THERMAL INACTIVATION OF PATHOGENS AND VERIFICATION OF ADEQUATE COOKING IN MEAT AND POULTRY PRODUCTS

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I. INTRODUCTION

Thermal processing is one of the most important means of assuring the safety and quality of the human food supply. The principal effects of the heat treatment include: (1) modification of the organoleptic properties of the raw product, such as color, flavor and texture, and improved digestibility, (2) inactivation of enzymes and spoilage microorganisms to increase the shelf life and (3) destruction of pathogenic bacteria and viruses that may be present in the raw material. It is desirable to verify the adequacy of the thermal treatment, both during the manufacturing process and during cooking preparation by consumers at home. This need can be a critical one as demonstrated by frequent cases of food poisoning arising from undercooked products.

This chapter reviews thermal processing as a means to eliminate microbial pathogens in meat and poultry. Besides discussing the evolution of thermal processing regulations in the US and listing official and alternative tests to verify compliance with the cooking requirements, an effort has been made to evaluate the advantages and disadvantages for each of the verification methods as well as the challenges to determine the thermal inactivation kinetics of microbial pathogens. Topics covered in this chapter include: a brief review of the thermal processing requirements currently implemented in the US; thermal inactivation of most common microbial pathogens found in meat and poultry products, and the use of thermometers, color determination, endpoint temperature indicators, and time-temperature integrators as means of verifying thermal processing adequacy.

II. HISTORY OF CURRENT THERMAL PROCESSING REGULATIONS FOR MEAT AND POULTRY PRODUCTS

Adequate thermal processing or cooking is the most important as well as one of the simplest methods to eliminate pathogenic bacteria and viruses from meat and poultry products to prevent foodborne diseases. Cooking is a commonly employed critical control point in the HACCP program of meat processors and is one of the last control points applied to a food product before it is eaten. Although cooking would seem to be a simple process for pathogen control, undercooked ground beef has been identified as the most frequent vehicle of *E. coli* O157:H7 infection in the United States since 1982 (Todd, 1996; CDC, 1997).

Title 9 of the Code of Federal regulations outlines the thermal requirements for the processing of several meat and poultry products (USDA-

FSIS, 1999). Regulations were provided to ensure destruction of pathogenic bacteria and viruses that may be present in the raw product. After the 1993 outbreak of *E. coli* O157:H7, the government implemented several new thermal processing regulations.

A. FOODSERVICE AND RETAIL OPERATIONS

The Food Code, published by the Food and Drug Administration (FDA), provides recommendations for cooking meat products in foodservice establishments (FDA, 1999). The Food Code recommends that pork products and comminuted meats be cooked to internal temperatures of either 63°C (145°F) for 3 min, 66°C (150°F) for 1 min or 68°C (155°F) for 15 sec. Roast beef and corned beef should be cooked to any of the following time/temperature protocols: 54°C (130°F)/121 min, 56°C (132°F)/77 min, 57°C (134°F)/47 min, 58°C (136°F)/32 min, 59°C (138°F)/19 min, 60°C (140°F)/12 min, 61°C (142°F)/8 min, 62°C (144°F)/5 min or 63°C (145°F)/3 min. Poultry products are to be cooked to 74°C (165°F) or above for 15 sec.

B. HOME COOKING

As far as consumers are concerned, earlier recommendations by USDA were to avoid eating rare ground beef and to cook hamburgers until the internal color was brown (USDA-FSIS, 1985). In 1990, the USDA advised consumers to cook hamburgers to a minimum temperature of 71°C (160°F) (USDA-FSIS, 1990). Because of growing concerns associated with internal color development in ground beef patties (see Section IV.B), more recently the USDA has advised against color measurements and recommended instead the use of a thermometer when cooking hamburgers (USDA-FSIS, 1997a).

C. MEAT PROCESSORS

Prior to 1993, USDA regulations required that cured and uncured poultry products be cooked to an internal temperature of 68.3°C (155°F) and 71.1°C (160°F), respectively. Cooked beef, roast beef and corned beef were required to be heat processed in any of 16 time/temperature schedules with holding time or to 63°C (145°F) with no holding time. No directives were specified for the processing of beef patties and each state was allowed to apply its own regulations.

In 1993, after a multi-state outbreak of *E. coli* O157:H7 in the Pacific US associated with consumption of undercooked ground beef patties,

commercial establishments that manufacture fully-cooked patties were required to use one of the following time/temperature protocols: 66.1°C (151°F)/41 sec, 66.7°C (152°F)/32 sec, 67.2°C (153°F)/26 sec, 67.8°C (154°F)/20 sec, 68.3°C (155°F)/16 sec, 68.9°C (156°F)/13 sec, and 69.4°C (157°F) (and up)/10 sec (USDA-FSIS, 1993). Also, commercial establishments that manufacture partially cooked patties were required to heat patties to a minimum internal temperature of 60°C (140°F) followed by cooling to a maximum internal temperature of 4°C (40°F) within 2 hr (USDA-FSIS, 1993). These schedules were based on destruction of *Salmonella* and *E. coli* O157:H7 (Goodfellow and Brown, 1978; Doyle and Schoeni, 1984).

The USDA-FSIS has recently changed the thermal processing regulations for meat products to include a lethality standard based on destruction of the pathogenic microorganism *Salmonella* (Federal Register, 1999). Performance standards require that commercial establishments produce safe products while allowing each processor to use their own specific plant-processing procedures. Three performance standards were proposed by FSIS: lethality, stabilization and handling. The purpose of the lethality standard was to effectively eliminate pathogenic microorganisms that could be present in the product. Lethality standards are based on destruction of *Salmonella* and are expressed in terms of specific *decimal reductions* or $\log_{10}$ reductions.

Traditionally, *Salmonella* was the microorganism of concern in cooked beef products because of the high number of outbreaks associated with consumption of "rare" roast beef (Goodfellow and Brown, 1978). Although *E. coli* O157:H7 has become of greater concern, especially in meat patties, it is less heat resistant than *Salmonella* (Doyle and Schoeni, 1984; Line *et al.*, 1991; Orta-Ramirez *et al.*, 1997). In addition, although *Listeria monocytogenes* is more heat resistant than *Salmonella*, its incidence in meat products is usually a result of recontamination. Thus, expected levels of *L. monocytogenes* are much lower than those of *Salmonella*. Therefore, the thermal inactivation of *Salmonella* in meat products is indicative of destruction of other pathogenic microorganisms.

Initially, FSIS proposed a process lethality resulting in a 7-$\log_{10}$ reduction in *Salmonella* for cooked beef, roast beef, cooked corned beef and cooked poultry products. It is important to notice that all time/temperature combinations previously required by USDA for cooked/roast beef, cooked corned beef and poultry products would result in a 7-$\log_{10}$ reduction in *Salmonella* (Federal Register, 1996). Therefore, establishments that were already employing any of the above-mentioned time-temperature schedules are producing cooked beef or poultry products that complied with the proposed lethality standards.

In the final rule published in 1999, the USDA required that manufacturers use a combination of thermal and non-thermal processes sufficient to achieve a 6.5-$\log_{10}$ reduction in *Salmonella* in ready-to-eat, cooked beef, roasted beef and cooked corned beef and a 7-$\log_{10}$ reduction in ready-to-eat poultry products (USDA-FSIS, 1999). Alternatively, compliance or safe harbor guidelines listing specific allowed times and temperatures may also be used. Thirty different time-temperature processing schedules are allowed for roast beef, ranging from a minimum internal temperature of 54.4°C with a holding time of 112 min to 71.1°C for 10 sec. Compliance guidelines for cured and uncured poultry products (68.3 and 71.1°C, respectively) remain the same as before.

A thermal process sufficient to cause a 5-$\log_{10}$ reduction in *Salmonella* was initially proposed as the lethality performance standard for fully cooked, uncured meat patties. However, this proposal was not implemented, as it was thought that a 5-$\log_{10}$ reduction process would not provide a sufficient margin of safety (Federal Register, 1999). In February of this year, the USDA-FSIS proposed to amend the meat and poultry regulations by establishing performance standards for all ready-to-eat and all partially heat-treated meat and poultry products, including meat patties. The proposed lethality standard requires "either a reduction in viable *Salmonella* of 6.5-$\log_{10}$ or alternatively one of the following probabilities that no more than small numbers of *Salmonella* would remain in any 100 gram sample of a finished product made from worst case product" (Federal Register, 2001):

> 0 surviving	> 1 surviving	> 2 surviving	> 3 surviving	> 4 surviving
39.4	9.06	1.45	0.177	0.0174

Any processor deviating from the compliance guidelines must provide evidence from "a reliable process authority" that the process selected meets the 6.5-$\log_{10}$ or 7-$\log_{10}$ lethality performance standard (USDA-FSIS, 1999). However, pathogen inactivation models based on isothermal laboratory tests in sealed containers may not predict results in commercial ovens or smokehouses (Marks *et al.*, 1999). Both the cooking environment (wet and dry bulb temperatures) and the water activity or water vapor pressure of a product have an impact on microbial inactivation kinetics. Consequently, inactivation models developed in a laboratory or pilot plant may not accurately predict pathogen lethality in an actual processing environment. More research is needed to develop and validate first-principle cooking models that accurately predict process lethality for a range of cooking conditions.

Another major factor limiting the processor's ability to prove process lethality is their inability to take pathogens into production facilities, and thereby conduct challenge studies on actual processing equipment. Instead, challenge studies need to be performed in a pilot plant that may not have the same ovens or smokehouse used by a particular plant.

According to the recent directive, processing compliance should be documented with a challenge study using a cocktail of *Salmonella* serotypes. The USDA does not specify specific serotypes or the number of serotypes that should be used in the cocktail. Instead, they state that the cocktail should consist of pathogenic strains of *Salmonella* that exhibit relatively high heat resistance and have been previously implicated in foodborne outbreaks (UDSA-FSIS, 1999b).

Although an increase in thermal processing enforcement can help reduce the incidence of foodborne disease, there is still the problem of confirming adequate cooking once the product has been processed. Even with modern and sophisticated technology, there is a need to verify that proper times and temperatures have been achieved for each process.

III. THERMAL INACTIVATION OF MICROBIAL PATHOGENS IN MEAT AND POULTRY PRODUCTS

Microbial contamination is considered the most important hazard associated with foodborne illnesses (Todd, 1996; Mead *et al.*, 1999). From 1988 to 1992, a total of 2423 foodborne outbreaks were recorded, of which 79% were caused by bacterial pathogens (Bean *et al.*, 1997). *Salmonella*, *E. coli* O157:H7 and *Campylobacter* are among the most common causative agents of foodborne illnesses in the US. Inadequate cooking and improper storage and holding temperatures are the most common errors that lead to foodborne outbreaks (Todd, 1996; Mead *et al.*, 1999).

The thermal inactivation of microorganisms has been historically expressed in terms of D and z values (Singh and Heldman, 1993; Hendrickx *et al.*, 1995). These values are calculated assuming microbial inactivation follows first order kinetics. The D value, or thermal reduction time, is defined as the time necessary to reduce a microbial population by 90% and is indicative of the thermal stability of a microorganism at a constant temperature. The z value is the temperature increase necessary to reduce the D value by 90% and measures the temperature dependence of microbial inactivation. The determination of D and z values for a particular microorganism is very useful in designing a thermal process that targets that specific microorganism (Pflug, 1997).

