EFFECT OF WATER ACTIVITY AND HUMIDITY ON THE THERMAL INACTIVATION OF *SALMONELLA* DURING HEATING OF MEAT

By

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ABSTRACT

EFFECT OF WATER ACTIVITY AND HUMIDITY ON THE THERMAL INACTIVATION OF *SALMONELLA* DURING HEATING OF MEAT

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The USDA-FSIS recently amended the regulations governing cooked meat and poultry products, creating a shift to lethality performance standards, and a need for inactivation models. Studies clearly show that many factors affect thermal inactivation of pathogens; however, water has not been previously isolated as an intrinsic or extrinsic factor. The objectives of this study were (1) to test the effects of meat moisture content/water activity on thermal inactivation of *Salmonella* in a sealed environment, (2) to test the effects of air humidity on thermal inactivation of *Salmonella* during convection heating, and (3) to demonstrate the inclusion of a water term into a secondary inactivation model. Ground turkey was inoculated with an 8-strain *Salmonella* cocktail and heated isothermally either in a waterbath or in air convection oven. Survivors were enumerated via serial dilutions and plated on Petrifilm®. The rate of thermal inactivation of *Salmonella* decreased with decreasing meat water activity; however, in the air convection oven, the same results were not observed for a corresponding decrease in relative humidity. In conclusion, the water effect lies in the intrinsic property of the meat (i.e., water activity), rather than the extrinsic process parameter (i.e., humidity), and should be accounted for in inactivation models used to validate commercial convection cooking systems.

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ABBREVIATIONS

A	frequency factor (Arrhenius equation)
a	slope of a line
ANOVA	analyses of variance
AVG	average
a_w	water activity
b(T)	temperature dependent constant (Peleg and Cole, 1998)
C_0 to C_4	empirical coefficients without biological significance (Cerf et al., 1996)
CFU	colony forming units
-dN/dt	rate of inactivation of viable cells
E_a	activation energy
HF	high fat
k	death rate (min^{-1})
LF	low fat
MC	moisture content
N	number of surviving cells
N_o	initial number of cells
n(T)	temperature dependent constants (Peleg and Cole, 1998)
$N(t)/N_o$	survival ratio
R	universal gas constant
SD	standard deviation
T	absolute temperature (K)

T_o	temperature when the line was extrapolated to k=0 (Zweitering et al., 1990)
t	time

CHAPTER 1

INTRODUCTION

1.1 Background and justification

In this section, four main points are emphasized. First, background information on *Salmonella* is presented. Then, the food industry, and more specifically the meat industry, is discussed. Thirdly, current changes in the federal regulations affecting the industry are addressed. Finally, the resulting scientific needs are described.

1.1.1 Foodborne disease

Campylobacter, Salmonella, and *Escherichia coli* O157:H7 are the most commonly recognized causes of foodborne illness in the US (CDC, 2001). Over 2000 *Salmonella* strains have been identified (Jay, 1996). According to the Centers for Disease Control and Prevention (CDC, 2001), there are 1.4 million cases of salmonellosis in the United States per year, and of these, approximately 40,000 are culture-confirmed cases that are reported to the CDC (CDC, 2000). People infected with *Salmonella* develop fever, abdominal cramps, and diarrhea (sometimes bloody), which occurs 12-72 h after exposure and usually lasts 4-7 days (CDC, 2000). Most people recover without treatment, although severe cases require hospitalization, and over 500 people die each year in the United States from acute salmonellosis (CDC, 2000). Additionally, cases of human salmonellosis impose a considerable economic burden on the economy. This responsibility falls upon the industry (retail and wholesale), the infected people, and their family (Roberts and Sockett, 1994).

Turkey is one of the most common vectors for pathogens, and *Salmonella* is one of the most prevalent pathogens found in turkey. FSIS reported combined prevalence (small and large plants) of *Salmonella* from July 1999 to June 2000; broiler chicken was 9.9%, ground chicken was 14.4%, ground beef was 5.0%, and ground turkey was 30.0% (USDA-FSIS, 2000).

Thermal processing is the main solution to eliminating bacteria in food products. Salmonellae are obviously sensitive to heat, but their sensitivity varies greatly. The composition of the heating menstrum has a strong influence on the thermal resistance of bacteria (Murphy et al., 2000). Occasionally, some salmonellae may survive standard food-processing techniques (Doyle and Mazzotta, 2000). This may result from outside factors that affect the thermal resistance. In addition, some strains of *Salmonella* are more heat resistant that others. Because of the various factors that affect thermal resistance, the need exists to evaluate inactivation in meat and not rely on data developed in model substrates.

1.1.2 The affected industry

The food industry is generally considered the nation's largest manufacturing sector and is one of the most stable. The meat and poultry industry contributes over $90 billion in annual sales to the U.S. Gross National Product (GNP) and is the largest component of the U.S. agriculture sector (AMI, 2000). The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) (2001) reported that there were 1,630 establishments producing ready-to-eat cooked or partially cooked meat and poultry product in 1997 with the value of shipments totaling over $28.2 billion for that

year. Given consumer preferences for convenience, it is likely that the market for fully-cooked products will continue to grow.

The focus of thermal processing is placed in three areas: 1) cooking methods in homes and commercial kitchens; 2) processing methods in plants producing fully cooked products; and 3) treatment of raw poultry (Doyle and Mazzotta, 2000). This thesis focuses on the processing methods in plants producing fully cooked products.

1.1.3 Regulatory trends

Regulations are aimed to ensure that pathogens are destroyed and not present in food products. For whole muscle products, the regulatory paradigm has shifted from command-and-control regulations to performance standards (USDA-FSIS, 1999). Performance standards require that commercial establishments meet specific food safety objectives. USDA-FSIS has set regulations in Title 9 of the Code of Federal Regulations for meat and poultry. The regulation states that any thermal processing procedure must achieve 7.0- or 6.5-$\log_{10}$ reduction in *Salmonella* for whole-muscle poultry or beef, respectively. Processors are not held to specific endpoint temperatures; however, they must validate new or altered process schedules by "scientifically supportable means" (USDA-FSIS, 1999).

A proposed regulation would extend these standards to all ready-to-eat products. This regulation allows either challenge studies (i.e., inoculation of real products with target organisms) or the use of models to document process lethality (USDA-FSIS, 2001). This regulation is advantageous because it allows flexibility in processing procedures. However, this creates a problem, because pathogens cannot be intentionally brought into processing facilities to conduct challenge studies. Furthermore, most

models are based on microbial thermal death time studies performed in a laboratory and may not be valid for commercial processes. In regard to models, the regulation states,

> "The establishment will need to demonstrate the relationships between the lethality treatments and the specific characteristics of a product, such as physical and chemical properties. This demonstration could involve the use of heat transfer equations and should account for **all variables** that would affect lethality (e.g., size of product, **humidity**, density, thermal conductivity, specific heat, shape, product composition and strain of organism" (USDA-FSIS, 2001).

1.1.4 Scientific needs

Studies clearly show that nearly "all variables," including fat, salts, pH, and additives (Chapter 2), affect the thermal inactivation of bacteria,. However, no current inactivation model accounts for the effects of water (i.e., moisture content, water activity, or humidity) on microbial inactivation in meat products. Water affects the lethality of *Salmonella* in meat products, and more organisms generally survive in a dry environment. However, the specific cause of the effect is unknown. Further research is needed to determine whether the effect is best related to moisture content, water activity, or process humidity. This effect must be fully understood to accurately model process lethality for commercial systems. Incorporating accurate terms into a secondary model would improve model performance and usefulness. Therefore, due to the regulatory changes and economic importance of this industry, there is a need to directly test these water effects.

1.2 Hypothesis and objectives

The hypothesis of this study was that the rate of thermal inactivation for *Salmonella* decreases with decreasing meat moisture content and/or process humidity. The objectives of this study were:

(1) To test the effects of meat moisture content/water activity on thermal inactivation of *Salmonella* in a sealed environment,

(2) To test the effects of air humidity on thermal inactivation of *Salmonella* during convection heating, and

(3) To demonstrate the inclusion of a water term into a secondary inactivation model.

CHAPTER 2

LITERATURE REVIEW

2.1 Salmonella

Salmonellae are a small, gram-negative, non-spore forming rod shaped bacteria that cause foodborne gastroenteritis (Jay, 1996). They are widely distributed in nature and humans, with the intestinal tract of domestic livestock and wild animals being their primary habitat (Jay, 1996). Salmonellae are excreted in feces, then transmitted to other living creatures in a variety of ways. The most common vectors associated with salmonellosis in humans are eggs, poultry, and meat products (Jay, 1996).

The temperature range for growth of salmonellae is between 5.5 and 45°C (Ng et al., 1969). The temperature where the salmonellae begin to die and the maximum temperature for growth depend on the strain, growth phase, food composition, test media, other physical conditions, and competing microflora (Doyle and Mazzotta, 2000). The pH for optimum growth is between 6.6-8.2, with values greater than 9.0 and less than 4.0 being bactericidal (Jay, 1996). Regarding moisture, *Salmonella* growth inhibition in laboratory media (pH 7.0) has been reported at water activity values below 0.94 (Jay, 1996). Due to variations in these parameters, it is often difficult to compare data from experiments using different conditions.

With only a few exceptions, most studies on pathogens in poultry were conducted with single strains. However, a 'real' process is not necessarily limited to one strain, because various pathogens may be concurrently encountered in products. Therefore, regulations require that data and/or models used to document compliance be based on a

combination of *Salmonella* serotypes, referred to as a cocktail. The USDA does not specify the serotypes to be used, but says that any blend should include strains that have been implicated in foodborne outbreaks as well as strains that show fairly high heat resistance (USDA-FSIS, 1999). Different cocktails result in different model parameters; however, this problem could be eliminated if a universal cocktail were defined.

2.2 Thermal inactivation modeling

Predictive microbial models are mathematical representations of the growth, survival, or inactivation of microbial populations. Such models can be used to describe the behavior of microorganisms under different physical or chemical conditions. As stated by Zwietering et al. (1990), "these models allow the prediction of microbial safety or shelf life of products, the detection of critical parts of the production and distribution process, and the optimization of products and distribution chains."

To be of practical value, predictive microbial models must account for the effects of time and the various intrinsic and extrinsic factors affecting the microbial response. Whiting and Buchanan (1993) classified microbial models into primary, secondary, and tertiary types. Primary models describe the response of the microorganism with time to a single set of conditions. Each population vs. time curve can be described by a set of specific values for each of the parameters in the model (Whiting and Buchanan, 1993). Secondary models describe the response of one or more parameters of a primary model to changes in one or more of the cultural conditions (Whiting and Buchanan, 1993). These models calculate the changes in primary model parameters with respect to changes in temperature, pH, water activities, etc. (Whiting and Buchanan, 1993). Tertiary models

are computer programs that calculate microbial responses to varying conditions, compare the effects of the conditions, or contrast the behavior of several microorganisms (Whiting and Buchanan, 1993). Tertiary models make primary and secondary models "user-friendly."

2.2.1 Primary model

Several means are available to describe the relationship between microbial populations and time during thermal inactivation, including reaction kinetics analogies, simple D-values, and population-based models.

Chick (1908) proposed the following model:

$$N=N_0e^{-kt} \quad (1)$$

where N_0=the initial number of cells, N=the number of surviving cells, t=exposure time, and k=death rate. The instantaneous rate of inactivation of viable cells is proportional to the number of viable cells present at that time (Chiruta et al., 1997).

$$dN/dt=-kN \quad (2)$$

where (-dN/dt)=rate of inactivation of viable cells, N=the number of surviving cells, and t=time. According to this model, when bacteria are exposed to a constant temperature, microbial death occurs following the kinetics of first-order reactions. Taking the logarithm of equation (1) yields:

$$\ln(N/N_0)=-kt, \quad (3)$$

which is a log-linear equation with a slope of k, with k depending on factors such as temperature, pH, or water activity.

The thermal reduction time, or "D-value," describes the time dependence of bacterial destruction at a given condition. Similar to reaction kinetics analogies, D-values

represent first-order, log-linear reduction models. The D-value is the time required to decrease a bacterial population by 90% at a given temperature. When the D-value increases, the culture becomes more heat resistant. From the equation above, the D-value can be calculated as (Chiruta et al., 1997):

$$D=2.303/k \quad (4)$$

where k=inactivation rate constant from equations 1 and 2. This measurement is often used, but the variability among reported values is high, depending on the organism and conditions. Also, this method has been criticized, because it can be confusing or can obscure what should be simple mathematics of a first-order equation (Chiruta et al., 1997). However, because D has the dimension of time, it is often better understood (than k) in the food industry.

An example of a population-based model, where a non-linear relationship occurs, is the Weibull distribution. Depending on the data, it can have a downward or upward concavity, a "shoulder," or sigmoidal shape (Peleg and Cole, 1998). Population-based models assume that each cell in a bacterial population has a discrete resistance to thermal inactivation. If resistance follows a Weibull distribution, then the number of survivors can be modeled via the following model (Peleg and Cole, 1998):

$$\log_{10}[N(t)/N_o]=-b(T)t^{n(T)} \quad (5)$$

where $N(t)/N_o$=survival ratio, and b(T) and n(T) are temperature dependent constants.

2.2.2 Secondary models

Various types of secondary models include Arrhenius, extended Arrhenius, and square-root. While these are just a few of the most common secondary models, many other secondary models (of various forms) exist that account for a variety of parameters.

The effect of temperature on the rate of microbial inactivation is often described using the Arrhenius equation:

$$k=A\ e^{-Ea/RT} \tag{6}$$

where A=frequency factor, E_a=activation energy, R=universal gas constant, and T=absolute temperature. However, this model only accounts for temperature, and it has been recognized for decades that other factors affect the death rate; however, few attempts have been made to develop multifactorial models.

Reichart (1994) was the first to consider water activity in a semi-empirical model for thermal inactivation of *E. coli*. Shortly after, Cerf et al. (1996) proposed another five-parameter, extended Arrhenius, model from the experimental data of Reichart (1994). The Cerf model extends Davey's (1978) model, and includes other parameters. The Cerf et al. (1996) model is as follows:

$$\ln(k)=C_0+(C_1/T)+C_2pH+C_3pH^2+C_4a_w^2 \tag{7}$$

where T=absolute temperature, and C_0 to C_4 are empirical coefficients without direct biological significance.

The square-root or Belehradek model is typically used for growth models, and is based on the linear relationship between the square-root of the growth rate and temperature (Zwietering et al., 1990). Biological zero, the value for temperature when the growth rate was extrapolated to zero, was introduced here. The simplest version of the model for temperatures below the optimum growth rate is:

$$\sqrt{k}=a(T-T_o) \tag{8}$$

where k is the growth rate or other rate term, such as the reciprocal of the lag time, T_o is the temperature when the line is extrapolated to k=0, and a is the slope (Zweitering et al., 1990).

2.3 Factors affecting thermal resistance

Variables affecting heat resistance of pathogens in meat include species, pH, fat content, salts, and other environmental factors (Jay, 1996). In addition, experimental approaches, serotypes, growth media, and enumerating procedures vary among laboratories, and this makes comparison difficult and causes data to be relevant only to the particular commodity tested (Skinner et al., 1994; Doyle et al., 2001; Doyle and Mazzotta, 2000).

2.3.1 Pathogen species and strains

Heat resistance differences among species and strains exist (Doyle and Mazzotta, 2000); for the purpose of this literature review, various pathogens are examined.

2.3.2 Inactivation media

Salmonella tends to be more thermally resistant in actual food products than in laboratory media (Murphy et al., 2002); moreover, food type also affects resistance (Ahmed et al., 1995; Murphy et al., 2002). Numerous studies show that bacteria are more resistant to heat when tested in food than in laboratory media (Doyle et al, 2001).

Bacteria attached to muscle tissue are more heat resistant than bacteria suspended in liquid media (Murphy et al., 2002). Murphy et al. (2000) compared D-values in meat to those in a semi-liquid medium and found that the D-values were higher in ground

chicken breast than in a peptone-agar solution at 55 to 70°C. Therefore, there is a need to evaluate *Salmonella* inactivation in meat and not rely on data (only) from model media.

