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**Verification of Indicator Proteins and Color
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Thermal Processing of Ground Beef Patties**

presented by

Arnie Sair

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Denise M. Smith

Major professor

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**VERIFICATION OF INDICATOR PROTEINS AND COLOR MEASUREMENTS AS
METHODS TO DETERMINE ADEQUATE THERMAL PROCESSING OF GROUND
BEEF PATTIES**

By

Arnie Sair

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ABSTRACT

VERIFICATION OF INDICATOR PROTEINS AND COLOR MEASUREMENTS AS METHODS TO DETERMINE ADEQUATE THERMAL PROCESSING OF GROUND BEEF PATTIES

By

Arnie Sair

The goal of this study was to determine whether triose phosphate isomerase (TPI) could be used as a marker protein to verify adequate thermal processing of ground beef patties. The objectives were 1) to determine the effect of muscle source, composition and storage on TPI activity and concentration in ground beef, and 2) to compare the use of TPI activity, lactate dehydrogenase (LDH) concentration and color to verify processing adequacy of ground beef patties. TPI activity differed between muscles in cows and steers. TPI activity and concentration fluctuated in cooked beef during storage at -13°C for 5 months. A maximum TPI activity of 6.2 U/kg meat indicated that ground beef (20% fat) was adequately cooked to 66.1°C/41 sec in a model system. In pilot studies, TPI activity of beef patties (24% fat) decreased between 65.6 and 71.1°C to a value of 6.28 U/kg meat. Lactate dehydrogenase (LDH) concentration decreased between 71.1 to 76.7°C. While color measurements were able to detect differences between undercooked and fully cooked beef patties, past research has shown color to be a poor indicator of adequate thermal processing. TPI could possibly be used as an indicator to verify the adequate processing of ground beef patties cooked to 71.1°C and above with no holding time.

This thesis is dedicated to my father and mother for providing me with the opportunities to excel in achieving my goals and to my sisters, for being the best sisters in the world.

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CHAPTER 1

INTRODUCTION

Heat processing is commonly used to ensure the destruction of pathogens in meat products. *Salmonella*, hemorrhagic *Escherichia coli*, *Campylobacter*, and *Staphylococcus* are the most prevalent pathogenic bacteria found in foodborne illness outbreaks associated with domestic and imported precooked meats. Inadequate cooking, or survival of the pathogen during cooking is a common cause of foodborne disease outbreaks associated with meat products (Bean and Griffin, 1990; Mermelstein, 1993). *Salmonella* has been the traditional pathogenic microorganism of concern in meat products, however, *E. coli* O157:H7 has emerged as a significant cause of foodborne disease. While most types of *E. coli* are normally found in the intestinal tract of humans and other animals, there are a limited number of pathogenic strains of *E. coli*. In 1982, *E. coli* O157:H7 was associated with two outbreaks of hemorrhagic colitis where ground beef was epidemiologically linked as the vehicle of transmission. Since 1982, undercooked ground beef has been identified as the most frequent vehicle of *E. coli* O157:H7 infection (CDC, 1996). Cattle have been identified as a major reservoir of *E. coli* O157:H7. An outbreak of foodborne disease in several western states from late 1992 to early 1993 resulted in more than 475 cases and several children's deaths. It was caused by the consumption of undercooked enteropathogenic *E. coli* O157:H7-contaminated ground beef patties (Mermelstein, 1993; Ahmed et al., 1995). There are an estimated

10,000 to 20,000 cases of *E. coli* O157:H7 infection in the United States each year (CDC, 1996). Infection often leads to bloody diarrhea and occasionally to kidney failure.

Foodborne illness is recognized as a significant public health problem in the United States (USDA-FSIS, 1997a). Data from varied sources suggests that each year, 24 to 81 million people become ill from pathogens in food resulting in an estimated 10,000 deaths. Of the foodborne illnesses and deaths resulting from microbial pathogens in food, 37 million illnesses and more than 5700 deaths may be associated with meat and poultry products. The annual cost of foodborne illness is estimated to be between \$7.7 and \$23 billion (FDA, 1995).

On May 12, 1997, a five-point Administrative plan was unveiled by Vice President Gore to strengthen and improve food safety for the American people (FDA, 1997). The plan detailed how President Clinton planned to use the \$43.2 million in new funds he requested in his 1998 budget to supplement the food safety precautions already in place. Among the measures developed by the Administration to reduce foodborne illness from microbial contaminants was increased research to develop new tests to detect foodborne pathogens and to assess risks in the food supply. An accurate and rapid method is currently needed to verify that meat products have received adequate thermal treatment to prevent foodborne disease.

The U.S. Department of Agriculture - Food Safety Inspection Service (USDA-FSIS) requires that meat products be cooked to safe endpoint temperatures (EPT) under Title 9 of the Code of Federal Regulations to ensure destruction of pathogenic bacteria and viruses. The USDA-FSIS (1997b) has required that patties labeled as "fully cooked"

be cooked to any one of seven time/temperature processing schedules that range from 66.1°C for 41 sec to 69.4°C for 10 sec.

On May 2, 1996, the USDA-FSIS proposed to amend the processing regulations of cooked beef, roast beef, and cooked corned beef; fully cooked, partially cooked, and char-marked uncured meat patties; and certain fully and partially cooked poultry products into performance standards (USDA-FSIS, 1996). The three performance standards which meat processing establishments would be required to meet are lethality, stabilization, and handling. To meet the lethality standard, establishments would be required to achieve a 5 log (5-D) or 7 log (7-D) reduction in *Salmonella*, depending on the product. While *E. coli* O157:H7 has recently emerged as a significant pathogen of concern in meat products, *Salmonella* is more resistant to heat than *E. coli* O157:H7. Therefore, a 7-D reduction in *Salmonella* in cooked beef products would eliminate all pathogenic microorganisms and provide a significant margin of safety. For cooked beef, roast beef, and cooked corned beef products, the USDA-FSIS has proposed a 7-D reduction in *Salmonella*.

A 5-D reduction in *Salmonella* has been proposed for fully cooked uncured meat patties. Any of the seven time/temperature processing schedules currently being used for the cooking of beef patties generate a 5-D lethality. Therefore, an establishment using one of the time/temperature combinations could continue to produce beef patties which meet the proposed lethality standards. However, in order to have an overall lethality standard, the USDA-FSIS may reduce the proposed lethality performance standard for roast beef, cooked beef, and cook corned beef to a 5-D reduction in *Salmonella* as well (Anderson, 1997). The performance standards would dictate the level of reduction in pathogenic

microorganisms needed to be achieved by the processing establishments, while allowing the use of processing procedures other than those prescribed in the current regulations.

A time-temperature integrator (TTI) is needed to verify whether fully cooked beef patties have been processed to meet the lethality standard. A TTI is a measuring device which undergoes a time/temperature dependent, irreversible change after being subjected to a variable temperature exposure (Van Loey et al., 1996). The irreversible change in the TTI should parallel that of the target attribute, such as *Salmonella*, also being exposed to the time/temperature process. A TTI should: 1) quantify the impact of a thermal process on a target attribute, 2) be easy to recover from the food after processing, and 3) be quickly and easily prepared for monitoring (Hendrickx et al., 1995). Through the use of a TTI with a similar z value as that of *Salmonella* in ground beef patties, the monitoring of adequate thermal processing can be achieved.

Current USDA methods to monitor endpoint temperature (EPT) are difficult to interpret, subjective, impractical in that they require the use of sophisticated scientific equipment, trained operators, and are time consuming. The methods currently in use by the USDA include the Bovine Catalase Test (USDA, 1989), the Coagulation Test (USDA, 1986a), and the Acid Phosphatase Activity Method (USDA, 1986b). The greatest disadvantage of the USDA methods for EPT determination is that none of them utilize an indicator with a z value that is similar to that of *Salmonella*, the microbial pathogen selected by the USDA-FSIS to define the proposed lethality standards. Therefore, it is not currently possible to determine whether beef products have been adequately processed after being cooked to one of the seven time/temperature processing schedules established by the USDA-FSIS.

In previous work (Orta-Ramirez et al., 1997; Wang et al., 1996b; Hsu, 1997), triosephosphate isomerase (TPI) was identified as a potential TTI in ground beef. Orta-Ramirez et al. (1997) found TPI to have a similar thermal inactivation rate as *E. coli* O157:H7 and *S. senftenberg* between 53 and 66°C. Hsu (1997) determined TPI activity in roast beef after processing to three adequate and three inadequate (decreased cook times) USDA processing schedules. The activity of TPI was found to be the same when compared within adequate cooking treatments and increased as cooking time decreased. The above results suggested that TPI could be used as a TTI in beef products to verify compliance to USDA processing schedules.

Lactate dehydrogenase (LDH) concentration, as quantified by ELISA, has been identified as a means for determining the EPT of cooked ground beef (Orta-Ramirez et al., 1996; Wang et al., 1996a). Wang et al. (1996a), using polyclonal antibodies against bovine muscle LDH, tested a sandwich ELISA to monitor the EPT of cooked ground beef. In a model system, the ELISA was able to detect differences in LDH concentration in ground beef at 2°C intervals within the temperature range of 66 to 74°C, suggesting it could be used to verify cooking adequacy of beef patties processed using the USDA-FSIS time/temperature combination of 69.4°C for 10 sec.

Orta-Ramirez et al. (1996) produced monoclonal antibodies to bovine muscle LDH and a sandwich ELISA was developed to determine the EPTs of cooked ground beef. The LDH content of commercial patties, cooked from 54.4 to 65.6°C, 68.3 to 71.1°C and 73.9 to 82.2°C decreased as cooking temperature was increased. Results of Orta-Ramirez et al. (1996) suggested that a maximum concentration of about 3 µg of

LDH/g meat might indicate that ground beef was processed to the USDA recommended EPT of 71°C.

The overall goal for this study was to determine whether TPI could be used as a marker protein to verify adequate thermal processing of ground beef patties. The first study was designed to determine the effect of several factors that may have an effect on TPI activity and concentration in ground beef, including; 1) muscle type and gender of the animal; 2) fat content of ground beef; 3) frozen storage of raw steer muscles; 4) frozen storage of cooked ground beef; 5) refrigerated storage of meat extracts; 6) repeated freeze-thaw cycles of raw and cooked ground beef; and 7) salt and phosphate concentration of beef. The second study was designed to determine whether the EPT of ground beef could be differentiated using residual TPI activity and LDH concentration and to compare the results to the internal cooked color of the beef patties. This study was completed in collaboration with Dr. Brad Berry at the United States Department of Agriculture - Agricultural Research Service Meat Science Research Lab in Beltsville, MD.

CHAPTER 2

LITERATURE REVIEW

2.1 UNITED STATES DEPARTMENT OF AGRICULTURE-FOOD SAFETY INSPECTION SERVICE THERMAL PROCESSING REGULATIONS

To assure the adequate cooking and safety of various cooked and partially cooked meat and poultry products, the U.S. Department of Agriculture - Food Safety Inspection Service (USDA-FSIS) has issued specific cooking regulations to meat processing establishments since 1972 (USDA-FSIS, 1996). The regulations ensured that all establishments were subject to the same rules and were using similar methods to kill harmful bacteria. The USDA-FSIS (USDA-FSIS, 1997b) requires that beef patties be cooked to any of seven time/temperature processing schedules that range from 66.1°C for 41 sec to 69.4°C for 10 sec (Table 2.1). These regulations do not take into account an establishment's individual processing procedures nor the distinct needs of the establishment. While large scale businesses are able to spread the costs of the command-and-control regulations over their high production volumes, the smaller businesses producing meat and poultry products often pay a high cost per unit when required to employ a specific process.

2.2 PROPOSED PERFORMANCE STANDARDS FOR THE PRODUCTION OF CERTAIN MEAT PRODUCTS

The USDA-FSIS has proposed to convert the command-and-control regulations to performance standards (USDA-FSIS, 1996). Establishments would be granted the

Table 2.1-Permitted heat-processing temperature/time combinations for fully-cooked patties

Minimum internal temperature (°C (°F)) at the center of each patty	Minimum holding time (sec) after temperature is reached
66.1 (151)	41
66.7 (152)	32
67.2 (153)	26
67.8 (154)	20
68.3 (155)	16
68.9 (156)	13
69.4 (157)	10

Code of Federal Regulations, Title 9 (USDA-FSIS, 1997b).

freedom to employ individually suitable processing procedures to produce safe products, while not being obligated to follow any of the specific time/temperature processing requirements established by the USDA-FSIS. Three performance standards are being proposed by the USDA-FSIS: lethality, stabilization, and handling. The lethality standard is to ensure that establishments eliminate pathogenic microorganisms from the food product. Reduction of pathogenic microorganisms is measured in terms of decimal reduction time, or D value. The D value (minutes) is defined as the time at constant temperature required to reduce the initial microbial population by 90% (Van Loey et al., 1996).

While in recent years *Escherichia coli* O157:H7 has been recognized as the pathogenic microorganism of concern in cooked beef products, the traditional microorganism of consequence has been *Salmonella*. *Salmonella* is generally more resistant to heat than *E. coli* O157:H7 (Doyle and Schoeni, 1984; Line et al., 1991; Orta-Ramirez, 1994). *Listeria monocytogenes* is more resistant to heat than *Salmonella* (Fain et al., 1991), however the presence of *Listeria monocytogenes* in a finished product is indicative of recontamination. Therefore, the expected levels of *Listeria monocytogenes* after heat treatment are much lower than that of *Salmonella*. Based on the above data, the destruction of *Salmonella* in beef products would be indicative of the destruction of the other two pathogens. For cooked beef, roast beef, and cooked corned beef products, the USDA-FSIS is proposing a 7-D reduction in *Salmonella*, while for fully cooked, uncured meat patties the lethality performance standard is proposed to be a 5 log, or 5-D reduction in *Salmonella* (Goodfellow and Brown, 1978; USDA-FSIS, 1996). The lower proposed lethality performance standard that would be required for fully cooked, uncured

meat patties would be sufficient from a food safety standpoint while also maintaining product flavor and texture. However, in order to have an overall lethality standard, the USDA-FSIS may reduce the proposed lethality performance standard for roast beef, cooked beef, and cook corned beef to a 5-D reduction in *Salmonella* as well (Anderson, 1997). The time/temperature guidelines for beef patties, developed by the USDA, were based on the destruction of *E. coli* O157:H7. When utilized, any of the seven time/temperature combinations generate a 5-D lethality. Therefore, an establishment currently applying one of the time/temperature combinations, could continue to produce beef patties that meet the proposed lethality standards.

2.3 CURRENT USDA METHODS FOR ENDPOINT TEMPERATURE DETERMINATION

Currently, the USDA-FSIS is using the protein "Coagulation Test", developed 30-35 years ago, to monitor the endpoint temperatures (EPT) of beef and pork products heat processed to temperatures lower than 65°C (Townsend and Blankenship, 1989). A residual "Acid Phosphatase Activity Method" for determining the EPT of canned hams, canned picnics and canned luncheon meat, and the "Bovine Catalase Test", for the determination of catalase which gives a pass/fail indication at a cooking temperature of 62.8°C for rare roast beef and cooked beef, are also in use. The Phosphatase Activity Method was developed 30-25 years ago, and the Bovine Catalase Test was developed approximately 10-11 years ago (Townsend and Blankenship, 1989).

2.3.1 Bovine Catalase Activity Test

The Bovine Catalase Test (USDA, 1989) is subjectively performed by observing the appearance of foam when a meat sample is immersed in a solution of hydrogen

peroxide and sodium lauryl sulfate (shampoo). Strong, medium, weak and no activity must be inferred from the quantity of foam produced. Errors can occur if the peroxide reagent is weak or inactive, if the analyst does not carefully observe the appearance of extra foam formed by the catalase reaction, or if the catalase activity is low.

2.3.2 Protein "Coagulation Test"

The "Protein Coagulation Test" is based on measurable loss in protein solubility as temperature of the product is increased (USDA, 1986a). The soluble muscle proteins are extracted with 0.9% saline, filtered, and heated. The temperature at which the first signs of cloudiness or turbidity appear in the filtrate is recorded, and is considered to be the maximum internal cooking temperature of the product. Because visible observation is involved in this test, the test is considered to be empirical and subjective in nature (Townsend and Blankenship, 1989). Operator experience and several testing steps are also required.

2.3.3 Acid Phosphatase Activity Test

The FSIS residual acid phosphatase activity method (USDA, 1986b) is based on the residual enzyme activity after cooking, and is expressed as the micromoles of phenol formed per 1,000 g sample, when the sample is allowed to react with the substrate, disodium phenylphosphate, for 60 min at 37°C and pH 6.5. The residual acid phosphatase activity method demonstrated poor correlation with actual internal temperature (Townsend and Blankenship, 1989; Kormendy et al., 1987; Cohen, 1969). Recently, Kormendy et al. (1987) reported that the residual activity of acid phosphatase in hams does not depend directly on temperature alone, but also on a combined time and temperature effect. The acid phosphatase test requires trained personnel, expensive

equipment, several reagents, and takes over one hour to perform. Recently, a new and more rapid fluorometric assay of acid phosphatase activity in cooked poultry meat was developed and field tested by the USDA (Davis and Townsend, 1994).

2.4 ALTERNATIVE METHODS FOR ENDPOINT TEMPERATURE DETERMINATION

The methods described above do not provide sensitive, rapid, and accurate results that are necessary when testing meat products to verify processing adequacy and to ensure safety. Therefore, alternative methods have been evaluated for testing the endpoint temperature (EPT) of meat and poultry products. These methods include the internal cooked patty color and color of expressible juices (Berry, 1994; Hague et al., 1994), the electrophoresis of meat extracts to monitor changes in protein composition (Lee et al., 1974; Caldironi and Bazan, 1980; Steele and Lambe, 1982), differential scanning calorimetry of muscle proteins (Parson and Patterson, 1986; Ellekjaer, 1992), near infrared spectroscopy (Ellekjaer and Isaksson, 1992), and residual enzyme activity (Townsend and Blankenship, 1989). It has been reported that lactate dehydrogenase (LDH) activity could be used as an endpoint heating indicator in turkey products (Wang et al., 1992, 1993; Abouzied et al., 1993), beef muscle model systems (Collins et al., 1991a; Stalder et al., 1991), and beef patties (Wang et al., 1996b).

2.4.1 Color of Cooked Ground Beef Patties as an Indicator of EPT

To assure that a safe internal temperature has been reached in cooked beef and poultry, the USDA-FSIS recommends that consumers use a meat thermometer when cooking meat and poultry. However, focus group research has shown that consumers are not likely to use a meat thermometer on products as small as hamburgers (USDA-FSIS,

1997c). The USDA-FSIS advises consumers who choose to not use a meat thermometer to evaluate the doneness of ground beef patties by several factors including: (1) the cook out juices should have no trace of pink, red, or cloudiness, (2) the cooked ground beef should be brown in the center, (3) the cooked ground beef should have a firm or flaky texture, as compared to the raw meat which has a soft, mushy texture, regardless of the color (USDA-FSIS, 1997d).

Two problems have recently been identified by the USDA-FSIS regarding its advice to consumers about using a color index, to test for doneness of ground beef, used to ensure the destruction of pathogenic microorganisms. One problem is that some ground beef loses all pink color before fully cooked (Hague et al., 1994). This preformation of cooked color could be due to storing meat for long periods of time, storing above recommended temperatures, or if the meat was ground from carcasses of older animals (Hague et al., 1994; USDA-FSIS, 1997d).

The second problem is related to a pink color that persists in beef patties cooked to 71°C (Mendenhall, 1989; Troutt et al., 1992; Hunt et al., 1995; Van Laack et al., 1996). Van Laack et al. (1996) cooked 17 different commercially prepared patty formulations to an internal temperature of 71°C and characterized the cooked color of the patties. Large differences in color were found among the different formulations after cooking. The a^* -values, a measure of redness in the cooked patty, ranged from 5.3 to 14.2. Eight of the 17 formulations were classified as red or pink after reaching 71°C (a^* -values ≥ 11.4). Spectral analysis of the cooked products showed undenatured myoglobin (Mb) to be responsible for the red color. Research has shown that the oxidative state of the myoglobin at the time of cooking plays a role in the final cooked color (Hunt et al., 1995).