A. THERMAL INACTIVATION KINETICS OF *SALMONELLA*

Salmonella is a microorganism commonly found in the intestinal tract of humans and animals. More than 2000 strains have already been identified. Of those, approximately 150 are associated with disease outbreaks. Although *Salmonella* can be transmitted from person to person, it is believed that the major source is contaminated foods of animal origin.

One method to eliminate *Salmonella* from foods is by cooking (Centers for Disease Control and Prevention, 1998). This microorganism is mildly resistant to high temperatures. Several factors influence the resistance of *Salmonella* to heat. Some strains are more resistant than others. For example, *S.* Senftenberg is unusually heat-resistant. It is considered to be 10–20 times more resistant than the average *Salmonella* spp (Doyle and Cliver, 1990). Other factors influencing heat resistance include composition, water activity and pH of the food that the *Salmonella* is in. This microorganism is more resistant to dry than moist heat and shows higher heat susceptibility at extreme pHs (Schuman and Sheldon, 1997).

The thermal inactivation of *Salmonella* in chicken (Table I), turkey (Table II) and ground beef (Table III) has been the subject of numerous studies. In general, D values of *Salmonella* at 60°C ranged from 5–6 min in chicken, 5–13 min in turkey and 3–5 min in beef. In most studies, the z value of *Salmonella* ranged from 5.0 to 6.5°C.

Goodfellow and Brown (1978) were one of the first to determine D values for *Salmonella* spp in ground beef. Thermal death time experiments were performed using a 6-strain cocktail, which included *S.* Typhimurium, *S.* Newport, *S.* Agona, *S.* Bovis-Morbificans and *S.* Muenchen. The D values at 51.6, 57.2, and 62.7°C were 61–62, 3.8–4.2, and 0.6–0.7 min, respectively, depending on the recovery method used (Table III). The z value was found to be 5.6°C. The results of Goodfellow and Brown were used to design the time-temperature combinations for the cooking of roast beef listed in the USDA thermal processing regulations (USDA-FSIS, 1990).

Orta-Ramirez *et al.* (1997) performed thermal inactivation studies using *S.* Senftenberg in ground beef. The calculated D values were 53.0, 15.72, 2.1 and 0.2 min at 53, 58, 63 and 68°C, respectively, with a z value of 6.2°C (Table III).

Murphy *et al.* (2000) calculated the thermal inactivation kinetics of a 6-strain *Salmonella* cocktail in ground chicken breast patties. The cocktail included *S.* Senftenberg, *S.* Typhimurium, *S.* Heidelberg, *S.* Mission, *S.* Montevideo and *S.* California. The calculated D and z values were in good accordance with those reported by Orta-Ramirez *et al.* (1997), suggesting that the overall heat resistance of a cocktail comprising of several strains

TABLE I

SUMMARY OF PUBLISHED *D* AND *z* VALUES OF *SALMONELLA* SPP IN CHICKEN MEAT

	Juneja *et al.* (2001)		Juneja *et al.* (2001)		Murphy *et al.* (2000)	
Strain(s)	Cocktail[1]		Cocktail[1]		Cocktail[2]	
Fat content (%)	7		7		0.12	
pH	6.0		6.0		NR[3]	
Recovery method	TSA Supplemented[4]		TSA Supplemented[4]		TSB-N Agar[5]	
Heating vessel	Stomacher bags		Stomacher bags		Thin wall metal tubes[5]	
Regression type	Linear		Non linear		Linear	
D values (min)	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*
	58.0	7.1	58.0	7.1	55.0	30.1
	60.0	5.2	60.0	5.2	57.5	12.9
	62.5	1.4	62.5	1.4	60.0	5.9
	65.0	0.6	65.0	0.5	62.5	2.5
					65.0	1.2
					67.5	0.3
					70.0	0.2
z value (°C)	6.1		8.8		6.5	

[1] *S.* Thompson, *S.* Typhimurium, *S.* Enteritidis, *S.* Hadar, *S.* Copenhagen, *S.* Montevideo and *S.* Heidelberg.

[2] *S.* Senftenberg, *S.* Typhimurium, *S.* Heidelberg, *S.* Mission, *S.* Montevideo and *S.* California.

[3] Not reported.

[3] Trypticase soy agar with 0.6% yeast extract and 1% sodium pyruvate.

[5] Containing 200 ppm nalidixic acid, sodium salt.

[6] 8.23 × 152.4 mm.

may be dictated by the strain showing the highest heat resistance (in this case, *S.* Senftenberg).

More recently, Juneja *et al.* (2001) completed thermal death time studies in chicken, turkey and beef using a *Salmonella* cocktail which included eight representative and appropriate strains according to USDA-FSIS recommendations (USDA-FSIS, 1999). These recommendations stipulated that the strains should be relatively heat resistant and among those historically implicated in foodborne outbreaks. The strains included were *S.* Thompson, *S.* Typhimurium, *S.* Enteritidis, *S.* Hadar, *S.* Copenhagen, *S.* Montevideo and *S.* Heidelberg. There was no correlation between heat resistance and strain origin due to the high variation in heat

TABLE II

SUMMARY OF PUBLISHED *D* AND *z* VALUES OF *SALMONELLA* SPP IN TURKEY MEAT

	Veeramuthu *et al.* (1998)	Veeramuthu *et al.* (1998)	Juneja *et al.* (2001)	Juneja *et al.* (2001)	Maurer *et al.* (2000)	Maurer *et al.* (2000)	Maurer *et al.* (2000)	Maurer *et al.* (2000)	Maurer *et al.* (2000)	Maurer *et al.* (2000)
Strain(s)	*S.* Senftenberg	*S.* Senftenberg	Cocktail[1]	Cocktail[1]	*S.* Senftenberg	*S.* Senftenberg	*S.* Senftenberg	*S.* Senftenberg	*S.* Senftenberg	*S.* Senftenberg
Type of meat	Thigh	Thigh			Thigh	Thigh	Breast	Breast	Breast + 1% NaCl	Breast + 2% NaCl
Fat content (%)	4.3	4.3	6.0	6.0	5.3	7.4	0.6	4.4	4.4	4.4
Recovery method	Coliform petrifilms	Phenol red sorbitol agar	TSA supplemented[2]	TSA supplemented[2]	Aerobic petrifilms	Aerobic petrifilms	Aerobic petrifilms	Aerobic petrifilms	Aerobic petrifilms	Aerobic petrifilms
Heating vessel	TDT tubes[3]	TDT tubes[3]	Stomacher bags	Stomacher bags	Plastic pouches[4]	Plastic pouches[4]	Plastic pouches[4]	Plastic pouches[4]	Plastic pouches[4]	Plastic pouches[4]
pH	6.3	6.3			6.2	6.2	5.8	5.7	5.7	5.7
Regression type	Linear	Linear	Linear	Non linear	Linear	Linear	Linear	Linear	Linear	Linear
D values (min)	*T* (°C) *D*	*T* (°C) *D*	*T* (°C) *D*	*T* (°C) *D*	*T* (°C) *D*	*T* (°C) *D*	*T* (°C) *D*	*T* (°C) *D*	*T* (°C) *D*	*T* (°C) *D*
	55.0 211.4	55.0 227.2	58.0 7.4	58.0 7.2	58 9.4	58 11.6	58 8.8	58 10.7	58 11.6	58 12.6
	60.0 13.2	60.0 13.6	60.0 4.8	60.0 4.8	61 3.1	61 4.6	61 2.6	61 4.2	61 3.3	61 4.6
	65.0 3.4	65.0 3.1	62.5 1.5	62.5 1.5	64 0.6	64 0.7	64 0.6	64 1.1	64 0.6	64 1.6
			65.0 0.8	65.0 0.7	66 0.2	66 0.2	66 0.2	66 0.4	66 0.3	66 0.5
z value (°C)	5.6	5.4	6.9	9.0	5.3	5.1	5.3	6.2	5.5	6.5

[1] *S.* Thompson, *S.* Typhimurium, *S.* Enteritidis, *S.* Hadar, *S.* Copenhagen, *S.* Montevideo and *S.* Heidelberg.

[2] Trypticase soy agar with 0.6% yeast extract and 1% sodium pyruvate.

[3] Thermal Death Time tubes (10 × 75 mm).

[4] 5 × 25.5 cm polyethylene laminated nylon bags.

TABLE III

SUMMARY OF PUBLISHED *D* AND *z* VALUES OF *SALMONELLA* SPP IN GROUND BEEF

	Goodfellow and Brown (1984)		Orta-Ramirez *et al.* (1997)		Juneja *et al.* (2001)		Juneja *et al.* (2001)		Smith *et al.* (2001)		Smith *et al.* (2001)	
Strain(s)	Cocktail[1]		*S.* Senftenberg		Cocktail[2]		Cocktail[2]		Cocktail[2]		Cocktail[2]	
Fat content (%)	NR[3]		4.3		12.5		12.5		19.1		19.1	
pH	NR[3]		6.3		6.0		6.0		5.7		5.7	
Growth phase	Log		Log		Log		Log		Log		Stationary	
Recovery method	PCA[4]/XL agar		Coliform petrifilms		TSA supplemented[5]		TSA supplemented[5]		Aerobic petrifilms		Aerobic petrifilms	
Heating vessel	TDT tubes[6]		TDT tubes[7]		Stomacher bags		Stomacher bags		Plastic pouches[8]		Plastic pouches[8]	
Regression type	Linear		Linear		Linear		Non linear		Linear		Linear	
D values (min)	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*
	55.0	61	53.0	53.0	58	8.7	58.0	8.9	55.0	16.3	55.0	18.7
	60.0	3.8	58.0	15.2	60	5.5	60.0	5.3	58.0	2.7	58.0	3.4
	65.0	0.6	63.0	2.1	62.5	1.5	62.5	1.5	61.0	0.4	61.0	0.6
			68.0	0.2	65	0.7	65.0	0.5	63.0	0.2	63.0	0.2
z value (°C)	5.6		6.2		6.0		9.1		3.9		4.1	

[1] *Salmonella*, *S.* Typhimurium, *S.* Newport, *S.* Agona, *S.* Bovis-morbificans and *S.* Muenchen.
[2] *S.* Thompson, *S.* Typhimurium, *S.* Enteritidis, *S.* Hadar, *S.* Copenhagen, *S.* Montevideo and *S.* Heidelberg.
[3] Non reported.
[4] Plate Count Agar.
[5] Trypticase soy agar with 0.6% yeast extract and 1% sodium pyruvate.
[6] Thermal Death Time tubes (10 × 60 mm).
[7] Thermal Death Time tubes (10 × 75 mm).
[8] 5 × 25.5 cm polyethylene laminated nylon bags.

resistance among strains. The calculated *D* values in ground beef (Table III) were lower and the *z* values were higher than those reported by Goodfellow and Brown (1978) and Orta-Ramirez *et al.* (1997), while the *D* values in chicken were lower than those reported by Murphy *et al.* (2000).

Differences in *D* values reported by various researchers are due to differences in the composition of the meat and to the use of different recovery methods employed for *Salmonella* enumeration. In addition, differences in size and volume of the inoculated meat samples may affect the calculation of the thermal inactivation parameters (see Section III.D.3).