2.3.3 Fat content

Fat content influences the thermal resistance of microorganisms in meat; however, some inconsistencies have been observed. Some studies have shown higher D-values in high fat meat, while other studies have shown the opposite (Table 2.1). However, in general, inconsistent trends between fat content and D-values have been reported in the literature.

TABLE 2.1 *Effect of fat on thermal inactivation of vegetative cells.*

Organism	Product	Reference	D-value (min)	Temp (°C)	Fat (%)
E. coli O157:H7	turkey	Ahmed et al., 1995	70.41	50	3
			6.37	55	
			0.55	60	
			115	50	11
			9.69	55	
			0.58	60	
	chicken		65.24	50	3
			8.76	55	
			0.38	60	
			105.5	50	11
			9.74	55	
			0.55	60	
E. coli O157:H7	beef	Line et al., 1991	78.2	51.7	2
			4.1	57.2	
			0.3	62.8	
			115.5	51.7	30.5
			5.3	57.2	
			0.5	62.8	
E. coli O157:H7	turkey	Kotrola and Conner, 1997	42.3*	52	3
			12.5*	55	
			2.8*	57	
			0.9*	60	
			38.5*	52	11
			11*	55	
			2.4*	57	
			0.9*	60	
Listeria monocytogenes Scott A	beef	Fain et al., 1991	81.3*	51.7	2
			2.6	57.2	
			0.6	62.8	
			71.1*	51.7	30.5
			5.8	57.2	
			1.2	62.8	

* An increased D-value was not observed with increased fat content.

In some studies, D-values for pathogens were higher in high fat meat than in low fat meat. According to Line et al. (1991), D-values for *E. coli* O157:H7 in beef increased in the heating range of 52 to 63°C as the fat content increased from 2.0 to 30.5%. Ahmed et al. (1995) used a single strain of *E. coli* O157:H7 and found that as the fat content increased (3-30%) in different meat products (chicken, turkey, beef, and pork sausage),

the D-values increased. Fain et al. (1991) inoculated ground beef with *Listeria monocytogenes* and generally found that D-values increased as the fat content increased (2-30.5%); however, this did not hold true on one occasion. Ben-Embarek and Huss (1993) also reported higher D-values for *L. monocytogenes* in salmon than in cod and attributed the greater heat resistance in salmon to the higher fat content.

Several explanations were given as to why D-values increased as the fat content increased. Ahmed et al. (1995) stated that the higher D-values were likely due to the decreased moisture content of the meat. They claimed that bacteria suspended in fat are more difficult to destroy than in aqueous medium, due to a reduction of water activity. Veeramuthu et al. (1998) observed higher D-values for *S.* Senftenberg in turkey containing increased levels of fat and attributed this finding to the effect of fat on water activity.

However, other authors did not find fat content to be a significant factor. Kotrola and Conner (1997) did not see an increase in D-values for *E. coli* O157:H7 as the fat content increased in ground turkey, with the opposite being observed. Kotrola and Conner (1997) reported D-values at 55°C ranging from 12.5 (3% fat) to 11 (11% fat) min at 60°C. Juneja and Eblen (2000) found that the D-values of an 8-strain *Salmonella* Typhimurium DT 104 cocktail in ground beef decreased with increasing fat content. Maurer (2001) observed that higher fat levels significantly affected the D-value of *S.* Senftenberg in turkey; however, no significant effect was observed with *E. coli* O157:H7 in turkey or beef, or with a *Salmonella* cocktail in beef.

Several explanations were given as to why D-values decreased as the fat content increased. Kotrola and Conner (1997) explained that finely grinding the meat and fat

together before heating could have affected the dispersal of the fat in the meat and allowed it to emulsify. This could, in turn, have increased the solubility of water in fat before the product was heated. Olson and Nottingham (1980) attributed not seeing an increase in D-values with increasing fat content to a protective effect in higher fat products.

2.3.4 pH

The pH describes the hydrogen ion concentration [H^+], and is often recognized as one of the most important factors influencing the heat resistance of bacteria. Juneja and Eblen (1999) showed that as the pH decreased, the D-values for *L. monocytogenes* decreased. Abdul-Raouf et al. (1993) showed that *E. coli* O157:H7 was less heat stable in acidified ground beef slurries, as compared to non-acidified slurries, with stability dependent on the type of acid used.

Davey et al. (1995) found that pH significantly affected the thermal inactivation rate for *E. coli*. When experiments were performed in a test carrier liquid over a temperature range of 54 to 62°C, the influence of pH was most significant at the lower temperatures. Overall, D-values were highest at pH 7, and decreased as pH was reduced below 7. Chiruta et al. (1997) tested the effect of pH on the rate constant for thermal inactivation and generally found results consistent with Davey et al. (1995) for *E. coli*, *L. monocytogenes*, and *P. fluorescens*. Temperatures ranged from 52 to 62°C, with the effect most significant at the lower temperatures.

However, Foster and Hall (1991) showed that *S.* Typhimurium could be induced to survive under more acidic conditions than expected. Also, Farber and Pagotto (1992)

demonstrated that HCl acidification actually increased thermal resistance of *L. monocytogenes*.

2.3.5 Salts and other common additives

Salts, lactates, and phosphates are common additives in meat products. Primary functions of salt in meat products are: (1) to solubilize muscle proteins to assist in binding meat, moisture and fat; (2) to serve as a flavoring agent; and (3) to inhibit growth of foodborne pathogens (Pearson and Gillett, 1996). In most cases, salt appears to act as a protective agent, resulting in higher heat resistance, but this does not always hold true.

Thermotolerance can be increased by incorporating salt or curing salt mixtures (Juneja and Eblen, 1999). Juneja and Eblen (1999) found that by adding NaCl, this protected *L. monocytogenes* against heat inactivation in beef gravy at 55 to 65°C. D-values increased 2- to 5-fold after curing salts were added to meat (Juneja and Eblen, 1999). Maurer et al. (2000) found that the D-values for *Salmonella* increased as the salt content increased from 0 to 2% in ground turkey.

Additional studies have assessed various combinations of additives. Kotrola and Conner (1997) found that both sodium chloride and sodium lactate enhanced survival of *E. coli* O157:H7 in cooked turkey meat as compared to meat without additives at 52 to 60°C, with the highest D-values (greatest survival) observed when three additives (sodium chloride, sodium lactate, and polyphosphate) were added to the turkey. The authors attributed this increase to the reduction of water activity caused by the additives binding water in the heating medium.

Other food additives, such as bacteriocins, EDTA, polyphosphates, hydrogen peroxide and the lactoperoxidase system, make *Salmonella* more heat sensitive (Doyle and Mazzotta, 2000). The effectiveness varies depending if the additive is in culture media or a complex food, because it may interact with fat and protein and thereby be less available to interact with bacterial cells (Doyle and Mazzotta, 2000). Goepfert et al. (1970) and Corry (1975) tested several solutes and found that heat resistance of bacteria varied widely with different solutes at the same water activities. Overall, sucrose had the greatest protective effect, compared to glycerol, glucose, polyethylene glycol (Goepfert et al., 1970) and glucose, fructose, sorbitol, and glycerol (Corry, 1975).

Because solutes and other additives affect the thermal resistance of bacteria, tests should be run specific to the meat product and solute and/or additive of interest.

2.3.6 Water

Water is essential for all living processes. Due to its chemical and physical properties, water is so unique that it is often considered one of the most important compounds on earth (Gailani and Fung, 1987).

Water availability has an influence on the heat resistance of *Salmonella* in meat products. However, the specific cause of the effect is unknown. There are several ways to quantify water in a food system. Many studies have looked at meat moisture content or water activity (intrinsic parameters), and a few have looked at humidity (extrinsic parameter). This section will further investigate this issue.

Throughout the literature, a common theme is seen regarding various other factors affecting thermal inactivation. Authors have often attributed the effects of other parameters (specifically fat and salt) to changes in water activity (Blankenship, 1978;

Ghazala et al., 1995; Kotrola and Conner, 1997; Shelef and Yang, 1991; and O'Donovan and Upton, 1999); however, others suggest that changes in water activity do not completely explain the effects of these other factors (O'Donovan and Upton, 1999). The present study includes tests specifically aimed at testing the impact of water activity on thermal inactivation without changing other factors.

2.3.6.1 Water activity

Water activity (a_w) describes the amount of available water and is defined as:

$$a_w = P_i / P_o \quad (9)$$

where P_i=vapor pressure of water in equilibrium with the material, and P_o=vapor pressure of pure water at the same temperature (Gailani and Fung, 1987). Water activity controls the movement of water between a food product and the environment (Gailani and Fung, 1987). The range of water activity for high moisture foods is 0.9 to 1.0 (Gailani and Fung, 1987), with meats classified as high moisture foods.

In general, as water activity decreases, thermal resistance of pathogens increases. However, most studies have been performed in sugar solutions rather than in actual food systems. As discussed in section 2.3.2, resistance varies depending on the media used; therefore, it is crucial to perform the studies in actual food products.

Goepfert et al. (1970) studied the effect of water activity in sucrose solutions (0.87-0.99), and found that heat resistance of *Salmonella* always increased as the water activity of the heating menstruum (0.75-0.99) decreased. Riemann (1960) also documented increased heat resistance with decreased water activity.

Cerf et al. (1996) used Reichart's (1994) experimental data for thermal inactivation of *E. coli* at both constant temperature (isothermic) and constantly varying

temperature (anisothermic). The tests were performed using laboratory media with glycerol added to distilled water to reach the targeted water activities (Reichart, 1994). The isothermic data encompassed the following conditions: 58°C, pH 3-9, and water activity 0.928-0.995. The anisothermic data contained the same parameters, except the temperature ranged between 52-63°C. Cerf et al. (1996) claimed that the additive, linear Arrhenius model accurately predicted the combined effect of sterilizing temperature, pH, and water activity on the thermal inactivation of *E. coli*. Cerf et al. (1996) suggested that these models could be extrapolated over a limited range of environmental values; however, sufficient published and independent data to test this were lacking. The isothermic model (58°C) was as follows:

$$\ln(k)s^{-1}=-6.021-2.377pH+0.1994pH^2+8.997a_w^2 \qquad (10)$$

The anisothermic model was as follows:

$$\ln(k)s^{-1}=86.49-0.3028*10^{-5}/T-0.5470pH+0.0494pH^2+3.067a_w^2 \qquad (11)$$

O'Donovan-Vaughan and Upton (1999) investigated the survival of *Salmonella* Typhimurium in four different carbohydrate solutions (glycerol, sucrose, glucose, and polyethylene glycol) at three different water activities (0.45, 0.70, and 0.90). They found that as the water activity of the solution was reduced, the heat resistance increased (55 and 65°C). Additionally, heat resistance depended on the nature of the solute used to reduce the water activity; sucrose gave the greatest protection. The conclusion was that the heat resistance depended on the solute used to reduce the water activity; however, this result was not entirely consistent in the data reported.

2.3.6.2 Humidity

While water activity is the means to quantify the state of water in a food product, humidity is the means to quantify the water state in the environment. Nevertheless, only limited research has focused on evaluating the effects of process humidity on thermal inactivation of foodborne pathogens.

Kirby and Davies (1990) evaluated humidity effects in a non-food system. *Salmonella* Typhimurium LT2 received dehydration treatment by being placed in an atmosphere controlled by a saturated salt solution of sodium bromide (BHD) (57% equilibrium relative humidity (ERH)) at 37°C for 48 h, with this dehydration treatment continued for up to 34 d (Kirby and Davies, 1990). After being heated at 135°C for 30 min, the thermal resistance of these dehydrated *Salmonella* cells were enhanced (Kirby and Davies, 1990). By increasing the length of the dehydration treatment, the initial count was reduced, but the shape of the curve was the same (triphasic death curve) (Kirby and Davies, 1990). In addition, populations remained relatively constant when heated at 100°C for 1 h (Kirby and Davies, 1990).

Lethality of *Salmonella* during roasting of beef has been studied, and research showed that the death rate depends on both where the bacteria are located and the heating conditions (Goodfellow and Brown, 1978; Blankenship, 1978). Dry roasting of meat will kill *Salmonella* on the interior, but allow for survival on the surface (Blankenship, 1978; Blankenship, 1980; and Goodfellow and Brown, 1978). Goodfellow and Brown (1978) found viable *Salmonella* on the surface of the meat after reaching an internal temperature of 57.2°C in a dry environment with the oven at 107°C for 5.5 h. However, no survivors were present after reaching an internal temperature of 54.4°C in a wet environment

(steam injection) at 79.4°C for 30 min. In a different study with dry heat, Blankenship (1978) observed *Salmonella* survivors in meat that attained an internal temperature of 64.2°C. Blankenship et al. (1980) hypothesized that a possible explanation was that the surface and near the surface of the meat probably had a lower water activity (compared to the center part), due to drying and crust formation during cooking.

Murphy et al. (2001c) studied thermal inactivation of *Salmonella* and *Listeria* in inoculated ground chicken patties (N_o~10^7CFU/g) under varying conditions in an air convection oven at an air temperature of 177°C. Thermal processing was conducted at wet bulb temperatures (humidity conditions) of 48 and 93°C, with the endpoint center temperature of the patties ranging from 65-75°C (Murphy et al., 2001c). Patties processed at a wet bulb temperature of 93°C (high humidity) in a wet environment showed no survivors. The patties processed at a wet bulb temperature of 48°C (low humidity) in a dry environment contained more than 100 CFU/g (both *Salmonella* and *Listeria*) at the entire endpoint temperature range (Murphy et al., 2001c). Therefore, bacterial survival was enhanced at a lower humidity. In a high humidity environment, the authors hypothesized that meat pores opened and the space was occupied with water vapor, which created a wet environment that enhanced for bacterial inactivation (Murphy et al., 2001c). In a low humidity environment, pores may have still opened, but the space would have been occupied with dry air, which would create a dry environment that was less effective in inactivating bacteria (Murphy et al., 2001c).

In another study, Murphy et al. (2001b) evaluated thermal inactivation of *Salmonella* and *Listeria* in ground chicken patties processed in the same oven as the previously reported study (Murphy et al., 2001c). The air humidity was controlled by

steam injection into the oven (Murphy et al., 2001b). Microbial inactivation decreased with decreasing wet bulb temperature (39-98°C) (Murphy et al., 2001b). However, this trend could be caused by the moisture content and water activity of the meat decreasing during cooking, and not necessarily be a direct effect of wet bulb temperature (i.e., process humidity).

Murphy et al. (2001a) also used laboratory-based inactivation models to calculate process lethality for chicken patties processed in an impingement oven (Murphy et al., 2000). The air temperature was 149°C, wet bulb temperature ranged from 39 to 98°C, and patty center temperature ranged from 55 to 80°C (Murphy et al., 2001a). The cooking conditions affected the time-temperature history of the patties; therefore, the cooking humidity affected predicted process lethality with a slight decrease in lethality seen at higher wet bulb temperatures (Murphy et al., 2001a). According to the authors, this occurred so that the same final product temperature could be reached; therefore, cooking time decreased with increasing wet bulb temperature (Murphy et al., 2001a).

CHAPTER 3

MATERIALS AND METHODS

3.1 Overview

This project was comprised of three different experiments (Table 3.1) involving isothermal inactivation trials. For simplicity, the different experiments will hereafter be referred to as Parts 1, 2, and 3. For Part 1, raw, ground, irradiated turkey breast was used. The moisture content was either increased or slightly decreased, and the samples were heated in a waterbath. However, after completing this experiment with a small range of moisture contents, moisture content did not appear to influence the thermal inactivation of *Salmonella*. Therefore, Part 2 consisted of a series of tests with a much wider moisture content range, using cooked ground turkey breast. For Part 3, the same meat was used as in Part 1, but the samples were heated in an air convection oven, with humidity as the primary factor, to determine if increasing the moisture in the environment affected the inactivation. See Appendix B for the details on treatment levels for every test in Parts 1, 2, and 3.