The more metmyoglobin (metMb), as opposed to oxymyoglobin (oxyMb), that the raw product has, the more the cooked product will appear to be done. Mendenhall (1989) demonstrated that the characteristic pink color of fresh meat remained in beef cooked to 71.6°C if the pH was 6.0 or higher. The higher the pH of the meat, the longer the cooking time and/or higher the final internal temperature required for the denaturation of myoglobin to be complete. The cooking times of the 17 patty formulations used by Van Laack et al. (1996) ranged from 4.1 to 7.7 min.

Van Laack et al. (1996) also examined the effect of frozen storage (-27°C) on the color of patties cooked to 71°C. After one year of frozen storage, 16 of the 17 patty formulations were red/pink when cooked to 71°C. Internal temperatures of 81 to 87°C were needed for complete disappearance of the red/pink color. The use of such high cooking temperatures to attain the cooked brown color can negatively affect the sensory qualities of the beef patties.

The amount of fat in beef patties can also affect the cooked ground beef color. Due to lower heat conduction in low-fat beef, low-fat beef patties require longer cooking times to reach equivalent internal temperatures of higher-fat patties (Troutt et al., 1992). Low-fat beef patties have maintained a pink color at temperatures of 71.1 to 74.5°C (Berry, 1994).

Many studies continue to use similar cooking times based on the false assumption that all beef patties cooked for the same amount of time have reached the same EPT. Liu and Berry (1996) documented the variability that exists in cooking properties of beef patties even when the cooking process is controlled. In the first study of Liu and Berry (1996), beef patties were processed in the same facility in two separate trials using similar

raw materials containing 10% fat by cooking to internal temperatures of 68, 71 or 74°C. Beef patties in the second trial required longer cooking times and had higher a^* -values than the first replication. In the second study, constant times were used to cook beef patties to target EPTs of 68 and 71°C. There was considerable variation found in the degree of doneness and final EPTs of the beef patties. The third study evaluated the variability of internal temperatures of beef patties of different formulations when cooked for constant times to attain an internal temperature of 71°C. Nine percent of the patties did not achieve an internal temperature of 68°C and 1.3% did not reach 60°C. The fourth study evaluated the temperature changes that beef patties undergo for a 1-min period after being removed from a griddle. The internal temperature of the ground beef patties did not change greatly within 40 sec of cooking. Forty seconds was determined to be was determined to be available after cooking to assess the EPT. All of the studies showed variability in cooking properties of beef patties due to cooking conditions. Further research was deemed necessary to determine mechanisms to control the cooking conditions of beef patties.

2.4.2 Enzymatic Methods for EPT Determination in Beef Products

Several enzymes have been evaluated as possible means for determining EPTs of beef products (Collins et al., 1991a; Stalder et al., 1991; Townsend and Davis, 1992; Wang et al., 1996b). Townsend and Davis (1992) investigated the use of a glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) combination test kit to assess the thermal treatment that had been applied to ground beef products. While substantial enzyme activity remained in the beef when cooked to 71.1°C, there was a distinct loss of enzyme activity when processed to 79.4°C. It was concluded that the

combination test kit could be used as a method to verify the adequacy of heat treatment given to imported cooked beef which must be heat processed to 79.4°C, but could not be used as an enzyme indicator for determining the EPT of domestic-type meat products heat processed to temperatures lower than 71.1°C.

Collins et al. (1991a) analyzed LDH activity in beef top round muscles that were processed using thermal treatments similar to those found within the meat industry. Results showed that a significant amount of LDH activity was lost upon heating to 63°C and there was minimal activity present at 66°C. Beef top rounds that underwent one freeze-thaw cycle had the same LDH activity as fresh beef muscle.

Stalder et al. (1991) reported the potential for LDH to indicate the minimum heating endpoint in precooked beef. The muscle-to-muscle variations, the influence of gender, age, pH, salt, phosphate, and cooking temperature on LDH activity was investigated. Significant differences were found in LDH activity between the 15 major muscles sampled from three bovine carcasses. The largest disparity in LDH activity was found between the *semimembranosus*, with 1476 U activity/g muscle, and the *vastus intermedius*, with 283 U activity/g muscle. Gender was found to have no effect on LDH activity, but as the maturity of the carcass increased, activity decreased. LDH activity was reduced from greater than 1000 U/g muscle in unheated samples to nearly undetectable levels at 66°C, regardless of pH. Between 57 and 63°C, at pH 5.6, LDH activity in the sample slurries decreased. There was a sharp decrease in LDH activity above 60°C at pH 6.4 in the sample slurries. Increasing the salt concentration (0 to 4.5% NaCl) reduced LDH activity at pH 5.6, but to a lesser degree at pH 6.4. The addition of NaCl and 0.3% sodium tripolyphosphate (STP) decreased LDH activity between 57 and 63°C at pH 5.6.

Wang et al. (1996b) examined five proteins that might be used as indicators to verify that ground beef patties have been cooked to the proper EPT. An ideal protein indicator was identified as one that was present in relatively large quantities at low internal temperatures and then disappeared or decreased markedly in intensity at higher temperatures. Ground beef patties were cooked to 63, 66, 68 and 71°C. Five proteins, acid phosphatase, bovine serum albumin, glyceraldehyde-3-phosphate dehydrogenase, LDH, phosphoglycerate mutase, and TPI were analyzed. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Western blotting and enzyme activity were used to monitor the changes in protein composition of extracts of cooked patties. The concentration or activity of each protein decreased as EPTs of the ground beef patties were increased. Therefore, each of the five proteins showed possible usefulness to verify processing temperatures of cooked ground beef patties.

2.4.3 Immunoassays for EPT Determination in Meat Products

Immunoassays, specifically enzyme-linked immunosorbent assays (ELISAs), have been used to determine the presence of pathogenic and toxigenic microorganisms in foods (Samarajeewa et al., 1991). Immunoassay test kits, currently in use within the food industry, have been developed for detecting antibiotics, pesticide residues, microorganisms, mycotoxins, and indicator proteins to verify minimum EPTs of meat and poultry products (Fukal, 1991; Samarajeewa et al., 1991; Wang et al., 1992; Abouzied et al., 1993; Orta-Ramirez et al., 1996). ELISAs have high specificity and sensitivity, are easy to use, and are rapid and adaptable to automation. An ELISA can be performed rapidly on a large number of samples by minimally trained personnel with small quantities

of solvent and does not require major scientific equipment in the field or processing plant (Smith, 1995).

2.4.3.1 Ground Beef Products

The use of immunoassays to determine the EPT of meat products (i.e. ground beef and poultry products) has been well documented (Wang et al., 1992; Abouzied et al., 1993; Wang et al., 1993, 1994, 1996a; Orta-Ramirez et al., 1996; Smith et al., 1996). Lactate dehydrogenase concentration, as quantified by ELISA, has been investigated as a means for determining the EPT of poultry products (Wang et al., 1992, 1993; Abouzied et al., 1993; Smith et al., 1996) and ground beef (Wang et al., 1996a; Orta-Ramirez et al., 1996). Wang et al. (1996a), using polyclonal antibodies against bovine muscle LDH, tested a sandwich ELISA to monitor the EPT of ground beef. In a model system, LDH content decreased ($p < 0.05$) as ground beef was cooked to temperatures ranging from 62 to 74°C at 2°C intervals. The ELISA was able to detect differences in LDH concentration at 2°C intervals within the temperature range of 66 to 74°C, suggesting it could be used to verify cooking adequacy of beef patties processed using the USDA-FSIS minimum time/temperature combination of 69.4°C for 10 sec.

Orta-Ramirez et al. (1996) produced monoclonal antibodies to bovine muscle LDH and a sandwich ELISA was developed to determine the EPTs of cooked ground beef. Fat concentrations and the effect of freeze-thaw cycles on LDH concentrations in ground beef were also investigated. The sandwich ELISA was able to detect differences in LDH content of ground beef between 64 and 74°C, at 2°C intervals, in a model system. The LDH content of commercial patties, cooked from 54.4 to 65.6°C, 68.3 to 71.1°C and 73.9 to 82.2°C also decreased as cooking temperature was increased. Similar

concentrations of LDH (3 µg/g meat) were detected in both the model system ground beef and commercially cooked patties at a cooking temperature of 70°C. Fat content had no effect on the concentration of LDH in ground beef cooked to 69.4°C. The LDH concentration of raw ground beef remained the same through five freeze-thaw cycles. The LDH concentration of cooked beef was decreased after undergoing one freeze-thaw cycle and was reduced by 70.4% after undergoing four freeze-thaw cycles.

2.5 THERMAL DEATH TIME (TDT) STUDIES

Due to the continued threat of foodborne illnesses caused by the consumption of meat products, research has been undertaken to establish the effects of thermal processing on the pathogenic microorganisms responsible for the outbreaks. The thermal inactivation kinetics of *Escherichia coli* O157:H7 in ground beef (Line et al., 1991), *Listeria monocytogenes* Scott A in ground beef and turkey (Fain et al., 1991), and *Salmonella* in beef products (Goodfellow and Brown, 1978) have been established.

2.5.1 Definitions

The decimal reduction time, or D value is the time (min) required to destroy 90% of the organisms in a food system at a specified temperature. A thermal resistance curve (TRC) can be constructed by plotting temperature against the D values for each temperature on a log scale. The negative reciprocal of the slope of the TRC is the z value. The z value is defined as the temperature change needed for the TRC curve to traverse one log cycle (Jay, 1992).

2.5.2 TDT Studies in Ground Beef

Goodfellow and Brown (1978) determined the D values of *Salmonella* serotypes in ground beef to establish time-temperature processes for manufacturers to use to insure

the elimination of 10^7 *Salmonella*/g in the center of beef roasts. *Salmonella typhimurium* strain TMI was a reference strain in the experiment as its D values had previously been reported (Ng et al., 1969). A mixture of six *Salmonella* serotypes including, *Salmonella newport*, *Salmonella agona*, *Salmonella bovis-morbificans* and *Salmonella muenchen* strains, all isolated from food poisoning outbreaks associated with meat, was inoculated into the ground beef. The thermal resistance curves for the *Salmonella* strains inoculated into ground beef were used to establish processing times necessary for a 7-D reduction of *Salmonella* in cooked beef. The data presented by Goodfellow and Brown (1978) was accepted by the USDA and was the agency's basis for the implementation of time-temperature schedules to govern the cooking of roast beef.

Fain et al. (1991) determined the D values and z values for *L. monocytogenes* Scott A in lean (2.0% fat) and fatty (30.5% fat) ground beef. The z value of *L. monocytogenes* was higher in fatty ground beef than the lean beef. At all three temperatures tested (52.0, 57.7, and 62.8°C), the D values for *L. monocytogenes* Scott A exceeded those for *Salmonella* spp. The z value of *L. monocytogenes* in fatty ground beef, 11.4°C, was higher than the 10°C reported for *Salmonella* spp. (Goodfellow and Brown, 1978). The Scott A strain of *L. monocytogenes* showed a higher heat resistance than that of *Salmonella* spp. in ground beef. Fain et al. (1991) proposed additional research to include other strains of *L. monocytogenes* to determine if the cooking procedures that were in use were sufficient to destroy populations of *L. monocytogenes* normally present in meat and poultry products.

In recent years, *E. coli* O157:H7 has been recognized as the pathogenic microorganism of concern in cooked beef products. To the chagrin of the meat and

poultry industry, Doyle and Schoeni (1987) found *E. coli* O157:H7 to be a common contaminant of fresh meats and poultry after assaying a total of 896 samples. *E. coli* O157:H7 was isolated from 6 (3.7%) of 164 beef, 4 (1.5%) of 264 pork, 4 (1.5%) of 263 poultry, and 4 (2.0%) of 205 lamb samples. Doyle and Schoeni (1984) compared the D values of *E. coli* O157:H7 to those of *Salmonella* spp. that were determined by Goodfellow and Brown (1978). *E. coli* O157:H7 in ground beef was more sensitive to heat than salmonellae. Therefore, at the same temperature, less time is needed to inactivate *E. coli* O157:H7 than the same number of salmonellae. Consequently, any processing schedule that is developed to inactivate *Salmonella* in beef products will ultimately inactivate *E. coli* O157:H7.

Recognizing *E. coli* O157:H7 as a microorganism of concern in beef products, the USDA-FSIS commissioned Line et al. (1991) to determine the thermal lethality of *E. coli* O157:H7 in lean (2.0% fat) and fatty (30.5% fat) ground beef. The D values for fatty ground beef exceeded those for lean ground beef at the temperatures tested (51.7, 57.2, and 62.8°C). This indicated that *E. coli* O157:H7 was killed more rapidly in the lean beef than fatty beef. Increasing the fat concentration decreased the water content of the product and protected the pathogen from heat.

2.6 TIME-TEMPERATURE INTEGRATORS AS INDICATORS OF ADEQUATE THERMAL PROCESSING

2.6.1 Methods of Thermal Process Evaluation

Currently, there are three methods used to evaluate the adequacy of thermal processes. The two most commonly used techniques are the *in situ* method and the

physical-mathematical method, while the use of time-temperature integrators (TTI) has been a rather recent research development (Van Loey et al., 1996).

The *in situ* method is used to monitor the concentration of the actual quality or safety attribute before and after thermal processing (Maesmans et al, 1993). Quality or safety attributes may include microorganisms, vitamins, or proteins. A disadvantage of the *in situ* method includes not being able to detect the attribute being measured after the thermal processing due its level not able to be detected with current analytical methods (Hendrickx, et al., 1995). As data is collected before and after the thermal processing, the *in situ* method can also be considered laborious, time-consuming, and expensive, making routine checks impractical.

The physical-mathematical method combines prior knowledge of the kinetic response of a food quality attribute with the time-temperature history curve imposed on the food. This method can only be used if the time-temperature data is available. In many thermal processes, i.e. rotating retort or a continuous aseptic system, time-temperature data acquisition is not practical in that the thermocouples could disrupt the free movement of the product, thereby leading to misinterpretations of the temperature history (Maesmans et al., 1993). The use of models to reconstruct the time-temperature history depends on the accuracy of the physical input parameters that are required (e.g., data on viscosity or conductivity at high temperatures, fluid-to-particle heat transfer coefficients) (Van Loey et al., 1996).

2.6.2 Time-Temperature Integrators

To overcome the limitations and disadvantages associated with the *in situ* and the physical-mathematical methods, TTIs have been, and are being developed as a means to

evaluate the adequacy of thermal processing. A TTI is a measuring device which undergoes a time/temperature dependent, irreversible change after being subjected to a variable temperature exposure (Van Loey et al., 1996). The irreversible change that the TTI undergoes should parallel that of a target attribute also being exposed simultaneously to the time/temperature process. The TTI should be able to quantify the impact of the process on a target attribute. It must be easy to recover after the processing and must be able to be incorporated into the food product without disturbing the heat transfer. A TTI should be economical and easy to prepare (Hendrickx et al., 1995).

The major advantage to the use of TTIs is the ability to quantify the impact of a time-temperature process on a target attribute without information regarding the actual time-temperature history of the product. To be considered an ideal indicator of thermal processing, an endogenous muscle protein should have a thermal inactivation rate constant (z value) that closely resembles the z value reported for the microorganism used for establishing the thermal process used for monitoring the safety of the food (Van Loey et al., 1996). The thermal inactivation rate constants of the TTI and the target should be equal in the relevant temperature range (Taoukis and Labuza, 1989).

TTIs can be grouped into three major categories: (1) biological (microbiological and enzymatic), (2) chemical, and (3) physical. The use of microbiological TTIs is the most common within the food industry (e.g. the use of *Bacillus* spp. and *Clostridium sporogenes* spores). TTIs can be further classified as either intrinsic or extrinsic to the food system. Intrinsic TTIs are compounds present in the food, while extrinsic TTIs are incorporated or added to the food prior to processing.

Mulley et al. (1975) added thiamin to pureed vegetables, beef and brine of canned peas to act as a chemical TTI to monitor thiamin reduction. The use of the thiamin as a TTI had advantages over typical quantitative microbiological techniques, such as enhanced accuracy in the assay, ease of handling and absence of contamination.

To monitor sterilization processes, the heat inactivation kinetics of α -amylase from *Bacillus amyloliquefaciens*, a possible enzymatic TTI, were studied at low moisture contents and in the presence of various organic solvents (De Cordt et al., 1992; Saraiva et al., 1993; De Cordt et al., 1994). After promising results were published regarding the development of TTIs based on α -amylase from *Bacillus* species, Van Loey et al. (1995) used α -amylase from *Bacillus subtilis* to develop enzymatic TTI systems to monitor the thermal death of *Clostridium botulinum* nonproteolytic type B in cod, *Streptococcus faecalis* in fish, and the denaturation of chlorophyllase in spinach.

2.6.3 Time-Temperature Integrators to Determine Thermal Adequacy of Ground Beef Processing

The current USDA methods cannot be used to verify whether beef products, cooked using the USDA-FSIS time/temperature schedules, were adequately processed. To verify adequate beef processing when any one of the seven USDA-FSIS time-temperature processing schedules are used, the use of a TTI is necessary. If the proposed USDA-FSIS lethality standard for beef patties (USDA-FSIS, 1996) is implemented, a new method, utilizing TTIs should be developed to accurately verify proper processing.

The thermal inactivation of *E. coli* O157:H7 and *Salmonella senftenberg* (*S. senftenberg*) were compared to that of several previously proposed protein markers in ground beef heated at four different temperatures (Orta-Ramirez et al., 1997). The z

values for *E. coli* O157:H7, *S. senftenberg*, and TPI were 5.59, 6.25, and 5.56°C, respectively. To be considered an ideal indicator of thermal processing, an endogenous muscle protein should have a thermal inactivation rate constant (z value) that closely resembles the z value reported for the microorganism used for monitoring the safety of the food (Van Loey et al., 1996). To calculate the z value for the hamburger processing schedule, Orta-Ramirez et al. (1997) plotted the log of the holding time vs. the temperatures stated in the USDA regulations. The calculated z value for the USDA-FSIS beef patty processing schedule was 5.48°C, therefore Orta-Ramirez et al. (1997) found that TPI could be used as an indicator of the adequacy of thermal processing of beef patties.

To identify a marker protein in ground and roast beef, Hsu (1997) determined the enzyme activities of peroxidase, acid phosphatase and TPI after roast beef was processed using three adequate (high, medium and low temperatures were 62.2°C/5 min, 58.3°C/24 min, and 54.4°C/121 min, respectively) and three inadequate (0.5 and 1 log reductions in adequate cook times at each processing temperature) processing schedules. The activity of TPI was the same when compared within adequate cooking treatments and increased as cooking time decreased. TPI was identified as the best marker protein in ground beef when using the medium to high temperature processes.

2.7 BIOCHEMICAL PROPERTIES OF TRIOSE PHOSPHATE ISOMERASE

Triose phosphate isomerase (TPI, D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1) is a glycolytic enzyme that catalyzes the interconversion of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde 3-phosphate (GAP) (Webb and Knowles, 1975). TPI is a homodimer (26,000 Da per monomer), however only the dimer is fully

active (Wierenga and Noble, 1992). TPI has been isolated and crystallized from 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). Recently, Hsu (1997) was successful in purifying TPI from bovine *semimembranosus* muscle (top round choice muscle).

The molecular mass of TPI from human skeletal muscle, as determined by gel filtration on a Sephadex G-100 column, was $57,400 \pm 3000$ Da (Dabrowska et al., 1978). This result was in agreement with molecular masses determined for TPI from other species, i.e. $52,900 \pm 2000$ Da in rabbit muscle (Norton et al., 1970), and 60,000 Da in rabbit liver (Krietsch et al., 1970). Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Hsu (1997) determined the subunit molecular weight of bovine TPI to be 23,000 Da. The subunit molecular weight of TPI from rabbit and human skeletal muscle was 26,500 Da (Norton et al., 1970) and 28,300 Da (Dabrowska et al., 1978), respectively.