The growth phase as well as the physiological state of the organisms (starvation, heat injury) may also influence the thermal inactivation kinetics of *Salmonella*. Overall, bacterial cells in stationary phase have shown more thermotolerance than those in exponential or log phase. Heddleson *et al.* (1991) observed maximum heat resistance of *Salmonella* in phosphate buffer during stationary phase. Smith *et al.* (2001) compared the thermal inactivation of *Salmonella* cocktail in log and stationary phase. The *D* values in 19.1% fat ground beef at 55, 58, 61 and 63°C were 16.3, 2.7, 0.4 and 0.2 min, respectively, for the log phase, and 18.7, 3.4, 0.6 and 0.2 min, respectively, for the stationary phase (Table III).

Cell starvation, thermal injury, and heat shocking prior to thermal inactivation studies can also affect the thermal inactivation of *Salmonella*. Little information can be found on the effect of starvation on the heat inactivation of microorganisms in meat or poultry products. Smith *et al.* (2001) stored a *Salmonella* cocktail in 0.1% peptone water at 4°C for 14 days. The starved cells showed reduced heat resistance in ground beef when compared with non-starved cultures.

Thermally injured cultures are those that can go undetected because they do not grow on selective media, but can be recovered on non-selective media. These cells can pose a hazard if present in a food product since they may not be detected with normal enumeration procedures. The effect of heat shock on thermal inactivation kinetics of *Salmonella* can also be of importance in thermally processed products. Heat shock occurs when the bacterial cells have been exposed for a short period to temperatures higher than the normal growth temperature range. Cells exposed to high but nonlethal temperatures develop or acquire some thermotolerance (Bunning *et al.*, 1990). It has been speculated that this increased thermotolerance is due to a specific set of proteins (i.e. heat shock proteins) that induce recovery of injured cells (Juneja *et al.*, 1998).

Bunning *et al.* (1990) reported a 1 to 2 min increase in the *D* value of *S.* Typhimurium in broth culture at 58.7°C when cells were heat shocked

for 30 min at 48°C prior to the thermal inactivation experiment. Furthermore, the *D* value at 52°C increased from 21.3 in non-heat shocked to 96.1 min in heat shocked cells. Mackey and Derrick (1987) also found increased thermotolerance in heat shocked *Salmonella* spp. in minced beef. Smith (2000) studied the effect of thermal injury and heat shock in an 8-strain *Salmonella* cocktail in 19.1% fat ground beef. The *D* values of the thermally injured and heat shocked cultures did not statistically differ from one another at the four temperatures tested. The *D* values at 55, 61 and 63°C for the heat shocked cultures did not differ from those of the non-heat shocked cocktail, but the *D* value at 58°C was lower in the heat shocked cocktail.

B. THERMAL INACTIVATION KINETICS OF *ESCHERICHIA COLI* O157:H7

Escherichia coli is commonly found in the intestinal tract of humans and most mammals. A few strains, however, are pathogenic and can cause gastrointestinal syndromes. Among them, *E. coli* O157:H7 has become very important due to its role in several foodborne disease outbreaks since the 1980s. Thus, several researchers have studied the thermal inactivation of this microorganism in chicken (Table IV), turkey (Table V) and ground beef (Table VI). *D* values at 60°C ranged from 0.4–1.6 min in chicken, 0.6–10 min in turkey and 0.5–3 min in beef. In most studies, the *z* value ranged from 4–6°C.

Differences in reported *D* values can be attributed to meat composition, strain of *E. coli* O157:H7 used and the plating method employed to recover the microorganism. Also, the size and volume of the inoculated meat samples may have an impact in the calculation of the thermal inactivation parameters (Section III.D.3). Doyle and Schoeni (1984) pioneered the thermal inactivation studies of *E. coli* O157:H7. They calculated the *D* and *z* values in 20% fat containing ground beef. The calculated *D* values ranged from 39.8 min at 54.4°C to 0.2 min at 64.3°C with a *z* value of 4.1°C (Table VI). The thermal inactivation parameters for *E. coli* O157:H7 in ground beef reported by Doyle and Schoeni (1984) were used to establish command and control regulations for processing of beef patties (USDA-FSIS, 1995).

Line *et al.* (1991) and Ahmed *et al.* (1995) studied the effect of fat content in meat on the thermal inactivation of *E. coli* O157:H7. Increasing the fat content of the meat resulted in higher *D* values at all temperatures tested (Table VI). Juneja *et al.* (1997) performed thermal death time studies using a 4-strain *E. coli* O157:H7 cocktail in chicken (Table IV) and ground beef (Table VI). The *D* values ranged from 11.6 min at 55°C to 0.2 min at 65°C, in chicken and 21.1 min at 55°C to 0.4 min at 65°C in

TABLE IV
SUMMARY OF PUBLISHED *D* AND *z* VALUES OF *ESCHERICHIA COLI* O157:H7 IN CHICKEN

	Ahmed *et al.* (1995)		Ahmed *et al.* (1995)		Juneja *et al.* (1997)		Juneja *et al.* (1997)	
Fat content (%)	3		11		NR[1]		NR[1]	
pH	NR[1]		NR[1]		6.0		6.0	
Recovery method	Phenol red sorbitol agar		Phenol red sorbitol agar		TSA[2]/Sorbitol McConkey		TSA[2]/Sorbitol McConkey	
Heating vessel	TDT tubes[3]		TDT tubes[3]		Whirl-pak bags[4]		Whirl-pak bags[4]	
Regression type	Linear		Linear		Linear		Non linear	
D values (min)	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*
	50.0	65.2	50.0	105.5	55.0	11.8	55.0	11.6
	55.0	8.8	55.0	9.7	57.5	3.8	57.5	3.3
	60.0	0.4	60.0	0.6	60.0	1.6	60.0	1.6
					62.5	0.8	62.5	0.5
					65.0	0.4	65.0	0.2
z value (°C)	4.5		4.4		6.8		5.8	

[1] Non reported.
[2] Trypticase soy agar.
[3] Thermal Death Time tubes (non-specified dimensions).
[4] 15 × 22.9 cm.

ground beef, respectively. The *D* values reported by Orta-Ramirez *et al.* (1997) in ground beef ranged from 46.1 min at 53°C to 0.12 min at 68°C, with a *z* value of 5.6°C.

When a 4-strain *E. coli* O157:H7 cocktail was heat shocked for 15–30 min at 46°C, there was a 6.4 log decrease in colony forming units (CFU)/g after 15 min at 60°C, while non-heat shocked cells decreased by 7.8 logs under same conditions. Heat shocking increased the *D* values by 37, 68 and 50% at 54, 58 and 62°C, respectively, in tryptic soy broth but did not affect the *D* value at 58°C in a ground beef slurry (Williams and Ingham, 1997).

C. THERMAL INACTIVATION KINETICS OF *LISTERIA* AND *CAMPYLOBACTER*

1. Listeria

Listeria microorganisms are motile Gram-positive non-sporeforming rod-like bacteria found in soil, water and other environmental sources.

TABLE V

SUMMARY OF PUBLISHED *D* AND *z* VALUES OF *ESCHERICHIA COLI* O157:H7 IN TURKEY

	Ahmed *et al.* (1995)	Ahmed *et al.* (1995)	Veeramuthu *et al.* (1998)	Veeramuthu *et al.* (1998)
Type of meat	NR[1]	NR[1]	Thigh	Thigh
Fat content (%)	3	11	4.3	4.3
pH	NR[1]	NR[1]	6.3	6.3
Recovery method	Phenol red sorbitol agar	Phenol red sorbitol agar	Coliform petrifilms	Phenol red sorbitol agar
Heating vessel	TDT tubes[2]	TDT tubes[2]	TDT tubes[3]	TDT tubes[3]
Regression type	Linear	Linear	Linear	Linear
D values (min)	*T* (°C) *D*	*T* (°C) *D*	*T* (°C) *D*	*T* (°C) *D*
	50.0 70.4	50.0 115.0	55.0 79.5	55.0 118.9
	55.0 6.4	55.0 9.7	60.0 5.5	60.0 10.0
	60.0 0.6	60.0 0.6	65.0 1.7	65.0 2.1
z value (°C)	4.7	4.4	6.0	5.7

[1] Non reported.

[2] Thermal Death Time tubes (non-specified dimensions).

[3] Thermal Death Time tubes (10 × 75 mm).

Although six species of *Listeria* have been recognized, *L. monocytogenes* is the primary pathogenic species (Jay, 1996).

Listeria can be eliminated from food by cooking or pasteurization. Boyle *et al.* (1990) calculated *D* values for *L. monocytogenes* Scott A in a meat slurry (20% ground beef, 80% water). The *D* values at 60, 65 and 70°C were 2.54, 0.75 and 0.23 min, respectively. Zaika *et al.* (1990) studied the thermal resistance of *L. monocytogenes* Scott A during the processing of frankfurters. The authors concluded that cooking of frankfurters for 70 min to achieve an internal temperature of 71°C was adequate to reduce *Listeria* populations by 3 log cycles. Fain *et al.* (1991) calculated *D* and *z* values for *L. monocytogenes* Scott A in lean (2.0% fat) and high fat (30.5%) ground beef. *D* values at 51.7, 57.2 and 62.8°C were 56.1 and 34.5, 2.4 in lean beef and 4.6, and 0.5 and 1.1 min in high fat beef. The *z* values were 5.4 and 7.3°C in lean and fatty beef, respectively. Doherty *et al.* (1998) reported *D* values of 3.14 and 0.33 min at 55 and 60°C, respectively, for *L. monocytogenes* in minced beef heated in vacuum bags. These results indicate that *L. monocytogenes* is less heat resistant than many *Salmonella* spp.

TABLE VI

SUMMARY OF PUBLISHED *D* AND *z* VALUES OF *ESCHERICHIA COLI* O157:H7 IN GROUND BEEF

	Doyle and Schoeni (1984)		Line *et al.* (1991)		Ahmed *et al.* (1995)		Juneja *et al.* (1997)		Juneja *et al.* (1997)		Orta-Ramirez *et al.* (1997)		Smith *et al.* (2001)		Smith *et al.* (2001)	
Fat content (%)	17–20		2		10		10		10		4		5		19	
pH	NR[1]		NR[1]		NR[1]		6.0		6.0		6.0		5.7		5.7	
Recovery method	TSA[2]/ McConkey		PCA[3]/1% sodium pyruvate		Phenol red agar/1% sorbitol		TSA[2]/ Sorbitol McConkey		TSA[2]/ Sorbitol McConkey		Coliform petrifilms		Aerobic petrifilms		Aerobic petrifilms	
Heating vessel	TDT tubes[4]		TDT tubes[5]		TDT tubes[6]		Whirl-pak bags[7]		Whirl-pak bags[7]		TDT tubes[5]		Plastic pouches[8]		Plastic pouches[8]	
Regression type	Linear		Linear		Linear		Linear		Non linear		Linear		Linear		Linear	
D values (min)	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*	*T* (°C)	*D*
	54.4	39.8	51.7	78.2	50.0	80.7	55.0	21.1	55.0	20.5	53.0	46.1	55.0	20.1	55.0	30.1
	57.2	4.5	57.2	4.1	55.0	15.3	57.5	5.0	57.5	2.7	58.0	4.1	58.0	1.2	58.0	1.2
	58.9	1.7	62.8	0.3	60.0	0.5	60.0	3.2	60.0	0.6	63.0	0.4	61.0	0.3	61.0	0.4
	60.0	0.8					62.5	0.9	62.5	0.4	68.0	0.1	63.0	0.2	63.0	0.1
	62.8	0.4					65.0	0.4	65.0	0.2						
	64.3	0.2														
z value (°C)	4.1		4.6		4.4		6.0		4.9		5.6		3.8		3.5	

[1] Not reported.
[2] Trypticase soy agar.
[3] Plate count agar.
[4] Thermal Death Time tubes (10 × 75 mm).
[5] Thermal Death Time tubes (10 × 60 mm).
[6] Thermal Death Time tubes (non-specified dimensions).
[7] 15 × 22.9 cm.
[8] 5 × 25.5 cm polyethylene.