TABLE 3.1 *Summary of experimental design.*

Part 1 (Moisture Effects-High Range)

Temperature (°C)	55, 60, and 65
Moisture Content (%)	70.9-76.3 (LF) and 64.5-68.5 (HF)
Fat Content (%)	1 and 13
Time (min)	5 durations (dependent on temperature)

Part 2 (Moisture Effects-Low Range)

Temperature (°C)	60
Moisture Content (%)	37.1, 54.4, and 72.5
Time (min)	0, 0.75, 1.5, 2.25, and 3
Fat (%)	2

Part 3 (Humidity Effects)

Temperature (°C)	60
Relative Humidity (%)	90 and 96
Fat Content (%)	1 and 13
Time (min)	0, 0.75, 1.5, 2.25, and 3

3.2 Part 1 – Moisture effects-high range

The purpose of Part 1 was to test the effect of meat moisture content (over a small range) on the inactivation of *Salmonella* in isothermal heating trials in a waterbath.

3.2.1 Inoculum

3.2.1.1 Bacterial strains

The inoculum consisted of eight *Salmonella* strains, obtained from Dr. V.K. Juneja (Agricultural Research Service, Eastern Regional Research Center, USDA-ARS, Philadelphia, PA). The strains were: *S.* Thompson FSIS 120 (chicken isolate), *S.* Enteriditis H3527 and H3502 (clinical isolates phage type 13A and 4, respectively), *S.* Typhimurium (DT104) H3380 (human isolate), *S.* Hadar MF0404 (turkey isolate), *S.* Copenhagen 8457 (pork isolate), *S.* Montevideo FSIS 051 (beef isolate), and *S.* Heidelberg F5038BG1 (human isolate). Each strain was preserved at –80°C in a vial containing tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI) with 10% glycerol.

3.2.1.2 Culture preparation

To propagate the cultures, one loop of frozen culture was transferred to 9 ml of TSB in 20 ml culture tubes. The cultures were transferred daily in TSB (37°C, 18-24 h), with a minimum of two consecutive transfers before subsequent inoculation. Each inoculum was prepared from an 18-24 h (assumed log phase (Maurer, 2001)) culture. The eight strains were grown in separate culture tubes, and then equal volumes were combined prior to centrifugation to produce a cocktail with a target total concentration of 10^8 CFU/ml. A new series of cultures from the frozen stock was initiated every week.

On the day of each experiment, cultures were pelleted by centrifugation at 6,000 x g for 20 min at 4°C and resuspended in sterile 0.1% peptone water. The cultures were enumerated by plating in duplicate on Petrifilm® aerobic count plates (3M, St. Paul, MN) and incubating at 37°C for 24-36 h.

3.2.2 Meat

3.2.2.1 Ground turkey preparation

Skinless turkey breast meat was obtained from Michigan Turkey Producers, Inc. (Wyoming, MI) on the day of slaughter and transferred to the Michigan State University Meat Laboratory at 0°C. The muscle was immediately chopped in a bowl chopper (Hobart Mfg. Co., Model 841810, Troy, OH) until the temperature reached 13°C. The turkey fat was chopped separately and then mixed back into half of the previously ground turkey to create two lots, one with lower and one with higher fat content. Keeping the two fat lots separate, the turkey was double-bagged in polyethylene-laminated nylon pouches, vacuum packaged in approximately 100 g portions, and stored at -12°C.

The frozen meat was transported overnight on dry ice to Iowa State University and irradiated to >30 kGy to eliminate indigenous microflora. The frozen meat was transported back to Michigan State University on dry ice overnight. Samples of irradiated turkey were tested for sterility to ensure negligible background microflora by plating a 1:10 dilution in 0.1% peptone on Petrifilm® aerobic count plates.

Proximate analysis was performed in triplicate from three sub-samples taken from each lot (i.e., low and high fat). Moisture, fat, and protein contents were determined by AOAC (1996) methods 991.36, 981.1, and 950.46B, respectively. To determine the pH, 10 g of ground turkey were added to 90 g of distilled water and homogenized using a Polytron homogenizer (Model PT 10/35, Brinkman Instruments, Westbury, NJ) for 30 s at speed setting 3. Three samples of both fat levels were prepared, and duplicate measures were taken of each, using a combination electrode (Model 145, Corning, Medfield, MA).

Twenty-four hours prior to performing each experiment, meat samples were thawed in a refrigerator at 4°C.

3.2.2.2 Moisture content alteration

The overall purpose of this experiment was to manipulate the moisture content of each sample before inoculation and thermal treatment, in the general range that might occur during thermal processing of a fresh product to a ready-to-eat state. For "native state" samples, no water was added or removed (other than that associated with the inoculum). For increased moisture samples, 0.1% sterile peptone was pipetted dropwise into the meat prior to inoculation. For decreased moisture samples, liquid was removed by centrifugation at 6,000-9,000 x g for 10-40 min at 4°C. Liquid was poured off the

samples, and samples were weighed to determine the amount of liquid. The centrifugation settings and time increments were varied in order to achieve the target moisture content. While centrifugation decreased the moisture content, the degree of reduction was fairly limited, because it was assumed that soluble proteins were also being extracted with the liquid. After the manipulations, the moisture content and water activity were determined for each sample using AOAC (1996) method 991.36 and an electronic water activity meter (accuracy is $\pm$0.003) (Decagon Devices, Inc., Model 3TE, Pullman, WA), respectively.

3.2.3 Inoculation

The inoculum (~1 ml) was added dropwise, using aseptic procedures, to obtain a target concentration of 10^8 CFU/g ground turkey. The inoculum added to the meat had a minimal effect on the moisture content (<0.2%). The meat was manually mixed (using sterile gloves) in a sterile bowl for 5 min to ensure even distribution of the inoculum. Uniform distribution was visually verified using green food dye (McCormick and Company, Inc., Hunt Valley, MD) in preliminary trials. Actual uniformity was verified by plating sub-samples of the inoculated meat (Chapter 4).

For each sample to be heated, 1 g of inoculated meat was aseptically placed into a 5 x 25.5 cm polyethylene laminated nylon bag (Butcher and Packer Supply Co., Detroit, MI). The bags were screened to ensure negligible background microflora by mixing 9 ml of 0.1% peptone water in 10 random bags and plating on Petrifilm® aerobic count plates. The bags containing meat were subsequently rolled between two guides, using a large glass test tube, to a uniform thickness of <1 mm (Figure 3.1). This procedure was used for two primary reasons; 1) to transfer heat as quickly as possible, thereby minimizing

the thermal lag time and 2) to consequently produce more accurate thermal inactivation parameters (Orta-Ramirez and Smith, 2002). The bags were heat-sealed using a soldering iron, refrigerated at 4°C, and subjected to thermal treatment within 4 h.

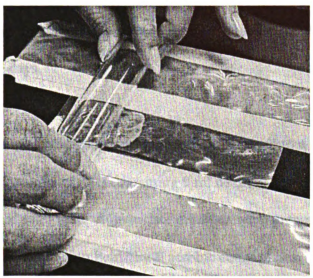

FIGURE 3.1 *Meat samples rolled between two guides to achieve a uniform thickness.*

3.2.4 Thermal inactivation

The sealed bags were placed in a rack and completely submerged in a temperature-controlled waterbath (NESLAB Instruments, Inc., Newington, NH) set at 55.5, 60.5, or 65.5°C. The waterbath was set at 0.5°C above the treatment temperature to obtain an actual water temperature of 55, 60, or 65°C.

The thermal lag time was defined as the time required for the meat temperature to reach within 0.5°C of the waterbath temperature. The lag time was determined by placing a T-type thermocouple in the geometric center of a sample, submerging the sample in the heated water, and logging the sample temperature with a DuaLogR™ thermocouple thermometer (Cole Parmer Instrument Company, Model # 01100-50, Vernon Hills, IL). The test was performed in triplicate, and a lag time of 8 s was

determined. The end of the thermal lag was defined as the initial test time for inactivation ("time zero").

Samples were removed at five specific time intervals for each test temperature, placed directly into an ice-water bath, and plated within 4 h. Samples at each moisture content were heated in three replicate batches at each temperature. Replicate batches were run on different days.

3.2.5 Enumeration

After treatment, each sample was aseptically transferred to a sterile Whirlpak™ bag (18 oz, Nasco, Ft. Atkinson, WI) containing 9 ml of 0.1% sterile peptone water, and manually homogenized for 1 min. Appropriate dilutions were prepared in 0.1% peptone water, then 1 ml was pipetted onto Petrifilm® aerobic count plates. All samples were plated in duplicate and incubated at 37°C for 24-36 h before enumeration. The minimum detection level was 10 CFU/g.

3.2.6 Statistics and modeling

Analysis of variance (ANOVA) was run to evaluate $\ln(N/N_o)$ of the cocktail as a function of the main effects of meat temperature, time, moisture content, and fat content, and all two-term interactions. N is number of survivors at the end of treatment, and N_o is the initial inoculum. Linear regressions were run with the raw data to obtain k values from the slope of equation 3. Then an ANOVA was conducted to evaluate the k values of the cocktail as a function of the main effects of temperature, moisture content, fat content, and all two-term interactions.

Statistical analyses were performed using JMP (version 4, copyright 2000-2001; SAS Institute, Inc., Cary, N.C.).

3.3 Part 2 – Moisture effects-low range

The purpose of Part 2 was to test the effect of meat moisture content (over a large range) on the inactivation of *Salmonella* in isothermal heating trials in a waterbath. In order to decrease the moisture content over a large range (as aseptically as possible), the meat was cooked/dried in a smokehouse.

3.3.1 Inoculum

3.3.1.1 Bacterial strains

The same bacterial strains were used as described in Section 3.2.1.1.

3.3.1.2 Culture preparation

The culture was prepared as described in Section 3.2.1.2.

3.3.2 Meat

3.3.2.1 Ground turkey preparation and moisture content alteration

Skinless turkey breast meat was obtained from Michigan Turkey Producers, Inc. (Wyoming, MI) on the day of slaughter and transferred to the Michigan State University Meat Laboratory at 0°C. The muscle was immediately chopped in a bowl chopper (Hobart Mfg. Co., Model 841810, Troy, OH) until the temperature reached 13°C.

The turkey was stuffed into either permeable or impermeable casing, using a hand stuffer (VOGT9, KOCH, Kansas City, MO). The meat to be held at native state moisture content was stuffed in a non-permeable casing (Faserin #2, Teepak, Kansas City, MO) measuring 6.5 cm in diameter and 68.58 cm in length. The meat to be dried was stuffed into a permeable casing (Fiberous Securex #2, Teepak, Kansas City, MO) measuring 4.0 cm in diameter and 76.2 in cm length.

Meat was dried/cooked in a smoke house (CGI, Model A28-RO101, Cicero, IL), to decrease the moisture content and to minimize the microbes in the product. All samples were heated to an internal temperature of 73.9°C. Native state samples were removed when the internal temperature reached 73.9°C; whereas, samples for the two decreased moisture content levels remained in the smokehouse (at an internal temperature of ~57°C) until the desired targeted moisture contents were obtained (Figure 3.2).

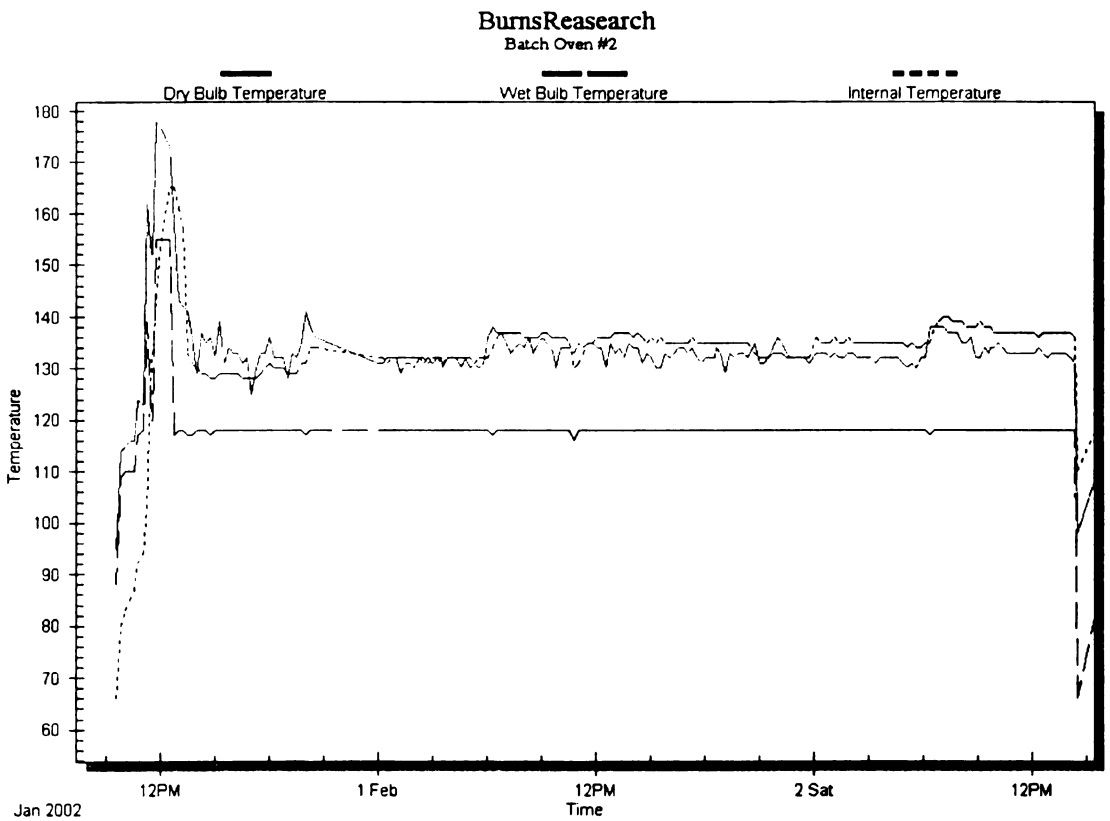

FIGURE 3.2 *Smokehouse operation schedule-temperature (°F).*

When the turkey was removed from the smokehouse, it was immediately chilled in a 2-3°C cooler, and placed in a polyethylene-laminated bag, vacuum packaged, and stored at 2-3°C for approximately 24 h. In an aseptic environment, the turkey was then

cut into approximately 20 g sub-samples, double packaged in polyethylene-laminated bags, vacuum-sealed, and stored at –12°C.

Indigenous microbial levels in the turkey were determined by manually homogenizing a 1 g sample in 9 ml of 0.1% sterile peptone water for 1 min. Appropriate dilutions were made in 0.1% peptone water, after which 1 ml was pipetted onto a Petrifilm® aerobic count plate. All samples were plated in duplicate and incubated at 37°C for 24-36 h before enumeration.

Proximate analysis was performed in triplicate from three sub-samples taken from each of the three lots. Moisture, fat, and protein contents were determined by AOAC (1996) methods 991.36, 981.1, and 950.46B, respectively. To determine the pH, 10 g of ground turkey were added to 90 g of distilled water and homogenized for 30 seconds using a Polytron homogenizer (Model PT 10/35, Brinkman Instruments, Westbury, NJ) at speed setting 3. Water activity was determined using an electronic water activity meter (Decagon Devices, Inc., Model 3TE, Pullman, WA). Each sample was thawed by placing in the refrigerator at 4°C for 4 h prior to performing the experiment.

3.3.2.2 Decreasing the particle size

Due to the low moisture content of the product, each sample was chopped aseptically in a high-speed grinder (Tekmar Company, Cincinnati, Ohio) to a particle size equivalent of powder.

3.3.3 Inoculation

The inoculum (~1 ml) was added dropwise (minimally affecting the moisture content), using aseptic procedures, to obtain a target concentration of 10^8 CFU/g ground turkey. The meat was manually mixed in a sterile bowl for 5 min using a sterile spatula

to ensure even distribution of the inoculum. Even distribution was visually verified using food dye in preliminary trials. Actual uniformity was verified by plating sub-samples of the inoculated meat (Chapter 4).

For each sample to be heated, 1 g of inoculated meat was aseptically placed into a 5 x 25.5 cm polyethylene laminated nylon bag (Butcher and Packer Supply Co., Detroit, MI). The bags were screened to ensure negligible background microflora by mixing 9 ml of 0.1% peptone water in 10 random bags and plating on Petrifilm® aerobic count plates. The bags containing meat were subsequently rolled between two guides, using a large glass test tube, to a uniform thickness of <1 mm (Figure 3.1). Again, this procedure was used for two primary reasons; 1) to transfer heat as quickly as possible, thereby minimizing the thermal lag time and 2) to consequently produce more accurate thermal inactivation parameters (Orta-Ramirez and Smith, 2002). The bags were heat-sealed using a soldering iron, refrigerated at 4°C, and subjected to thermal treatment within 1 h.