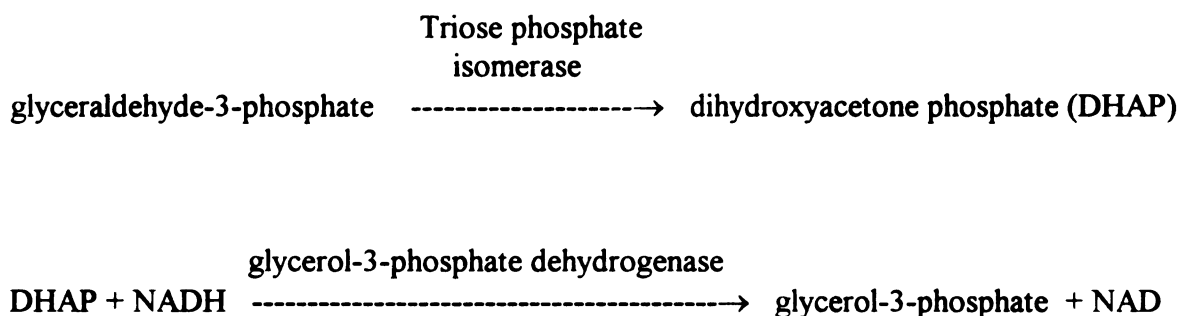
Multiple forms of TPI have been documented (Eber and Krietsch, 1980). Rabbit muscle TPI may have eight electrophoretic forms (Sawyer et al., 1972). Lee et al. (1971) resolved rabbit muscle, horse liver, and human liver TPI into five, three and three isozymes, respectively, by polyacrylamide gel electrophoresis. Using DEAE-cellulose chromatography, Eber and Krietsch (1980) resolved purified human skeletal muscle TPI into three principal forms (A, B and C). The three major forms of TPI accounted for 97% of the total activity. The A and C forms of TPI were classified as homodimers, $\alpha\alpha$ and $\beta\beta$, while the B form was grouped as a heterodimer, $\alpha\beta$. All three forms (A, B and C) were assumed to be immunologically identical after gel diffusion tests, and

immunotitration produced no observable differences. The amino acid composition, molecular mass and antigenicity of the α and β polypeptide chains were determined to be identical.

Using electrofocusing in a sucrose stabilized, pH 5-8 gradient, Norton et al. (1970) resolved TPI into two major and two minor components. The isoelectric points (pI) of the minor species were 5.55 and 5.95, and the major components had pIs of 6.75 and 6.85. Sawyer et al. (1972) studied human TPI from erythrocytes. The human TPI was resolved into three active forms (I, II, III) by isoelectric focusing. Variant I, with 1-5% of total activity, had an isoelectric point of 6.7. Variants II and III, 70-75% and 20-25% of total activity, respectively, had isoelectric points of 6.5 and 6.1, respectively.

2.7.1 Triose Phosphate Isomerase Activity Assay

The TPI activity is determined through the following two coupled reactions:



Glyceraldehyde-3-phosphate (GAP) is used as the substrate and the glycerol-3-phosphate dehydrogenase (GDH) as the coupling enzyme (Beisenherz, 1955). The activity of TPI is twice that in triethanolamine-HCl buffer as in bicarbonate-CO₂ buffer at pH 7.5 (Beisenherz, 1955). Between the optimal pH range of 7 to 8, the activity of TPI varies little, however, at pH 6.3, the activity is about half (Beisenherz, 1955). Triose

phosphate isomerase is inhibited by phosphate ions. A buffer containing 0.05 M phosphate reduced the activity of TPI by 75% (Oesper and Meyerhof, 1950; Beisenherz, 1955).

2.8 BEEF FOR MODEL SYSTEM STUDIES

2.8.1 Carcass Selection

In 1995, the total production of ground beef in the United States was estimated at 3.22 billion kg (AMI, 1996). Seventy five percent of an average cow carcass and 25.8% of an average steer carcass account for the trimmings plus whole muscle/cuts ground by the meat packer. The "commercial" USDA quality grade is the classification that is assigned to a cow of D/E maturity (Hale, 1994). Research of Smith et al. (1988) supported claims by the USDA of declining palatability in beef with advancing maturity. Meat from D/E maturity cows is not sold as "prime" cuts, but is used primarily as ground beef. Meat packers report that approximately 49.4% of their trimmings sales are marketed in a final form as ground beef, as is 54.9% of their whole muscle/cuts sales (AMI, 1996).

Steers of B maturity produce cuts of meat that are classified as "prime". Therefore, the trimmings from the young steers are used in ground beef production. Approximately one-third of the trimmings of what meat packers sell from steers is marketed as ground beef. An average of 38.8 kg of ground beef is produced per steer carcass, up from 30.5 kg in 1990 (AMI, 1996). The activity of several glycolytic enzymes including, phosphorylase a and a+b, glycogen synthetase I and I+D, hexokinase, LDH and glyceraldehyde-3-phosphate dehydrogenase, change very little after the age of 6 months in bovine carcasses (Talmant et al., 1986). Therefore, the maturity of a carcass does not influence the activity of the enzyme being investigated.

2.8.2 Muscle Selection

Most supermarkets sell ground beef from many sources, including ground chuck, ground round, or ground sirloin (Evans and Greene, 1973). It is estimated that 30.3% of retail ground beef, up from 21.3% five years ago, was labeled and marketed as a ground cut (ground chuck, ground round, ground sirloin) (AMI, 1996). The beef chuck from both cows and steers contains many small muscles and large quantities of intramuscular fat and connective tissue (Ruiz et al., 1993). Therefore, the chuck is used primarily for ground beef, and lower-priced steaks and roasts.

CHAPTER 3

FACTORS AFFECTING THE ACTIVITY AND CONCENTRATION OF TRIOSE PHOSPHATE ISOMERASE IN GROUND BEEF

3.1 ABSTRACT

In previous research, triose phosphate isomerase (TPI, E.C. 5.3.1.1) was identified as a potential indicator to verify adequate thermal processing of ground beef. The objective of this study was to investigate different factors that may affect TPI activity and concentration in beef. TPI activity differed in four muscles obtained from cows and steers, although animal maturity had no effect. When beef was cooked, TPI activity and concentration decreased with increasing temperatures. TPI activities were similar when ground beef was inadequately processed by reducing the required USDA cook times at the low (66.1°C) and high (69.4°C) temperatures by 1.0 log, but different when the ground beef was fully cooked. Frozen storage (-13°C) of raw steer muscles for 10 months had no effect on TPI activity. During storage at -13°C for 5 months, TPI activity increased after two months, however returned to a level similar to the initial after 5 months. TPI activity in raw and cooked beef extracts fluctuated during the first 8 hours of refrigerated (4°C) storage, however remained constant from 24 to 48 hours. Five freeze-thaw cycles had no effect on TPI concentration of raw beef. The TPI activity of raw beef increased by 10% after five freeze-thaw cycles. The TPI activity of the cooked beef increased by 13% after four freeze-thaw cycles and then decreased. Cooked beef (62.8°C) with 0.5%

tetrapotassium diphosphate had increased TPI activities and cooked beef (69.4°C) with 1.0% NaCl had lower TPI activities than the control. Further investigation into the factors having a significant effect on TPI activity is warranted if TPI is to be used as an indicator of adequate thermal processing of ground beef patties.

Key words: Ground beef, cooking temperature, frozen storage, triose phosphate isomerase, endpoint temperature

3.2 INTRODUCTION

To assure the adequate cooking and safety of various cooked and partially cooked meat and poultry products, the USDA-FSIS has issued specific cooking regulations to meat processing establishments since 1972 (USDA-FSIS, 1996). The USDA-FSIS (USDA-FSIS, 1997c) requires that patties labeled as "fully cooked" be cooked to any of seven time/temperature processing schedules that range from 66.1°C for 41 sec to 69.4°C for 10 sec. In May of 1996, the USDA-FSIS proposed to convert the command-and-control regulations to performance standards (USDA-FSIS, 1996). The lethality standard is to ensure that establishments eliminate pathogenic microorganisms from the food product. *Salmonella* is generally more resistant to heat than *E. coli* O157:H7 (Doyle and Schoeni, 1984; Line et al., 1991; Orta-Ramirez, 1994) and was selected on that basis as the organism to indicate the destruction of pathogens in beef products. For cooked beef, roast beef, and cooked corned beef products, the USDA-FSIS is proposing that processors use any thermal process to achieve a 7-D reduction in *Salmonella*, while for fully cooked, uncured meat patties the lethality performance standard is proposed to be a 5-D reduction in *Salmonella* (Goodfellow and Brown, 1978; USDA-FSIS, 1996). However, in order to have an overall lethality standard, the USDA-FSIS may reduce the

proposed lethality performance standard for roast beef, cooked beef, and cook corned beef to a 5-D reduction in *Salmonella* (Anderson, 1997).

Current USDA methods to monitor endpoint temperature (EPT) are difficult to interpret, subjective, impractical in that they require the use of sophisticated scientific equipment and trained operators, and time consuming (Smith and Orta-Ramirez, 1995). The greatest disadvantage of the USDA methods for EPT determination is that none of them are based on the thermal inactivation of *Salmonella*, the microbial pathogen selected by the USDA-FSIS to define the proposed lethality standards. Therefore, it is not possible to determine whether beef products have achieved adequate heat processing after being cooked to one of the seven time/temperature processing schedules established by the USDA-FSIS.

A time-temperature integrator (TTI) is a measuring device which undergoes a time/temperature dependent, irreversible change after being subjected to a variable temperature exposure (Van Loey et al., 1996). The irreversible change that the TTI undergoes should parallel that of a target attribute also being exposed simultaneously to the time/temperature process. To be considered an ideal indicator of the thermal process in ground beef, an endogenous muscle protein should have a thermal inactivation rate constant (z value) that closely resembles the z value for *Salmonella* (Van Loey et al., 1996). The thermal inactivation of *E. coli* O157:H7 and *Salmonella senftenberg* (*S. senftenberg*) were compared to that of several previously proposed protein markers in ground beef heated at four different temperatures (Orta-Ramirez et al., 1997). The z values for *E. coli*, *S. senftenberg*, and TPI were 5.59, 6.25, and 5.56°C, respectively. With the calculated z value for the USDA-FSIS beef patty processing schedule 5.48°C,

Orta-Ramirez et al. (1997) found that TPI could be used as a TTI to verify the adequacy of thermal processing of beef patties.

To identify a TTI in ground and roast beef, Hsu (1997) determined the enzyme activity of TPI after roast beef was processed using three adequate (high, medium and low temperatures were 62.2°C/5 min, 58.3°C/24 min, and 54.4°C/121 min, respectively) and three inadequate (0.5 and 1 log reductions in adequate cook times at each processing temperature) USDA processing schedules. The activity of TPI was the same when compared within adequate cooking treatments and increased as cooking time decreased using the medium and high temperature schedules. TPI was identified as a possible TTI in ground beef when using the medium to high temperature processes.

The objective of this study was to investigate different factors that may affect TPI activity and concentration in beef. The effects of muscle type, maturity of the animal, fat concentration, and frozen storage of beef on TPI activity were investigated. The thermal inactivation of TPI in four different steer muscles heated from 48.9°C to 76.7°C was characterized. Frozen storage of cooked ground beef, refrigerated storage of raw and cooked beef extracts, repeated freezing and thawing of raw and cooked ground beef, and the effect of salt and phosphate in ground beef were studied to determine how these conditions affect TPI activity and concentration.

3.3 MATERIALS AND METHODS

3.3.1 Source and preparation of beef for model system studies

The effect of muscle type and maturity on TPI activity in fresh and frozen beef was determined using four different muscles from three cows (D/E maturity) and three steers (B maturity) obtained on the day of slaughter from Ada Beef (Ada, MI 49301). The

thermal inactivation of TPI was also determined in selected steer muscles. The following muscles were analyzed: 1) *trapezius* (T, predominantly red muscle); 2) *longissimus dorsi* (LD, predominantly white muscle); 3) *vastus intermedius* (VI, predominantly red muscle); and 4) *semimembranosus* (S, predominantly white muscle). The muscles were stored in Ziploc bags (Gordon Food Service, Inc., Grand Rapids, MI 49501), packed in ice filled coolers and transported to Michigan State University. In order for use in future studies, each muscle sample was divided into three equal portions prior to freezing. The muscle samples were vacuum packaged and frozen to -13°C the same day.

The effect of fat on TPI activity was determined using a 115 - two piece boneless chuck (34.9 kg), purchased from a local wholesaler and vacuum packaged the same day. The chuck (4°C) was transported to Michigan State University Food Stores and processed within one hour in a cutting room at 14°C. The lean and fat portions of the chuck were divided and ground through a 4.8 mm diameter grinder plate in a Hobart grinder (Model 4146, Hobart Mfg. Co., Troy, OH 45374). Each portion was then separately mixed in a Keebler mixer (Type VAV-TF-GR TE, Keebler Mfg. Co., Chicago, IL) at speed setting 0.1 (57 rpm) for 2 min and ground through a 3.2 mm diameter grinder plate in the Hobart grinder. The fat content of the lean and fat portions was determined by AOAC method 991.36 (AOAC, 1990). The fat and lean beef were combined to achieve fat concentrations of 10, 20 and 30%. The fat and lean portions were blended 3 times at each fat concentration for a total of 9 replicates in a 4°C refrigerator using a Hobart mixer (Model K5-A, Hobart Mfg. Co., Troy, OH, 45374) at speed 2 for 2 min. The ground beef was vacuum packaged and frozen to -80°C.

Ground beef containing 10% fat was also utilized to determine the effect of the phosphate and salt concentrations on TPI activity. The 10% fat ground beef underwent one freeze-thaw cycle and was stored at -13°C for two weeks prior to this study.

All other experiments were completed using chuck roasts (choice roast, A/B maturity) purchased from a local store within 6 hr of purchase. The *longissimus dorsi* (LD) muscles were excised and trimmed of external connective tissue. The muscles were ground twice through a 3.175 mm diameter grinder plate in a Hobart grinder (Model K5-A, Hobart Mfg. Co., Troy, OH 45374). The ground beef was then mixed within a Ziploc bag for 5 min to obtain a homogeneous sample and aliquoted prior to cooking.

3.3.2 Initial TPI in raw cow and steer muscles

After storage at -13°C for one week, the vacuum packaged raw cow and steer muscles (3 replicates/muscle) were thawed overnight in a 4°C refrigerator. The muscles that were not immediately used were assayed for TPI activity after 5 and 10 months of storage at -13°C. Each muscle was sampled in triplicate. After trimming excess fat and connective tissue, 25 g of beef was cut with a knife into 2.5 cm x 2.5 cm pieces and then blended using 3 volumes (w/v) cold phosphate buffer saline (PBS, 0.15 M NaCl, 0.01 M Na phosphate buffer, pH 7.2) in a Waring blender™ (Model 1120, Winsted, CT 06057) for a total of 90 sec (45 sec on, 45 sec off, repeated two times). The extract was stirred for 15 min using a magnetic stir bar. The homogenate was immediately centrifuged at 4,500 x g for 10 min at 4°C. The supernatant was collected after filtration (Whatman No. 1) and stored on ice until assayed for TPI activity as described below.

3.3.3 Model system cooking of steer muscles

Due to the small sample size (30 - 60 g) of each frozen steer muscle, dry ice was used to grind the meat (Bunch et al., 1995). Each muscle was sampled in triplicate. Meat was frozen overnight at -80°C. Meat was then hammered into pieces weighing about 5 g. Dry ice was chiseled into 20 g portions. The 5 g pieces of meat were ground with about 40 g dry ice in an Oster Blender for 15 sec (Model 869-18, Schaumburg, IL 60173). The meat/dry ice mixture was transferred to aluminum foil and the dry ice was allowed to evaporate for 45 min.

The ground beef was packed into a 60 mL syringe (Becton Dickinson and Co., Franklin Lakes, NJ, 07417). Two grams of beef were compressed through 7 cm of plastic tubing into 10 x 75 mm borosilicate glass thermal death time (TDT) tubes (Fisher Scientific®, Pittsburgh, PA 15219). The tubes were sealed with Teflon tape and heated in a Polystat circulator bath (Model 1268-52, Cole-Parmer Instrument Company, Chicago, IL) connected to a temperature programmer (Model 1268-62, Cole-Parmer). To monitor the temperature of the meat, a resistance temperature detector (RTD, 1.6 mm diameter, +/- 0.5°C) connected to a Solomat MPM 2000 Modumeter (Solomat Partners LP, Glenbrook Industrial Park, Stanford, CT), was inserted into the center of a control tube containing 2.0 g of meat. For each of the three replicates of the four steer muscles, three tubes packed with meat were heated to internal temperatures of 48.9°C (120°F), 54.4°C (130°F), 60°C (140°F), 65.6°C (150°F), 71.1°C (160°F), and 76.7°C (170°F). The temperature of the water bath was set to 82.2°C (180°F). Once the control tube reached the target temperature, the tubes were removed and immediately placed in an ice-water

bath. Protein was extracted from the meat and assayed for TPI activity and concentration as described below.

3.3.4 Effect of fat on triose phosphate isomerase activity in cooked ground beef

Ground beef was cooked using two adequate USDA processing schedules including a low and high temperature cook [66.1°C (151°F) for 41 sec, 69.4°C (157°F) for 10 sec]. Inadequate processing schedules were designed by reducing the adequate processing time at each temperature by 0.5 log and 1.0 log (Table 3.1).

For each fat concentration, two grams of meat were packed into eighteen 10 x 75 mm glass thermal death time (TDT) tubes and placed on ice. Nine of the test tubes were cooked to the low temperature processing schedules (3 test tubes at each processing time) and nine tubes were cooked to the high temperature schedules (3 test tubes at each processing time). Type-T (copper-constantan) thermocouples (Part # TJ48-SCPSS-032G-3.25-OST-M, Omega Engineering, Inc., Stamford, CT 06907) were used to monitor ground beef temperature during cooking and cooling. Thermocouples were inserted into the first 9 test tubes to be cooked. The thermocouples were connected to 9 channels of a DaqBook™ Data acquisition system equipped with a DBK 19 Thermocouple Card (Omega Engineering, Inc., Stamford, CT 06907). The DaqBook™ was connected to a Zenith Data Systems 466X+ computer (Zenith Data Systems, St. Joseph, MI 49085) loaded with Omegasoft® Daqview software, version 4.1 (Omega Engineering, Inc., Stamford, CT 06907). Temperatures of the thermocouples were scanned and data was acquired at a rate of 1 scan/sec. To increase the effective accuracy of a noisy signal the averaging function was set to 50 (Omega Engineering, Inc., 1994). Once the starting temperature of $1.2 \pm 0.3^\circ\text{C}$ was attained, the test tube connected to the first channel being

Table 3.1-Thermal processing schedules used to prepare adequately and inadequately processed ground beef in a model system

	Low temp process	High temp process
Adequate cook ^a	66.1°C (151°F)/41 sec	69.4°C (157°F)/10 sec
Inadequate cook (0.5 log reduction in time)	66.1°C (151°F)/13 sec	69.4°C (157°F)/3 sec
Inadequate cook (1.0 log reduction in time)	66.1°C (151°F)/4 sec	69.4°C (157°F)/1 sec

^a Adequate cook schedules were based on the USDA-FSIS fully-cooked patty processing schedules (USDA-FSIS, 1997b).

utilized was submersed into the circulating water bath, as previously described. After undergoing the desired thermal process, the test tube was immediately placed in an ice-water bath. The procedure was continued for all remaining test tubes in both the low and high temperature processes. Protein was extracted and assayed for TPI activity and protein concentration as described below.

3.3.5 Ground beef cooking for storage studies

Thirty two 15 mL polypropylene centrifuge tubes (430052, Corning Costar Corp., Oneonta, NY 13820) were each packed with 10 g of meat for the frozen cooked storage study. Ten grams of meat were packed in four 15 mL centrifuge tubes for the refrigerated storage studies. The tubes containing beef were placed in a test tube rack and were held in an ice-water bath until cooked. A resistance temperature detector (RTD, 1.6 mm diameter, $\pm 0.5^{\circ}\text{C}$) connected to a Solomat MPM 2000 Modumeter (Solomat Partners LP, Glenbrook Industrial Park, Stanford, CT) was inserted into the center of a control tube containing meat which was placed in the middle of the rack. The test tube rack was submersed in the Polystat circulator bath (Model 1268-52, Cole-Parmer Instrument Company, Chicago, IL) connected to a temperature programmer (Model 1268-62, Cole-Parmer) and was removed and placed in an ice-water bath once the control tube reached 62.8°C (145°F). The temperature of the water bath was set to 71.1°C (160°F). Protein was extracted from the meat and assayed for TPI activity and concentration as described below.