Juneja and Eblen (1999) studied the interaction of temperature, pH, NaCl and sodium pyrophosphate on the heat inactivation of a 4-strain cocktail of *L. monocytogenes* in beef gravy. NaCl had a protective effect, whereas sodium pyrophosphate and low pH decreased the heat resistance of the microorganism. Overall, the combination of all four factors affected the thermal inactivation of *L. monocytogenes*.

Thermal injury and heat shock may also affect heat resistance of *L. monocytogenes*. Farber and Brown (1990) inoculated a sausage mix with *L. monocytogenes* that had been previously heat shocked at 48°C for 30, 60 or 120 min. While cells that were heat shocked for 30 or 60 min did not exhibit an increased thermotolerance, those heat shocked for 120 min showed up to 2.4-fold increase in the *D* value at 64°C.

Stephens *et al.* (1994) tested the effect of heating rate on the thermal inactivation of *L. monocytogenes* in tryptic phosphate broth. Heating rates ranged from 5.0 to 0.3°C/min. Maximum thermotolerance was observed at heating rates ≤ 0.7/min. The authors suggested that the mechanism of this acquired thermotolerance was similar to that induced by the heat shock proteins.

2. *Campylobacter*

Campylobacter has become the leading agent of diarrheal disease. It has been estimated that 5–14% of diarrheal disease worldwide is due to *Campylobacter jejuni*, *C. fetus* and *C. coli* (National Institute of Allergy and Infectious Diseases, 1998). The disease is mostly associated with handling raw poultry or consumption of undercooked meat and poultry products.

Campylobacteriosis is easily prevented by thorough cooking, avoiding cross-contamination, and safe food handling (USDA-FSIS, 1997b). The microorganism is sensitive to heat, but freezing may not effectively destroy bacteria present in a food (USDA-FSIS, 1997b). The *D* values for *C. jejuni* strain H-840 in ground chicken at 49, 51, 53, 55 and 57°C were 20.5, 8.77, 4.85, 2.12 and 0.79 min, respectively (Blankenship and Craven, 1982). Since *C. jejuni* has lower *D* values than *Salmonella*, the authors concluded that cooking procedures that destroy *Salmonella* would also destroy *C. jejuni*. Stern and Kotula (1982) reported complete inactivation of *C. jejuni* when ground beef was cooked to an internal temperature of 70°C. Gill and Harris (1984) studied the thermal destruction of *C. jejuni* in hamburgers and chicken. The authors remarked that the microorganism showed little thermotolerance and minimal cooking of beef patties eliminated the microorganism.

D. CHALLENGES IN DETERMINING THERMAL INACTIVATION KINETICS OF MICROORGANISMS

The determination of thermal inactivation parameters for a particular microorganism is very useful in designing a thermal process that targets this specific microorganism (Pflug, 1997). Proper calculation of microbial inactivation is necessary to ensure safety during thermal processing of meat products. To comply with the new lethality performance standards, the thermal inactivation parameters of a *Salmonella* cocktail must be established in the food of interest prior to selecting a thermal process. There are several challenges that must be considered when determining the thermal inactivation parameters of microorganisms in food systems.

1. The shape of microbial curves

Traditionally, the thermal inactivation of microorganisms has been assumed to follow first-order kinetics, and methods for estimating the safety of commercial thermal processes are based on this assumption (Kormendy and Kormendy, 1997; Peleg and Cole, 1998). Previously reported D and z values for *Salmonella* (Goodfellow and Brown, 1978) and *E. coli* O157:H7 (Doyle and Schoeni, 1984; Line *et al.*, 1991; Orta-Ramirez *et al.*, 1997) were calculated assuming first-order kinetics (Tables I–VI). More recently, however, research has shown that, in many cases, microbial inactivation kinetics deviate from linearity (Kormendy and Kormendy, 1997; Peleg and Cole, 1998). Whiting (1993) explained that, frequently, the presence of a subpopulation of more heat-resistant bacteria (i.e., inactivation rate is slower than the rest of the population) might account for the tailing of the survival curve, although many other causes for non-linearity have been cited. Peleg and Cole (1998) analyzed previously published survival curves of several microorganisms, including *S.* Typhimurium, and demonstrated that microbial inactivation did not follow first-order kinetics, and, in fact, the reaction orders for most of the cases were different from unity.

Peleg and Cole (1998) also showed that when the order of the reaction (n) is less than 1 ($n < 1$) the semilogarithmic survival curve has an upward concave shape, while if $n > 1$ a downward concave curve results (Figure 1). If the linear approach to calculate D values is to be applied to concave curves, the D value will be overestimated when $n > 1$, while underestimation will occur in cases when $n < 1$ (Peleg and Cole, 1998). Underestimation of a D value could lead to foodborne illness due to undercooking, whereas overestimation could lead to a decrease in product quality due to overcooking.

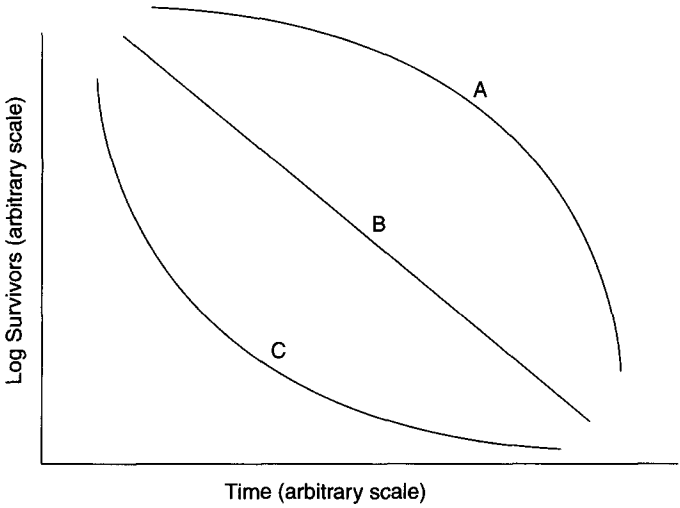

FIG. 1. Shape of survival curves of microbial populations: (A) with reaction order $n > 1$; (B) with $n = 1$; (C) with $n < 1$.

The recent USDA directive describing the new lethality standards for roast beef (6.5-$\log_{10}$ reduction) and poultry (7-$\log_{10}$ reduction), and the proposed standards for beef patties (5-$\log_{10}$ reduction) are designed assuming all *Salmonella* serotypes will be inactivated following first order reaction kinetics. This problem is exacerbated by the suggestion to use a cocktail of *Salmonella* serotypes when verifying USDA processes. Each *Salmonella* serotype has distinctive inactivation kinetics and thus, different D and z values. In a mixed cocktail of *Salmonella* the inactivation kinetics may not be linear (Kormendy *et al.*, 1998). This lack of linearity can lead to over- or underestimation of process lethality and potential for incomplete destruction of pathogens in cooked beef patties. Obviously, this has serious implications for both consumers and meat processors. Underestimation may pose a hazard to the consumer because of the possible foodborne contamination. But, also, overestimation and subsequently, over cooking, may result in loss of quality and nutritional value of the final product, while increasing the cost of production due to unnecessary higher energy consumption.

2. *The use of mathematical models*

Mathematical models have been used to describe bacterial growth, inactivation and survival or to predict effects of environmental conditions on microorganisms during storage of foods (Whiting and Buchanan, 1994).

Whiting (1993) and Buchanan *et al.* (1994) described a curve-fitting model to follow microbial survival under unfavorable conditions of salt and pH. The model was designed to explain microbial lag periods and/or tailing of more resistant subpopulations which result in non-linear curves. Using this model, recent studies reported *D* and *z* values for *E. coli* O157:H7 (Juneja *et al.*, 1997) and an 8-strain *Salmonella* cocktail (Juneja *et al.*, 2001) in meat and poultry products. The mathematical expression involved a combination of two linear regression analyses to determine the *D* value of the main microbial population and a more resistant subpopulation. Large differences in both *D* and *z* values for *Salmonella* (Table III) and *E. coli* O157:H7 (Table VI) in ground beef occurred when results were calculated using both linear and non-linear models.

Linton *et al.* (1995) tested a modified Gompertz equation to model survival curves of *L. monocytogenes* Scott A at different temperatures, pHs and NaCl concentrations. According to the authors, the equation was capable of fitting both linear and non-linear survival curves. Blackburn *et al.* (1997) used a log-logistic function to fit non-linear survival curves of *S.* Enteritidis and *E. coli* O157:H7 as affected by temperature and NaCl concentration. When the models were compared to previously published thermal destruction data, 70% of linear kinetics studies predicted faster inactivation for *S.* Enteritidis and 93% for *E. coli* O157:H7. The authors concluded that the log-logistic function was able to account for deviations from linearity of microbial survival curves.

Zanoni *et al.* (1997) developed a computer program combining Whiting and Buchanan's microbial survival model and heat and mass transfer equations to model thermal processing of bologna sausages. The model was tested in thermal processing experiments using bologna inoculated with *Enterococcus faecium*. According to the authors, the model was able to predict thermal inactivation of the microorganism more accurately than was the linear kinetics equation. Peleg and Cole (1998) have defined survival curves as "cumulative forms of a temporal distribution of lethal events." Following this approach, they described previously published thermal inactivation data for different microorganisms using the Weibull distribution.

Non-linear models may be more valuable and accurate than traditional linear equations in describing destruction of pathogenic microorganisms during cooking. However, the success of any model depends on accuracy of parameter estimates used in such a model. In addition, one must be aware of limitations due to the type of heat resistance data collected to develop the model (i.e. no extrapolations should be made outside the range of collected data). More research is needed to develop quantitative population based models to describe the thermal inactivation of *Salmonella* in meat and poultry products.

3. The size, volume and composition of samples in thermal inactivation studies

Earlier studies of thermotolerance of *Salmonella* (Goodfellow and Brown, 1978; Orta-Ramirez *et al.*, 1997; Veeramuthu *et al.*, 1998) and *E. coli* O157:H7 (Doyle and Schoeni, 1984; Ahmed *et al.*, 1995; Orta-Ramirez *et al.*, 1997; Veeramuthu *et al.*, 1998) were performed in thermal death time tubes (Tables I–VI). These tubes are usually 10 mm in diameter. The heating lag time (i.e. time necessary for the center of the sample to reach the target temperature) during isothermal experiments can be considerable at the low temperatures. On the other hand, at high temperatures, destruction of microorganisms occurs in the outside of the sample close to the tube wall, while the center has not yet reached the target temperature. Thus the D and z values calculated using 10 mm diameter tubes may lead to miscalculation of microbial lethality.

