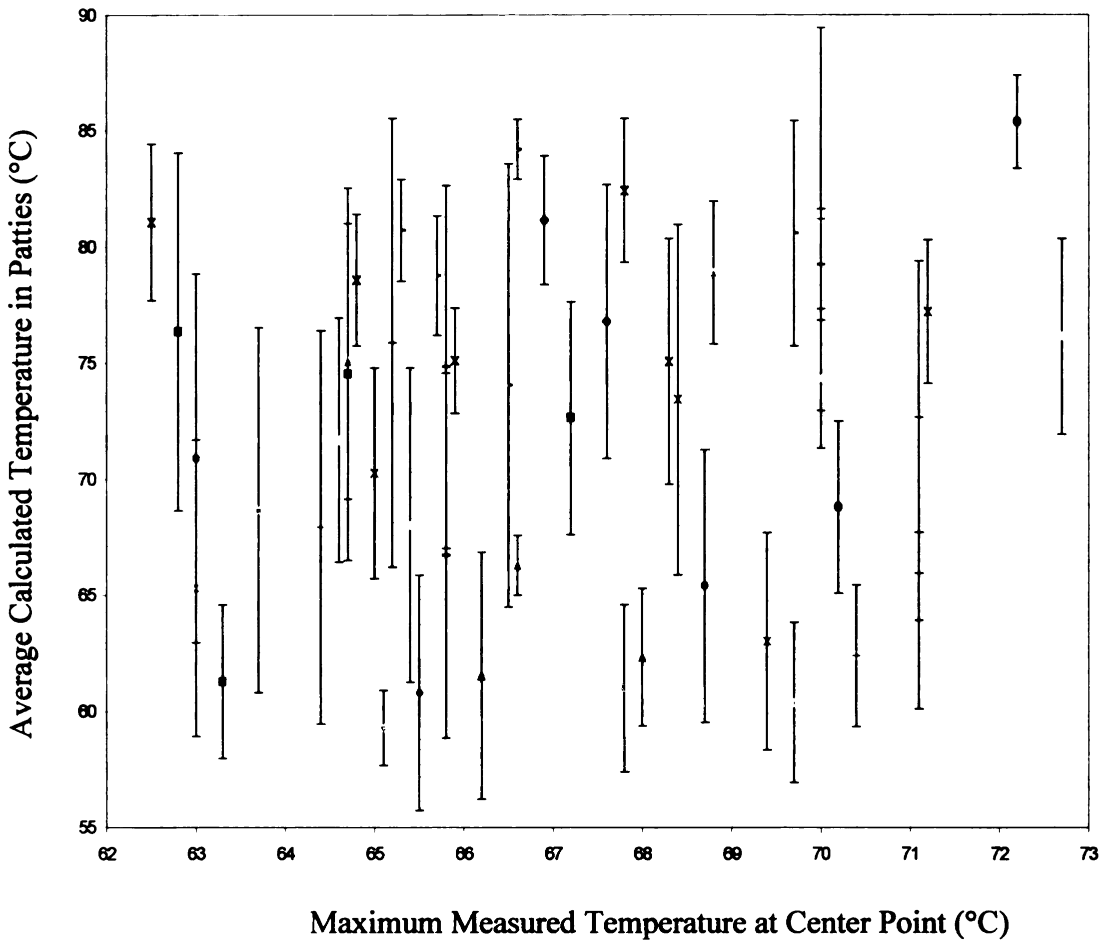


Figure 5.8. The effect of maximum measured internal temperature of the center point of ground beef patties on the average calculated internal temperature error bars indicate standard deviation.

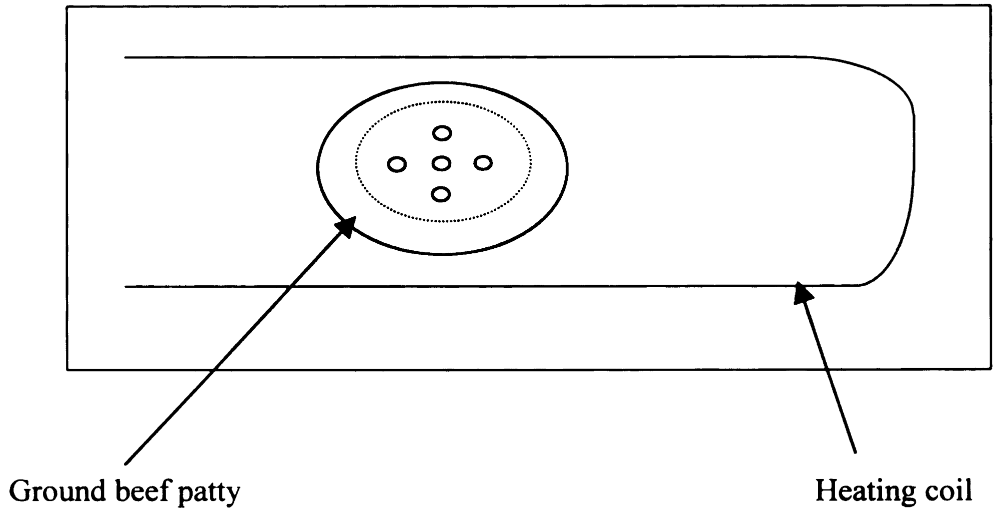


Figure 5.9. Location of the heating coil and ground beef patty on the cooking surface of the griddle.

reasonable that the maximum center temperatures did not correlate to the average calculated temperatures within the patties.