3.3.5.1 Frozen storage stability of TPI in cooked ground beef

After cooking, ground beef was removed from the 32 centrifuge tubes, combined and placed into a Ziploc bag. The meat, about 320 g, was hand mixed for 5 min to obtain

a homogeneous mixture. Approximately 10 g of cooked meat was aliquoted into each of 24 vacuum packaging bags. Meat from three of the bags was immediately assayed for TPI activity and concentration. The concentration of protein was also determined. The 21 remaining bags were stored in a -13°C freezer to be assayed over time. Three samples were thawed at 4°C and were assayed for TPI and protein concentration, and TPI activity after four days of frozen storage as an initial check. Each month, for a total of five months, three samples were removed and meat assayed for extractable protein concentration, TPI concentration, and TPI activity as described below.

3.3.5.2 Refrigerated storage of meat extracts from cooked ground beef

After cooking, ground beef was removed from 4 centrifuge tubes and placed into a Ziploc bag. The meat was hand mixed for 5 min to obtain a homogeneous mixture. Protein was immediately extracted, as described in section 3.3.8, from three 5 g aliquots of cooked meat and three 5 g aliquots of raw meat. Triose phosphate isomerase activity and protein concentration were assayed and the results were used as the zero time point. Raw and cooked meat extracts were assayed for TPI activity and protein concentration during storage at 4°C for 1, 2, 3, 4, 8, 24, and 48 hr.

3.3.6 Effect of five freeze-thaw cycles on TPI activity and concentration of raw and cooked ground beef

Raw ground meat was stuffed into 48 TDT tubes. The tubes were sealed with rubber stoppers and Parafilm® (American National Can™, Chicago, IL 60631) and were frozen at -13°C. The following day, all tubes were removed from the freezer and the meat was thawed in a 25°C reciprocal shaking water bath (Model 25, Precision Scientific, Chicago, IL 60647) for 45 min. Five tubes of meat were cooked to a temperature of

62.8°C (no holding time) using the DaqBook™ Data acquisition system equipped with a DBK 19 Thermocouple Card (Omega Engineering, Inc., Stamford, CT 06907) as previously described, and then cooled in an ice-water bath. Three tubes containing thawed raw meat and the tubes containing the cooked meat were placed on ice, while the remaining thawed tubes were refrozen. The freeze-thaw cycles were repeated four more times at 24 hr intervals. After each freeze-thaw cycle, the raw and cooked meat were assayed for extractable protein concentration, TPI concentration, and TPI activity as described below.

3.3.7 Effect of phosphate and salt content on TPI activity in ground beef

Beef was thawed overnight in a 4°C refrigerator. Two hundred and fifty grams of ground beef (9.3% fat) were blended in a Hobart mixer (Model K5-A, Hobart Mfg. Co., Troy, OH, 45374) to obtain mixtures of either 1.0 % NaCl (2.5 g NaCl), 0.5% tetrapotassium diphosphate (TPDP, 50% Food Grade Liquid Potassium Diphosphate, Butcher & Packer Supply Co., Detroit, MI 48207) (2.5 mL TPDP), or 1% NaCl and 0.5% TPDP. A control sample was prepared the same way by adding 2.5 mL H₂O before blending. Ground beef was blended 3 times for each mixture at 4°C.

Two grams of meat from each ground beef mixture were packed into six 10 x 75 mm TDT tubes and placed on ice as previously described. Three of the test tubes were cooked to 62.8°C and three test tubes were cooked to 69.4°C and monitored using the DaqBook™ Data acquisition system equipped with a DBK 19 Thermocouple Card (Omega Engineering, Inc., Stamford, CT 06907) as previously described, and then cooled in an ice-water bath. Protein was extracted and assayed for TPI activity and protein concentration as described below.

3.3.8 Extraction of protein from cooked beef

The 2.0 g cooked beef was transferred from the TDT tubes into scintillation vials (Research Products International Corp., Mount Prospect, IL 60056). Meat was blended with 3 volumes (w/v) of PBS buffer by mixing for 30 sec on a Fisher Vortex Genie2™ (Scientific Industries, Inc., Bohemia, NY 11716). Extracts were then stirred on a magnetic stir plate, without foaming, for 15 min at 4°C. Extracts were immediately centrifuged at 4,500 x g for 10 min at 4°C. Supernatants were collected after filtration (Whatman No. 1) and were held on ice until assayed for TPI activity and concentration.

3.3.9 TPI activity

Triose phosphate isomerase activity was determined as described by Bergmeyer (1984) except that 1.0 mL triethanolamine buffer (TEA, 0.2M, pH 8.0), 0.2 mL 15mM glyceraldehyde-3-phosphate (GAP), 10 µL β-nicotinamide adenine dinucleotide (NADH, 10 mg/mL), 10 µL glycerol-3-phosphate dehydrogenase (GDH, 1.5 mg/mL), and 10 µL of protein extract were used in the assay solution. The change in absorbance was read at 340 nm for 1 min at 25°C. Meat extracts were diluted in water so that the change in absorbance per min was less than 0.2 (Beisenherz, 1955; Norton et al., 1970). One unit (U) of activity was defined as the conversion of 1 µmol GAP into dihydroxyacetone phosphate per minute (Norton et al., 1970). TPI activity was calculated based on the following formula:

$$\text{Activity (U/L)} = \frac{1000 \times A \times V}{t \times \epsilon \times d \times (\phi/D)}$$

A = absorbance

V = assay volume, mL

t = time (min)

ϵ = extinction coefficient (6.310), L x mmol⁻¹ x mm⁻¹

d = distance of light path, 10 mm

ϕ = sample volume, mL

D = dilution of sample

3.3.10 TPI concentration

The TPI concentration was quantified using a sandwich ELISA (Hsu, 1997). The sandwich ELISA was performed by coating Immulon® 2 Removawell Strips (Dynatech Laboratories, Inc., Chantilly, VA 22021) with 100 µL of bovine muscle TPI polyclonal antibody (PAb), the capture antibody, diluted (1/250) in 0.1 M carbonate buffer, pH 9.6, and drying overnight at 37°C. Plates were washed four times with PBS containing 0.05% Polyoxyethylene sorbitan monolaurate (Tween 20) (PBS-T). Nonspecific binding sites were blocked by adding 300 µL of PBS containing 0.5% casein and 0.1% Tween (PBS-CT) to each well and incubating at 37°C for 30 min. Plates were washed four times and 50 µL of serially diluted bovine muscle TPI or diluted ground beef extracts was added to each well and incubated at 37°C for 30 min. After four washings, biotin-labeled bovine muscle TPI PAb, for detection, was diluted (1/100) in PBS-CT, and 50 µL was added to each well and incubated at 37°C for 30 min. After incubation, the plate was washed four times with PBS-T and 100 µL of avidin-horseradish peroxidase (HRP) conjugate

(Sigma[®]) diluted (1/8000) in PBS-CT was added to each well. Plates were incubated for 30 min at 37°C and washed eight times with PBS-T. Peroxidase binding was determined with ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) substrate (Pestka, 1982). After 30 min, absorbance was read at 405 nm using a microplate reader (THERMOmax, Molecular Devices Co., Menlo Park, CA). On each plate, purified bovine muscle TPI (Hsu, 1997) was used to prepare a standard curve ranging from 0 to 40 µg TPI/mL. Results were expressed as micrograms of TPI per gram of meat.

3.3.11 Bradford extractable protein determination

Protein concentration of meat extracts was determined using the Bradford method with bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO 63178) as the standard (Bradford, 1976). The BSA standard curve ranged from 10 to 913 µg protein/mL. Ten microliters of diluted meat extract and 200 µL of Coomassie blue G-250 dye reagent (500-0006, Bio-Rad[®]) were added to each microwell (Immulon[®] 1 Removawell Strips, Dynatech Laboratories, Inc., Chantilly, VA 22021) and incubated at room temperature for 5 min. Absorbance was read at 590 nm using a microplate reader (THERMOmax, Molecular Devices Co., Menlo Park, CA).

3.3.12 Proximate composition

The moisture, fat, and protein contents of the beef samples were determined according to AOAC methods 950.46B, 991.36, and 981.10, respectively (AOAC, 1990). For pH determination, 10 g meat was homogenized with 90 g deionized water using a Polytron homogenizer (Model PT 10/35, Brinkmann Instrument, Co., Westbury, NJ 11590) equipped with a PTA 10TS generator. The sample was stirred for two periods of

30 sec at 5.0 speed setting and the pH was measured while the solution was continuously stirred over a magnetic stirrer.

3.3.13 Statistical analysis

Data were analyzed using analysis of variance in the General Linear Model Program of the Statistical Analysis System (SAS Institute, Inc., 1996). Least square means procedures were used to separate means at $P < 0.05$. Animal was a random effect when analyzing the effects of muscle type and maturity of the animal on TPI activity. Analysis of covariance was used to determine if TPI concentration was an accurate predictor of TPI activity. The linear or curvilinear change in TPI activity in different fat contents of beef was determined using the orthogonal polynomial contrast test by SAS software (Release 6.12). Tukey's test was used to test significant differences among means at $P < 0.05$ for storage and freeze-thaw studies.

3.4 RESULTS AND DISCUSSION

3.4.1 Effect of muscle type and maturity on TPI activity in muscle

The proximate composition and the pH of 4 muscles from cows and steers varied by muscle and gender. The cow *longissimus dorsi* (LD) contained 8.4% fat, the highest among the four cow muscles (Table 3.2). The *vastus intermedius* (VI) in steer had 7.6% fat, the highest among the four steer muscles. The VI muscle in cow contained the highest moisture content of 74.2%. All four steer muscles contained similar moisture concentrations, ranging from 70.6 to 71.8%. The *semimembranosus* (S) in steer had the highest percent protein of 24.6%. The *trapezius* (T) muscle in cow had the highest percent protein of 24.8%. The steer and cow VI had the highest pH of 6.2 and 6.8, respectively.

Table 3.2-Proximate composition and pH of cow and steer muscles^a

Muscle ^b	Cow	Steer
	<u>Fat (% w/w)</u>	
Longissimus dorsi	8.4 ± 1.0	5.2 ± 0.8
Semimembranosus	5.2 ± 4.5	3.7 ± 1.4
Trapezius	3.7 ± 2.0	6.3 ± 3.3
Vastus intermedius	4.5 ± 0.6	7.6 ± 1.7
	<u>Moisture (% w/w)</u>	
Longissimus dorsi	69.9 ± 0.6	71.8 ± 1.4
Semimembranosus	73.1 ± 1.2	71.3 ± 1.2
Trapezius	70.5 ± 0.9	70.6 ± 2.5
Vastus intermedius	74.2 ± 0.8	71.1 ± 1.2
	<u>Protein (% w/w)</u>	
Longissimus dorsi	21.4 ± 0.4	22.6 ± 1.4
Semimembranosus	24.4 ± 1.9	24.6 ± 1.6
Trapezius	24.8 ± 1.1	22.3 ± 1.1
Vastus intermedius	20.3 ± 1.3	20.1 ± 2.0
	<u>pH</u>	
Longissimus dorsi	6.2 ± 0.4	5.4 ± 0.1
Semimembranosus	5.3 ± 0.1	5.1 ± 0.1
Trapezius	6.1 ± 0.3	5.7 ± 0.3
Vastus intermedius	6.8 ± 0.1	6.2 ± 0.0

^a Means ± standard deviation of 3 replicate values.

^b Longissimus dorsi and semimembranosus are white muscles. Trapezius and vastus intermedius are red muscles.

The above results are in agreement with others who reported the proximate composition and pHs of muscles from cow and steer carcasses. Browning et al. (1990) reported the percent moisture, fat and protein of a composite of 10 muscles from eight typical steers to be 72.69, 5.89 and 20.80%, respectively. Talmant et al. (1986) reported the pHs of *trapezius*, *longissimus dorsi* and *semimembranosus* muscles from cows (D/E maturity) and steers (B maturity) to be 5.8, 5.6 and 5.6, respectively. The proximate composition and pHs of the selected cow and steer muscles used in this study were found to be similar to those found in typical beef carcasses.

Differences ($P < 0.05$) in TPI activity were found between muscles in both the cows (D/E maturity) and steers (B maturity) (Table 3.3). The cow *trapezius* had a TPI activity of 2213 U/kg meat, the highest among the four cow muscles. The steer *trapezius*, *semimembranosus*, and *longissimus dorsi* muscles all had greater TPI activities than the *vastus intermedius*. The cow and steer *vastus intermedius* had the lowest TPI activities of 595 and 796 U/kg meat, respectively. In both cows and steers, the largest difference in TPI activity was in the round muscles. The steer and cow *semimembranosus* had TPI activities of 3704 and 1985 U/kg meat, respectively, while the steer and cow *vastus intermedius* had TPI activities of 796 and 595 U/kg meat, respectively. Stalder et al. (1991) found a large difference in LDH activities in the round muscles with the bovine *semimembranosus* having 1476 U activity and the *vastus intermedius* having 283 U activity. This data is in agreement with Talmant et al. (1986) who reported that muscle type has a large influence on glycolytic enzyme activity.

Differences in TPI activity were nonsignificant for carcasses differing by maturity. Talmant et al. (1986) reported that glycolytic enzyme activities do not change after the

Table 3.3-Triose phosphate isomerase activity (U/kg meat) of muscles from cows and steers

Muscle	Cow	Steer
Chuck trapezius	2213.4 ± 792.4 ^a	1950.3 ± 282.3 ^a
Round semimembranosus	1984.6 ± 667.6 ^b	3703.6 ± 945.2 ^a
Chuck longissimus dorsi	1754.4 ± 398.4 ^b	2926.9 ± 417.9 ^a
Round vastus intermedius	595.0 ± 231.0 ^c	796.3 ± 154.3 ^b

^{a-c} Mean ± standard deviation in the same column followed by the same

letter are not different (P>0.05).

age of six months. Previous research indicates that gender of the animal has no effect on the activities of glycolytic muscle enzymes (Talmant et al., 1986; Stalder et al., 1991). Therefore, the effect of gender on TPI activity was not investigated.

To use TPI as a TTI to monitor the adequate thermal processing of beef patties, the TPI activity within the raw beef should initially be high so that a measurable decrease can be detected after cooking. High levels of TPI activity were found within both the round and chuck muscles, ones commonly used for the production of ground beef. The average TPI activity was 1636 and 2344 U/kg meat for the cow and steer muscles, respectively. Due to a variety of muscles used for the production of ground beef, TPI activity of raw ground beef should be similar to the average activity from a number of muscles.

3.4.2 Frozen storage of steer muscles

Triose phosphate isomerase activity did not change ($P>0.05$) in the raw steer muscles during frozen storage at -13°C for 10 months (Table 3.4). Frozen storage had no effect ($P>0.05$) on the TPI specific activity of the *longissimus dorsi* and *vastus intermedius* muscles during 10 months of frozen storage. The TPI specific activity in the *trapezius* and *semimembranosus* muscles did not change after the initial 5 months of storage, but increased in the *trapezius* and decreased in the *semimembranosus* after an additional 5 months of frozen storage. The discrepancy between the constant activity and changing specific activity of these two muscles can be attributed to the change in extractable protein content during frozen storage. Between 5 and 10 months of frozen storage, the *trapezius* showed a decrease ($P<0.05$) in extractable protein, which increased the ratio of activity to milligrams of extracted protein, which resulted in the increased

Table 3.4-Stability of triose phosphate isomerase activity and extractable protein concentration in selected steer muscles during frozen storage at -13°C

Time (months)	Steer muscle			
	Longissimus dorsi	Trapezius	Semimembranosus	Vastus intermedius
TPI activity (U/kg meat)				
0	2926.8 ± 417.9 ^a	1950.3 ± 265.7 ^a	3703.6 ± 854.6 ^a	796.3 ± 141.0 ^a
5	2870.1 ± 587.9 ^a	2381.4 ± 307.1 ^a	4513.0 ± 1131.0 ^a	893.4 ± 151.6 ^a
10	3349.7 ± 862.4 ^a	2366.8 ± 971.3 ^a	3771.5 ± 996.8 ^a	953.1 ± 123.8 ^a
TPI specific activity (U/g protein)				
0	86.6 ± 11.3 ^a	59.4 ± 10.7 ^{ab}	105.2 ± 27.2 ^a	26.9 ± 5.7 ^a
5	66.3 ± 16.8 ^a	53.7 ± 7.9 ^b	90.0 ± 15.4 ^{ab}	25.9 ± 5.5 ^a
10	81.7 ± 19.8 ^a	71.2 ± 24.4 ^a	72.2 ± 22.4 ^b	31.3 ± 10.1 ^a
Extractable protein concentration (mg/mL)				
0	11.3 ± 0.7 ^a	11.0 ± 0.7 ^{ac}	11.8 ± 0.5 ^a	9.9 ± 0.5 ^a
5	14.6 ± 1.9 ^b	14.9 ± 1.8 ^b	16.6 ± 2.5 ^b	11.6 ± 1.1 ^a
10	13.7 ± 1.3 ^{ab}	11.1 ± 2.1 ^c	17.7 ± 2.3 ^b	10.9 ± 2.8 ^a

^{a-c} Mean ± standard deviation in the same column followed by the same letter are not different (P>0.05).

specific activity. The extractable protein in the *semimembranosus* increased during 10 months of frozen storage, which decreased the ratio of activity to milligrams of extracted protein and resulted in the decreased specific activity.

The changes in the extractable protein concentration of the steer muscles could have been caused by the microstructural alterations that the bovine muscles underwent during frozen storage (Awad et al., 1968). Ice crystal formation within the muscle tissue could have led to the denaturation of the beef muscle proteins. The changes in the extractability of the muscle proteins would be expected if protein denaturation had occurred.

3.4.3 Thermal inactivation of triose phosphate isomerase in selected steer muscles

TPI activity in the four steer muscles decreased as cooking temperature was increased from 48.9 to 76.9°C (Fig. 3.1). There were significant effects on TPI activity due to muscle, animal and cooking temperature. Muscle x animal and muscle x temperature were significant interactions, however animal x temperature was not. TPI activity in the two chuck muscles, *trapezius* and *longissimus dorsi*, decreased from 48.9 to 71.3°C. The TPI activity in the round muscles was more heat labile as the *semimembranosus* and *vastus intermedius* had their lowest TPI activities when heated to 65.7°C and above. The *semimembranosus* muscle had the greatest decrease in TPI activity of 861 U/kg meat between 48.9 to 54.5°C. In all four muscles, there was no difference in TPI activity between the 71.3 and 76.9°C cooking temperatures.