In an effort to produce more accurate thermal inactivation parameters, more recently researchers have adopted the use of meat-filled plastic pouches that are flattened to a thickness of 1–2 mm (Juneja *et al.*, 1997; Juneja *et al.*, 2001; Smith *et al.*, 2001; Maurer *et al.*, 2000). Thus the heat transfer across the pouches is optimized while the heating lag time is minimized.

The size of samples in heat inactivation studies has to be considered as well: the bigger the sample, the higher the risk that heating may not be homogeneous. Hence, variations in temperature across the sample may lead to cooler spots where microorganisms are not destroyed, therefore interfering with proper calculation of kinetic parameters. In addition, composition, water activity and pH of the meat utilized in the studies are of great importance. Variations in these parameters will result in alteration of heat inactivation kinetics of microorganisms.

IV. VERIFICATION OF THERMAL PROCESSING ADEQUACY

A. THERMOCOUPLES AND THERMOMETERS

Current regulations for commercial establishments require that the temperature and time of cooking be verified on one patty from each production line each hour using a temperature measuring device accurate within ±1°F (USDA-FSIS, 1998a). It is extremely difficult to accurately use thermometers or thermocouple probes in beef patties. It has been found that the heterogeneous nature of ground beef leads to large differences in heat and mass transfer and thus different heating rates within a

patty (Pan and Singh, 1998). Berry *et al.* (1999) found that the internal temperature of beef patties may vary as much as 18°C when the center temperature was 71°C. Thus a patty cooked to 71°C may have a cold spot as low as 53°C.

Taken together, these results suggest that single point temperature determinations taken at 1 hr intervals in a commercial processing environment can result in large errors leading to the incomplete destruction of pathogens and an unsafe food product. To overcome this problem, commercial processors are overcooking beef patties by as much as 20°C to ensure destruction of pathogens. Unfortunately, overcooking often leads to tough, dry patties or other quality problems.

Currently, the USDA-FSIS advises consumers to use a meat thermometer to ensure proper cooking of meat and poultry products (USDA-FSIS, 1997a). More recently, the USDA-FSIS (1998b) has released guidelines for the use of thermometers to cook ground beef patties, including procedures for the calibration of the thermometer before use. However, research by a focus group revealed certain barriers that limit use of these devices by consumers (USDA-FSIS, 1998b). According to the study, consumers are more likely to use thermometers when cooking big products, such a turkey or roast, rather than for smaller ones like hamburger patties.

B. COLOR DETERMINATION

Typically, internal meat color changes from pink to gray-brown with cooking. Although consumers seem to rely on cooked color to evaluate degree of doneness, research has shown that residual color may not be a good indicator of proper meat processing. Two major problems associated with ground beef patties have been identified: (1) prevalence of pink color regardless of endpoint temperature during cooking, and (2) premature browning.

Several authors have demonstrated a condition in which internal color of ground beef patties appears pink after cooking well above the USDA recommended temperature of 71°C. Mendenhall (1989) reported persistence of red color in beef patties cooked to 71°C when the pH of the meat was higher (6.2) than the normal pH range (5.3–5.7). The red-pink color was also more pronounced in patties with high concentration of total pigment, such as bull meat. Van Laack *et al.* (1996a) studied variations in internal color of commercially formulated beef patties cooked to 71°C. Eight out of 17 patties showed pink color which was attributed to incomplete denaturation of myoglobin at high pH. When the same products

were analyzed after one year of frozen storage, 16 out of 17 had pink/red color after cooking to 71°C. The red/pink color disappeared after cooking to 81–87°C. It was concluded that several unknown factors may influence color formation and myoglobin denaturation during cooking. In a related study, Van Laack *et al.* (1997) studied the residual color of patties prepared from normal or high pH beef and lean finely textured beef. Addition of lean finely textured beef to high pH beef resulted in a more well-done color that was associated with increased myoglobin denaturation. According to the authors, since the textured beef has a relatively high pH (6.2), results confirmed that although pH influences cooked color, other factors may have an effect as well. Berry (1998) investigated the effect of fat content and frozen/thawed state on color development in high pH beef patties cooked to 71°C. Neither fat content nor cooking from the frozen stated had much influence in brown color formation. All patties in the study had pink color. The author suggested that processors should minimize the use of high pH meat for ground beef processing. Because high temperatures are needed to reduce pink color that remains after cooking to 71°C, Berry and Bigner-George (1999) evaluated the properties of ground beef patties cooked to internal temperatures of 81–85°C. Although cooking yields and juiciness were lower than those of patties cooked to 71°C, no major detrimental changes in sensory properties or tenderness were found.

Premature browning is a condition in which the internal color of cooked patties is brown at temperatures below the USDA recommended temperature of 71°C. Marksberry (1990) observed early formation of cooked color in ground beef from old animals (D/E maturity carcasses) as compared to young animals (A maturity). When patties from D/E-maturity carcasses were evaluated using instrumental measurements, color did not differ at cooking temperatures between 55–77°C. The authors concluded that color was not an accurate indicator of adequate cooking for ground beef from old carcasses. Hague *et al.* (1994) evaluated internal meat color and color of expressible juices of ground beef patties as influenced by endpoint temperature and raw meat source. Redness decreased with increasing temperature from 55 to 77°C. Some patties, however, showed premature browning and, in general, juices never ran clear. Raw meat source had little effect on internal cooked color. The authors concluded that visual evaluation of ground beef color was not a reliable indicator of degree of doneness.

Ang and Huang (1994) studied color changes in chicken leg patties cooked to different endpoint temperatures. Although some differences could be seen among patties, both packaging method and storage time influenced color values markedly. Van Laack *et al.* (1996b) evaluated the

effect of precooking (thawing and packaging) conditions on color of ground beef patties cooked to 71°C. Internal color was influenced by thawing and packaging of patties and the effects were attributed to changes in the chemical form of myoglobin. Premature browning was observed in patties that had been thawed before cooking, but not in those cooked while still frozen. The authors strongly recommended against the use of color as an indicator of endpoint temperature.

Warren *et al.* (1996) looked at the myoglobin oxidative state in beef patties cooked to 55°C. Normal and prematurely brown patties contained oxidized or reduced myoglobin before cooking. The oxidative state of myoglobin influenced internal color of patties but did not affect the color of expressible juices. Oxidation resulted in a brown color whereas reduction yielded a red/purplish color. The authors concluded that conditions promoting myoglobin oxidation would result in premature browning of ground beef during cooking. Hunt *et al.* (1999) studied the effects of myoglobin forms on cooked color development in ground beef patties cooked between 55 and 75°C. Patties containing mainly oxy- or metmyoglobin showed brown color at temperatures as low as 55°C. It was concluded that internal cooked color could be used to assess proper cooking only when deoxymyoglobin was the predominant pigment in ground beef at the time of cooking. Limited effects due to cooking at high temperatures were attributed to the high pH of patties in the study.

Safety risks associated with premature browning will depend, however, on the prevalence of this condition: the higher the incidence, the higher the risk. Although no actual numbers have been found in the literature, a study by Killinger *et al.* (1995) estimated the incidence of premature browning between 25 and 62% in ground beef purchased at retail.

C. ENDPOINT TEMPERATURE INDICATORS

Because of foodborne outbreaks associated with consumption of inadequately cooked meat and/or poultry products, there has been increased interest in finding accurate methods to assure safety of thermally processed meat and poultry products. One approach that has been suggested is the use of endpoint cooking temperature indicators. According to this approach, a parameter, usually a protein or an enzyme, is identified in the product of interest and a relationship between this marker and the endpoint temperature reached during cooking is established. Most methods suggested in the literature are based on the use of endogenous protein markers in which the residual concentration, enzymatic activity or even spectra are measured after cooking.

1. USDA tests

The USDA-FSIS has published three different assays to verify proper thermal processing of meat products. The residual acid phosphatase test (USDA-FSIS, 1986a) is used for canned hams, picnics and luncheon meats. This assay is based on the residual enzyme activity in water-soluble protein extracts of cooked meat. Positive reactions show a blue color that can be read at 610 nm. One problem with this assay is that there is a great loss in acid phosphatase activity during frozen storage (Townsend, 1989) that must be taken into consideration when analyzing samples that have been kept under freezing conditions.

The coagulation test (USDA-FSIS, 1986b) is used for beef and pork products. The method is based on loss of protein solubility during heat processing of the meat product. Protein extracts of cooked samples are heated until cloudiness appears. The temperature at which the turbidity occurs is considered the internal temperature the product reached during cooking. For products cooked to the 63–71°C the assay can differ 8–10°C from the real temperature achieved during processing (Townsend and Blankenship, 1989). This test is thus very subjective and only gives a gross estimation of the thermal process applied.

The Bovine Catalase test (USDA-FSIS, 1989) is used to verify processing adequacy in roast beef and cooked beef. The assay is based on the production of foam when catalase reacts with oxygen. Bovine catalase is destroyed between 60.5–61.1°C. Thus, at temperatures above this range this assay becomes useless. In addition, the visual detection of foam is very subjective.

None of these assays are sensitive and reliable. In addition, the thermal inactivation kinetics of these markers are not known, and, therefore, are not suitable to monitor processes based on lethality of *Salmonella*. Moreover, with the introduction of new processing techniques, it would be desirable to identify an indicator whose inactivation depends on time-temperature only and not on the technique applied.

2. Enzymatic methods

Different enzymes have been tested for their potential as indicators of endpoint temperature of cooking. Townsend and Blankenship (1987) used the APIZYM enzyme system to monitor decrease in enzymatic activity in heated aqueous and saline meat extracts. The APIZYM system could detect 19 different enzymatic activities in a variety of specimens. As a result, the authors suggested that the enzyme leucine aminopeptidase had potential to act as an endpoint temperature indicator. The method,

however, was not tested in whole muscle samples. Davis *et al.* (1988) reported loss of pyruvate kinase activity in both a model system and a commercial canned cured pork product. The enzyme became inactive between 69.5 and 70°C. The method was based on the loss of fluorescence when NADH is oxidized which could be observed under long-wave ultraviolet light. The assay, however, required visual determination, thus is likely to be subjective. Townsend and Davis (1992) evaluated the potential of glutamic oxalacetic transaminase and glutamic pyruvic transaminase as indicators of endpoint temperature in ground beef using a commercial test kit. The authors suggested the use of both enzymes to monitor thermal processing of imported cooked beef that must be heated to 79.4°C. The test, however, should not be used in domestic meat products cooked to less than 71.1°C. Bogin *et al.* (1992) assayed the activity of 12 enzymes in turkey breast samples heated to different temperatures and concluded that L-aspartate-2-oxoglutarate aminotransferase, creatine phosphotransferase, isocitric dehydrogenase, aldolase, malate dehydrogenase, and lactate dehydrogenase (LDH) could be used as indicators for verification of heat treatment.

Collins *et al.* (1991a) measured LDH activity in samples of beef muscle cooked to different temperatures. They observed a sharp decline when samples were heated to 63°C and only marginal activity could be detected at 66°C. The same authors evaluated LDH in cured and uncured pork products (Collins *et al.*, 1991b). For uncured products, activity decreased markedly at 63°C and was almost undetectable at 68°C. No activity was detected in cured products at either 63 or 68°C. Stalder *et al.* (1991) studied the effect of pH, salt, phosphate, cooking temperature, muscle variation, carcass sex and maturity on LDH activity from heated beef extracts. The authors concluded that LDH showed potential to be used as an endpoint temperature indicator. Hsu (1993) screened enzymatic activity of 26 enzymes from extracts of turkey muscle for their suitability as indicators of proper heat processing. Of the 26, the author reported LDH and malate dehydrogenase to have potential to be used as markers in poultry products.