3.3.4 Thermal inactivation

To prevent water from entering the bags (through possible leaks at the seal), the bags were sealed at the top, and the tops were held above the water line during treatment. The samples were placed in a rack that was completely submerged in a temperature-controlled waterbath (NESLAB Instruments, Inc., Newington, NH) set at 60.5°C, to obtain an actual water temperature of 60°C.

The thermal lag time was defined as the time required for the meat temperature to reach within 0.5°C of the target temperature (60°C). To determine this time, a thermocouple was placed in the geometric center of a meat sample. The test was performed in triplicate, and a lag time was determined. The lag times were 15, 30, and

60 s, for moisture contents of 73, 55, and 37%, respectively. The end of the thermal lag was defined as the initial test time for inactivation ("time zero").

Samples were removed from the waterbath at five specific time intervals and placed directly into an ice-water bath, with the seals remaining above the ice-water line. Duplicate batches were run on different days.

3.3.5 Enumeration

Within 1 h of treatment, each sample was aseptically transferred to a sterile Whirlpak™ bag containing 9 ml of 0.1% sterile peptone water, and manually homogenized for 1 min. Appropriate dilutions were made in 0.1% peptone water after which 1 ml was pipetted onto Petrifilm® aerobic count plates. The experiment was performed in duplicate, and all samples were plated in duplicate and incubated at 37°C for 24-36 h before enumeration. The minimum detection level was 10 CFU/g.

3.3.6 Statistics and modeling

Analysis of variance (ANOVA) was run to evaluate $\ln(N/N_o)$ of the cocktail as a function of the main effects of time, moisture content/water activity, and all two-term interactions. Linear regressions were run with the raw data to obtain k values from the slope of equation 3. Then an ANOVA was conducted to evaluate the k values of the cocktail as a function of the main effects of moisture content/water activity.

Statistical analyses were performed using JMP (Version 4, copyright 2000-2001; SAS Institute, Inc., Cary, N.C.).

3.4 Part 3 – Humidity effects

The purpose of Part 3 was to test the effect of humidity on the inactivation of *Salmonella* in isothermal heating trials in an air convection oven.

3.4.1 Inoculum

3.4.1.1 Bacterial strains

The same bacterial strains were used as described in Section 3.2.1.1.

3.4.1.2 Culture preparation

The culture was prepared as described in Section 3.2.1.2.

3.4.2 Meat

3.4.2.1 Ground turkey preparation

The ground turkey was prepared as described in 3.2.2.1.

3.4.3 Inoculation

The inoculum (~1 ml) was added dropwise (minimally affecting the moisture content), using aseptic procedures, to obtain a target concentration of 10^8 CFU/g ground turkey. The meat was manually mixed in a sterile bowl for 5 min using sterile gloves to ensure even distribution of the inoculum. Even distribution was visually verified using food dye in preliminary trials. Actual uniformity was verified by plating sub-samples of the inoculated meat (Chapter 4).

For each sample to be tested, 1 g of inoculated meat was aseptically spread onto an ~8 x 8 cm piece of sterile fiberglass screen (New York Co., Mt Wolf, PA) (Figure 3.3) to a uniform thickness of <1 mm. For sterility testing, ten screens were placed in a sterile bag containing 9 ml of 0.1% peptone water with 0.1 ml plated on Petrifilm® aerobic count plates.

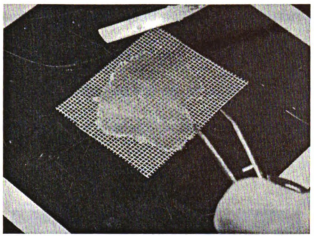

FIGURE 3.3 *Meat sample being spread to a uniformly thickness onto a sterile screen.*

3.4.4 Thermal inactivation

The samples were heated in a custom air convection oven at 60°C and 90 or 96% relative humidity (Figure 3.4). The oven was capable of producing dry bulb temperatures ranging from 25 to 200°C (± 1°C) and relative humidities ranging from 0 to 90% (±1%) and 90 to 100% (±2%). The unique heating system consisted of a sample chamber connected to a mixing chamber, which supply an electronically controlled air/vapor mixture for a programmed sample exposure.

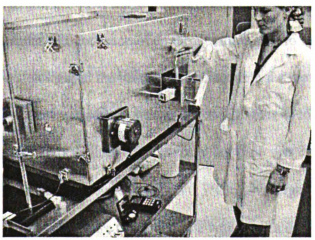

FIGURE 3.4 *Meat sample entering the custom air convection oven.*

The oven contained 6 heat strips (350 watts each). Moisture was added from a steam generator that injected steam in short bursts until the desired humidity was reached. A centrifugal fan circulated air inside the heating chamber. The sample was placed in the heating chamber on a stand, so that air was blown across the top and bottom surfaces of the sample (Figure 3.5).

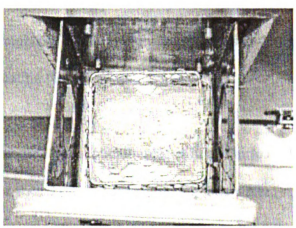

FIGURE 3.5 *Sample in the heating chamber on a stand, which allows air to be blown across the top and bottom surface of the sample.*

In Parts 1 and 2, the thermal lag time was defined as the time required for the meat sample to reach within 0.5°C of the waterbath temperature. However, in Part 3, using the oven, the samples did not reach oven temperature, due to the effects of evaporative cooling, which limited the sample temperature to the oven wet bulb temperature. Therefore, the oven setting was adjusted (Chapter 4) to ensure that the samples reached the target temperature. The thermal lag time was defined as the time required for the meat temperature to reach within 0.5°C of the target temperature (60°C). To determine the lag time, a thin-wire thermocouple was woven in and out of the screen in the middle of the meat sample. The test was performed in triplicate, with the thermal

lag time determined to be 20 s. The end of the thermal lag was defined as the initial test time for inactivation ("time zero").

Samples were removed after five specific test durations, aseptically placed directly into sterile Whirlpak™ bags containing 9 ml of chilled 0.1% peptone water (4°C), and plated within 30 min. The samples were heated in two replicate batches at 60°C and 90 or 96% relative humidity. Each replicate batch occurred on a different day. The initial and final weights of each sample were recorded to determine the amount of moisture lost during heating (Chapter 4 and Appendix A).

3.4.5 Enumeration

Each treated sample was aseptically placed in a Whirlpak™ bag and manually homogenized for 1 min with 9 ml of 0.1% sterile peptone water. Appropriate dilutions were made in 0.1% peptone water with 1 ml pipetted onto Petrifilm® aerobic count plates. The experiment was performed in duplicate, and all samples were plated in duplicate and incubated at 37°C for 24-36 h before enumeration. The minimum detection level was 10 CFU/g.

3.4.6 Statistics and modeling

Analysis of variance (ANOVA) was run to evaluate $\ln(N/N_o)$ of the cocktail as a function of time, relative humidity, final moisture content, and fat, and all two-term interactions. Linear regressions were run with the raw data to obtain k values from the slope of equation 3. Then an ANOVA was conducted to evaluate the k values of the cocktail as a function of the main effects of relative humidity, final moisture content and fat, and all two-term interactions.

Statistical analyses were performed using JMP (version 4, copyright 2000-2001; SAS Institute, Inc., Cary, N.C.).

CHAPTER 4

RESULTS AND DISCUSSION

As described in Chapter 3, this project was comprised of three parts. Section 4.1 will give some background information common to all three parts, related to the inoculum, proximate composition, initial counts, inoculum distribution, lag time, and then some background information pertinent for each specific part. Subsequent sections (4.2.1 to 4.2.4) focus on the inactivation results specific to each of the three respective parts, including graphs of the results, analyses of variance (both raw data and k values), and inactivation modeling.

While much of the data obtained in this study appeared to be non-linear (e.g., Figure 4.9), the amount of data generated was insufficient to fit non-linear models. The results and conclusions of this work would most likely be unaffected by this; however, the precision and accuracy of the models would likely be affected.

4.1 General background information

4.1.1 Salmonella cocktail

The inoculum culture for each strain was plated in duplicate (Table 4.1). The overall average, before mixing the cocktail, of all the strains was 1.40×10^9 CFU/ml in the inoculum.

TABLE 4.1 *Salmonella counts.*

Strain	CFU/ml Individual reps	AVG
S. Thompson FSIS 120	1.13E+09 1.20E+09	1.17E+09
S. Enteritidis H3527	1.55E+09 1.73E+09	1.64E+09
S. Enteritidis H3502	3.95E+09 3.91E+09	3.93E+09
S. Typhimurium H3380	1.22E+09 1.25E+09	1.24E+09
S. Hadar MF60404	8.50E+08 8.50E+08	8.50E+08
S. Copenhagen 8457	4.30E+08 6.40E+08	5.35E+08
S. Montevideo FSIS 051	6.70E+08 9.40E+08	8.05E+08
S. Heidelberg F5038BG1	8.40E+08 1.23E+09	1.04E+09

4.1.2 Proximate composition

The proximate composition of meat used (Part 1-3) is listed in Table 4.2.

TABLE 4.2 *Proximate composition of ground turkey.*

Meat type	Part	Fat (%)		Moisture (%)		Protein (%)		pH	
		Avg	SD	Avg	SD	Avg	SD	Avg	SD
Low Fat	1 and 3	1.1	0.09	72.3	0.15	23.9	0.91	6.3	0.01
High Fat		13.0	0.42	64.5	0.27	19.3	1.20	6.2	0.01
Native State	2	1.8	0.01	72.5	0.66	25.8	0.55	6.1	0.02
Dry		3.0	0.02	54.4	1.26	41.6	2.62	6.2	0.03
Driest		4.3	0.02	37.1	1.79	56.6	0.77	6.2	0.01

4.1.3 Initial counts

4.1.3.1 Part 1—Moisture effects-high range

Samples of irradiated turkey were tested to ensure negligible background microflora by plating a 1:10 dilution in 0.1% peptone water on Petrifilm® aerobic count plates. All plate counts showed no growth.

4.1.3.2 Part 2—Moisture effects-low range

Samples of cooked turkey were tested for initial counts (immediately before inoculation) by plating a 1:10 dilution in 0.1% peptone water on Petrifilm® aerobic count plates. The average initial count was ~547.9 (SD=±1248.27) CFU/g (Appendix E). Compared to the amount of inoculum added into the ground turkey, the initial count was very small. Because non-selective Petrifilm® plates were used, it was not confirmed what specific bacteria were actually in the ground turkey prior to inoculation. However, only *Salmonella*-like colonies were observed and counted.

4.1.3.3 Part 3—Humidity effects

The same meat was used as in Part 1 (See 4.1.2.1).

4.1.4 Inoculum distribution

Uniformity of inoculation was verified by plating unheated inoculated meat samples diluted in 0.1% peptone water on Petrifilm® aerobic count plates. Sub-samples were plated for all three parts to determine the uniformity of mixing. The means (±SD) of the unheated inoculated meat samples in Part 1, 2, and 3 were 7.9 (±0.27), 6.9 (±0.51), and 7.8 (±0.11) log(CFU/g), respectively.

The targeted total concentration was 10^8 CFU/ml. Raw ground turkey in Parts 1 and 3 was close to the targeted concentration; however, the cooked meat used in Part 2, was approximately 1 log lower than the targeted concentration, and also more variable.

4.1.5 Thermal lag times

The thermal lag time was the time required for the meat temperature to reach within 0.5°C of the target temperature. The end of the thermal lag was the initial test time for inactivation ("time zero"). The thermal lag time for Part 1 and 3 were 8 and 20 s, respectively, whereas for Part 2, the thermal lag times for 73, 55, and 37% moisture content were 15, 30, and 60 s, respectively.

4.1.6 Additional test information for Part 1

4.1.6.1 Changes during the experiment

In Part 1 and 3, raw ground turkey was used. From the time the bag of meat was opened until the experiment was completed, some moisture was lost from the meat during processing. In order to evaluate this loss of moisture, 100 g of low fat ground turkey (native state) sat at room temperature (22°C) for 2.5 h. During that time, 2.47 g of moisture was lost. However, during actual inactivation trials, the meat was refrigerated in a covered vessel, whenever it was not being used, in order to minimize moisture loss.

During the experiment, on any given day, meat inoculum levels fluctuated ~0.5 log(CFU/g) from the time the experiment started until completion. However, all of the inactivation analyses were based on actual "time zero" counts for each specific test.

4.1.6.2 Moisture content alteration

The moisture content ranged from 70.96 to 76.34% in the low fat ground turkey; the water activity ranged from 0.993 to 0.997. The moisture content ranged from 64.49 to 68.49% in the high fat ground turkey; the water activity ranged from 0.991 to 0.994.

4.1.7 Additional test information for Part 2

4.1.7.1 Moisture content alteration

The moisture contents were 37.13, 54.37, and 72.51% in cooked ground turkey, with water activities of 0.95, 0.98, and 0.99, respectively.

4.1.8 Additional test information for Part 3

4.1.8.1 Moisture lost during heating

Moisture lost during oven heating was determined by comparing the mass of the sample before and after heating (Appendix A). During heating, the samples lost an average of 0.0313 g ($\pm$0.10) of moisture per ~1 g of sample. For low fat ground turkey, the average final moisture content was 71.5% ($\pm$0.01). For high fat ground turkey, the average final moisture content was 62.4% ($\pm$0.08).

4.1.8.2 Oven adjustments

During convection heating, the wet bulb temperature of the air limited the temperature of the meat. Because the goal was to keep the sample temperature the constant for all treatments, the dry bulb temperature of the oven was adjusted to achieve equal wet bulb (and therefore sample) temperatures for each treatment (Table 4.3). These sample temperatures were verified by using a thin-wire thermocouple in preliminary tests (as described for determining the thermal lag time, Chapter 3).

TABLE 4.3 *Oven settings for inactivation trials.*

T_{db} (°C)	RH (%)	T_{sample} (°C)
62.3	90	60
60.6	96	60

4.2 Inactivation results

The inactivation results are presented as: graphs of the average raw results, as analyses of variance (both raw data and k values), and as a secondary inactivation model. Sections are also included to compare results from Parts 1 (raw) and 2 (cooked) at the native state moisture content and to compare results from Part 1 (waterbath) to Part 3 (oven).

4.2.1 Part 1 – Moisture effects-high range

4.2.1.1 Data

Figures 4.1 to 4.6 depict the mean survivor data from all inactivation tests in Part 1 (Appendix B). The lines are linear regressions (with slope=k). The goodness of fit (R^2) ranged from 0.05 to 0.99, with an average of 0.74 ($\pm$0.250). In Figures 4.2 and 4.5, the data fit the line well. In Figures 4.1, 4.4, and 4.6, the data vary and even show an increase in the number of survivors. In Figures 4.3, 4.4, and 4.6, the data exhibit "tailing."

4.2.1.1.1 Low fat:

Figures 4.1 to 4.3 depict the mean survivor data for low fat ground turkey heated at 55, 60, and 65°C.