The TPI concentration of the four steer muscles decreased as the cooking temperature of the beef was increased (Fig. 3.2). There were differences in TPI concentration due to muscle and cooking temperature, however animal had no effect

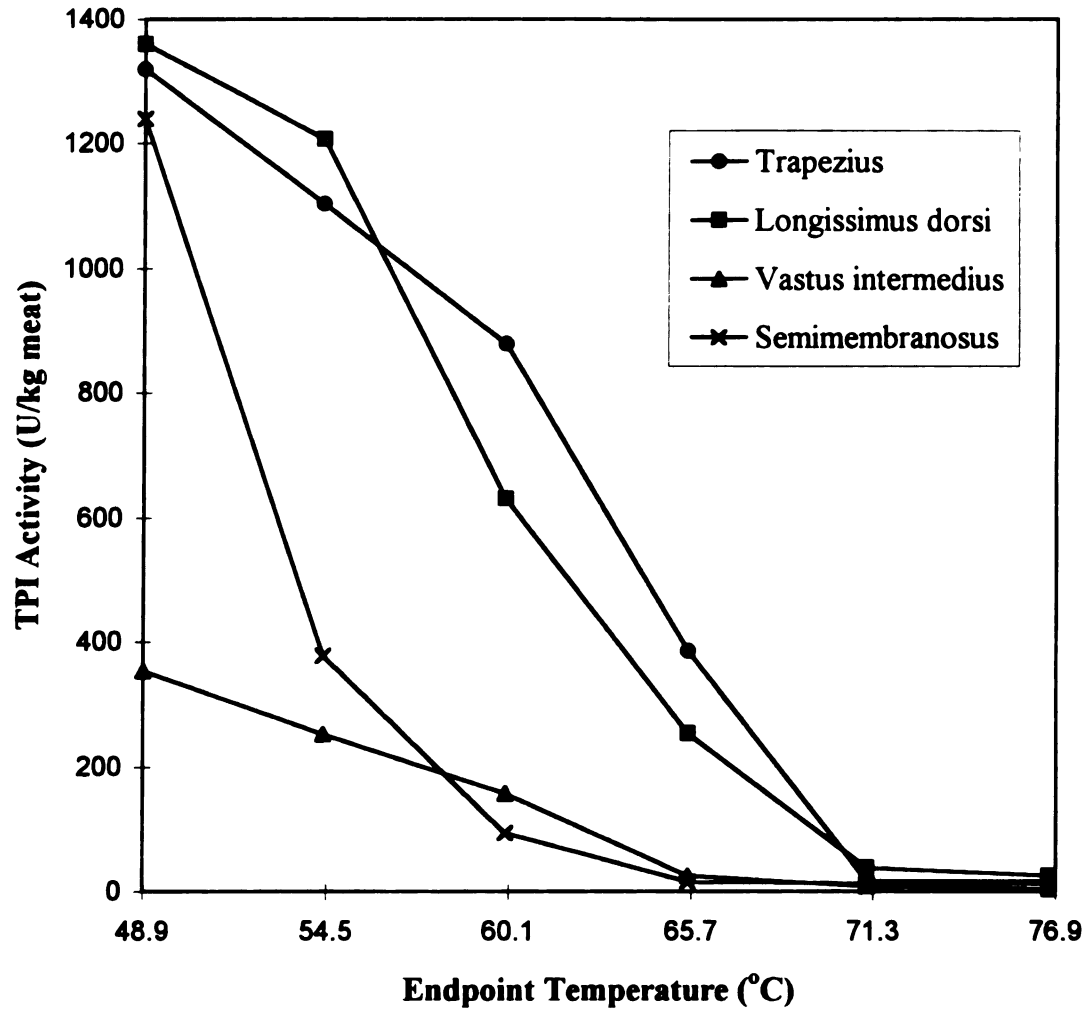


Fig. 3.1-Effect of heating temperature on triose phosphate isomerase activity (U/kg meat) of selected steer muscles. Standard error of the means is ± 120.9 .

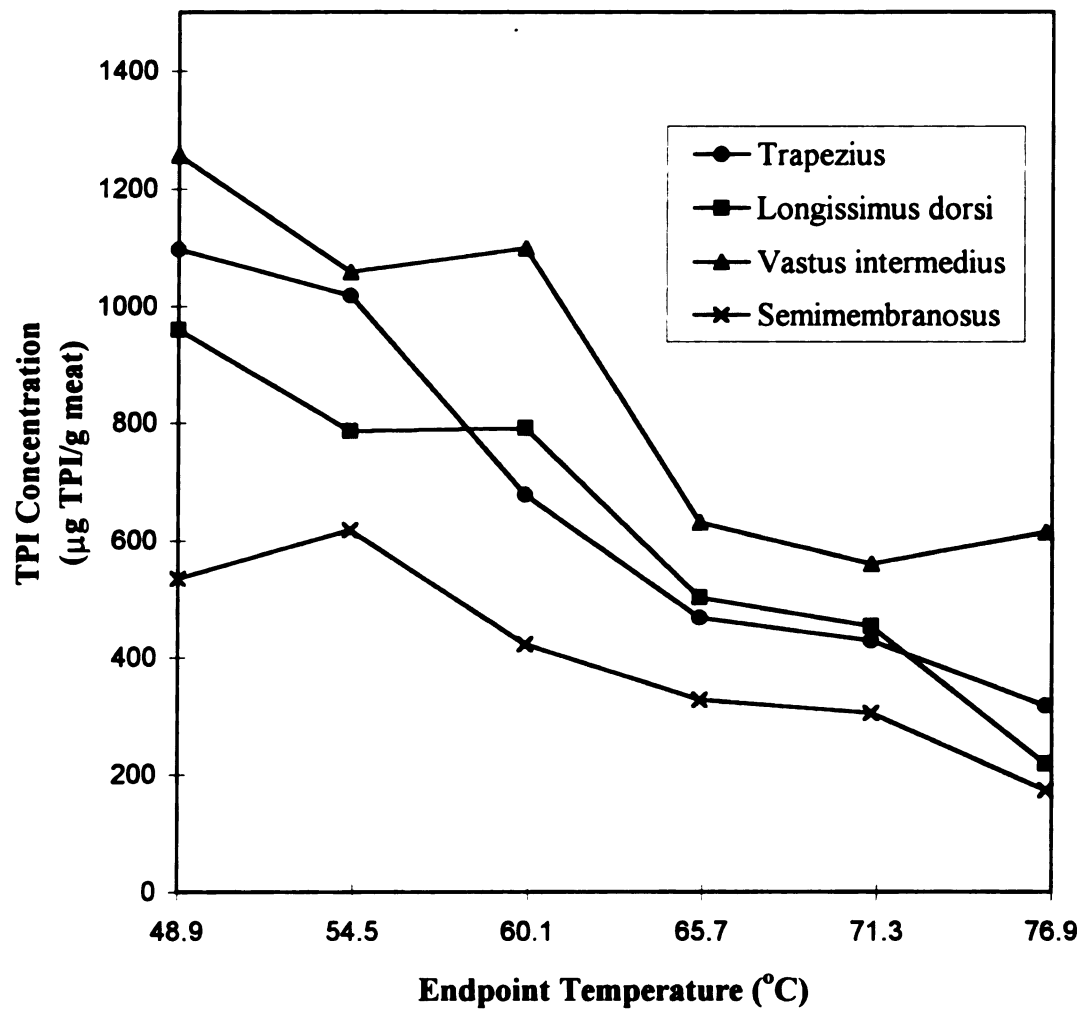


Fig. 3.2-Effect of heating temperature on triose phosphate isomerase content (µg TPI/g meat) of selected steer muscles. Standard error of the means is ± 34.8 .

($P>0.05$). Muscle x animal and animal x temperature were significant interactions, however muscle x temperature was not. At 76.9°C, the lowest concentration of TPI was 173.7 µg/g meat in the *semimembranosus* muscle. No differences were found in TPI concentrations in all four steer muscles between the cooking temperatures of 48.9 to 54.5°C. The *trapezius* showed a decrease in TPI concentration from 48.9 to 60.1°C. TPI concentration decreased only in the *longissimus dorsi* from 65.7 to 76.9°C.

Triose phosphate isomerase concentration was not a good predictor ($P>0.75$) of TPI activity in the ground beef model system. Both the TPI activity and concentration decreased as the cooking temperature was increased, however, the difference in TPI activity was more evident. The average decrease in TPI activity among all four muscles was 99.4%, while only a 73.5% decrease in TPI concentration on heating from 48.9 to 76.9°C was observed. The *vastus intermedius*, while having among the lowest TPI activities of the four steer muscles assayed, had the highest concentration of TPI over all cooking temperatures. The TPI activity of the *semimembranosus* muscle decreased by 1227 U/kg meat as the temperature was increased from 48.9 to 76.9°C, the largest decrease in activity seen among all four muscles. However, the TPI content of the *semimembranosus* only decreased by 361 µg TPI/g meat from 48.9 to 76.9°C, the smallest decrease in TPI content among all four muscles. When Hsu (1997) measured the TPI activity and concentration of ground beef after heating to EPTs ranging from 48.9 to 76.9°C, the change in TPI activity with cooking temperature was more apparent than the change in TPI concentration.

Hsu (1997) produced anti-bovine TPI PAb to be used in the sandwich ELISA. The poor specificity related to the use of PAb could have contributed to the less apparent

change in TPI content vs. TPI activity. The development of monoclonal antibodies that recognize an epitope related to the conformational changes that TPI undergoes during heating might enhance the specificity of the assay.

3.4.4 Effect of fat on triose phosphate isomerase activity in ground beef

The fat content of ground beef was found to have an effect on TPI activity of the cooked product (Table 3.5). At both the low and high temperature processing schedules, the TPI activities in the cooked ground beef containing 9.3 and 20.0% fat did not differ, but activities were higher than cooked beef containing 30% fat. TPI activity varied due to the batch of meat used, although identical procedures were maintained from batch to batch. The temperature and time of cook were significant effects on TPI activity, as was the interaction (temperature x time) of the two.

Within all three fat contents, the TPI activity was similar at 66.1 and 69.4°C when the ground beef was inadequately processed by reducing cook time by 1.0 log, but different at the full cook and 0.5 log reduction in holding time processes. At 66.1°C, TPI activity linearly decreased as the cook time was increased. Time had a quadratic effect on TPI activity in beef cooked to 69.4°C. Smith et al. (1991) reported TPI activity when ground beef was heated to 70°C, but not at 80°C. Orta-Ramirez et al. (1997) reported that TPI was inactivated in ground beef before reaching 68°C. Research showing TPI to be close to its thermal inactivation point at 69.4°C may explain the quadratic, rather than linear decrease in TPI activity in beef cooked to 69.4°C.

The results in the model system show that TPI could not be used as a TTI to determine the adequate thermal processing of ground beef because activities were different at the lowest and highest temperatures of the USDA-FSIS processing schedules for beef

Table 3.5- Triose phosphate isomerase activity (U/kg meat) of ground chuck of differing fat contents processed using two USDA approved schedules for ground beef and inadequately processed by reducing the holding time by 0.5 and 1.0 log cycles

	Low temperature cook ^A (66.1°C; 151°F)	High temperature cook ^B (69.4°C; 157°F)
9.3% Fat		
Full cook	7.3 ± 2.0 ^a	9.7 ± 4.6 ^b
Inadequate cook (0.5 log reduction in time)	9.1 ± 1.6 ^a	12.4 ± 3.1 ^b
Inadequate cook (1.0 log reduction in time)	10.5 ± 2.6 ^a	10.5 ± 1.9 ^a
20.0% Fat		
Full cook	6.2 ± 1.7 ^a	9.6 ± 1.9 ^b
Inadequate cook (0.5 log reduction in time)	8.9 ± 2.2 ^a	9.8 ± 3.8 ^b
Inadequate cook (1.0 log reduction in time)	9.6 ± 2.4 ^a	9.5 ± 3.3 ^a
29.4% Fat		
Full cook	5.1 ± 2.4 ^a	6.6 ± 2.0 ^b
Inadequate cook (0.5 log reduction in time)	6.1 ± 2.9 ^a	8.2 ± 1.9 ^b
Inadequate cook (1.0 log reduction in time)	6.1 ± 2.9 ^a	6.2 ± 2.6 ^a

^A Means ± standard deviation in the same column for each fat content showed a linear response to temperature (P<0.05).

^B Means ± standard deviation in the same column for each fat content showed a quadratic response to temperature (P<0.05).

^{a-b} Means in the same row followed by the same letter are not different (P>0.05).

patties. In order for TPI to function as a TTI, TPI activity should be similar within equivalent adequately and inadequately schedules at the lowest and highest temperatures. Results of Wang et al. (1996b), Orta-Ramirez et al. (1997) and Hsu (1997) were used in selecting TPI as a possible TTI to determine the adequate thermal processing of ground beef. Wang et al. (1996b) reported that several proteins including, TPI, LDH, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate mutase (PGAM), and acid phosphatase (AP) might be useful in assays to verify the adequate cooking of ground beef patties. Hsu (1997) investigated several enzymes as possible indicators of the adequate thermal processing of roast beef and reported TPI activity to be the same in roast beef when equivalent processes were used at low and high temperatures. Orta-Ramirez et al. (1997) showed the temperature dependence of TPI to be similar to those of *E. coli* O157:H7 and *S. senftenberg*. TPI was found to have a similar z value to that of *Salmonella*, the indicator organism used by the USDA-FSIS to create the lethality performance standards (Orta-Ramirez et al, 1997). The z value of TPI was determined over the temperature range of 53 to 66°C, while the USDA-FSIS beef patty time/temperature processing schedule covers 66.1 to 69.4°C. The temperature range from which the z value for TPI was determined may have contributed to the lack of similarity between the low and high temperature TPI activities within the model system (Taoukis and Labuza, 1989; Van Loey et al., 1996).

The beef in the model system was cooked to the lowest and highest temperatures of the USDA approved processing schedules for ground beef. The fully cooked ground beef had lower TPI activities when cooked to the lowest temperature of 66.1°C for 41 sec (equivalent to 71°C instantaneously) at all fat contents. While TPI was determined to be

unsuitable for use as a TTI in a ground beef model system, TPI might be a suitable EPT indicator in ground beef. The residual TPI activities at 66.1°C for 41 sec determined for each fat content of ground beef could be proposed as the maximum allowable activities to verify the adequate thermal processing of beef patties. For example, the 20.0% fat ground beef cooked to the USDA-FSIS low temperature schedule, 66.1°C (151°F) for 41 sec, had a TPI activity of 6.2 U/kg meat. The TPI activity of the 20% fat ground beef processed to the high temperature schedule, 69.4°C (157°F) for 10 sec, was 9.6 U/kg meat. Therefore, it is suggested that any 20% fat beef patty having a TPI activity less than 6.2 U/kg meat could be considered fully cooked.

Further studies are needed to establish a maximum TPI activity for ground beef patties cooked in a commercial setting to the recommended USDA endpoint processing temperature of 71.1°C. Once a maximum TPI activity is established for fully cooked beef patties, TPI activity can possibly be used as an indicator of the adequate thermal processing of ground beef patties.

3.4.5 Effect of frozen storage of cooked ground beef

The effect of frozen storage (-13°C) on TPI activity and concentration of ground beef (9.5% fat) cooked to 62.8°C was studied for 5 months (Table 3.6). After four days of frozen storage, the TPI activity increased ($P < 0.05$), while the TPI concentration decreased as compared to the unfrozen cooked beef. Both the TPI activity and concentration increased after two months of frozen storage, and then decreased after an additional month of storage. After three months of frozen storage, TPI concentration did not change. TPI activity did not change from three to four months of frozen storage, but increased after an additional month. A TPI activity similar to that of the unfrozen cooked

Table 3.6-Effect of frozen storage (-13°C) on triose phosphate isomerase in cooked (62.8°C) ground beef

	TPI activity (U/kg meat)	TPI conc (µg TPI/g meat)
Initial cook	21.5 ± 1.9 ^{ab}	1582.2 ± 544.7 ^a
4 Day	34.1 ± 0.9 ^c	881.4 ± 122.4 ^{bc}
One month	26.8 ± 1.0 ^d	987.7 ± 111.4 ^{bd}
Two month	33.1 ± 2.8 ^c	1373.3 ± 492.6 ^{ad}
Three month	17.6 ± 4.7 ^b	486.1 ± 189.3 ^{ce}
Four month	17.2 ± 1.9 ^b	312.0 ± 124.7 ^e
Five month	22.8 ± 2.5 ^{ad}	432.3 ± 188.8 ^{ce}

^{a-e} Mean ± standard deviation in the same column followed by the same letter are not different (P>0.05).

beef was seen after five months of frozen storage. The changes in activity and concentration could have been caused by the microstructural and chemical alterations that the bovine muscle underwent during frozen storage (Awad et al., 1968). These changes can cause the leakage of drip, containing amino acids, salts, proteins, and peptides, from thawed muscle (Forrest et al., 1975).

3.4.6 Effect of refrigerated storage of protein extracts

Due to time constraints, TPI activity was not always assayed immediately following protein extraction. As the meat extracts were held at 4°C until assayed, the effect of refrigerated (4°C) storage of raw and cooked beef protein extracts on TPI activity was investigated. The TPI activity in the raw extracts did not change ($P>0.05$) during the first 4 hr of storage (Fig. 3.3). After one hr of refrigerated storage, the TPI activity of the cooked beef extract decreased as compared to the TPI activity observed at the zero time point. The cooked beef extract increased in TPI activity after 3 hr of storage. The lowest TPI activities of both the raw and cooked beef extracts, 1502 and 7.2 U/kg meat, respectively, were after 8 hr of refrigerated storage. During 8 to 24 hr of refrigerated storage, both the TPI activity of raw and cooked beef extracts increased to levels similar to that of the zero time point and did not change thereafter. These results suggest that TPI activity may fluctuate during the initial hours of storage in refrigerated (4°C) conditions. Therefore, the TPI activity of meat extracts was determined after an 8 hr holding period throughout our studies.

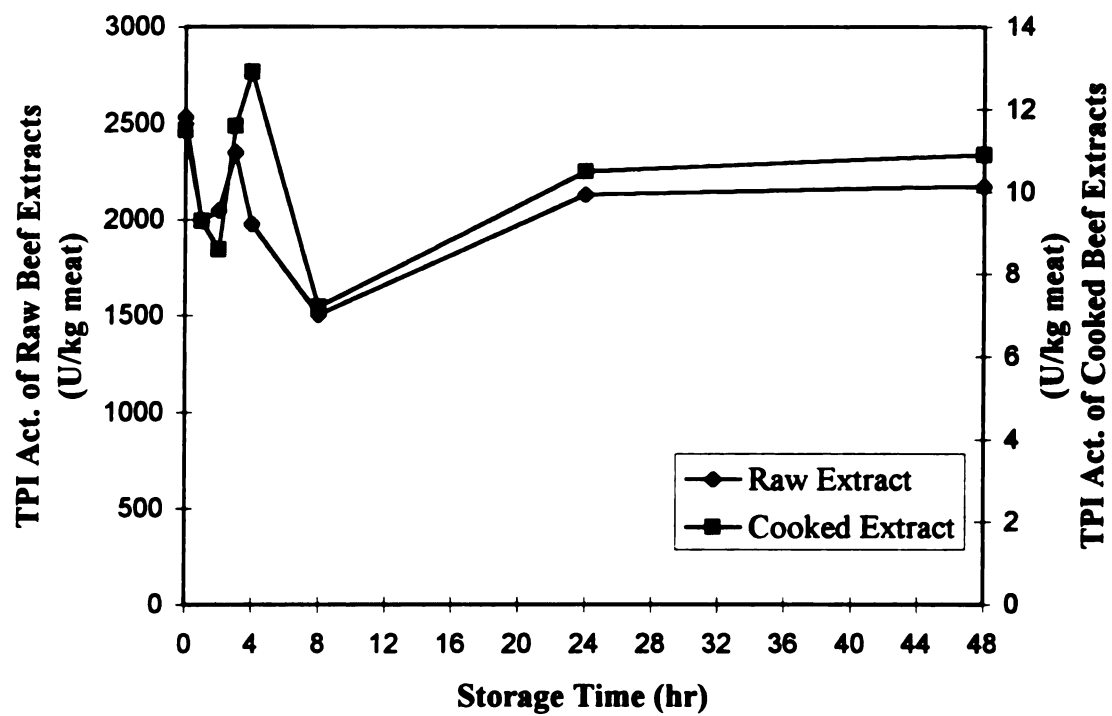


Fig. 3.3-Effect of refrigerated storage (4°C) of protein extracts on triose phosphate isomerase activity (U/kg meat). Standard error of the means for raw beef extracts is ± 119.5 . Standard error of the means for cooked beef extracts is ± 0.4 .

3.4.7 Effect of freeze-thaw cycles on TPI activity and concentration of raw and cooked ground beef

Five freeze-thaw cycles had no effect ($P>0.05$) on TPI concentration or extractable protein concentration of raw ground beef (Table 3.7). Orta-Ramirez et al. (1996) reported that five freeze-thaw cycles had no effect on the LDH concentration of raw ground beef. The TPI activity of the raw and cooked beef decreased after one freeze-thaw cycle. After the second freeze-thaw cycle, TPI activity increased in the raw beef and did not change thereafter. Collins et al. (1991b) reported one freeze-thaw cycle to decrease LDH activity in raw ham muscles.