Townsend *et al.* (1993) reported loss in N-acetyl-β-D-glucosaminidase activity in samples of beef, pork and poultry heated from 40° to 70°C. Townsend *et al.* (1994) studied creatine phosphokinase activity as a possible indicator of endpoint temperature using a commercial test kit. Although enzymatic activity in meat and poultry products was reduced with increasing processing temperatures, the test was very product dependent, and the authors suggested that it should not be used as a regulatory assay.

Ang *et al.* (1994) modified the bovine catalase test (Eye, 1982) to be used as endpoint temperature determination method in poultry products.

The modified assay was used to estimate endpoint temperatures between 69 and 71°C within ±0.5°C. However, because temperature gradients occur within the product during cooking, the endpoint temperatures determined with this method could be more than 0.6°C above the actual temperature reached in the center of the product. Liu *et al.* (1996) studied the effect of process lethality on residual catalase activity in cooked chicken breast. Residual catalase activity varied with product endpoint temperature. For products heated to the same endpoint temperature, catalase activity decreased with increasing cooking time, regardless of heating rate. The authors developed a mathematical relationship between process lethality and catalase activity ($R^2 = 0.84$) and concluded that the assay could be used to estimate total process lethality in cooked chicken. In a related study, Liu *et al.* (1997) investigated the effects of fat and water content of chicken breast on residual catalase activity. Both fat and water influenced catalase activity but not total process lethality for different endpoint temperatures. The authors concluded that fat and water composition should be calculated before using the catalase method to determine endpoint temperature in chicken meat.

Kormendy *et al.* (1992) developed a modified acid phosphatase assay and suggested that it could be successfully used to verify heating in canned hams. Acid phosphatase activity was used to determine the endpoint processing temperature in boneless broiler and turkey meat, and ground turkey dark meat (Davis and Townsend, 1994). Acid phosphatase activity decreased in a curvilinear response to endpoint temperature and was dependent on muscle type. A fluorometric acid phosphatase test for cooking verification of broiler breast and thigh meat has been published as an AOAC peer verified method (Davis, 1998).

Wang *et al.* (1996) screened different enzymes to be used as indicators of proper heat processing in ground beef patties, using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Western blot and enzymatic assays. They concluded that triose phosphate isomerase (TPI), LDH and phosphoglycerate mutase showed potential to be used as endpoint temperature indicators. Sair *et al.* (1999) investigated the use of TPI activity as a means to determine adequate cooking of ground beef patties. TPI activity decreased with increasing endpoint temperatures between 60 and 71.1°C, but no differences in enzymatic activity were found between patties cooked to 71.1°C and above. In addition, freezing and thawing of meat did not affect residual TPI activity. Since the assay could differentiate between undercooked and adequately cooked patties, it was concluded that TPI could be used as a marker of proper cooking in ground beef.

These tests are useful for verification of command-and-control regulations stating specific processing times and temperatures. One

disadvantage of these methods is the need to identify a marker for each product type and size, even within the same species (i.e. a specific protein may work for turkey breast rolls but not necessary for turkey thigh rolls). Similarly each marker will need to be verified for each process used. In addition, if the marker is completely depleted during processing, the actual endpoint temperature cannot be determined. Another disadvantage of enzymatic assays is that, unless they are made into an analysis kit, they can be tedious and time consuming. The endogenous protein or enzyme will need to be extracted before the assay is performed. With the exception of TPI, the thermal inactivation kinetics of most of the enzymes described above are not known or do not match those of *Salmonella* (Orta-Ramirez *et al.*, 1997; Veeramuthu *et al.*, 1998). Therefore, most endpoint temperature indicators are not suitable for validating adequate thermal processing when using the lethality standard based on *Salmonella* reduction.

3. Immunoassays

In recent years, immunoassays have been replacing other methods of food analysis due to their sensitivity, specificity and ease of application. One great advantage of these tests is that they can be optimized into a kit to reduce testing time and instrumentation. On the other hand, a marker must be identified and antibodies must be developed against this marker (antigen). Development of antibodies with the greatest affinity, avidity and specificity is highly desirable. Polyclonal antibodies are produced when different lymphocyte clones respond to the same antigen. The resulting immune serum will show a broad range of antibodies against different antigenic determinants (Smith and Orta-Ramirez, 1995). Advantages of using polyclonal antibodies include: versatility, stability in frozen or lyophilized conditions and lower production cost than monoclonal antibodies. Disadvantages include limited supply and the fact that extremely pure antigen is required to elicit high affinity antibodies.

Monoclonal antibodies result from a single cell or clone that produces a specific antibody against one particular site or epitope of an antigen (Galfre and Milstein, 1981). The advantages of using monoclonal over polyclonal antibodies are their specificity and selectivity for individual antigenic determinants, and unlimited supply. The main disadvantages are: longer time and higher cost of production, higher physicochemical variability and biological activity than polyclonal antibodies, and possibility of cross-reactions. In addition, mutations can reduce antibody production and cell lines can be lost due to fungal contamination.

The use of immunoassays for determination of endpoint temperature in meat and poultry products is well documented (Smith and Orta-Ramirez,

1995; Smith and Desrocher, 1996). Wang *et al.* (1992) developed an indirect competitive enzyme-linked immunosorbent assay (ELISA) to verify proper cooking of uncured turkey breast rolls based on detection of LDH using polyclonal antibodies. The ELISA accurately distinguished endpoint temperature within ±1.1–1.2°C in the temperature range of 68.3 to 72.1°C. In a related study, Abouzied *et al.* (1993) developed monoclonal antibodies to be used in a sandwich ELISA. Minimum detection limits for turkey and chicken LDH were 1 ng/ml. Effects of formulation, storage and processing of poultry products on LDH were also studied (Wang *et al.*, 1993). Salt concentration, cooking protocol and type of casing did not markedly influence LDH content. Based on these studies, it was suggested that a maximum residual concentration of 310 ng LDH/g meat indicated that turkey breast rolls had been processed to 71.1°C or above.

Wang *et al.* (1994) studied the efficacy of LDH, turkey serum albumin and immunoglobulin G ELISAs for determining endpoint temperatures of uncured turkey thigh rolls. The TSA ELISA could differentiate between products cooked to 68.9 and 71.1°C. The LDH and immunoglobulin G ELISAs, however, could not distinguish between products heated to 68.9 and 71.1°C, and 68.9 and 72.2°C, respectively. The LDH, turkey serum albumin and immunoglobulin G ELISAs were also verified for their applicability in endpoint temperature determination in turkey ham processed between 67 and 73°C (Smith *et al.*, 1996). It was estimated that maximum concentration of 330 ng LDH/g meat, 51,852 ng turkey serum albumin/g meat and 1221 ng immunoglobulin G/g meat indicated that turkey ham had been processed to 68.3°C or above.

Veeramuthu *et al.* (1997) studied the effect of species, muscle type and product formulation on residual concentration of LDH, turkey serum albumin and immunoglobulin G after cooking turkey bologna, turkey pastrami, turkey sausage and turkey frankfurters using ELISAs. The residual concentration of all three enzymes decreased as the endpoint temperature increased. The authors concluded that a minimum residual concentration should be established for each product before the ELISAs could be used to verify endpoint temperatures.

Polyclonal (Wang *et al.*, 1995) and monoclonal antibodies (Orta-Ramirez *et al.*, 1996) against bovine muscle LDH were developed and used in a sandwich ELISA. This ELISA could differentiate endpoint temperatures at 2°C in the temperature range of 66 to 74°C in a ground beef model system. The residual LDH concentration decreased from 122 μg/g meat at 68°C to 3 μg/meat at 70°C. When the ELISA was tested in commercially cooked beef patties, residual LDH content averaged 3.4 μg/g in the 68.3 to 71.1°C range. The authors concluded that the LDH

ELISA had potential to monitor proper cooking in ground beef products cooked at or above 69.4°C.

4. Physical methods

Another approach to monitor endpoint temperature reached during thermal processing of meat products is the use of physical techniques. These include electrophoretic and chromatographic separation, differential scanning calorimetry, near infrared spectroscopy, loss of protein solubility and nuclear magnetic resonance.

Caldironi and Bazan (1980) used sodium dodecyl sulfate-polyacrylamide gel electrophoresis to monitor disappearance of low-salt soluble proteins when beef muscle samples were heated at different temperatures. They observed a gradual loss in some bands after reaching 60°C until their complete disappearance above 80°C. Steele and Lambe (1982) used gradient gel electrophoresis to separate polypeptides formed during heating of different muscle extracts. The intensity of the bands decreased with increasing heating temperatures. McCormick *et al.* (1987) used reverse phase high performance liquid chromatography to monitor the effect of heating on porcine extracts.

Isaksson *et al.* (1989) observed changes in spectra of samples of beef with increasing temperature when analyzed by differential scanning calorimetry. This same method was used to determine the cooking temperature of beef within ±0.6°C (Ellekjaer, 1992). Ellekjaer and Isaksson (1992) verified thermal processing of beef samples that had been cooked to nine different temperatures using near infrared reflectance and transmittance. Both methods could detect endpoint temperature within 2.0–2.1°C. Chen and Marks (1997) detected the endpoint temperature with 2.5°C accuracy when previously cooked chicken patties were evaluated using a near-infrared technique, but noted that sample thickness would probably affect the results.

Davis *et al.* (1985) measured the amount of water-extractable proteins in pork and beef muscles heated following different time/temperature schedules using the biuret method. They observed that loss of solubility was time/temperature dependent but concluded that more information was needed to determine an extractable biuret-positive ratio to be used in a mathematical model. In a related study, Davis *et al.* (1987) monitored loss of protein solubility in canned cured pork by isoelectric focusing on polyacrylamide gels. Again the authors remarked about the difficulty of establishing a method to verify endpoint temperature based on a measure of extractable protein.

More recently, Walton and McCarthy (1999) used nuclear magnetic resonance to determine fat content and internal temperature of hamburger, sausage and chicken products during cooking. Internal temperature was calculated from the frequency difference between water and fat lines within the nuclear magnetic resonance spectrum. The accuracy of determination was ±1°C for hamburger and sausage and ±5°C for chicken products. The authors speculated that differences in accuracy could be due to differences in fat content of products.

The main disadvantages of some of these methods are that they require expensive equipment and well-trained personnel. In addition, some of the techniques are cumbersome and/or very time consuming and could not be applied for routine monitoring. Moreover, many of these methods cannot be used to verify compliance to the new process lethality standards based on *Salmonella* destruction, as the kinetics of the marker was not tested.

D. TIME-TEMPERATURE INTEGRATORS

1. Background and theoretical considerations

Methods to evaluate the accuracy of thermal processes are classified according to three different approaches: *in situ* methods, physical-mathematical methods, and time-temperature integrators (TTIs) (Hendrickx *et al.*, 1995; Van Loey *et al.*, 1996).