5.5. CONCLUSIONS

The sampling method revealed large variations in TPI activity within ground beef patties. Temperature differences as large as 25.7°C were estimated within a 113g patty. Heat transfer within a beef patty is affected by fat content, initial temperature of the patty, method of cooking, and air and fat pockets formed during cooking. Therefore, it would be very difficult to develop methodologies to accurately measure temperatures inside beef patties. A safety factor could be incorporated into thermal processing regulations to account for temperature variation within patties. The lethality performance standard for uncured, fully cooked beef patties has not been finalized. The results of this study would recommend raising the lethality performance standard from the proposed 5.0 $\log_{10}$ to a 6.5 $\log_{10}$ reduction in *Salmonella* to ensure safely cooked ground beef patties.

CHAPTER 6

CONCLUSIONS

Lethality performance standards are in the process of being established for ground beef patties to add flexibility to processing schedules allowed in the meat industry. The USDA is recommending that lethality standards be verified through challenge studies with a *Salmonella* cocktail comprised of heat resistant strains that have been previously implicated in foodborne outbreaks.

Heat resistance of an 8-strain *Salmonella* cocktail in stationary growth phase decreased after being inoculated into ground beef and frozen for 7 days. The thermal stability of the cocktail also decreased ($P<0.05$) at 55, 61, and 63°C after 14d of starvation in 0.1% peptone water at 4°C. The D values of the thermally injured cocktail were not different ($P<0.05$) from the *Salmonella* cocktail in log phase. The heat shocked cocktail was not different ($P<0.05$) from the *Salmonella* cocktail in log phase except for 58°C at which it was less heat resistant. The z values of the *Salmonella* cocktail ranged from 4.20 to 4.64°C. Based on our findings, this 8-strain *Salmonella* cocktail may not be able to adequately represent a 5-log reduction for some pathogens including more heat resistant strains of *S.* Typhimurium DT104 at low temperatures (≤55°C).

New lethality performance standard regulations require that processors validate the efficacy of their processing schedules. Difficulties arise since *Salmonella* cocktails cannot be used in commercial processing plants for verification. However, a time-temperature integrator that could accurately predict reduction in the number of *Salmonella* survivors, it could be used in a plant for validation. A normalized PE fluorescence of 0.31 was correlated with a 5 or greater $\log_{10}$ decrease in *Salmonella* within 113g ground beef patties (19.1% fat). A similar normalized fluorescence of 0.29 was determined as a cut-off point for proper thermal processing in low fat ground beef patties (4.8%). A normalized PE fluorescence of 0.21 indicated proper thermal processing of 60g beef patties. However, due to methodology limitations, the correlation between normalized PE fluorescence and the log decrease in *Salmonella* was low. PE was consistent in monitoring process lethalities in ground beef patties and does have potential for actual use in the meat industry.

Many factors affect heat transfer in ground beef due to its heterogeneous nature, resulting in large temperature differences throughout the patty during cooking. TPI, an endogenous enzyme found in ground beef, was able to detect temperature differences within a beef patty. Internal temperatures were estimated from activity levels measured at 5 locations within a beef patty. Temperature differences within beef patties ranged from 3.1 to 25.7°C. These temperature differences could have a large impact on final endpoint temperature and lethality of beef patties. Lethality standards are currently being determined for ready-to-eat ground beef patties. Results presented here indicate the need to incorporate a safety factor into lethality performance standards. For example, estimated temperature variations exceeded 25°C, meaning that an internal temperature of 40°C could be detected in a patty cooked to a target temperature of 65°C. Results reported here support an increase in lethality performance standards from 5.0 $\log_{10}$ to 6.5 $\log_{10}$ decrease in *Salmonella* due to temperature variations within patties.

CHAPTER 7

RECOMMENDATIONS FOR FUTURE RESEARCH

The thermal inactivation parameters of a *Salmonella* cocktail were determined under different environmental conditions. The cocktail was thermally injured and heat shocked in tryptic soy broth before thermal death time studies. If sub-lethal heat treatments were performed in ground beef, the resulting thermal inactivation parameters could be used to evaluate effects of temperature abuse in meat products.

The experiments with PE were performed using glass capillary tubes. In a real processing situation, this would be unacceptable for safety reasons. Additional research is needed to find a suitable material to encapsulate PE for insertion into beef patties. The encapsulating material should not change the heat transfer properties of the meat system or interact with the PE.

Methodologies have been developed that accurately record lethality, maximum center temperature and the destruction of PE in a patty system. Thus these methodologies could be applied to other meat patty systems, including turkey and chicken, whose target pathogen for proper thermal processing is also *Salmonella*.

Phycoerythrin is just one of three proteins in the class of phycobiliproteins. Thermal inactivation parameters could be determined for the other two pigments to investigate their possible application as a TTI in food systems.

Limitations were seen in the ability to accurately measure the internal center temperature of the beef patty during cooking. Theoretical heat transfer calculations could be used to predict the internal temperature without inaccuracies due to thermocouple placement.

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