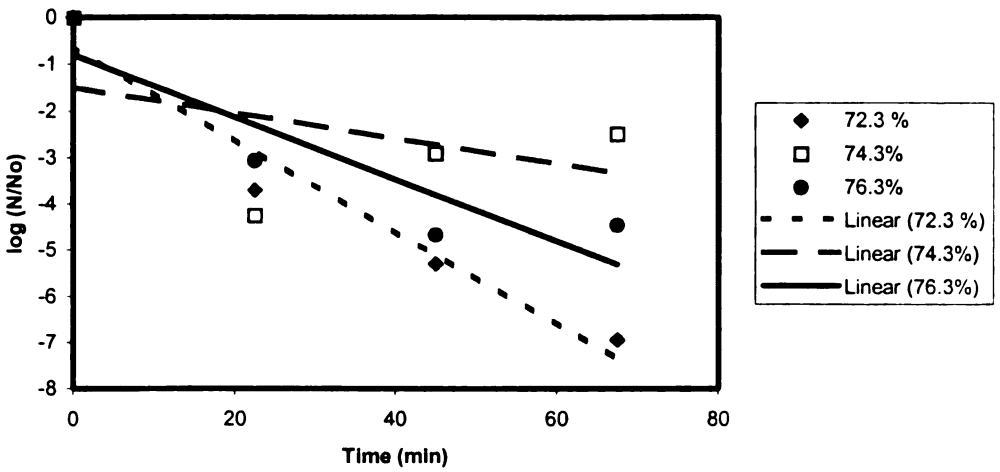

FIGURE 4.1 *Thermal inactivation of Salmonella in low fat ground turkey at 55 °C and three different moisture contents (72.3-76.3%).*

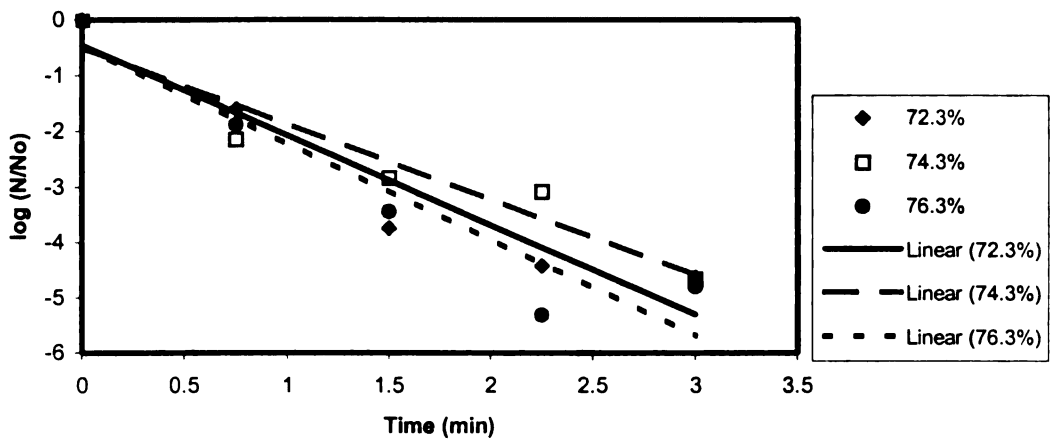

FIGURE 4.2 *Thermal inactivation of Salmonella in low fat ground turkey at 60 °C and three different moisture contents (72.3-76.3%).*

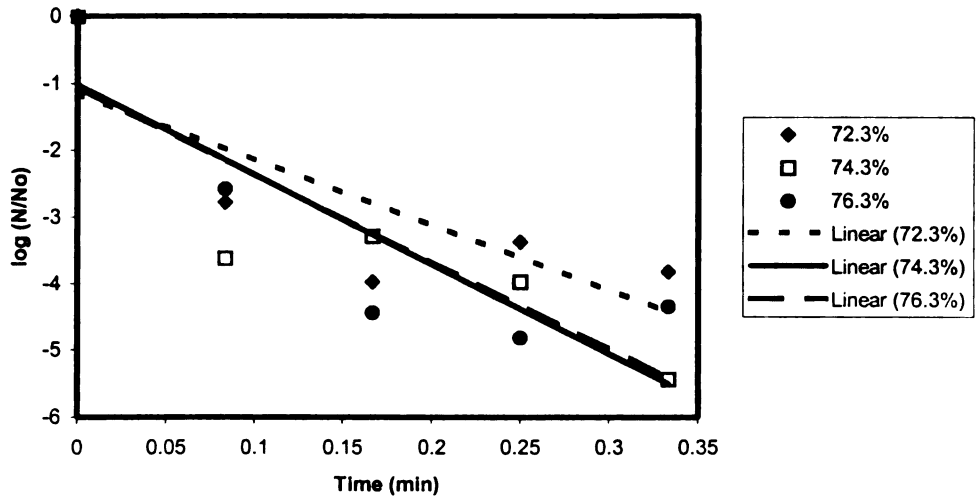

FIGURE 4.3 *Thermal inactivation of Salmonella in low fat ground turkey at 65 °C and three different moisture contents (72.3-76.3%).*

4.2.1.1.2 High fat

Figures 4.4 to 4.6 represent mean survivor data for high fat ground turkey heated at 55, 60, and 65°C.

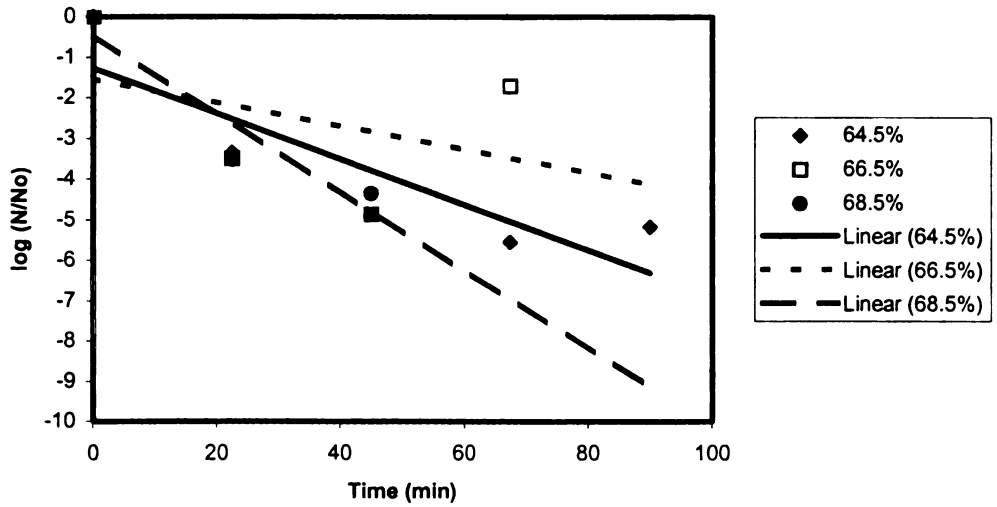

FIGURE 4.4 *Thermal inactivation of Salmonella in high fat ground turkey at 55 °C and three different moisture contents (64.5-68.5%).*

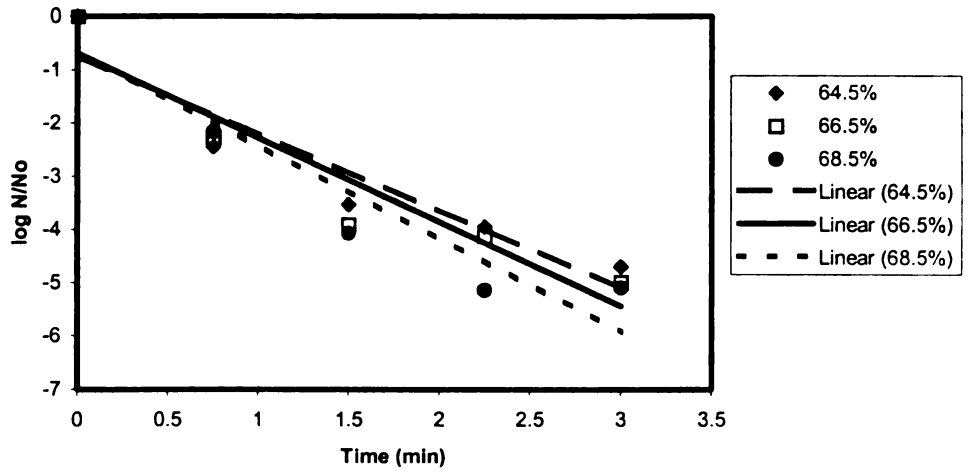

FIGURE 4.5 *Thermal inactivation of Salmonella in high fat ground turkey at 60 °C and three different moisture contents (64.5-68.5%).*

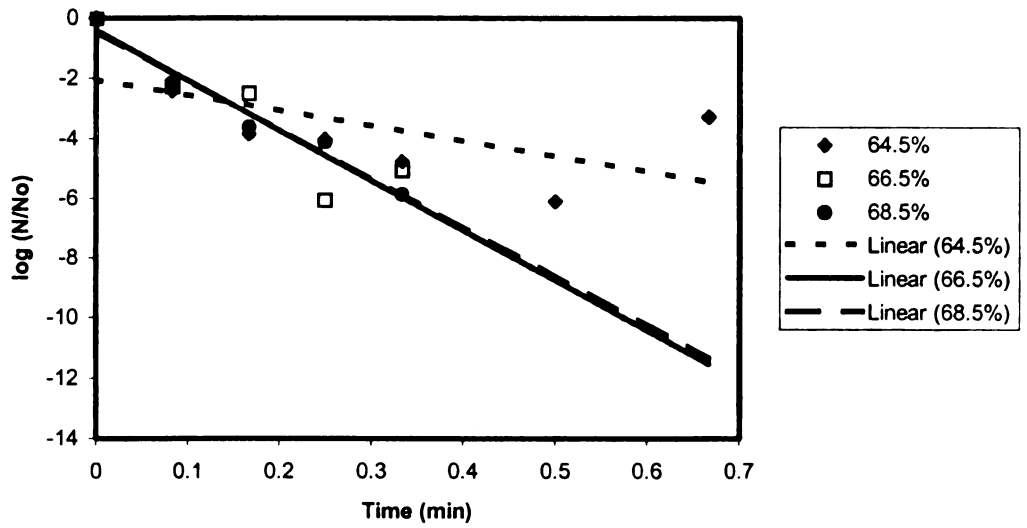

FIGURE 4.6 *Thermal inactivation of Salmonella in high fat ground turkey at 65 °C and three different moisture contents (64.5-68.5%).*

Variability occurred in the high fat meat samples at 55 and 65°C and the low fat meat samples at 55°C. This variability could be caused because the fat may prevent the inoculum from mixing well into the meat, as compared to the low fat meat. Fat "pockets" present in the high fat meat could have protective properties, as well. This could also

explain why an *increase* in CFU/g was observed over time (Figure 4.4 at 66.5% MC and Figure 4.6 at 64.5% MC). However, this was not always true in the high fat meat heated at 60°C, since variability was small. Furthermore, this did not explain the variability in the low fat meat (Figure 4.1), and the increased CFU/g in low fat meat (Figure 4.1 at 74.3% MC).

While performing the trials in Part 2, it was noticed that moisture was leaking into the pouches during the waterbath heating treatment. For Part 2, the problem was corrected; however, it remains uncertain whether moisture was leaking into the pouches in Part 1. If so, it was not noticed because of the moist state of the meat (compared to the dry meat in Part 3), but the possibility cannot be eliminated. If water was actually leaking into some bags, this could account for the high variability.

4.2.1.2 ANOVA

4.2.1.2.1 Raw data

For Part 1, the raw data were analyzed via analyses of variance (ANOVA) with both fat levels together (Table 4.4, column a), and then at the low (Table 4.4, column b) and high fat (Table 4.4, column c) levels independently. With all samples included, the ANOVA included time, temperature, moisture content, and fat content, and all two-term interactions (Table 4.4, column a). Time, temperature, moisture content, fat, and the time * temperature interaction were significantly related to *Salmonella* survival (α=0.05).

With only low fat samples included, the ANOVA included time, temperature, and moisture content, and all two-term interactions (Table 4.4, column b). Time and temperature, and the time * temperature interaction were significantly related to *Salmonella* survival (α=0.0001); however, moisture content did not affect inactivation.

With only high fat samples included, the ANOVA included time, temperature, and moisture content, and all two-term interactions (Table 4.4, column c). Time, temperature, moisture content, and the time * temperature interaction was significantly related to *Salmonella* survival (if α=0.10).

Moisture content ranged from 64.5 to 76.3% (~12% range), and the maximum moisture content of the high fat meat never exceeded the minimum moisture content of the low fat meat ($MC_{HF\text{-}max}<MC_{LF\text{-}min}$). Therefore, fat, rather than moisture content, probably was the controlling factor for the observed difference in the ANOVA that included both fat levels, given that fat was a significant factor in the lumped data set (Table 4.4, column a). As fat increased, the lethality rate decreased, with others reporting similar trends (Ahmed et al., 1995; Line et al., 1991; Fain et al., 1991; and Ben-Embarek and Huss, 1993) (Chapter 2). However, for the high fat meat, moisture content was significantly related (if α=0.10) to k values (Table 4.5, column d); therefore, fat may not have been the lone factor affecting inactivation.

TABLE 4.4 *P values from analyses of variance of the raw data in high fat and low fat ground turkey at varying moistures, fats, times, and temperatures.*

TEST FACTORS	(a) HF and LF	(b) Only LF	(c) Only HF
Time	**<0.0001**	**<0.0001**	**<0.0001**
Temperature	**<0.0001**	**<0.0001**	**<0.0001**
MC	**0.0177**	0.1398	**0.0772**
Fat	**0.0038**	n/a*	n/a
Time*Temp	**<0.0001**	**<0.0001**	**<0.0001**
Time*MC	0.9520	0.9585	0.7847
Time*Fat	0.8592	n/a	n/a
Temp*MC	0.7762	0.3289	0.1057
Temp*Fat	0.4424	n/a	n/a
Fat*MC	0.6481	n/a	n/a

*n/a=not included in the particular analysis represented by that column.

For Part 1, water activity was not a parameter in the analysis, because water activity ranged from only 0.993 to 0.997. In these tests, moisture content was increased by pipetting sterile 0.1% peptone water into the sample *or decreased* by centrifuging liquid out of the sample. Controlling added moisture was uncomplicated and consistent; however, removing moisture was more difficult. The *entire* water activity range for Part 1 was 0.004, including the increased *and* decreased moisture content range of 70.9 to 76.3%. Also, a single water activity reading was not possible for the decreased moisture samples, because every sample was different, and the range of water activities was very small due to limited ability to remove water via centrifugation.

4.2.1.2.2 K values

The k values, rates of thermal inactivation (assuming first order kinetics), were calculated by linear regression of the $\ln(N/N_o)$ data over time for each replication (Appendix B).

For Part 1, the k values were analyzed via analyses of variance (ANOVA) with both fat levels together (Table 4.5, column a), and then with low (Table 4.5, column b) and high fat (Table 4.4, column c) levels independently. With all data incorporated into the model, the first ANOVA included temperature, moisture content, and fat, and all two-term interactions (Table 4.5, column a). Temperature and the moisture content * fat interaction significantly affected the k values (α=0.05). The second ANOVA (Table 4.5, column b) included only low fat samples and evaluated temperature, moisture content, and the interaction of temperature * moisture content into the model, and both temperature and moisture content were significantly related to k (if α=0.10). The third ANOVA included only high fat samples and evaluated temperature, moisture content, and the interaction of temperature * moisture content into the model, and only temperature significantly affected the k values (if α=0.10).

Therefore, these results are consistent with the ANOVA of the raw data. It appears, given a very narrow range of moisture content (and therefore water activity), that moisture content might affect thermal inactivation of *Salmonella* in turkey; however, the results were not conclusive. Therefore, the next test series (Part 2) widened the range of the moisture content/water activity in the meat.

TABLE 4.5 *P values from analyses of variance of the k values in high and low fat ground turkey at varying moistures, fats, times, and temperatures.*

TEST FACTORS	(a) HF and LF	(b) Only LF	(c) Only HF
Temperature	**<0.0001**	**<0.0001**	**<0.0001**
MC	0.4985	**0.0901**	0.1310
Fat	0.5349	n/a	n/a
Temp * MC	0.7305	0.6377	0.1278
Temp * Fat	0.8119	n/a	n/a
MC * Fat	**0.0395**	n/a	n/a

*n/a=not included in the particular analysis represented by that column.

4.2.2 Part 2 – Moisture effects-low range

4.2.2.1 Data

Figure 4.7 depicts the mean survivor data from the entire inactivation trial in Part 2 (Appendix B). The lines are linear regressions (with slope=k). The goodness of fit (R^2) ranged from 0.95 to 0.97 with an average of 0.97 ($\pm$0.011). The R^2 range in Part 2 was much smaller than in Part 1.