In the *in situ* method, a safety or quality parameter is chosen and its concentration is measured before and after the treatment. Such parameters can be sensory factors, nutritional quality attributes or microbial contaminants. In practice, however, the determination of these parameters, especially after the treatment, can be very difficult, even impossible in some cases, because of the detection limits, analytical techniques and/or sampling requirements. Moreover, these methods are often unsuitable for routine monitoring due to their complexity (Hendrickx *et al.*, 1995). An *in situ* method would not be appropriate for verifying the processing adequacy of meat, as pathogens cannot be introduced into the processing plant environment.

In the physical-mathematical approach, the impact of the heat treatment on the attribute of interest is estimated based on the time-temperature profile and the known kinetic parameters for such an attribute. The need for time-temperature data often limits the practical applicability of this approach. In some cases, direct recording is not possible due to processing conditions; on the other hand, if the time-temperature history is reconstructed by mathematical modeling, the accuracy of the estimation by model parameters will limit the accuracy of the process evaluation.

Conservative estimates used in these models often result in unrealistically severe heat treatment requirements which in turn lead to over processing, significant nutrient loss and reduction of quality of the product.

Time-temperature integrators are another alternative for monitoring of thermal processes based on microbial lethality standards and can be adapted for use in a meat processing plant or food service environment. A TTI is defined as a marker that responds to time-temperature history by undergoing an irreversible and precisely measurable change in a manner that mimics the changes of a target attribute exposed to the same thermal history (Hendrickx *et al.*, 1995). TTIs allow fast and reliable validation of a process without the need for detailed information on the actual time-temperature profile within the product.

A reliable TTI should be simple and inexpensive to prepare, easy to recover from the food, give an accurate and easily detectable response, be readily incorporated into the food product without interfering with the heat transfer and must quantify the process impact on the target attribute

The thermal death time (F value) is the time required to achieve a particular reduction in a target attribute (quality or safety index) (Hendrickx *et al.*, 1995). For a first order process, the F value can be computed from:

$$F = D_T \log (C_0/C) \tag{1}$$

where F has units of time, D_T is the decimal reduction time at a given temperature T, and C_0 and C are the initial and final values, respectively, of the target attribute. Thus, C_0 and C may refer to the concentration of a microorganism, activity of an enzyme, or other measurable parameter specific to the target attribute of interest.

A TTI must show the same time-temperature dependent response as that of the target attribute when the temperature is the only rate-determining factor. Mathematically, this can be written as:

$$F_{\text{Target}} = F_{\text{TTI}} \tag{2}$$

Assuming a general nth order reaction, the rate equation can be expressed as:

$$dC/dt = -kC^n \tag{3}$$

where k is the reaction constant and n is the reaction order. For a first order reaction, the equation can be integrated to give:

$$\ln (C_0/C) = kt \tag{4}$$

where C_0 is the initial value of the target attribute, and C is the value of the target attribute at any time t.

For a non-first order reaction ($n \neq 1$), Eqn (3) can be integrated, assuming that k is neither a function of time or reaction order, to give the expression:

$$[1/(n-1)]\,(C^{1-n} - C_0{}^{1-n}) = kt \tag{5}$$

In many cases, the rate constant can be expressed by the Arrhenius equation:

$$k = k_0 \exp\,(-E_A/RT) \tag{6}$$

where k_0 is the pre-exponential factor, E_A is the activation energy, R is the universal gas constant and T is the absolute temperature.

In the general case where the reaction order is other than unity, and the reaction rate constant is independent of the order of reaction, the F value for an isothermal process can be written as (Hendrickx *et al.*, 1995):

$$(F_{\text{Tref}})_x = \int_0^t \exp \frac{E_A}{R}\left(\frac{1}{T_{\text{ref}}} - \frac{1}{T}\right) \mathrm{d}t \tag{7}$$

where F_{Tref} represents the equivalent heating time at a reference temperature resulting in the same lethality as the time-varying temperature profile.

This equation is valid for the target attribute as well. Therefore Eqn (2) will only be satisfied when the activation energies of both the TTI and the target attribute are the same. In other words, for a parameter to be used as a TTI, it must have identical activation energy to that of the attribute of interest. Mathematically this can be written as:

$$E_{A_{\text{TTI}}} = E_{A_{\text{Target}}} \tag{8}$$

Since z values are equivalent to activation energies, Eqn (6) can also be expressed as:

$$Z_{\text{TTI}} = Z_{\text{Target}} \tag{9}$$

A TTI could be used to verify that the new lethality performance standards are met during meat processing. In this case, the target attribute is *Salmonella*, or more specifically a cocktail of *Salmonella* serotypes (see Section II.C above). Thus if a first order process is assumed, a TTI that has

the same z value as *Salmonella* in a particular product could be used to verify the adequacy of any thermal process. However, if thermal inactivation is not a linear process, the first step is to accurately determine the kinetics of *Salmonella* in the product of interest. Then a potential TTI can be identified and its thermal inactivation kinetics determined. Once the thermal inactivation kinetics of both *Salmonella* and the TTI are accurately characterized, a model could be developed to use the TTI to predict *Salmonella* destruction during thermal processing.

2. *Types of time-temperature integrators*

TTIs can be classified according to their working principle (biological, chemical or physical), origin (intrinsic or extrinsic to the food product), application in the food material (dispersed, permeable or isolated) and location in the food (volume average or single point) (Van Loey *et al.*, 1996). Most currently existing TTIs are based on microbiological or enzymatic assays.

a. Microbiological TTIs Microbiological TTIs mainly employ "calibrated" microorganisms or spores (i.e. they have been validated against a known standard). A thorough review on bacteriological evaluation for thermal process design has been published by Yawger (1978). Microbial spores can be used to monitor thermal processing using either a survivor curve or an endpoint method. In a survivor curve or "count reduction procedure", the number of microorganisms surviving the heat treatment is related through a calibration curve to obtain a sterilizing value. Usually, ten or more cans are inoculated with 30–50 million spores of a heat-resistant microorganism. A series of thermal treatments of varying time or temperature are applied. The initial population is calculated from an unprocessed container and survivor counts are obtained from each processed can. The D value for the microorganism is calculated for the series of treatments tested in the assay (Yawger, 1978; Pflug *et al.*, 1980).

In the endpoint procedure, also called quantal response, several units are subjected to each treatment. After incubation, each unit is tested for either growth or no growth, and the number of survivors is determined from the quantal response. An example of endpoint method is the inoculated pack system (Yawger, 1978). This system consists of a series of cans (usually 100) that are inoculated with a definite number of spores of known resistance. This method is generally used to validate a calculated food sterilization treatment.

The main disadvantages of microbiological TTIs are assay time, because of the microorganism incubation period required for enumeration, and the likelihood of microbial contamination, as well as cost, labor and the need to properly calibrate the spores. In addition, if the initial population of microorganisms is completely destroyed during processing, the actual lethality derived from the heat process cannot be determined.

b. Enzymatic TTIs Different enzymes, mainly of microbial origin, have also been proposed as TTIs. Weng *et al.* (1991a) investigated the thermal behavior of peroxidase, and studied its application as a bioindicator for thermal processing. The z value of peroxidase was modified from 26.3°C to 14.1°C by immobilization of the enzyme in glass beads. Furthermore, the z value was lowered to 11.1°C by modification of the environment with organic solvents. In a different study, Weng *et al.* (1991b) verified the use of the immobilized peroxidase in a dodecane environment as an indicator for pasteurization processes. The peroxidase system had a z value of 10.1°C. When evaluated at processing temperatures between 70 and 80°C, the lethalities of the indicator agreed with those calculated according to the Bigelow's General Method. In a related study, Hendrickx *et al.* (1992a) studied the thermal denaturation of peroxidase as a function of water activity. Lyophilized horseradish peroxidase was equilibrated over standard salt solutions to achieve a water activity between 0.11 and 0.88. The enzyme was much more thermostable in the dry state than in aqueous solution, and both D and z values changed with water activity. Inactivation temperatures were in the range 140–160°C at low water activities (compared to 70–85°C in aqueous solution). Later, Hendrickx *et al.* (1992b) studied the application of immobilized peroxidase as an indicator in a can model system under pasteurization conditions. Plastic spheres containing peroxidase were placed in a can and processed at 85°C. The lethalities calculated by integration of the resulting time-temperature profiles coincided very well with those read from the peroxidase system. The authors suggested that the validated bioindicator could be encapsulated in a small vial and used to calculate lethality in the center of the unit.

DeCordt *et al.* (1992a) studied the application of the enzyme α-amylase as a TTI in thermal processes. The group studied the thermal inactivation kinetics of α-amylase from *Bacillus licheniformis* in the temperature range 90–108°C. They also looked at the influence of immobilization, pH, ionic strength, Ca^{2+}, and concentration of enzyme as effective means to manipulate the thermal stability of the enzyme. The D values at 95°C differed between free and covalently immobilized enzyme. Extrinsic Ca^{2+} conferred stability, but there was a saturation level after which the enzyme

was destabilized. The optimum pH to lower thermal inactivation rates was found at pH of 8.5. Also, increasing the concentration of the enzyme resulted in higher thermoresistance. The authors concluded that with manipulation of the environment, the immobilized enzyme showed potential to be used as a TTI for monitoring thermal inactivation of *Clostridium botulinum* spores in food.

In a related paper, DeCordt *et al.* (1992b) tested biphasic and *n*th-order models to fit experimental inactivation data of *B. licheniformis* α-amylase immobilized on glass beads. Both isothermal and non-isothermal experiments were used to estimate model parameters (E_A, k and n) using a non-linear regression procedure. The authors calculated an E_A of 293 kJ mole^{-1} for the system, which fell within the range of that of *C. botulinum* (265–340 kJ mole^{-1}). They concluded that the system had potential to be used as a TTI in thermal processes in the temperature range of 96–108°C.

The general effects of polyols and carbohydrates on thermal denaturation kinetics of α-amylase were also studied (DeCordt *et al.*, 1993). The results showed that all polyols (glycerol, mannitol and sorbitol) and carbohydrates (starch and sucrose) tested were powerful thermostabilizers, at least at the temperature range used in the study.

DeCordt *et al.* (1994) studied the thermostability of α-amylase and peroxidase using differential scanning calorimetry. The endothermic peak temperature was used as a measure of protein stability. The thermal inactivation kinetics of α-amylase from two *Bacillus* spp and horseradish peroxidase were determined as a function of the concentration of glycerol, sorbitol and sucrose. *B. amyloliquefaciens* α-amylase was very stable in presence of either polyols and carbohydrate or combination of both, and inactivation temperatures were as high as 127°C. The authors suggested that the use of the endothermic peak area could be used as a TTI-response.

Maesmans *et al.* (1994a) conducted a theoretical study of the possibilities of combined mathematical model and TTI to monitor fluid-to-particle convective heat transfer coefficient. The authors concluded that although, theoretically, the combined use of both could be suitable, there is a need to very carefully examine experimental design considerations. These considerations are specific for each processing condition, TTI employed and type of material, therefore making the combined use of a mathematical model and TTI a rather cumbersome methodology. Also, Maesmans *et al.* (1994b) evaluated the efficacy of an α-amylase-based TTI to monitor the spatial distribution of thermal processing values in a food model system using a pilot retort. The TTI in this particular case was used to determine particle-to-particle variation in processing values. One question that was raised was whether the use of two TTIs, characterized by different z values, could determine the "coldest point" at the same

position when applied in the same heat process. The authors suggested that results from a TTI should not be extrapolated to another TTI with a different z value without experimental verification. Furthermore, the authors concluded that for an enzyme system to be used as a TTI for the monitoring of heat distribution in foods, it is critical to calibrate the system in terms of the kinetic inactivation parameters (D–z or k–E_A values).