Three freeze-thaw cycles decreased TPI activity by 57.2% in the cooked beef. However, after a fourth freeze-thaw cycle, TPI activity in the cooked beef increased. After two freeze-thaw cycles, the TPI concentration of the cooked meat was greater than the unfrozen cooked meat, but returned to concentrations similar to the unfrozen meat after additional freeze-thaw cycles. The changes in TPI activity and concentration of the cooked beef can be attributed to the damage brought upon by the formation of ice crystals within the muscle tissue. Ice crystal formation within the meat can damage cell membranes allowing TPI to be lost into the drip (Collins et al., 1991b; Orta-Ramirez et al., 1996). The location of the drip, as well as the amount of drip within the test tube, will dictate how heat susceptible TPI will be when cooking the beef in the water bath. The ground beef in test tubes containing greater amounts of drip loss, and therefore more TPI in the drip, would be expected to have lower TPI activities after cooking. Another factor contributing to changes in TPI activity and concentration of the cooked beef may be

Table 3.7-Effect of five freeze-thaw cycles on triose phosphate isomerase and extractable protein concentration in raw and cooked (62.8°C) ground beef

Cycle	Raw meat		Cooked meat	
	TPI activity (U/kg meat)	Percent of initial activity	TPI activity (U/kg meat)	Percent of initial activity
Initial	2131.1 ± 368.2 ^{ab}	100.0	68.4 ± 15.6 ^{ab}	100.0
1	1774.4 ± 224.2 ^b	89.2	54.0 ± 21.2 ^b	79.4
2	2235.3 ± 238.3 ^a	109.0	56.4 ± 11.0 ^{ab}	82.4
3	1902.1 ± 169.9 ^{ab}	90.8	29.3 ± 8.5 ^c	42.8
4	1916.6 ± 154.6 ^{ab}	92.5	77.4 ± 25.8 ^a	113.2
5	2289.9 ± 250.9 ^a	110.5	53.5 ± 10.3 ^b	78.2

	TPI concentration (µg/g meat)	Percent of initial conc.	TPI concentration (µg/g meat)	Percent of initial conc.
Initial	510.7 ± 262.3 ^a	100.0	313.4 ± 64.5 ^{ab}	100.0
1	478.8 ± 216.4 ^a	93.9	384.4 ± 81.8 ^{ab}	122.3
2	520.6 ± 137.8 ^a	102.1	778.5 ± 241.1 ^c	248.3
3	250.6 ± 188.8 ^a	49.0	302.4 ± 122.8 ^b	96.5
4	486.3 ± 248.7 ^a	95.3	500.5 ± 262.5 ^{ab}	159.6
5	552.8 ± 298.2 ^a	108.2	524.5 ± 258.2 ^a	167.3

	Extractable protein (mg/mL)	Percent of initial conc.	Extractable protein (mg/mL)	Percent of initial conc.
Initial	11.4 ± 0.5 ^a	100	5.3 ± 0.3 ^a	100
1	11.5 ± 0.8 ^a	100.9	5.9 ± 0.6 ^{ab}	111.3
2	11.8 ± 0.3 ^a	103.5	6.0 ± 0.1 ^{ab}	113.2
3	12.3 ± 0.7 ^a	107.9	5.4 ± 0.4 ^{ab}	101.9
4	12.7 ± 0.9 ^a	111.4	6.1 ± 0.4 ^b	115.1
5	11.1 ± 0.1 ^a	97.4	5.5 ± 0.3 ^{ab}	103.8

^{a-c} Means ± standard deviation in the same column followed by the same letter are not different (P>0.05).

changes in the extractability of the ground beef muscle proteins caused by the denaturing of the proteins during each freeze-thaw cycle.

3.4.8 Effect of phosphate and salt content in ground beef on TPI activity

The cooking temperature (62.8 and 69.4°C), phosphate and salt content had significant effects on TPI activity of the ground beef (9.3% fat), as did the temperature x ingredient (phosphate and salt) interaction. Wang et al. (1996b) and Hsu (1997) reported cooking temperature to have a significant effect on TPI activity in ground beef. At 69.4°C, the ground beef with 1.0% NaCl had a lower TPI activity than the control (Table 3.8). Stalder et al. (1991) reported a decrease in LDH activity in *semimembranosus* slurries as the salt concentration was increased. At 62.8°C, ground beef with 0.5% tetrapotassium diphosphate (TPDP) and 1.0% NaCl, and the ground beef with only 0.5% TPDP, had higher TPI activities than the control. The addition of TPDP increased the pH of the beef from 5.9 to 6.2.

The increased extractability of the beef muscle proteins in the presence of the TPDP might explain the increased TPI activities as compared to the control beef. Extractable protein increases as the pH of the beef is increased above the isoelectric point of the muscle proteins (Saffle and Galbreath, 1964; Prusa and Bowers, 1984). Van Den Oord and Wesdorp (1978) found an increase in extractability of pork muscle proteins in the presence of increasing pyrophosphate (0.01-1%) concentrations. Similarly, Fukazawa et al. (1961) showed that protein extracted from beef and rabbit muscle myofibrils increased in the presence of sodium pyrophosphate, tripolyphosphate, and hexametaphosphate.

Table 3.8-Effect of NaCl, tetrapotassium diphosphate (TPDP) and heating temperature on triose phosphate isomerase activity (U/kg meat) in ground beef

	62.8°C	69.4°C
Control	31.9 ± 11.0 ^a	8.4 ± 3.2 ^a
1.0% NaCl	5.9 ± 1.2 ^a	2.7 ± 0.5 ^b
0.5% TPDP	841.2 ± 211.9 ^b	7.2 ± 0.9 ^a
1.0% NaCl and 0.5% TPDP	135.5 ± 50.7 ^c	8.4 ± 2.4 ^a

^{a-c} Means ± standard deviation in the same column followed by the same letter are not different (P>0.05).

At 69.4°C, the ground beef with 0.5% TPDP and 1.0% NaCl, and the ground beef with 0.5% TPDP had similar TPI activities to the control. Past studies showing TPI to be close to its thermal inactivation point at 69.4°C may explain why the addition of 0.5% TPDP had no effect on the TPI activity of the ground beef cooked to 69.4°C (Smith et al., 1991; Orta-Ramirez et al., 1997). Current USDA-FSIS regulations require that beef patties be cooked to 69.4°C for 10 sec. The addition of phosphate salts and NaCl to beef should not affect the TPI activity of ground beef patties cooked to an internal temperature of 69.4°C.

3.5 CONCLUSIONS

Muscle type had a significant effect on TPI activity. However, the assaying of TPI activity from a ground beef patty would be similar to taking an average TPI activity from a number of muscles. Therefore, the significant muscle effect would not negatively inhibit the use of TPI activity as an indicator of the adequate thermal processing of ground beef patties. During storage at -13°C for 5 months, the TPI activity in cooked ground beef increased and decreased from the initial cook levels. As the fat content of ground beef was increased, TPI activity decreased. TPI was found not to be able to be used as a TTI to verify the adequate thermal processing of ground beef in this model system because activities were different at the lowest and highest temperatures of the USDA-FSIS processing schedules for beef patties. However, TPI might be a suitable indicator to determine the EPT of cooked ground beef. The residual TPI activities at 66.1°C for 41 sec (equivalent to 71°C instantaneously) for each fat content of ground beef could be proposed as the maximum allowable activities to verify the adequate thermal processing of beef patties. The USDA advises consumers to cook beef patties to an internal EPT of

71.1°C. Further study is needed to determine a maximum allowable TPI activity for ground beef patties that are cooked in a commercial setting to the recommended USDA processing temperature.

CHAPTER 4

**INDICATOR PROTEINS AND COLOR MEASUREMENTS AS METHODS TO
DETERMINE ADEQUATE THERMAL PROCESSING OF GROUND BEEF
PATTIES**

4.1 ABSTRACT

The objective of this study was to determine whether the EPT of ground beef could be differentiated using TPI activity and LDH concentration and to compare the results to the internal color of the beef patties. Ground beef patties (24.4% fat) were cooked to 5 endpoint temperatures (EPT) ranging from 60 to 82.2°C. Triose phosphate isomerase (TPI) activity, lactate dehydrogenase (LDH) concentration and the internal color of the beef patties were quantified to determine if these assays could be used to differentiate between adequately and undercooked beef patties. TPI activity of beef patties decreased from 20.26 to 6.28 U/kg meat between 65.6 and 71.1°C. LDH concentration decreased from 3.93 to 0.02 µg/g meat from 71.1 to 76.7°C. Beef patties cooked to 65.6 and 71.1°C had different primary degree of doneness scores, as determined by a sensory panel. Both the a* and b* values of the ground beef patties decreased as the EPT was increased, however temperature had no effect on the L* values. While the primary degree of doneness scores, a* and b* values were able to detect differences between undercooked and fully cooked beef patties in this study, past research has shown visual and instrumental color measurements to be poor indicators of adequate thermal

processing. Using TPI activity, undercooked ground beef patties were able to be distinguished from those that had been adequately cooked. TPI may be useful as an EPT indicator to verify the adequate thermal processing of ground beef patties.

Key words: Ground beef, endpoint temperature, triose phosphate isomerase, lactate dehydrogenase, internal color

4.2 INTRODUCTION

Since 1982, undercooked ground beef has been identified as the most frequent vehicle of *E. coli* O157:H7 infection (CDC, 1996). An outbreak of foodborne disease in several western states from late 1992 to early 1993, which resulted in more than 475 people becoming seriously ill and several children's deaths, was caused by the consumption of undercooked enteropathogenic *E. coli* O157:H7-contaminated ground beef patties (Mermelstein, 1993; Ahmed et al., 1995).

The USDA-FSIS (1997b) has required that meat processing establishments cook beef patties to any one of seven time/temperature processing schedules that range from 66.1°C for 41 sec to 69.4°C for 10 sec. In May of 1996, the USDA-FSIS proposed to convert the current meat and poultry processing regulations into performance standards (USDA-FSIS, 1996). A thermal process sufficient to cause a 5 log, or 5-D reduction in *Salmonella* was proposed by the USDA-FSIS as the lethality performance standard for fully cooked, uncured meat patties. When utilized, any of the seven currently used USDA time/temperature combinations generate a 5-D lethality.

The USDA-FSIS recommends that consumers use a meat thermometer to cook beef patties to an internal EPT of 71.1°C. If a meat thermometer is not available, consumers are advised to evaluate the doneness of ground beef patties by one of several

factors including: (1) the cook out juices should have no trace of pink, red, or cloudiness, (2) the cooked ground beef should be brown in the center, (3) the cooked ground beef should have a firm or flaky texture, as compared to raw meat that has a soft, mushy texture, regardless of the color (USDA-FSIS, 1997d).

Two problems have recently been identified by the USDA-FSIS regarding its advice to consumers about using a color index to test for doneness of ground beef. One problem is that some ground beef loses all pink color before it is fully cooked.

Alternatively, the pink color may persist in some adequately cooked beef patties (Mendenhall, 1989; Hague et al., 1994; Van Laack et al., 1996). The characteristic pink color of fresh meat can remain in beef cooked to 71.6°C if the pH is 6.0 or higher. The amount of fat in beef patties can also affect the cooked ground beef color.

Triose phosphate isomerase (TPI) was identified as a potential time-temperature indicator (TTI) in beef (Hsu, 1997; Orta-Ramirez et al., 1997). TPI was found to have similar thermal inactivation kinetics as that of *Salmonella*, the microbial pathogen selected by the USDA-FSIS to define the proposed lethality standards in ground beef.

Sair (Chapter 3) determined TPI activity in 9.3, 20.0, and 29.6% fat ground beef after cooking to the USDA-FSIS time/temperature schedules in a water bath. The 20.0% fat ground beef cooked to the USDA-FSIS low temperature schedule, 66.1°C (151°F) for 41 sec, had a TPI activity of 6.2 U/kg meat. The TPI activity of the 20% fat ground beef processed to the high temperature schedule, 69.4°C (157°F) for 10 sec, was 9.6 U/kg meat. Therefore, it was suggested that any 20% fat beef patty having a TPI activity less than 6.2 U/kg meat could be considered fully cooked.

Wang et al. (1996b) recognized LDH as a potential protein indicator for ground beef patties. Orta-Ramirez et al. (1996) prepared monoclonal antibodies (MAb) to bovine muscle LDH and used them to develop an enzyme-linked immunosorbent assay (ELISA) to determine the endpoint cooking temperature of ground beef. Commercially cooked ground beef patties with an EPT ranging from 68.3 to 71.1°C averaged 3.38 µg LDH/g of meat. It was suggested that ground beef patties with a maximum concentration of 3 µg LDH/g meat might indicate that the ground beef was processed to 70°C or above.

The objective of this study was to determine whether the EPT of ground beef could be differentiated using TPI activity and LDH concentration and to compare the results to the internal color of the beef patties. The subjective and objective color evaluation of the cooked beef patties was completed in collaboration with Dr. Brad Berry at the United States Department of Agriculture - Agricultural Research Service Meat Science Research Lab in Beltsville, MD.

4.3 MATERIALS AND METHODS

4.3.1 Patty formulation

Ground beef patties for this study were obtained from a meat supplier to fast food restaurants. U.S. cutter and canner grades of beef were used as the lean source and U.S. choice and select grades of beef as the fat source. Beef was formulated to contain 18 to 24% fat. Lean and fat sources were initially ground through a 0.95 cm plate, followed by grinding through a 0.24 cm plate. All processing operations were completed at approximately 4°C or below. The meat temperature at forming was -2.2°C. The ground beef was processed into patties (113.4g) using a Formax (Model 24, Formax, Inc., Mokena, IL) patty machine. Patties were individually quick frozen in a mechanical spiral

freezer, boxed, and immediately shipped by overnight courier to the Meat Science Research Lab at the Beltsville Agricultural Research Center - East (Beltsville, MD 20705-2350).

4.3.2 Cooking procedure

Dr. Brad Berry at the United States Department of Agriculture - Agricultural Research Service - Meat Science Research Lab at the Beltsville Agricultural Research Center - East cooked the patties and evaluated the internal color. Patties were immediately placed in a -18°C freezer. Forty patties were cooked the next day. The remaining 35 patties were cooked the following day. Immediately prior to cooking, each patty was removed from the freezer and weighed. Patties were individually cooked on electric griddles (Farberware Model 350A, Walter Kidde and Co., Bronx, NY) at a temperature of 163°C. Patties were turned after 2 min, turned again at 4 min and then at 1 min intervals until the desired temperature was reached. Fifteen patties were cooked to endpoint temperatures of 60, 65.6, 71.1, 76.7, or 82.2°C.

Preliminary cooking tests at all five temperatures were conducted to determine approximate cooking times to establish times for thermocouple insertion. An iron/constantan thermocouple, made by welding TT-J-36 special limits of error iron-constantan wire (Omega Engineering, Inc., Stamford, CT 06907) inside 19 gauge syringe needles, was connected to a recorder (Model 202, Honeywell Co., Ft. Washington, PA). During cooking, the thermocouple was inserted into the side of the patty to the thickest portion without any wiggling or pressure from the top of the patty (USDA, 1993). The thermocouple was inserted into the patty about 90 sec before the endpoint temperature

was attained. Cooking times were recorded. Immediately following cooking, patties were blotted with paper towels, reweighed to determine cooking yields and evaluated for color.

Patties were placed in the open air for 10 min to allow surfaces to reach room temperature, after which they were placed in freezer bags. Patties were placed in a 3°C cooler before shipping overnight, on ice, to the Department of Food Science and Human Nutrition at Michigan State University. The temperature of the patties after shipping to Michigan State University was 4°C.

4.3.3 Color

Cooked patties were immediately divided into two portions (perpendicular to the flat surface of the patty) and their interior cut surfaces were assessed for degree of doneness by two trained evaluators using the Kansas State University Ground Beef Patty Cooked Color Guide; A score of 1 = medium rare, 3 = medium, and 5 = well done (Marksberry et al., 1993). Secondary degree of doneness scores were recorded for beef patties having substantial areas of color beyond the primary score. Patty degree of doneness evaluations were performed using G.E. Deluxe Warm White lighting set to be 100 foot candles at the surface of the exposed patties. A Minolta Chroma Meter (Minolta Camera Co., Ltd. Osaka, Japan) CR-200 (illuminant C, calibrated with brown plate, $C=26.5$, $X=0.366$ and $Y=0.340$) was used for determining instrumental color values of L^* , a^* , b^* , saturation index (chroma) and hue angle (C.I.E., 1976; Van Laack et al., 1996). Three readings were obtained from different locations on each cut surface of the patty. The time from removal of patties from the grill to initiation of instrumental color measurement was approximately 30 sec.

4.3.4 Extract preparation

Patties were removed from the freezer bags and the center of the patty was removed using a 7 cm diameter Cookie & Donut Cutter (Norpro[®], Everett, WA 98203). Three volumes of PBS buffer (0.15M NaCl, 0.01 M sodium phosphate buffer, pH 7.2) were used to extract the protein. The beef samples were blended for 30 sec (15 sec on, 10 sec off, 15 sec on) in an Oster Blender (Model 869-18, Schaumburg, IL 60173) and then stirred on a magnetic stir plate for 15 min at 4°C. Extracts were immediately centrifuged at 4,500 x g for 10 min at 4°C. Supernatants were collected after filtration (Whatman No. 1) and were held on ice until assayed for TPI activity and LDH concentration (Orta-Ramirez et al., 1996). Triose phosphate isomerase activity was determined as described by Bergmeyer (1984) except that 1.0 mL triethanolamine buffer (TEA, 0.2M, pH 8.0), 0.2 mL 15mM glyceraldehyde-3-phosphate (GAP), 10 µL β-nicotinamide adenine dinucleotide (NADH, 10 mg/mL), 10 µL glycerol-3-phosphate dehydrogenase (GDH, 1.5 mg/mL), and 10 µL of protein extract were used in the assay solution (Sair, Chapter 3).

4.3.5 LDH concentration

Lactate dehydrogenase concentration was quantified using a sandwich ELISA (Orta-Ramirez et al., 1996). Lactate dehydrogenase monoclonal antibodies (MAb) were produced by Orta-Ramirez et al. (1996) following the procedures of Abouzied et al. (1990). Polyclonal antibodies (PAb) against beef muscle LDH were prepared as described by Wang et al. (1996a). The sandwich ELISA was performed by coating Immulon[®] 2 Removawell Strips (Dynatech Laboratories, Inc., Chantilly, VA 22021) with 100 µL of LDH MAb, the capture antibody, diluted (1/3000) in 0.1 M carbonate buffer, pH 9.6, and drying overnight at 37°C. Plates were washed four times with PBS containing 0.05%

Polyoxyethylene sorbitan monolaurate (Tween 20) (PBS-T). Nonspecific binding sites were blocked by adding 300 μ L of PBS containing 2% ovalbumin (PBS-OA) to each well and incubating at 37°C for 30 min. Plates were washed four times and 50 μ L of serially diluted bovine muscle LDH or ground beef patty extracts was added to each well and incubated at 37°C for 30 min. After four washings, 50 μ L of LDH specific PAb in 2% PBS-OA (1:500) was added to each well and incubated at 37°C for 30 min. After incubation, the plates were washed four times with PBS-T and 100 μ L of goat anti-rabbit IgG peroxidase conjugate diluted in 2% PBS-OA (1:500) was added to each well. Plates were incubated for 30 min at 37°C and washed eight times with PBS-T. Peroxidase binding was determined with ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) substrate (Pestka, 1982). After 30 min, absorbance was read at 405 nm using a microplate reader (THERMOmax, Molecular Devices Co., Menlo Park, CA). Results were expressed as micrograms of LDH per gram of meat.

4.3.6 Proximate composition

The moisture, fat, and protein contents of raw ground beef patties were determined according to AOAC methods 950.46B, 991.36, and 981.10, respectively (AOAC, 1990). For pH determination, 10 g meat was homogenized with 90 g deionized water using a Polytron homogenizer (Model PT 10/35, Brinkmann Instrument, Co., Westbury, NJ 11590) equipped with a PTA 10TS generator. The sample was stirred for two periods of 30 sec at 5.0 speed setting and the pH was measured while the solution was continuously stirred over a magnetic stirrer.