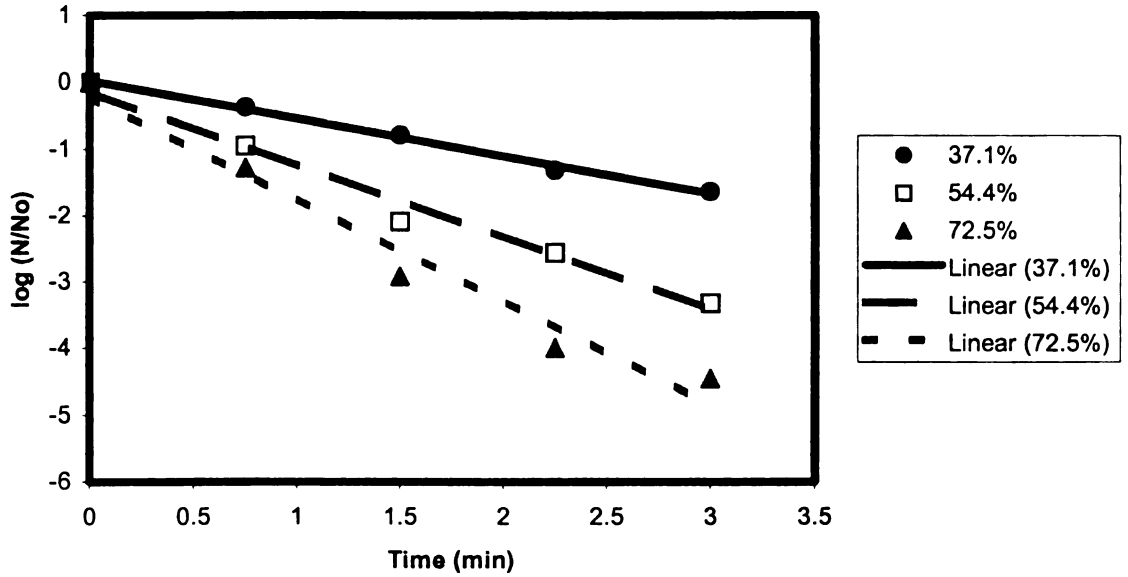

FIGURE 4.7 *Thermal inactivation of Salmonella in low fat ground turkey at 60°C and three different moisture contents (37.1-72.5%).*

From the graph, the data appear to be fairly linear; therefore, it appears that any background microflora (Section 4.1.3.2) did not affect these results; otherwise, a tailing phenomenon would be expected.

4.2.2.2 ANOVA

4.2.2.2.1 Raw data

For Part 2, one ANOVA was run with time and moisture content as the test variables (Table 4.6, column a), and a second ANOVA was run with time and water activity (Table 4.6, column b) as the test variables. Time, moisture content/water activity, and their interactions all affected inactivation of *Salmonella* (α=0.0001) (Table 4.6).

In contrast to Part 1, water activity in Part 2 was a parameter in the analysis, because the experimental design contained three well-controlled water activities (0.950, 0.981, and 0.996). Therefore, an accurate water activity reading was possible for the

decreased moisture samples. The range of moisture content in Part 2 (35.4%) was much larger than the range of moisture content in Part 1 (5.38%).

TABLE 4.6 *P values from analyses of variance of the raw data in low fat ground turkey at varying moistures, water activities, and times, at a sample temperature of 60 °C.*

TEST FACTORS	(a) LF-60°C	(b) LF-60°C
Time	**<0.0001**	**<0.0001**
MC	**<0.0001**	n/a
Time*MC	**<0.0001**	n/a
a_w	n/a*	**<0.0001**
Time *a_w	n/a	**<0.0001**

*n/a=not included in the particular analysis represented by that column.

4.2.2.2.2 K values

The k values, rates of thermal inactivation (assuming first order kinetics), were calculated by linear regression of the $\ln(N/N_o)$ data over time for each replication (Appendix B).

For Part 2, the k values were analyzed by analysis of variance (ANOVA) with moisture content (Table 4.7, column a) and water activity (Table 4.7, column b). The rate of inactivation was significantly increased at both high moisture content and high water activity (α=0.01) (Figure 4.7 and Table 4.7).

TABLE 4.7 *P values from analyses of variance of the k values in low fat ground turkey at varying moistures, water activities, and times, at a sample temperature of 60 °C.*

TEST FACTORS	(a) LF	(b) LF
MC	**0.0022**	n/a
a_w	n/a*	**0.0019**

*n/a=not included in the particular analysis represented by that column.

Three previous studies have reported an effect of water activity on thermal inactivation of *Salmonella* or *E. coli* (Goepfert et al., 1970; Reichart, 1994; and O'Donovan-Vaughan and Upton, 1999). Goepfert et al. (1970) evaluated the relationship between the heat resistance of *Salmonella* and water activity (0.75-0.99) in different sugar solutions. Reichart (1994) and Cerf et al. (1996) attempted to model the destruction of *E. coli* in a glucose solution as a function of pH (3-9) and water activity (0.928-0.995). Lastly, O'Donovan-Vaughan and Upton (1999) assessed the survival of *Salmonella* Typhimurium at three extreme water activities (0.45-0.90) and two temperatures, in sucrose solution. All three studies were performed in carbohydrate solutions (not food systems); and only one study attempted to model the data (while the other two just reported data). All three studies showed that water activity caused an effect, but other parameters (e.g., solute, pH) also affected the outcome.

First, Goepfert et al. (1970), used seven different *Salmonella* strains (*S.* Infantis, *S.* Alachua, *S.* Typhimurium, *S.* Anatum, *S.* Anatum GF, *S.* Montevideo, *S.* Senftenberg 775W, and *S.* Tennessee), and evaluated the effects of water activity in different carbohydrate solutions. Sucrose clearly provided a protective effect. Water activity ranged from 0.87 to 0.99 in sucrose (pH 6.9) and 0.75 to 0.99 in glycerol (pH 6.9). The media was heated in a flask to 57.2°C. In the study with glycerol, the same *Salmonella* strains were used, except for *S.* Infantis. For the purpose of comparing the data from Goepfert et al. (1970) with the current study, *S.* Senftenberg was removed from the Geopfert et al. (1970) data prior to computing averages, because of the strain's extreme heat resistance (Goepfert et al., 1970) (Table 4.8 and Figure 4.8).

The current work was also compared to earlier data of Reichart (1994). *E. coli* B 200 was inoculated into a glycerol-water mixture to obtain three water activities (0.995-0.928) and heated at 58°C. The pH of the heating menstrum in that study ranged from 3-9; however, only results obtained at pH 6 and 7 are being cited, because these values were closest to the pH in the current work (pH 6.2) (Table 4.8 and Figure 4.8).

Lastly, O'Donovan-Vaughan and Upton (1999) tested the survival of *S.* Typhimurium at water activity range of 0.45 to 0.9, at 55 and 65°C, and in different solutes. Again, consistent with Goepfert et al. (1970), sucrose was most protective, with heat resistance increasing as the water activity decreased. The overall conclusion was that heat resistance depended on the nature of the solute used to lower the water activity. In the following table (Table 4.8) and graph (Figure 4.8), the results of O'Donovan-Vaughan and Upton (1999) are not included, because the water activity range was too large (0.90-0.45), and minimum water activity was too small (0.45) to compare to the current study.

The direct comparison between previously reported data and the current work is limited, because heating occurred at different temperatures. When equal water activity changes were compared (~0.95 to 0.99), k value reductions of 64, 42, 64, and 92% were observed by the following: the current study, Reichart (1994) (pH 6), and Reichart (1994) (pH 7), and Goepfert et al. (1970), respectively. Overall, heat resistance increased as the water activity decreased, which was in agreement with this study.

To compare the current work with the previous work at similar temperatures, the k values (at 60°C) needed to be transformed to equivalent values at 57.6°C. This was

accomplished by linear regression of ln(k) vs. 1/T data from Part 1 to get the Arrhenius parameters (per equation 6); that regression (R^2=0.9485) yielded:

$$\ln(k)=169.64-56185(1/T_{abs}) \quad (12)$$

From this model, a ratio was determined at two temperatures (57.6 and 60°C) and one water activity, as follows:

$$k_{60°C}/k_{57.6°C}=e[(-56185/333) + (56185/330.6)] = 3.4 \quad (13)$$

The actual k values for Part 3 at 60°C were divided by this ratio to obtain transformed k values at 57.6°C, in order to make a more direct comparison with the published data (Table 4.8 and Figure 4.8).

TABLE 4.8 *k values (min^{-1}) as a function of water activity.*

Test solute/product	Citation	a_w	Temp (°C)	pH	Avg k value for 8 strain *Salmonella* cocktail (min^{-1})
ground turkey	Current work (2002)	0.996	60	6.2	3.569
		0.981	60	6.2	2.648
		0.95	60	6.2	1.301
ground turkey	*Current work (2002)	0.996	57.6	6.2	1.05
		0.981	57.6	6.2	0.779
		0.95	57.6	6.2	0.383
sucrose	Goepfert et al. (1970)				Avg k value for 6 *Salmonella* strains (min^{-1})
		0.99	57.2	6.9	2.302
		0.96	57.2	6.9	0.184
		0.93	57.2	6.9	0.071
glycerol		0.99	57.2	6.9	2.224
		0.9	57.2	6.9	0.972
glycerol	Reichart (1994)				Avg k value *E. coli* (min^{-1})
		0.995	58	6	0.762
		0.956	58	6	0.444
		0.928	58	6	0.288
		0.995	58	7	1.314
		0.956	58	7	0.468
		0.928	58	7	0.306

*Transformed data

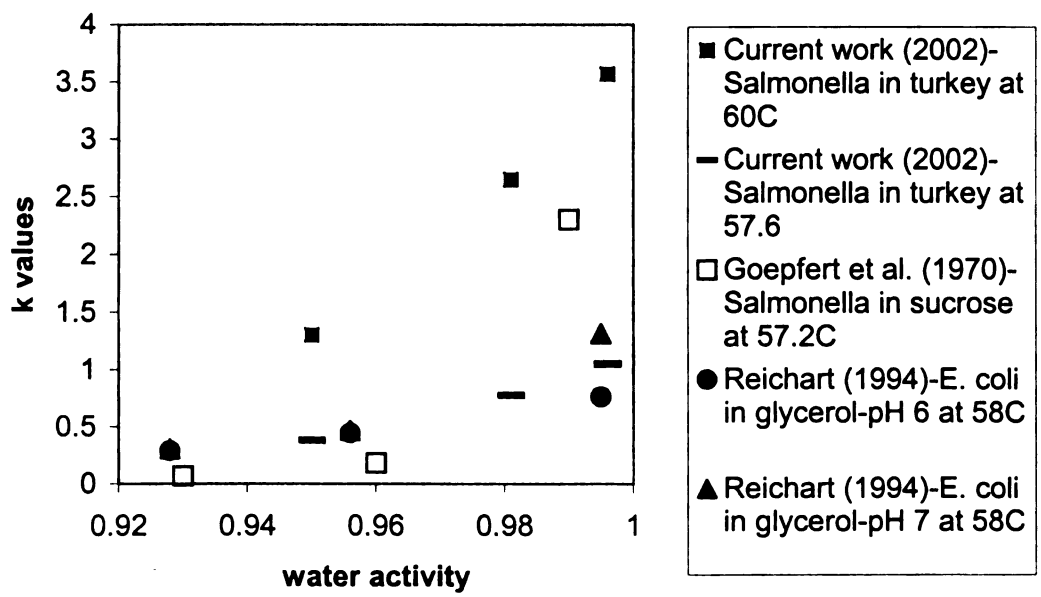

FIGURE 4.8 *Comparison of k values as a function of water activity.*

Obviously, among these cited studies, tests were performed at different temperatures, pH values, and water activities. The current study and that of Goepfert et al. (1970) used *Salmonella*; however, the current study used a *Salmonella* cocktail, and Goepfert et al. (1970) ran tests with individual *Salmonella* strains. Reichart's (1994) work was performed with *E. coli*, in glycerol, at different pH's. The current study was the only one that conducted tests in a food system and not a carbohydrate solution. In the meat product, the solutes in the aqueous solution are probably dominated by electrolytes (i.e., non-carbohydrate components).

However, in all these studies, the heat resistance of the organism increased as the water activity decreased. The present data (in meat) were generally consistent with the previous data in the carbohydrate system. However, meaningful differences between the current data and those of Geopfert suggest that inactivation models should not be derived from o*nly* media inactivation studies. In addition, specific substrate and test conditions must also be considered when determining thermal inactivation models.

4.2.2.3 Modeling

Because water activity was significantly related to the survival of *Salmonella* (Table 4.6 and 4.7), the data were applied to a secondary modified (additive) Arrhenius-type model. Cerf et al. (1996) applied this model to previously published data, and accounted for other parameters (Equation 7). The model used in the current work was as follows:

$$\ln(k)=\ln(k_o)-(E_a/R)(1/T)+\text{"water term"} \qquad (14)$$

where k=the inactivation rate, E_a=activation energy, R=universal gas constant, and T=absolute temperature. The three "water terms" added to the equation were as follows:

$1/a_w$, a_w, and $a_w+a_w^2$. Part 2 was only run at one temperature (60°C). Consequently, a model parameter for the temperature term (E_a) could not be estimated from regression of the present data, and a constant was arbitrarily applied for the activation energy (E_a) (Table 4.9). All three "water terms" resulted in a root meat square error of the model of less than 1 $\log_{10}$ (CFU/g) (Appendix D).

TABLE 4.9 *Effect of the "water term" form on the root mean square error for a first-order, modified Arrhenius-type model.*

"water term"	**Error $\log_{10}$ (CFU/g)**
$1/a_w$	0.747
a_w	0.749
$a_w+ a_w^2$	0.750

4.2.3 Part 3 – Humidity effects

4.2.3.1 Data

Figure 4.9 depicts the mean survivor data collected from the entire inactivation trial in Part 3 (Appendix B). The lines are linear regressions (with slope=k). The goodness of fit (R^2) ranged from 0.77 to 0.98, with an average of 0.89 ($\pm$0.081). The R^2 range in Part 3 was greater than Part 2, but less than Part 1. In Figure 4.11, the data appears to tail slightly.

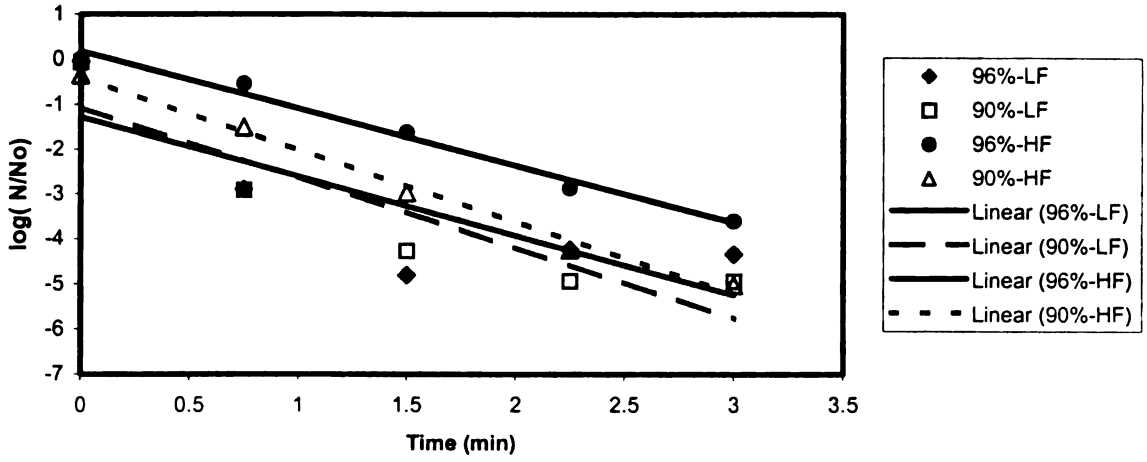

FIGURE 4.9 *Thermal inactivation of Salmonella in low and high fat ground turkey at 60 ℃ and 90 and 96% relative humidity.*

The hypothesis (Chapter 1) was that the rate of thermal inactivation of *Salmonella* decreases with decreasing meat moisture and/or process humidity. However, it can be seen (Figure 4.9) that, regarding humidity, the opposite was generally true. Therefore, the part of the hypothesis that referred to humidity was rejected (based on statistical analyses in the next section), because as the relative humidity increased, the thermal inactivation was not increased, but rather, actually decreased (Figure 4.9). Consequently, in the following analyses of variance of the raw data and k values, when the relative humidity was significantly related to *Salmonella* survival, it was not significant in the direction hypothesized.