Van Loey *et al.* (1997a) tested the efficacy of a *B. amyloliquefaciens* α-amylase-based TTI to evaluate in-pack lethalities in a pasteurized food model system. Thermal inactivation of the enzyme was measured as a function of reaction enthalpy using differential scanning calorimetry. The reaction enthalpy followed a log-linear reduction at constant temperature. Therefore, a first order reaction was assumed and a z value of 7.6°C was obtained. When tested in a heat processed food model system, the processing values determined from the TTI read-out coincided very well with the actual integrated processing values calculated using the general method, suggesting that this enzyme could be used to monitor pasteurization treatments (up to 90°C). Van Loey *et al.* (1997b) studied the heat inactivation kinetics of *B. subtilis* α-amylase under steady and non-steady conditions by following enthalpy changes associated with the thermal denaturation of the enzyme. When equilibrated at a water activity of 0.76, the enzyme had a z value of 9.7°C. Because of the similarity to the z value (10°C) of *C. botulinum*, the authors suggested that this enzyme system could be used to monitor the efficacy of sterilization treatments in foods.

c. Chemical and physical TTIs Only very few methods based on either *chemical or physical indexes* are currently available. The first chemical markers that were studied were compounds in foods for which analytical methods were already established, such as thiamin, pantothenic acid, vitamin C and methylmethionine sulfonium salt (Kim and Taub, 1993). One limitation of most of these methods is the need for additional steps (recovery, post treatment assay) after the thermal process, which results in lengthy procedures and technical manipulations (Van Loey *et al.*, 1996). Kinetically, other challenges have to be faced when dealing with chemical TTIs: first, the rate constant of a chemical reaction is usually smaller than that of microbial destruction; secondly, the z values are usually greater (the z value for thiamin is 48°C while the one for *C. botulinum* is 10°C) (Mulley *et al.*, 1975).

According to Kim and Taub (1993), compounds formed during food reactions show more potential to be used as TTIs, since one could determine the gain in concentration of such product as the heat process takes place. However, it is not easy to find such a thermally produced compound that can be easily assayed and whose concentration after the process can be used to verify sterility. Kim and Taub (1993) identified and characterized

three chemicals that showed potential to be used as TTIs. They reasoned that, since carbohydrates are present in mostly all foods, changes in carbohydrate profile could give an indication of potential markers. To screen for the compounds, they first monitored formation of chemicals by following changes in UV spectra in different food products, including meats, fruits and vegetables. They observed formation of three different compounds (depending on the food product) which they called M-1 (found in heated meats, fruits and vegetables), M-2 (found primarily in heated meats) and M-3 (found in heated fruits and vegetables), and identified (or partially identified) both the markers and their precursors: M-1 (2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one) and M-3 (5-hydroxymethylfurfural) are products from the degradation of D-fructose, while M-2 was associated with protein. Once identified, the authors proceeded to study the kinetic parameters of these compounds. They established a linear relationship between M-1 concentration and the sterility index, F, using a non-isothermal process, and developed a model relating microbial destruction to marker formation. They acknowledged the need to do more studies using a more controlled time exposure over a wider range of temperatures to validate the model and to calculate the actual precision with which the F value of the thermal process could be determined.

d. Triose phosphate isomerase as an intrinsic TTI to verify processing adequacy of beef Endogenous muscle proteins can be used as TTIs to monitor the impact of a thermal process (Hendrickx *et al.*, 1995; Van Loey *et al.*, 1996). An endogenous muscle enzyme must meet two criteria to function as a TTI to verify processing adequacy in meat. First, its concentration or activity should decrease as processing time at the same temperature is increased. Second, the residual enzyme activity across all adequate time-temperature cooking combinations should be constant. This will occur if the z value of the TTI matches that of the microorganism used to establish the cooking process (Hendrickx *et al.*, 1995).

Orta-Ramirez *et al.* (1997) determined the z value of six potential marker enzymes in bovine *semitendinosus* muscle. The z value of triose phosphate isomerase (TPI) (z = 5.7°C) was similar to that of *Salmonella* (z = 5.6°C) (Goodfellow and Brown, 1978) in beef. Thus, the authors postulated that TPI might be used as an endogenous TTI to determine the adequacy of thermal processing of roast beef.

To test this hypothesis, ground beef was cooked in a water bath using three different USDA-FSIS (1998) roast beef time-temperature combinations (54.4°C for 121 min, 58.3°C for 24 min, and 62.3°C for 5 min) that produce a 7 log reduction in *Salmonella* (Hsu *et al.*, 2000). TPI activity decreased as holding time was increased at each processing

temperature, meeting the first criterion stated above. This marker also met the second criterion for a TTI. Residual TPI activity was similar in ground meat adequately cooked at each temperature and averaged 2.1 U/g meat. Roast beef was also processed in a pilot scale oven using two USDA time-temperature combinations, representing the highest (62.2°C) and the lowest (54.4°C) temperatures allowed by the USDA prior to 1999. TPI activity decreased as cooking time increased using both processes, however residual TPI activity was the same when roasts were adequately cooked at each temperature. The lowest TPI activity of 1.6 U/g in the adequately processed roasts in the pilot scale oven was similar to that measured in the adequately cooked ground beef (2.1 U/g) cooked in the water bath. Although more verification work is needed, the authors suggested that cooked beef with a TPI activity at or below about 2 U/g could be considered to have met the 7-$\log_{10}$ lethality standard.

The similarity in TPI activity of the adequately processed beef cooked to widely different temperatures indicated that the z values representing thermal inactivation of TPI and *Salmonella* in beef were similar as reported by Orta-Ramirez *et al.* (1997), providing evidence that TPI might function as a TTI to verify processing adequacy. Hsu *et al.* (1999) developed a sandwich ELISA using polyclonal antibodies against bovine muscle TPI with a detection limit of 1 ng/ml. The development of a rapid, self-contained test to quantify TPI, such as an ELISA, might allow for the practical use of TPI as an endogenous TTI to verify processing adequacy when questions arise about a process.

Smith (2000) tested the efficacy of residual TPI activity after cooking as an indicator of thermal process adequacy in ground beef patties. A large variation in TPI residual activity was observed within each individual patty and as much as 25.7°C temperature difference was estimated within a 113 g patty. Thus, the residual TPI activity after cooking was highly affected by the heat transfer within a patty, sampling method and location of the patty on the grill.

e. Phycoerythrin as an extrinsic TTI to verify processing adequacy of beef Orta-Ramirez (1999) investigated the protein R-phycoerythrin (R-PE) for its application as a TTI to monitor adequate thermal processing in beef products. R-PE is a natural pigment-protein derived from an edible alga. The alga has abundant pigment (up to 20% of dry weight) that is easily extracted and purified. Encapsulation in a defined chemical environment allows known kinetics for use in products of different composition. When compared with enzymes, R-PE shows a wider extent of applications because of its tolerance over a broader range of chemical conditions. Finally, the fluorescence loss of R-PE is directly and immediately detectable, without the need for additional steps prior to analysis.

R-PE was selected as an extrinsic, single component, fluorescent-based TTI based on the calculated z value of 5.99°C determined when the protein was dissolved in 0.012 M borate buffer, pH 9.0. This z value was also found to fall within the range of the reported z value for *Salmonella* in beef (5.6–6.2°C) (Table III).

R-PE was isolated from algal tissues of *Porphyra yezoensis*, an edible seaweed, used as an ingredient of sushi. Under neutral conditions, R-PE has a characteristic absorption spectrum showing two maximum peaks at 498 and 565 nm, and a shoulder at about 540 nm. In fluorescent measurements and under neutral conditions, R-PE shows excitation and emission maxima in the visible range at 493 and 578 nm, respectively.

Isothermal and non-isothermal experiments were conducted to determine the thermal inactivation parameters of R-PE under different conditions of pH and additives (Orta-Ramirez *et al.*, 2000; Orta-Ramirez *et al.*, 2001). Thermal inactivation parameters were calculated on the basis of fluorescence loss at the maximum emission wavelength. Modifying the R-PE solution conditions altered the inactivation parameters of this protein.

A non-linear mathematical expression was used to fit the heat inactivation kinetics of R-PE. Kinetic parameters (n and k) were calculated using non-linear regression analysis. The lethalities determined using the non-linear model were highly predictable, suggesting that a mathematical relationship could be established between destruction of *Salmonella* and R-PE fluorescence decay. Thus, it was concluded that R-PE could be used as the detector component of a TTI to predict destruction of this microorganism during thermal processing of beef products.

This system was tested during cooking of ground beef patties inoculated with an 8-strain *Salmonella* cocktail (Smith, 2000). Encapsulated R-PE was inserted in the center of ground beef patties (113 g and 60 g) and the patties were cooked according to the USDA beef thermal processing recommendations (USDA-FSIS, 1999). In 113 g patties, a R-PE normalized fluorescence value of 0.30 or below after cooking indicated that both low and high fat beef patties had been adequately processed. The authors concluded that, upon optimization of the methodologies, R-PE showed potential as a TTI to verify proper thermal processing in beef products.

V. SUMMARY AND FUTURE RESEARCH

Adequate thermal processing is the most important method to eliminate pathogenic microorganisms from meat and poultry products to prevent foodborne poisoning. Government agencies provide guidelines for cooking meat and poultry products in food service, retail operations, commercial

establishments and the home. However, there is still a need to verify that the cooked products have been processed in an adequate and safe manner. Thermometers, color evaluation and endpoint temperature indicators have been developed to verify cooking adequacy when products are cooked to specific temperatures. However, thermometers and color determination pose several disadvantages in their use while endpoint temperature indicators are not suitable for validating adequate thermal processing when using the lethality performance standard based on specific log reductions in *Salmonella.*

The new performance standards require establishments to validate the efficacy of their processes to reduce microbial contamination if the procedure varies from published compliance or safe harbor guidelines. Since *Salmonella* cannot be taken into a processing plant, a TTI could be used to verify that the new lethality performance standards are met during meat processing. Although some TTIs are under development, more research is needed to develop and test TTIs for use in meat and poultry products.

First-principle models could also be developed and validated to accurately predict the lethality outcome of a thermal process given information about the product composition, size, and shape, and the dynamic process parameters (e.g., temperature, humidity, and air velocities). With this type of fundamental model (combining principles of heat and mass transfer and predictive microbiology), a processor could evaluate the efficacy of a new or modified process prior to ever implementing that process on a pilot or commercial scale. Only limited microbiological data would be needed from challenge studies to validate the model. Thereafter, operating data for the process parameters could be the primary means of assuring that a process is continuing to meet the lethality performance standards in combination with other tools, such as TTIs. Unfortunately, no such models are yet available to meet this industrial need; therefore, interdisciplinary research and development is needed to develop, validate, and disseminate such models.

Processors, retailers and consumers will benefit from improved product safety through the increase in the frequency, accuracy and convenience of thermal process verification. More frequent routine testing will yield safer meat and poultry products, while avoiding overcooking, reduced food quality and unnecessarily high energy consumption.

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