4.3.7 Statistics

Data were analyzed using analysis of variance in the General Linear Model Procedure of the Statistical Analysis System (SAS Institute, Inc., 1996). Least square means procedures were used to separate means at $P < 0.05$. Fifteen ground beef patties were cooked at each EPT. The linear or curvilinear change in selected variables was determined using the orthogonal polynomial contrast test by SAS software (Release 6.12). Tests revealed that the assumptions of the general linear model were not met for TPI activity and LDH content of cooked beef patties. Triosephosphate isomerase activity and LDH content data underwent a log transformation prior to analyses.

4.4 RESULTS AND DISCUSSION

4.4.1 TPI activity and LDH concentration

The raw ground beef patties contained $24.4 \pm 0.3\%$ fat, $62.5 \pm 5.7\%$ moisture and $17.0 \pm 0.6\%$ protein. The pH of the raw patties was 5.8 ± 0.1 . Raw ground beef patty extracts contained 2203 U TPI/kg meat. The TPI activity of the cooked beef patties linearly decreased ($P < 0.05$) as the processing temperature was increased from 60.0 to 82.2°C (Table 4.1). Triose phosphate isomerase activity decreased from 52.50 U/kg meat at 60.0°C to 5.36 U/kg meat at 82.2°C. Differences were detected in TPI activity from 60.0 to 65.6°C, however no difference ($P > 0.05$) in TPI activity was detected between 71.1 and 82.2°C. Ground beef patties had 6.28 U TPI/kg meat when processed to 71.1°C; the endpoint temperature recommended by the USDA to consumers to ensure the safe preparation of cooked ground beef (USDA, 1994). Wang et al. (1996b) reported a TPI activity of 5.2 U/g meat in ground beef patties processed to 71°C. Ground beef cooked to

Table 4.1-Influence of temperature on triose phosphate isomerase (TPI) activity and lactate dehydrogenase (LDH) content of cooked beef patties (24.4% fat)

Temp (°C)	LDH content ($\mu\text{g/g}$ meat)	TPI activity (U/kg meat)
60.0	2.96 ± 0.66^a	52.50 ± 12.69^a
65.6	3.80 ± 1.03^a	20.26 ± 6.40^b
71.1	3.93 ± 1.40^a	6.28 ± 0.26^c
76.7	0.02 ± 0.01^b	5.27 ± 0.28^c
82.2	0.00 ± 0.00^b	5.36 ± 0.38^c

^{a-c} Means $\pm$ standard error of the mean in the same column with the same letter were not different ($P > 0.05$).

66.1°C for 41 sec (equivalent to 71.1°C instantaneously) in model system studies had a TPI activity of 6.2 U/kg meat (Sair, Chapter 3). Results in both the model system and ground beef patties suggest that a maximum TPI activity of about 6 U/kg meat (mean minus one standard deviation) might indicate that ground beef was processed to 71°C or above.

Three cooked ground beef patties (113.4 g raw weight) were purchased from a local fast food restaurant. After removing the center of each patty with the Cookie & Donut Cutter (7 cm diameter), the protein was extracted and TPI activity determined, as previously described. The TPI activity of the cooked ground beef patties averaged 1.8 ± 0.3 U/kg meat, suggesting they were cooked above the recommended EPT of 71°C.

The LDH content decreased ($P < 0.05$) as cooking temperature of ground beef patties was increased from 60.0 to 82.2°C (Table 4.1). No differences ($P > 0.05$) were detected in the LDH concentration of ground beef patties processed from 60.0 to 71.1°C or 76.7 to 82.2°C. The LDH concentration of patties processed to 71.1°C averaged 3.93 µg/g meat. The LDH content decreased from 3.93 µg/g meat at 71.1°C to 0.02 µg/g meat at 76.7°C. Orta-Ramirez et al. (1996) reported LDH concentrations of 3.38 and 0.30 µg/g meat for commercially cooked beef patties processed within the temperature ranges of 68.3 to 71.1°C and 73.9 to 82.2°C, respectively. Wang et al. (1996a) reported a LDH concentration of 0.48 µg/g meat for commercially cooked beef patties cooked within the temperature range of 73.9 to 82.2°C. In our study, the LDH concentration of the cooked beef patties was similar at 65.6 and 71.1°C. However, the LDH concentration of the beef patties cooked to 76.7°C was different from those cooked to 71.1°C. Beef patties had 0.02 µg LDH/g meat when processed to 76.7°C, an EPT above that recommended to

consumers by the USDA. As the EPT of cooked beef patties is increased, reduced palatability results (Liu and Berry, 1996). To determine the lowest EPT above 71.1°C needed to significantly decrease the LDH concentration in cooked beef patties, LDH concentrations need to be established in beef patties cooked to EPTs between 71.1 and 76.7°C.

4.4.2 Internal color

The primary degree of doneness scores increased (less red) as the EPT of beef patties was increased (Table 4.2). Differences were seen in the primary degree of doneness scores of patties cooked to 60.0 and 65.6°C. Patties cooked from 71.1 to 82.2°C had similar primary degree of doneness scores. At 65.6°C, patties were evaluated as moderately pink and could be visually distinguished ($P < 0.05$) from patties cooked to 71.1°C, the temperature on which the USDA (1994) cooking recommendations are based. Therefore, using primary degree of doneness scores, undercooked ground beef patties could be distinguished from those that had reached the desired endpoint cooking temperature of 71.1°C.

However, temperature had no effect ($P > 0.05$) on the L^* values (measure of lightness) of the ground beef patties. No difference in L^* values was detected between the 65.6°C (undercooked) and 82.2°C (overcooked) EPTs. Hague et al. (1994) reported no difference in the L^* values of ground beef patties cooked to 60 and 77°C. The use of the L^* values as a measure of adequate cooking could potentially lead to the consumption of undercooked patties.

As expected, both the a^* and b^* values decreased (less red and less yellow, respectively) as the EPT of the patties was increased. These color changes agreed with

Table 4.2-Instrumental color values of ground beef patties (24.4% fat) cooked to several endpoint temperatures

	Endpoint temperature (°C)				
	60.0	65.6	71.1	76.7	82.2
Degree of doneness ^c	2.3 ^a	2.8 ^b	3.9 ^c	4.1 ^c	4.1 ^c
L* value	50.2 ^a	50.4 ^{ac}	51.4 ^b	51.5 ^b	51.3 ^{bc}
a* value	10.7 ^a	8.3 ^b	6.4 ^c	6.0 ^c	5.9 ^c
b* value	14.1 ^a	13.7 ^b	13.1 ^c	12.9 ^{cd}	12.8 ^d
Chroma ^f	17.8 ^a	16.1 ^b	14.6 ^c	14.2 ^c	14.2 ^c
Hue angle ^g	53.3 ^a	59.2 ^b	64.2 ^c	65.2 ^c	65.3 ^c
a*/b* ratio	0.76 ^a	0.6 ^b	0.49 ^b	0.46 ^c	0.46 ^c

^{a-d} Means within a row with the same letter are not different (P>0.05).

^c Degree of doneness score: 1 = medium rare, 3 = medium, 5 = well done.

^f Chroma = $(a^2 + b^2)^{0.5}$.

^g Hue angle = $\arctan(b/a)$.

the results of Hague et al. (1994) who reported decreasing a^* and b^* values in ground beef patties as the EPT was increased. The beef patties became less red and less intense in color, illustrated by a decreasing chroma and a^*/b^* ratio and an increasing hue angle, as the EPT was increased. Through the use of these instrumental color measurements, patties cooked to 65.6 could be distinguished from those cooked to 71.1°C. However, the chroma, a^*/b^* ratio and hue angle values were all similar for the patties cooked from 71.1 to 82.2°C. Consumers probably would not be able to differentiate the minor differences detected in the instrumental color measurements of the cooked beef patties at similar EPTs.

Hague et al. (1994) reported that assigning visual scores to the internal color of cooked beef patties as a method to determine adequate processing was not accurate. No difference was found between the internal colors of beef patties cooked to 66 and 71°C (Hague et al., 1994). Using internal color visual scores, some ground beef patties can be perceived to be well-done at internal temperatures as low as 55°C (Hunt et al., 1995). Van Laack et al. (1996) reported a pink color to persist in eight out of seventeen commercially prepared patty formulations when cooked to an internal temperature of 71°C. Therefore, the use of color values to detect differences in cooked patties is not a practical option for consumers or the food industry to ensure food safety.

4.4.3 Variability in degree of doneness scores

Larger standard errors were seen with TPI activity and LDH concentration at the lower endpoint processing temperatures (Table 4.1). Similarly, larger variations in degree of doneness scores were observed when beef patties were processed to lower temperatures (Table 4.3). At 60.0°C, 14 out of the 15 patties received secondary degree

Table 4.3-Degree of doneness (d/d) scores^a of beef patties cooked to 5 endpoint temperatures

Temp (°C)	Patty #	Degree of doneness	
		Primary	Secondary
60.0	1	2	3
	2	3	4
	3	3	4
	4	2	1,3
	5	3	
	6	2	1,3
	7	3	2,4
	8	3	2,4
	9	2	3,4
	10	2	1,3
	11	2	3,4
	12	2	1,3,4
	13	2	1
	14	1	2
	15	2	3
65.6	1	3	2,4
	2	3	4
	3	2	3
	4	3	4
	5	3	4
	6	3	
	7	3	4
	8	3	4
	9	3	4
	10	3	
	11	3	4
	12	3	4
	13	3	4
	14	2	1,3,4
	15	2	3
71.1	1	4	3
	2	4	
	3	4	3
	4	3	4
	5	4	3
	6	4	3
	7	4	

Table 4.3-(cont'd).

Temp (°C)	Patty #	Degree of doneness	
		Primary	Secondary
71.1	8	4	3
	9	4	
	10	4	
	11	4	3
	13	4	3
	14	4	3
	15	4	3
76.7	1	4	
	2	4	
	3	4	
	4	4	
	5	4	
	6	4	
	7	4	
	8	4	
	9	4	
	10	4	5
	11	4	5
	12	4	
	13	4	5
	14	4	5
	15	5	4
82.2	1	4	
	2	4	
	3	4	
	4	4	
	5	4	5
	6	4	5
	7	4	5
	8	4	
	9	4	5
	10	4	
	11	5	
	12	4	5
	13	5	
	14	4	5
	15	4	5

^a d/d score: 1 = medium rare, 3 = medium, 5 = well done.

of doneness scores which indicates another color, different from the primary degree of doneness score, was detected within the processed patty. Eight of the patties processed to 60.0°C received two secondary degree of doneness scores suggesting there were three areas of substantial color in the cooked beef patties. One patty received three secondary degree of doneness scores indicating large variations in color throughout the patty. Different regions within a single ground beef patty can be cooked to distinct EPTs, as illustrated by the variation within the degree of doneness scores at the lowest EPT.

Liu and Berry (1996) documented the wide variability in the EPTs of beef patties following cooking. Beef patties were cooked to 2 EPTs using constant cooking times. There was considerable variability in the degree of doneness scores and internal temperatures of the cooked beef patties when constant cooking times were used. The TPI activity and LDH concentration will have larger standard errors when multiple temperature gradients occur within individual beef patties. As the EPT was increased, the number of secondary degree of doneness scores assigned to the ground beef patties decreased. At 71.1°C, 10 of the 15 ground beef patties, and at 76.7°C only 5 of the ground beef patties received one secondary degree of doneness score. Smaller standard errors were reported for TPI activities and LDH concentrations for the ground beef patties that were given fewer secondary degree of doneness scores at the higher EPTs. The smaller standard errors indicate more uniform cooking at the higher EPTs. Methods need to be developed to achieve more uniform cooking of beef patties at all EPTs in order to establish, with the greatest accuracy and precision, TPI activities and LDH concentrations to be used as indicators of adequate processing.

4.5 CONCLUSIONS

Lactate dehydrogenase concentration in cooked beef patties decreased between 71.1 to 76.7°C and could possibly be used to verify the adequate processing of ground beef patties. The primary degree of doneness scores showed a difference between patties cooked to 65.6 and 71.1°C, however the L* values were not an accurate indicator of patty doneness as those cooked to 65.6°C had similar values to those cooked to 82.2°C. The a* and b* values both showed differences between patties cooked to 65.6 and 71.1°C. While the primary degree of doneness scores, a* and b* values were able to detect differences between undercooked and fully cooked beef patties in this study, past research has shown visual and instrumental color measurements of cooked beef patties to be poor indicators of adequate thermal processing (Hague et al., 1994; Hunt et al., 1995; Van Laack et al., 1996). Triose phosphate isomerase activity decreased from 20.26 to 6.28 U/kg meat in ground beef patties between the EPTs of 65.6 and 71.1°C; the EPT recommended by the USDA to consumers to ensure the safe preparation of cooked ground beef. In this study, TPI activity proved to be the most effective method for the determination of the adequate thermal processing of beef patties. In order to use TPI as an EPT indicator to verify the adequate thermal processing of beef patties, additional factors that may have an effect on TPI activity in cooked beef patties need to be investigated. These factors include: the effects of patty size, whether the beef patties were cooked from the frozen or raw state, cooking method, cooking rate, and other formulation and processing variables.

CHAPTER 5

CONCLUSIONS

In previous research, triose phosphate isomerase (TPI) and lactate dehydrogenase (LDH) were identified as possible means for determining the adequate thermal processing of cooked ground beef. Current recommendations of the United States Department of Agriculture - Food Safety Inspection Service (USDA-FSIS) are to evaluate the doneness of beef patties by the cooked ground beef color.

The frozen storage (-13°C) of raw steer muscles for 10 months had no effect on TPI activity, however TPI activity and concentration changed in cooked (62.8°C) beef during frozen storage. Ground beef had different TPI activities when processed to the low (66.1°C/41 sec) and high (69.4°C/10 sec) temperatures of the required USDA processing schedules in a model system. While, TPI was found not to be a suitable time-temperature integrator in ground beef, TPI was determined to be a possible endpoint temperature (EPT) indicator.

In pilot studies, lactate dehydrogenase (LDH) concentration decreased in cooked ground beef patties (24.4% fat) between 71.1 to 76.7°C. Beef patties had 0.02 µg LDH/g meat when processed to 76.7°C, an EPT above that recommended to consumers by the USDA. LDH concentrations need to be established in beef patties cooked to EPTs between 71.1 and 76.7°C to determine the lowest EPT above 71.1°C needed to significantly decrease the LDH concentration in cooked beef patties.

TPI activity of beef patties was less when cooked to 71.1°C as compared to 65.6°C. TPI activity was 6.28 U/kg meat when processed to 71.1°C; the EPT recommended by the USDA to consumers to ensure the safe preparation of cooked ground beef. A maximum TPI activity of about 6 U/kg meat might indicate that ground beef was processed to 71°C or above.

The primary degree of doneness scores showed a difference between patties cooked to 65.6 and 71.1°C, however the L* values were not an accurate indicator of patty doneness as those cooked to 65.6°C had similar values to those cooked to 82.2°C. The a* and b* values both showed differences between patties cooked to 65.6 and 71.1°C. While the primary degree of doneness scores, a* and b* values were able to detect differences between undercooked and fully cooked beef patties in this study, past research has shown visual and instrumental color measurements of cooked beef patties to be poor indicators of adequate thermal processing.

Therefore, TPI and LDH could possibly be used as indicator enzymes to determine the adequate thermal processing of ground beef patties. Sair (Chapter 3) examined factors that might have an effect on TPI activity in ground beef. The fat content of ground beef had an effect on TPI activity. The frozen storage of cooked (62.8°C) ground beef for 5 months caused changes in TPI activity. Cooked beef (69.4°C) with 1.0% NaCl had lower TPI activities than the control. These factors could influence the use of TPI as an indicator enzyme in ground beef patties. Further research needs to be undertaken to study the effects of the above factors and additional ones that may have an impact on the TPI activity and LDH concentration in cooked ground beef patties.

CHAPTER 6

FUTURE RESEARCH

This study was designed to determine whether triose phosphate isomerase (TPI) could be used as a time-temperature integrator (TTI) to determine adequate thermal processing of ground beef patties. Further investigation into this line of research is warranted and is described below.

The thermal inactivation rates of TPI, *E. coli* O157:H7 and *Salmonella senftenberg* were determined in ground beef by Orta-Ramirez et al. (1997) using *semitendinosus* muscle from the eye of the round. The muscles used in the production of ground beef primarily include those from the chuck (*trapezius* and *longissimus dorsi*). A similar thermal inactivation study to determine the D and z values of enzymes and microorganisms in chuck muscles should be completed.

The USDA-FSIS time/temperature processing schedule for fully cooked beef patties ranges from 66.1°C for 41 sec to 69.4°C for 10 sec. The D and z values of six protein markers in ground beef were determined over the range of 53°C to 66°C (Orta-Ramirez et al., 1997). In order to be used as a TTI, an endogenous enzyme should have a similar thermal inactivation rate as that of the target microorganism over a similar temperature range. The D and z values of the previously identified marker proteins should be re-determined over the temperature range established by the USDA-FSIS to assure adequate processing of beef patties.

The come-up times associated with the cooking of ground beef in thermal death time (TDT) tubes are about 2.5 to 4 min. As means to achieve instantaneous heating and to avoid the thermal inactivation of the enzymes during come up times that are incurred when using the 10 x 75 mm TDT tubes, the use of smaller diameter tubes should be considered.

The sandwich ELISA used to measure the TPI concentration in beef products used polyclonal antibodies for both capture and detection of bovine muscle TPI. The sensitivity and specificity of the sandwich ELISA may be improved through the use of monoclonal antibodies specific to native TPI.

Both TPI and lactate dehydrogenase (LDH) were found to be possible endpoint temperature indicators in ground beef patties. Additional factors that may have an effect on TPI activity and LDH concentration in cooked ground beef need to be investigated. These factors include: effects of patty size, cooking the beef patties from the frozen or thawed state, cooking method, heating rate, and other formulation and processing variables.

REFERENCES

- Abouzied, M.M., Asghar, A., Pearson, A.M., Miller, E.R., Gray, J.I., and Pestka, J.J. 1990. Hybridoma based enzyme-linked immunosorbent assay for C₁₉ Δ^{16} -steroids in sera of boars. *J. Agric. Food Chem.* 38:331-335.
- Abouzied, M.M., Wang, C.H., Pestka, J.J., and Smith, D.M. 1993. Lactate dehydrogenase as safe endpoint cooking indicator in poultry breast rolls: development of monoclonal antibodies and application to sandwich enzyme-linked immunosorbent assay (ELISA). *J. Food Protec.* 56: 120-124,129.
- Ahmed, N.M., Conner, D.E., and Huffman, D.L. 1995. Heat-resistance of *Escherichia coli* O157:H7 in meat and poultry as affected by product composition. *J. Food Sci.* 60:606-610.
- AMI. 1996. Relative ground beef contribution to the United States beef supply. Am. Meat Institute Foundation, Arlington, VA.
- Anderson, W.L. 1997. Lethality performance standards may change in FSIS final rule. *Food Chem. News.* 39(7):9.
- AOAC. 1990. *Official Methods of Analysis*, 15th ed. Association of Official Analytical Chemists, Arlington, VA.
- Awad, A., Powrie, W.D., and Fennema, O. 1968. Chemical deterioration of frozen bovine muscle at -4°C. *J. Food Sci.* 33:227-234.
- Bean, N.H., and Griffin, P.M. 1990. Foodborne disease outbreaks in the United States, 1973-1987: Pathogens, vehicles, and trends. *J. Food Protec.* 53:804-817.
- Beisenherz, G. 1955. Triosephosphate isomerase from calf muscle. Ch. 57, In *Methods in Enzymology*, S.P. Colowick and N.O. Kaplan (Ed.), p. 387-391. Academic Press, Inc. New York, N.Y.
- Bergmeyer, H.U. 1984. *Methods of Enzymatic Analysis*, Vol. 1, 2nd ed. Verlag Chemie International, Deerfield Beach, FL.
- Berry, B.W. 1994. Fat level, high temperature cooking and degree of doneness affect sensory, chemical and physical properties of beef patties. *J. Food Sci.* 59:10-14.