4.2.3.2 ANOVA

4.2.3.2.1 Raw data

For Part 3, the raw data were analyzed by analyses of variance (ANOVA) with both fat levels together (Table 4.10, column a), and then at the low (Table 4.10, column b) and high fat (Table 4.10, column c) levels independently. With all samples included, the ANOVA included time, relative humidity, final moisture content, and fat, and all two-

term interactions (Table 4.10, column a). Time, relative humidity, and the two-term interaction between time * relative humidity affected *Salmonella* survival (if α=0.1).

With the low fat samples only, the ANOVA included time, relative humidity, and final moisture content, and all two-term interactions (Table 4.10, column b). Time and relative humidity, and all two-term interactions significantly affected *Salmonella* survival (α=0.05). With only high fat samples included, the ANOVA was run with time, relative humidity, and final moisture content, and all two-term interactions (Table 4.10, column c). Time, relative humidity, and the interaction between the two significantly affected *Salmonella* survival (α=0.05).

TABLE 4.10 *P values from analyses of variance of the raw data in low and high fat ground turkey at varying relative humidities, at 60°C.*

TEST FACTORS	(a) HF and LF	(b) Only LF	(c) Only HF
Time	**<0.0001**	**<0.0001**	**<0.0001**
RH	**<0.0001**	**0.0181**	**<0.0001**
Final MC	0.6459	0.4740	0.2531
Fat	0.1710	n/a*	n/a
Time * RH	**0.0982**	**0.0462**	**0.0265**
Time * Final MC	0.3238	**0.0282**	0.8280
Time * Fat	0.3119	n/a	n/a
RH * Final MC	0.2423	**0.0285**	0.2450
RH * Fat	0.2234	n/a	n/a
Final MC * Fat	0.3488	n/a	n/a

*n/a=not included in the particular analysis represented by that column.

A direct explanation cannot be given as to why the rate of thermal inactivation increased with increasing relative humidity. However, the moisture lost was a controlled parameter, and only a minimal amount of water was lost during heating (Appendix A). In addition, thermal inactivation did not decrease with decreased humidity (as was hypothesized). Therefore, it appears that meat water activity (Part 2), rather than process humidity (Part 3), is the controlling water parameter affecting *Salmonella* resistance to thermal inactivation in a meat product.

The experimental design (Table 4.3) of the humidity test could possibly explain the significance in the results opposite of the hypothesis. The dry bulb temperature was increased at the lower relative humidity (90%) to provide a consistent sample temperature (60°C). In preliminary tests, the temperature set points and sample temperatures were determined by using a thermocouple inserted in the samples. However, the thin-wire thermocouple was nearly as thick as the meat sample. Hence it is possible that dry bulb temperature set points did not achieve a sample temperature of exactly 60°C, and even the slightest change in temperature could alter the inactivation rate. In order to maintain an aseptic environment, using a thermocouple for every sample was not possible.

As discussed earlier in Chapter 4, a small amount of moisture was lost during heating. When the humidity was increased from 90 to 96%, the average moisture lost during the heating process decreased from 0.041 to 0.025 g. The slightly decreased moisture content corresponded to an increased rate of thermal inactivation, which is inconsistent with results from Part 2.

4.2.3.2.2 K values

The k values, rates of thermal inactivation (assuming first order kinetics), were calculated by linear regression of the $\ln(N/N_o)$ data over time for each replication (Appendix B).

The k values were analyzed by analyses of variance (ANOVA) with both fat levels included (Table 4.11, column a), and then with low (Table 4.11, column b) and high fat (Table 4.11, column c) levels independently. The first ANOVA included data from both fat levels, and relative humidity and fat, and the two-term interaction, and none of these factors were related to k (Table 4.11, column a). When low fat meat was analyzed independently (Table 4.11, column b), the ANOVA included only relative humidity, and it was not related to k. However, when the model was run with only high fat meat, the ANOVA included relative humidity, which was related to k (α=0.05) (Table 4.11, column c). However, k increased with decreasing humidity, which was opposite of the hypothesis.

TABLE 4.11 *P values from analyses of variance of k values in low and high fat ground turkey at varying relative humidities, at 60 °C.*

TEST FACTORS	(a) HF and LF	(b) Only LF	(c) Only HF
RH	0.1708	0.8580	**0.0147**
Fat	0.4044	n/a	n/a
RH * MC	0.2735	n/a	n/a

*n/a=not included in the particular analysis represented by that column.

Murphy et al. (2001b, c) evaluated thermal inactivation of *Salmonella* and *Listeria* in ground chicken patties that were processed in an air-impingement oven. In both studies, microbial inactivation decreased with decreasing wet bulb temperatures. A

reasonable explanation was given (Chapter 2); however, one very important parameter was neglected. In all of these studies that assessed the effects of humidity, humidity was not isolated as the sole factor. During cooking, the *moisture content* and *water activity* of meat decreases; therefore, it was possible this was actually causing the effect, instead of humidity.

The current study isolated the intrinsic and extrinsic water variables to determine which parameters were actually controlling the effect. During the humidity test, the moisture content changed minimally (0.03 g), and the samples were heated at an isothermal dry bulb temperature of 60°C and two different humidities (90 and 96%). The rate of thermal inactivation did not decrease with decreasing process humidity.

4.2.4 Parts 1 and 2 combined

Parts 1 and 2 were combined to determine if the methodology (raw vs. cooked meat) affected the inactivation of *Salmonella*.

4.2.4.1 Data

The low fat ground turkey data, heated to 60°C, was combined from Parts 1 and 2. The one main difference between Parts 1 and 2 was that Part 1 utilized raw, irradiated ground turkey, whereas cooked, non-irradiated ground turkey was used in Part 2 (Figure 4.10).

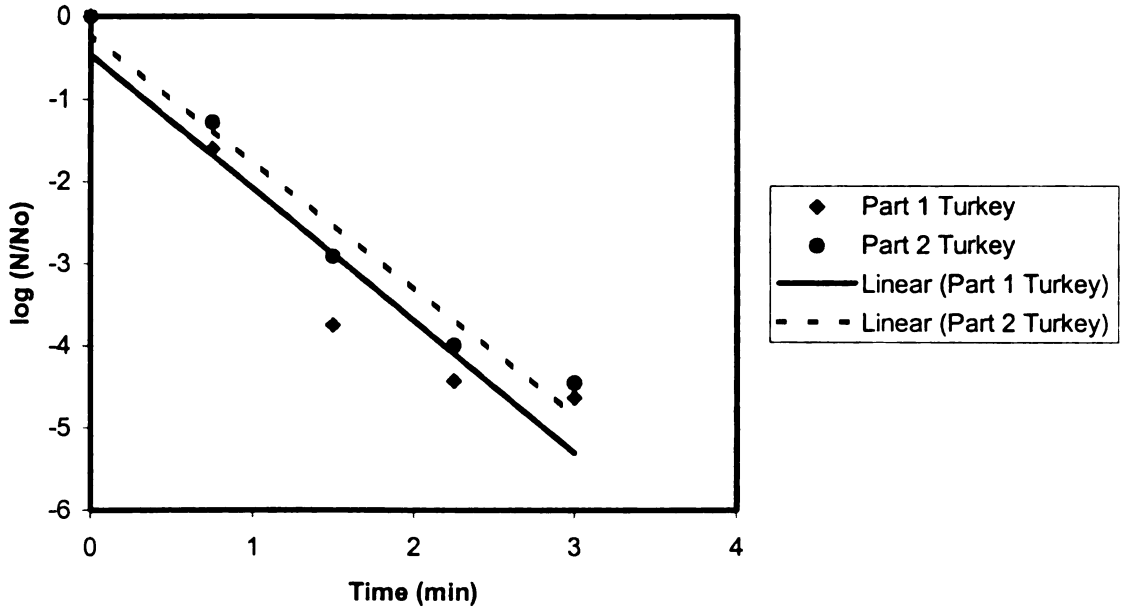

FIGURE 4.10 *Thermal inactivation of Salmonella in low fat ground turkey at 60°C in raw vs. cooked meat.*

4.2.4.2 ANOVA

Ideally, a difference between the meat used in Part 1 and the meat used in Part 2, would be indistinguishable; however, in the current study, that was not the case.

4.2.4.2.1 Raw data

A slight, but significant difference existed between the raw turkey vs. cooked turkey (only if α=0.1), in terms of *Salmonella* inactivation (Table 4.12). Most likely a difference was observed because the cooked meat contained a small amount of background microflora, and the raw meat was irradiated. However, that was not the only possible explanation. When the meat was dried in the smokehouse, the chemical properties of the meat may have been altered in such a way that a protective mechanism was afforded to the bacteria (possibly via fat), and this effect may not have occurred when the raw meat was irradiated. This may have led to a slightly greater survival in cooked meat compared to raw meat. The observed fat level at the native state moisture

content was 1.09 and 1.78% in the raw and cooked meat, respectively. Raw and cooked meat were both obtained from the same source, but from different lots. The difference in the fat level was approximately 0.7%.

TABLE 4.12 *P values from analyses of variance of the raw data in low fat ground turkey (raw vs. cooked) at similar moisture contents and water activities, and heated at 60°C.*

TEST FACTOR	LF-60°C
Time	<0.0001
Raw or cooked	0.0606

4.2.4.2.2 K values

The k values, rates of thermal inactivation (assuming first order kinetics), were calculated by linear regression of the $\ln(N/N_o)$ data over time for each replication. The difference between the raw vs. cooked ground turkey did not significantly affect the k value (Table 4.13), but it appeared to show a slight effect on the raw data (Figure 4.10). Therefore, it appears that the use of different meat pre-treatments did not have a large effect on thermal resistance of *Salmonella*.

TABLE 4.13 *P values from analyses of variance of k values in low fat ground turkey (raw vs. cooked) at similar moisture contents and water activities, and heated at 60 °C.*

TEST FACTOR	LF-60°C
Raw or cooked	0.5505

4.2.5 Parts 1 and 3 combined

Parts 1 and 3 were combined to determine if the different heating methods (waterbath vs. air oven) affected the inactivation of *Salmonella.*

4.2.5.1 Data

The low and high fat ground turkey data (60°C) were combined from Parts 1 and 3 in order to test whether the different heat treatments affected inactivation. The one main difference between Parts 1 and 3 was that samples in Part 1 were heated in a waterbath, whereas in Part 3, samples were heated in an air convection oven.

Figure 4.11 shows an example of a graph of mean data for low fat ground turkey, heated in a waterbath and air convection oven.

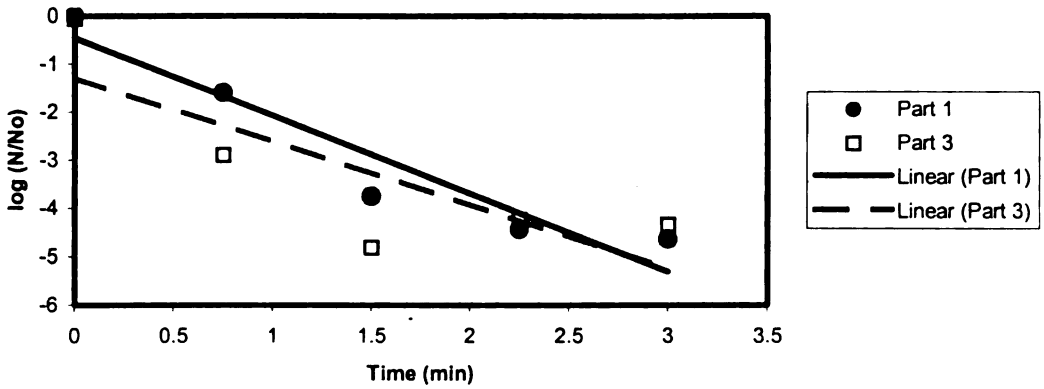

FIGURE 4.11 *Thermal inactivation of Salmonella in low fat ground turkey at 60°C in waterbath vs. air convection oven.*

4.2.5.2 ANOVA

No significant difference was observed between Parts 1 and 3, in terms of *Salmonella* inactivation (data not shown). The k values, rates of thermal inactivation (assuming first-order kinetics), were calculated by linear regression of the $\ln(N/N_o)$ data over time for each replication. The difference between Parts 1 and 3 did not significantly affect the k values (data not shown). Therefore, no significant effect of heating method on thermal inactivation could be determined from these results.

CHAPTER 5

CONCLUSIONS

The USDA-FSIS recently amended the regulations governing cooked meat and poultry products, creating a shift to lethality performance standards. Studies clearly show that many variables affect the thermal inactivation of bacteria. However, no current inactivation models incorporate the effect of water on microbial inactivation in meat products, and in particular, the intrinsic properties or extrinsic process. Therefore, the current work isolated two very important variables, water activity/moisture content and relative humidity, and studied the effect of both parameters on thermal inactivation.

The hypothesis of the current work was that the rate of thermal inactivation decreases with decreasing meat moisture content and/or process humidity. The objectives of the study were to test the effects of each parameter in either a sealed environment (intrinsic properties) or air convection oven (extrinsic properties). The experiment was divided into three parts to study thermal inactivation of *Salmonella* in ground turkey (Part 1-3).

In Part 1, the moisture content was altered in raw turkey within a narrow range, and a clear effect was not observed. Therefore, the moisture content/water activity range was widened by drying/cooking the meat (Part 2), and as the moisture content/water activity decreased, thermal inactivation decreased. Previous studies reported the same trend; however, these earlier studies were performed in carbohydrate solutions, and when compared to a food system, the k values differed. The data from the current study were

applied to a secondary modified Arrhenius-type model. The data fit the model, and resulted in a error of less than 1 $\log_{10}$ (CFU/g).

Previous studies have shown that the thermal inactivation rate decreased with decreasing humidity; however, the effects were not isolated. It remained unclear whether the intrinsic or extrinsic parameters were actually causing the effect. Therefore, the effect of relative humidity was isolated (Part 3), and the rate of thermal inactivation did not decrease with decreasing process humidity.

In conclusion, decreased moisture content/water activity, and *not* relative humidity in the process environment, resulted in a decreased rate of thermal inactivation. The intrinsic properties of meat should be included in inactivation models for commercial convection cooking systems.

CHAPTER 6

RECOMMENDATIONS FOR FUTURE RESEARCH

The current study examined whether moisture content/water activity or relative humidity decreased the thermal inactivation rate by decreasing the "water parameters." The following is a list of recommendations for future research:

- A method should be developed to test the effects of moisture content (alone) in a meat sample, with only a *small* range of *decreasing* moisture. Decreasing the moisture content would be more applicable to industry. The current study tested the moisture content primarily by increasing moisture over a small range. The range was broadened, and moisture was decreased; however, a small, decreased range should be examined.
- The thermal inactivation rate should be tested, isolating moisture content/water activity and humidity, in a "real world" setting using processed turkey products.
- The oven test should be performed at more temperatures. The current study only tested the humidity effect at 60°C.
- A means should be designed to aseptically monitor the temperature while undergoing oven/humidity testing. Preliminary tests were performed to determine set temperatures at which the experiment was run. However, the sample temperature per *each* individual sample was unknown.
- Both moisture content/water activity and humidity should be tested using a single strain of *Salmonella*, instead of the cocktail, to reduce the range of resistance and decrease tailing.

- A larger data set at different water activities and temperatures (relevant to commercial processes) should be created in order to develop and validate secondary inactivation modeling.