- Bonner, D.W., Bloomer, A.C., Petsko, G.A., Phillips, D.C., and Wilson, I.A. 1976. Atomic coordinates for triose phosphate isomerase from chicken muscle. *Biochem. Biophys. Res. Commun.* 72:145-155.
- Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
- Browning, M.A., Huffman, D.L., Egbert, W.R., and Jungst, S.B. 1990. Physical and compositional characteristics of beef carcasses selected for leanness. *J. Food Sci.* 55:9-14.
- Bunch, E.A., Altwein, D.M., Johnson, L.E., Farley, J.R., and Hammersmith, A.A. 1995. Homogeneous sample preparation of raw shrimp using dry ice. *J. AOAC* 78:883-887.
- Caldironi, H.A., Bazan, N.G. 1980. Quantitative determination of low-salt soluble protein patterns of bovine muscles cooked at different temperatures, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Food Sci.* 45:901-904.
- CDC. 1996. "Preventing Foodborne Illness: *Escherichia coli* O157:H7." <http://www.cdc.gov/ncidod/diseases/foodborn/e_coli.htm> (7 June. 1997).
- CIE. 1976. International Commission on Illumination, Colorimetry: Official Recommendations of the International Commission on Illumination. Publication CIE No. 15 (E-1.3.1.), Bureau Central de la CIE, Paris.
- Cohen, E.H. 1969. Determination of acid phosphatase activity in canned hams as an indicator of temperature attained during cooking. *Food Technol.* 23(961):101-104.
- Collins, S.S., Keeton, J.T., and Smith, S.B. 1991a. Lactate dehydrogenase activity in bovine muscle as a potential heating endpoint indicator. *J. Agric. Food Chem.* 39:1291-1293.
- Collins, S.S., Keeton, J.T., and Smith, S.B. 1991b. Lactate dehydrogenase enzyme activity in raw, cured, and heated porcine muscle. *J. Agric. Food Chem.* 39:1294-1297.
- Czok, R., and Buecher, T. 1960. Crystallized enzymes from the myosin of rabbit skeletal muscle. *Adv. Protein Chem.* 15:315.
- Dabrowska, A., Kamrowska, I., and Baranowski, T. 1978. Purification, crystallization and properties of triosephosphate isomerase from human skeletal muscle. *Acta. Biochim. Polonica.* 25:247-255.

- Davis, C.E., and Townsend, W.E. 1994. Rapid fluorometric analysis of acid phosphatase activity in cooked poultry meat. *J. Food Protec.* 57:1094-1097.
- De Cordt, S., Hendrickx, M., Maesmans, G., and Tobback, P. 1992. Immobilized α -amylase from *Bacillus licheniformis*: a potential enzymic time-temperature integrator for thermal processing. *Int. J. Food Sci. Technol.* 27:661-673.
- De Cordt, S., Hendrickx, M., Maesmans, G., and Tobback, P. 1994. The influence of polyalcohols and carbohydrates on the thermostability of α -amylase. *Biotechnol. Bioeng.* 43:107-114.
- Doyle, M.P., and Schoeni, J.L. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. *Appl. Environ. Microbiol.* 48:855-856.
- Doyle, M.P., and Schoeni, J.L. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. *Appl. Environ. Microbiol.* 53:2394-2396.
- Eber, S.W., and Krietsch, K.G. 1980. The isolation and characterization of the multiple forms of human skeletal muscle triosephosphate isomerase. *Biochim. Biophys. Acta.* 614:173-184.
- Ellekjaer, M.R. 1992. Assessment of maximum cooking temperatures of previously heat treated beef. Part 2: Differential scanning calorimetry. *J. Sci. Food Agric.* 60:255-261.
- Ellekjaer, M.R., and Isaksson, T. 1992. Assessment of maximum cooking temperatures in previously heat treated beef. Part 1: Near infrared spectroscopy. *J. Sci. Food Agric.* 59:335-343.
- Evans, T.M., and Greene, D. 1973. Chopped beef - everyone's favorite. Ch. 7, In *The Meat Book*, p. 69-76. Charles Scribner's Sons, New York, N.Y.
- Fain, Jr., A.R., Line, J.E., Moran, A.B., Martin, L.M., Lechowich, R.V., Carosella, J.M., and Brown, W.L. 1991. Lethality of heat to *Listeria monocytogenes* Scott A: D-value and Z-value determinations in ground beef and turkey. *J. Food Protec.* 54:756-761.
- FDA. 1995. Recommendations of the U.S. Public Health Service Food and Drug Administration, Food Code. Preface. Food and Drug Administration, Washington, DC.
- FDA. 1997. "Food Safety Initiative Fact Sheet."
<<http://www.fda.gov/opacom/foodsafety/fsfact.htm>> (16 May. 1997).

- Forrest, J.C., Aberle, E.D., Hedrick, H.B., Judge, M.D., Merkel, R.A. 1975. *Principles of Meat Science*, 1st ed. Plenum., New York.
- Fukal, L. 1991. Modern immunoassays in meat-product analysis. *Die Nahrung* 35:431-448.
- Fukazawa, T., Hashimoto, Y., and Yasiu, T. 1961. The relationship between the components of myofibrillar protein and the effect of various phosphates that influence the binding quality of sausage. *J. Food Sci.* 26:550.
- Goodfellow, S.J., and Brown, W.L. 1978. Fate of *Salmonella* inoculated into beef for cooking. *J. Food Protec.* 41:598-605.
- Hague, M.A., Warren, K.E., Hunt, M.C., Kropf, D.H., Kastner, C.L., Stroda, S.L., and Johnson, D.E. 1994. Endpoint temperature, internal cooked color, and expressible juice color relationships in ground beef patties. *J. Food Sci.* 59:465-470.
- Hale, D.S. 1994. Grading. Ch. 7, In *Muscle Foods: Meat, Poultry, and Seafood Technology*, Kinsman, D.M., Kotula, A.W., and Breidenstein, B.C. (Ed.), p. 186-223. Chapman and Hall, New York.
- Hendrickx, M., Maesmans, G., De Cordt, S., Noronha, J., Van Loey, A., and Tobback, P. 1995. Evaluation of the integrated time-temperature effect in thermal processing of foods. *Crit. Rev. Food Sci. Nutr.* 35:231-262.
- Hsu, Y.C. 1997. Identification and verification of an endogenous time-temperature indicator to determine processing adequacy of roast beef. Ph.D. Dissertation. Michigan State University, East Lansing, MI.
- Hunt, M.C., Warren, K.E., Hague, M.A., Kropf, D.H., Waldner, C.L., Stroda, S.L., and Kastner, C.L. 1995. Cooked ground beef color is unreliable indicator of maximum internal temperature. Presented to Amer. Chem. Soc., Manhattan, KS, April 6.
- Jay, J.M. 1992. High-temperature food preservation and characteristics of thermophilic microorganisms. Ch. 14, In *Modern Food Microbiology*. 4th ed., p. 335-355. Chapman & Hall, New York.
- Kormendy, L., Rehasi, E., and Fetter, I. 1987. Determination of the extent of heat treatment in canned hams by use of the phosphatase test. *Meat Sci.* 19:77-79.

- Krietsch, W.K.G., Pantchew, P.G., Klingenberg, H., Hofstetter, T., and Buecher, T. 1970. The isolation and crystallization of yeast and rabbit liver triose phosphate isomerase and a comparative characterization with rabbit muscle enzyme. *Eur. J. Biochem.* 14:289-300.
- Lee, E.W., Barriso, J.A., Pepe, M., and Snyder, R. 1971. Purification and properties of liver triose phosphate isomerase. *Biochim. Biophys. Acta.* 242: 261-266.
- Lee, Y.B., Rickansrud, D.A., Hagberg, E.C., and Briskey, E.J. 1974. Application of SDS-acrylamide gel electrophoresis for determination of the maximum temperature to which bovine muscles have been cooked. *J. Food Sci.* 39:428-429.
- Line, J.E., Fain, Jr., A.R., Moran, A.B., Martin, L.M., Lechowich, R.V., Carosella, J.M., and Brown, W.L. 1991. Lethality of heat to *Escherichia coli* O157:H7: D-value and Z-value determinations in ground beef. *J. Food Protec.* 54:762-766.
- Liu, M.N., and Berry, B.W. 1996. Variability in color, cooking times, and internal temperature of beef patties under controlled cooking conditions. *J. Food Protec.* 59:969-975.
- Maesmans, G., Hendrickx, M., De Cordt, S., and Tobback, P. 1993. Theoretical considerations on design of multicomponent time temperature integrators in evaluation of thermal processes. *J. Food Proc. Pres.* 17:369-389.
- Marksberry, C.L., Kropf, D.H., Hunt, M.C., Hague, M.A., and Warren, K.E. 1993. Ground beef patty cooked color guide. *Kansas Agric. Exp. Sta., Manhattan, KS.*
- Mendenhall, V.T. 1989. Effect of pH and total pigment concentration on the internal color of cooked ground beef patties. *J. Food Sci.* 54:1-2.
- Mermelstein, N.H. 1993. Controlling *E. coli* O157:H7 in meat. *Food Technol.* 47(4):90-91.
- Mulley, A., Stumbo, C., and Hunting, W. 1975. Thiamine: a chemical index of the sterilization efficacy of thermal processing. *J. Food Sci.* 40:993-996.
- Ng, H., Bayne, H.G., and Garibaldi, J.A. 1969. Heat resistance of *Salmonella*: the uniqueness of *Salmonella seftenberg* 775W. *Appl. Microbiol.* 17:78-82.
- Norton, I.L., Pfuderer, P., Stringer, C.D., and Hartman, F.C. 1970. Isolation and characterization of rabbit muscle triose phosphate isomerase. *Biochem.* 9:4952-4958.

- Oesper, P., and Meyerhof, O. 1950. The determination of triose phosphate isomerase. *Arch. Biochem. Biophys.* 27:223.
- Omega Engineering, Inc. 1994. *Data Acquisition Hardware & Software for Notebook and Desktop PCs*, Omega Engineering, Inc., Stamford, CT.
- Orta-Ramirez, A. 1994. Lactate dehydrogenase as indicator of proper heat processing and death of *Escherichia coli* O157:H7 and *Salmonella* in ground beef patties. M.S. Thesis. Michigan State University, East Lansing, MI.
- Orta-Ramirez, A., Wang, C.H., Abouzied, M.M., Veeramuthu, G.J., Price, J.F., Pestka, J.J., and Smith, D.M. 1996. Lactate dehydrogenase monoclonal antibody sandwich ELISA to determine cooking temperature of ground beef. *J. Agric. Food Chem.* 44:4048-4051.
- Orta-Ramirez, A., Price, J.F., Hsu, Y.C., Veeramuthu, G.J., Cherry-Meritt, J.S., and Smith, D.M. 1997. Thermal inactivation of *Escherichia coli* O157:H7, *Salmonella senftenberg* and enzymes with potential as endpoint temperature indicators in ground beef. *J. Food Protec.* 60:471-475.
- Parson, S.E., and Patterson, R.L.S. 1986. Assessment of previous heat treatment given to meat products in the temperature range 40-90°C. Part 2: Differential scanning calorimetry, preliminary study. *J. Food Technol.* 21:123-131.
- Pestka, J.J., Lee, Y.K., and Chu, F.S. 1982. Reactivity of aflatoxin B₂ antibody with aflatoxin B₁ modified DNA and related metabolites. *Appl. Environ. Microbiol.* 44:1159-1165.
- Prusa, K.J., and Bowers, J.A. 1984. Protein extraction from frozen, thawed turkey muscle with sodium nitrite, sodium chloride, and selected sodium phosphate salts. *J. Food Sci.* 49:709-713.
- Ruiz, C.F., Higginbotham, D.A., Carpenter, J.A., Resurreccion, A.V.A., and Lanier, T.C. 1993. Use of chuck muscles and their acceptability in restructured beef/surimi steaks. *J. Anim. Sci.* 71:2654-2658.
- Saffle, R.L., and Galbreath, J.W. 1964. Quantitative determination of salt-soluble protein in various types of meat. *Food Technol.* 18(12):119-120.
- Samarajeewa, U., Wei, C.I., Huang, T.S., and Marshall, M.R. 1991. Application of immunoassay in the food industry. *Critical Rev. Food Sci. Nutr.* 29:403-434.

- Saraiva, J., De Cordt, S., Hendrickx, M., Oliveira, J., and Tobback, P. 1993. Inactivation of α -amylase from *Bacillus amyloliquefaciens* at low moisture contents. 459-466. Cited in van den Tweel, W.J.J., Harder, A., and Buitelaar, R.M. *Stability and Stabilization of Enzymes*. Elsevier Science Publishers, Amsterdam.
- SAS Institute, Inc., 1996. *SAS User's Guide: Basic Statistical Analysis*, Version 6.12, SAS Institute Inc., Cary, NC.
- Sawyer, T.H., Tilley, B.E., and Gracy, R.W. 1972. Studies on human triosephosphate isomerase - nature of the electrophoretic multiplicity in erythrocytes. *J. Biol. Chem.* 247: 6499-6504.
- Smith, D.M. 1995. Immunoassays in process control and speciation of meats. *Food Tech.* 49(2):116-119.
- Smith, D.M., and Orta-Ramirez, A. 1995. Enzyme-linked immunosorbent assay technology to verify endpoint cooking temperatures of meat products. *J. Clin. Ligand Assay.* 18:161-165.
- Smith, D.M., Desrocher, L.D., Booren, A.M., Wang, C.H., Abouzied, M.M., Pestka, J.J., and Veeramuthu, G.J. 1996. Cooking temperature of turkey ham affects lactate dehydrogenase, serum albumin and immunoglobulin G as determined by ELISA. *J. Food Sci.* 61:209-212, 234.
- Smith, G.C., Berry, B.W., Savell, J.W., and Cross, H.R. 1988. USDA maturity indices and palatability of beef rib steaks. *J. Food Qual.* 11:1-13.
- Smith, G.L., Alford, E.S., and Keeton, J.T. 1991. Screening of endogenous enzyme activity as a means of assessing previous heat treatment in bovine muscle. Paper no. 454, presented at the 51st Annual Meeting of the Inst. of Food Technologists, Dallas, TX, June 2-5.
- Stalder, J.W., Smith, G.L., Keeton, J.T., and Smith, S.B. 1991. Lactate dehydrogenase activity in bovine muscle as a means of determining heating endpoint. *J. Food Sci.* 56: 895-898.
- Steele, P., and Lambe, W.J. 1982. SDS-gradient gel electrophoresis separation of muscle polypeptides for the estimation of maximum cooking temperatures in meat. *J. Food Protec.* 45:59-62.
- Talmant, A., Monin, G., Briand, M., Dadet, M., and Briand, Y. 1986. Activities of metabolic and contractile enzymes in 18 bovine muscles. *Meat Sci.* 18:23-40.
- Taoukis, P.S., and Labuza, T.P. 1989. Applicability of time-temperature indicators as shelf life monitors of food products. *J. Food Sci.* 54:783-788.

- Townsend, W.E., and Blankenship, L.C. 1989. Methods for detecting processing temperatures of previously cooked meat and poultry products - a review. *J. Food Protec.* 52:128-135.
- Townsend, W.E., and Davis, C.E. 1992. Transaminase (AST/GOT and ALP/GPT) activity in ground beef as a means of determining end-point temperature. *J. Food Sci.* 57:555-557.
- Troutt, E.S., Hunt, M.C., Johnson, D.E., Claus, J.R., Kastner, C.L., Kropf, D.H. 1992. Characteristics of low-fat ground beef containing texture-modifying ingredients. *J. Food Sci.* 57:19-24.
- USDA. 1986a. Determination of internal cooking temperature (Coagulation). In Revised Basic Chemistry Laboratory Guidebook No. 3.019, pp. 3-55. Science Chemistry Div., Food Safety and Inspection Service, U.S. Dept. of Agriculture, Washington, D.C.
- USDA. 1986b. Determination of internal cooking temperature (Acid phosphatase activity). In Revised Basic Chemistry Laboratory Guidebook No. 3.018, pp. 3-49. Science Chemistry Div., Food Safety and Inspection Service, U.S. Dept. of Agriculture, Washington, D.C.
- USDA. 1989. Performing the catalase enzyme test. A self-instruction guide. Technical Services Training Div., Food Safety and Inspection Service, U.S. Dept. of Agriculture, Washington, D.C.
- USDA. 1993. Instructions for verifying internal temperature and holding time of meat patties. FSIS Directive 7370.1, Food Safety Inspection Service, U.S. Department of Agriculture, Washington, DC.
- USDA. 1994. A quick consumer guide to safe food handling and labels. Home Garden Bull. No. 254. Food Safety Inspection Service, U.S. Department of Agriculture, Washington, DC.
- USDA-FSIS. 1996. Performance standards for the production of certain meat and poultry products. Federal Register. 61(86), 19564. U.S. Department of Agriculture, Food Safety Inspection Service, Washington, DC.
- USDA-FSIS. 1997a. "Protecting the Public from Foodborne Illness: The Food Safety and Inspection Service." <<http://www.usda.gov/agency/fsis/mission.htm>> (16 May. 1997).

- USDA-FSIS. 1997b. Heat-processing procedures, cooking instructions, and cooling, handling and storage requirements for uncured meat patties. In *Code of Federal Regulations*, Food Safety & Inspection Service (Meat and Poultry), Ch. III, Part 318.23. Office of the Federal Register, National Archives and Records, Washington, D.C.
- USDA-FSIS. 1997c. "Addendum to Color of Cooked Ground Beef and Juices as it Relates to Doneness: Technical Information from FSIS, April 1996." <<http://www.usda.gov/agency/fsis/befcolr2.htm>> (8 May. 1997).
- USDA-FSIS. 1997d. "Color of Cooked Ground Beef and Juices as it Relates to Doneness." <<http://www.usda.gov/agency/fsis/befcolor.htm>> (8 May. 1997).
- Van Den Oord, A.H.A., and Wesdorp, J.J. 1978. The specific effect of pyrophosphate on protein solubility of meat. 24th European Meeting of Meat Research Workers, Zevenaar, Netherlands, D 13:1(Abstract).
- Van Laack, R.L.J.M., Berry, B.W., and Solomon, M.B. 1996. Variations in internal color of cooked beef patties. *J. Food Sci.* 61:410-414.
- Van Loey, A., Hendrickx, M., Ludikhuyze, L., Weemaes, C., Haentjens, T., De Cordt, S., and Tobback, P. 1995. Potential *Bacillus subtilis* α -amylase-based time-temperature integrators to evaluate pasteurization processes. *J. Food Protec.* 59:261-267.
- Van Loey, A., Hendrickx, M., De Cordt, S., Haentjens, T., and Tobback, P. 1996. Quantitative evaluation of thermal processes using time-temperature integrators. *Trends Food Sci. Tech.* 7(1):16-26.
- Wang, C.H., Abouzied, M.M., Pestka, J.J., and Smith, D.M. 1992. Antibody development and enzyme-linked immunosorbent assay for the protein marker lactate dehydrogenase to determine safe cooking end-point temperatures of turkey rolls. *J. Agric. Food Chem.* 40:1671-1676.
- Wang, C.H., Booren, A.M., Abouzied, M.M., Pestka, J.J., and Smith, D.M. 1993. ELISA determination of turkey roll endpoint temperature: effects of formulation, storage, and processing. *J. Food Sci.* 58:1258-1261, 1264.
- Wang, C.H., Pestka, J.J., Booren, A.M., and Smith, D.M. 1994. Lactate dehydrogenase, serum protein, and immunoglobulin G content of uncured turkey thigh rolls as influenced by endpoint cooking temperature. *J. Agric. Food Chem.* 42: 1829.
- Wang, C.H., Abouzied, M.M., Pestka, J.J., and Smith, D.M. 1996a. Lactate dehydrogenase polyclonal antibody sandwich ELISA for determination of endpoint heating temperatures of ground beef. *J. Food Protec.* 59:51-55.

- Wang, S.F., Abouzied, M.M., and Smith, D.M. 1996b. Proteins as endpoint temperature indicators for ground beef patties. *J. Food Sci.* 61:5-7.
- Webb, M.R., and Knowles, J.R. 1975. The orientation and accessibility of substrates on the active site of triosephosphate isomerase. *Biochem.* 14:4692-4698.
- Wierenga, R.K., and Noble, M.E.M. 1992. Comparison of the refined crystal structures of liganded and unliganded chicken, yeast and trypanosomal triosephosphate isomerase. *J. Mol. Biol.* 224:1115-1126.

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