APPENDIX

APPENDIX A: Moisture lost during oven heating (Part 3)

LF-96%-60C

Final MC	Moisture lost (g)
72.3	0
72.3	0
72.5216	-0.01
73.4352459	-0.05
72.3	0
71.72886598	0.02
70.58659794	0.06
69.22222222	0.13
71.746	0.02
71.16938776	0.04
68.92195122	0.1
71.0015625	0.03

LF-90%-60C

Final MC	Moisture lost (g)
71.80973451	0.02
72.56132075	-0.01
68.56516854	0.12
72.84851485	-0.02
72.55648148	-0.01
71.42526316	0.03
71.04090909	0.04
70.73207547	0.06
70.78909091	0.06
69.73518519	0.1
69.64787234	0.09
70.43781513	0.08

APPENDIX A, con'd

LF-96%-60C

Final MC	Moisture lost (g)
72.02574257	0.01
72.3	0
72.52892562	-0.01
73.408	-0.05
72.8893617	-0.03
72.52520325	-0.01
72.92954545	-0.03
71.82241379	0.02
73.82677165	-0.07
72.08854962	0.01
71.79636364	0.02
70.52110092	0.07

LF-90%-60C

Final MC	Moisture lost (g)
72.00531915	0.01
71.45204082	0.03
73.57356322	-0.04
72.91102941	-0.03
71.75686275	0.02
72.87113402	-0.02
70.67058824	0.05
72.3	0
70.90336134	0.06
71.42063492	0.04
70.37304348	0.08
70.62121212	0.06

APPENDIX A, con'd

HF-96%-60C

Final MC	Moisture lost (g)
64.11827957	0.01
63.20909091	0.04
64.5	0
64.5	0
12.48837209	0.63
78.61180124	-0.64
63.88793103	0.02
63.70224719	0.02
63.89830508	0.02
63.1728972	0.04
64.22265625	0.01
53.18115942	0.22

HF-90%-60C

Final MC	Moisture lost (g)
63.74468085	0.02
63.71111111	0.02
64.88172043	-0.01
65.99473684	-0.04
64.20661157	0.01
64.14851485	0.01
63.38188976	0.04
63.09126984	0.05
63.83850932	0.03
63.86036036	0.02
60.24	0.12
60.89453125	0.13

APPENDIX A, con'd

HF-96%-60C

Final MC	Moisture lost (g)
63.69924812	0.03
64.5	0
64.87368421	-0.01
64.16190476	0.01
62.20967742	0.06
64.19130435	0.01
58.99137931	0.18
67.828125	-0.09
63.06565657	0.04
63.02083333	0.04
61.37912088	0.08
61.8375	0.09

HF-90%-60C

Final MC	Moisture lost (g)
63.76041667	0.02
64	0.01
64.0617284	0.01
64.91764706	-0.01
63.8826087	0.02
63.81730769	0.02
62.41176471	0.05
62.10674157	0.06
62.74257426	0.05
60.55555556	0.08
60.76315789	0.1
59.53763441	0.13

APPENDIX B: Inactivation data (Part 1-3)

PART 1

Temp C	MC %	Fat %	K value (min -1)	R2	Regression of y=ln(N/No) against x=time (min)
65	72.3	1.0915	25.688	0.9227	y = -25.688x - 0.3578
65	72.3	1.0915	10.971	0.0939	y = -10.971x - 3.58
65	72.3	1.0915	11.653	0.6613	y = -11.653x - 0.8947
65	72.3	1.0915	61.31	0.998	y = -61.31x + 0.133
65	72.3	1.0915	47.106	0.6752	y = -47.106x - 1.572
65	72.3	1.0915	65.652	0.9959	y = -65.652x - 0.2037
65	64.5	13.02	17.3	0.9769	y = -17.3x - 0.128
65	64.5	13.02	15.877	0.5652	y = -15.877x - 2.1077
65	64.5	13.02	16.976	0.8049	y = -16.976x - 1.1852
65	64.5	13.02	17.822	0.4428	y = -17.822x - 2.1011
65	64.5	13.02	32.822	0.5998	y = -32.822x - 4.3425
65	64.5	13.02	13.847	0.4125	y = -13.847x - 3.9158
65	72.3	1.0915	47.106	0.6752	y = -47.106x - 1.572
65	64.5	13.02	32.822	0.5998	y = -32.822x - 4.3425
65	72.3	1.0915	11.873	0.471	y = -11.873x - 4.2585
65	64.5	13.02	13.847	0.4125	y = -13.847x - 3.9158
55	72.3	1.0915	0.2914	0.9999	y = -0.2914x + 0.0279
55	72.3	1.0915	0.2169	0.8154	y = -0.2169x - 2.7798
55	74.3	1.0915	0.0674	0.268	y = -0.0674x - 3.0599
55	74.3	1.0915	0.0926	0.1798	y = 0.0926x - 5.1375
55	76.3	1.0915	0.1753	0.8002	y = -0.1753x - 0.6317
55	76.3	1.0915	0.2069	0.6564	y = -0.2069x - 1.945
55	64.5	13.02	0.2925	0.9917	y = -0.2925x + 0.3475
55	64.5	13.02	0.1213	0.6634	y = -0.1213x - 3.3312
55	66.5	13.02	0.2807	0.8321	y = -0.2807x - 1.6381
55	66.5	13.02	0.2102	0.9998	y = -0.2102x - 0.0372
55	66.5	13.02	0.0675	0.1544	y = -0.0675x - 3.6031
55	68.5	13.02	0.2332	0.9499	y = -0.2332x - 0.6955
55	68.5	13.02	0.2128	0.8028	y = -0.2128x - 1.3698
60	72.3	1.0915	3.6189	0.8311	y = -3.6189x - 1.4108
60	72.3	1.0915	3.5743	0.9146	y = -3.5743x - 0.4979
60	74.3	1.0915	4.4387	0.9613	y = -4.4387x - 1.2101
60	74.3	1.0915	1.9279	0.7621	y = -1.9279x - 0.6053
60	74.3	1.0915	5.3411	0.9613	y = -5.3411x - 0.464
60	76.3	1.0915	3.7027	0.8571	y = -3.7027x - 1.464
60	76.3	1.0915	3.906	0.8625	y = -3.906x - 1.071
60	76.3	1.0915	4.4181	0.9175	y = -4.4181x - 0.7706

APPENDIX B, Con'd

60	64.5	13.02	3.6836	0.9024	y = -3.6836x - 1.6104
60	64.5	13.02	3.5938	0.8881	y = -3.5938x - 0.9464
60	66.5	13.02	3.9753	0.924	y = -3.9753x - 1.6031
60	66.5	13.02	3.3075	0.8319	y = -3.3075x - 1.7134
60	66.5	13.02	5.6719	0.9859	y = -5.6719x - 0.2937
60	68.5	13.02	3.9887	0.8877	y = -3.9887x - 1.2742
60	68.5	13.02	5.5219	0.9399	y = -5.5219x - 1.1154
60	68.5	13.02	4.2253	0.9531	y = -4.2253x - 0.9784
65	72.3	1.0915	43.066	0.926	y = -43.066x - 1.1669
65	72.3	1.0915	18.294	0.153	y = -18.294x - 6.3279
65	74.3	1.0915	41.404	0.8778	y = -41.404x - 0.5975
65	74.3	1.0915	20.572	0.4096	y = -20.572x - 4.095
65	76.3	1.0915	29.201	0.7374	y = -29.201x - 2.4432
65	76.3	1.0915	44.03	0.9458	y = -44.03x - 1.5877
65	76.3	1.0915	17.433	0.2957	y = -17.433x - 3.2266
65	64.5	13.02	31.382	0.6846	y = -31.382x - 2.0978
65	64.5	13.02	29.027	0.8822	y = -29.027x - 1.6609
65	66.5	13.02	49.924	0.961	y = -49.924x + 0.0631
65	66.5	13.02	7.2754	0.0539	y = -7.2754x - 1.9004
65	68.5	13.02	37.727	0.9817	y = -37.727x - 0.6134
65	68.5	13.02	43.006	0.9059	y = -43.006x - 1.1311
65	68.5	13.02	32.87	0.9581	y = -32.87x - 1.1007
60	72.3	1.0915	3.9537	0.9264	y = -3.9537x - 1.2963
60	64.5	13.02	2.7795	0.6323	y = -2.7795x - 2.5999
55	72.3	1.0915	0.2435	0.9623	y = -0.2435x - 0.6258
55	64.5	13.02	0.1263	0.7552	y = -0.1263x - 3.0386
65	72.3	1.0915	61.31	0.998	y = -61.31x + 0.133
65	64.5	13.02	17.822	0.4428	y = -17.822x - 2.1011
55	72.3626	1.0915	0.1474	0.5748	y = -0.1474x - 1.6467
55	71.454	1.0915	0.1564	0.9102	y = -0.1564x - 1.6007
55	71.9273	1.0915	0.1623	0.8964	y = -0.1623x - 1.4992
55	71.4248	1.0915	0.1971	0.9294	y = -0.1971x - 1.488
55	71.9598	1.0915	0.2335	0.9329	y = -0.2335x + 1.2427
55	71.7505	1.0915	0.0589	0.2791	y = -0.0589x - 4.0281
65	72.3626	1.0915	45.217	0.969	y = -45.217x - 0.0752
65	71.9273	1.0915	43.831	0.8184	y = -43.831x - 1.9866
65	71.9598	1.0915	10.722	0.1122	y = -10.722x - 4.3436
65	71.7505	1.0915	27.914	0.536	y = -27.914x - 3.8563
55	72.493	1.0915	0.1917	0.8413	y = -0.1917x - 2.0347
55	72.2029	1.0915	0.1433	0.7011	y = -0.1433x - 4.1714

APPENDIX B, con'd

55	72.3925	1.0915	0.2216	0.8617	y = -0.2216x - 2.4289
55	71.8723	1.0915	0.1874	0.8174	y = -0.1874x - 2.0627
55	70.9637	1.0915	0.1519	0.5911	y = -0.1519x - 3.3367
55	71.4545	1.0915	0.1633	0.7563	y = -0.1633x - 3.891
65	72.493	1.0915	41.059	0.8091	y = -41.059x - 2.8638
65	72.2029	1.0915	45.015	0.8666	y = -45.015x - 1.7957
65	72.3925	1.0915	38.849	0.8797	y = -38.849x - 2.1896
65	71.8723	1.0915	32.627	0.6878	y = -32.627x - 3.3337
65	70.9637	1.0915	45.986	0.8769	y = -45.986x - 1.7454
65	71.4545	1.0915	33.519	0.7139	y = -33.519x - 2.7798

PART 2

Temp C	MC %	Fat %	aw	K value (min -1)	R2
60	37.12522	4.285198	0.95	1.315	0.9729
60	54.3668	3.036015	0.981	3.0081	0.9469
60	72.51162	1.780543	0.996	3.7763	0.9589
60	37.12522	4.285198	0.95	1.2877	0.9662
60	54.3668	3.036015	0.981	2.2889	0.9733
60	72.51162	1.780543	0.996	3.362	0.9746

PART 3

Temp C	MC %	Fat %	RH %	K value (min -1)	R2
60	72.3	1.0915	96	4.0202	0.8843
60	72.3	1.0915	90	3.5024	0.769
60	72.3	1.0915	96	3.0003	0.981
60	72.3	1.0915	90	3.7301	0.809
60	64.5	13.02	96	2.8326	0.9698
60	64.5	13.02	90	3.6867	0.8529
60	64.5	13.02	96	3.0196	0.9822
60	64.5	13.02	90	3.7248	0.9019

APPENDIX B, con'd

Regression of y=ln(N/No) against x=time (min)
y = -1.315x - 0.1762
y = -3.0081x - 0.118
y = -3.7763x - 0.5398
y = -1.2877x + 0.2682
y = -2.2889x - 0.3405
y = -3.362x - 0.403

Regression of y=ln(N/No) against x=time (min)
y = -4.0202x - 1.3034
y = -3.5024x - 3.0254
y = -3.0003x + 0.3819
y = -3.7301x - 1.8851
y = -2.8326x + 0.3463
y = -3.6867x - 0.739
y = -3.0196x + 0.4625
y = -3.7248x - 1.1297

APPENDIX C: Output from statistical analyses (Part 1-3)

#1 – Part 1 – Raw data
(For Table 4.4, column a)

Response ln N/No

Summary of Fit

RSquare	0.403525
RSquare Adj	0.387273
Root Mean Square Error	3.992153
Mean of Response	-6.87545
Observations (or Sum Wgts)	378

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	10	3956.9374	395.694	24.8282
Error	367	5848.9832	15.937	Prob > F
C. Total	377	9805.9207		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	169	3905.5658	23.1099	2.3545
Pure Error	198	1943.4174	9.8152	Prob > F
Total Error	367	5848.9832		<.0001
				Max RSq
				0.8018

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	399.26394	36.40635	10.97	<.0001
Time (min)	-4.27912	0.365657	-11.70	<.0001
Temp C	-6.97236	0.590094	-11.82	<.0001
MC %	0.3124975	0.131121	2.38	0.0177
Fat %	0.270407	0.092909	2.91	0.0038
(Time (min)-9.8093)*(Temp C-61.1243)	-0.671289	0.059006	-11.38	<.0001
(Time (min)-9.8093)*(MC %-70.1789)	0.0005768	0.009572	0.06	0.9520
(Time (min)-9.8093)*(Fat %-5.85659)	0.001097	0.006179	0.18	0.8592
(Temp C-61.1243)*(MC %-70.1789)	0.0116665	0.041011	0.28	0.7762
(Temp C-61.1243)*(Fat %-5.85659)	0.0201702	0.026229	0.77	0.4424
(MC %-70.1789)*(Fat %-5.85659)	0.0101322	0.02218	0.46	0.6481

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time (min)	1	1	2182.6007	136.9493	<.0001
Temp C	1	1	2225.0077	139.6102	<.0001
MC %	1	1	90.5238	5.6800	0.0177
Fat %	1	1	134.9998	8.4707	0.0038
Time (min)*Temp C	1	1	2062.7271	129.4278	<.0001
Time (min)*MC %	1	1	0.0579	0.0036	0.9520
Time (min)*Fat %	1	1	0.5023	0.0315	0.8592
Temp C*MC %	1	1	1.2897	0.0809	0.7762
Temp C*Fat %	1	1	9.4249	0.5914	0.4424
MC %*Fat %	1	1	3.3259	0.2087	0.6481

APPENDIX C, con'd

#2 – Part 1 – Raw data
(For Table 4.4, column b)

Response ln N/No
Whole Model

Summary of Fit

RSquare	0.417101
RSquare Adj	0.401204
Root Mean Square Error	3.915196
Mean of Response	-7.17878
Observations (or Sum Wgts)	227

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	2413.1140	402.186	26.2373
Error	220	3372.3277	15.329	Prob > F
C. Total	226	5785.4417		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	129	2736.1469	21.2104	3.0340
Pure Error	91	636.1808	6.9910	Prob > F
Total Error	220	3372.3277		<.0001
				Max RSq
				0.8900

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	471.62286	57.51517	8.20	<.0001
Time (min)	-3.857806	0.463262	-8.33	<.0001
Temp C	-8.126511	0.962308	-8.44	<.0001
MC %	0.2497615	0.168525	1.48	0.1398
(Time (min)-11.9431)*(Temp C-60.7489)	-0.641911	0.07979	-8.05	<.0001
(Time (min)-11.9431)*(MC %-73.049)	0.0005812	0.011151	0.05	0.9585
(Temp C-60.7489)*(MC %-73.049)	-0.049699	0.050796	-0.98	0.3289

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time (min)	1	1	1063.0018	69.3469	<.0001
Temp C	1	1	1093.1682	71.3148	<.0001
MC %	1	1	33.6688	2.1964	0.1398
Time (min)*Temp C	1	1	992.1099	64.7221	<.0001
Time (min)*MC %	1	1	0.0416	0.0027	0.9585
Temp C*MC %	1	1	14.6739	0.9573	0.3289

APPENDIX C, con'd

#3 – Part 1 – Raw data
(For Table 4.4, column c)

Response ln N/No

Summary of Fit

RSquare	0.405
RSquare Adj	0.380208
Root Mean Square Error	4.049244
Mean of Response	-6.41946
Observations (or Sum Wgts)	151

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	1607.1183	267.853	16.3361
Error	144	2361.0778	16.396	Prob > F
C. Total	150	3968.1961		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	37	1053.8413	28.4822	2.3313
Pure Error	107	1307.2365	12.2172	Prob > F
Total Error	144	2361.0778		0.0004
				Max RSq
				0.6706

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	287.42852	40.45771	7.10	<.0001
Time (min)	-5.014653	0.592644	-8.46	<.0001
Temp C	-5.103426	0.606911	-8.41	<.0001
MC %	0.3706851	0.208294	1.78	0.0772
(Time (min)-6.60155)*(Temp C-61.6887)	-0.723031	0.087321	-8.28	<.0001
(Time (min)-6.60155)*(MC %-65.8642)	-0.005029	0.01837	-0.27	0.7847
(Temp C-61.6887)*(MC %-65.8642)	0.1124595	0.069086	1.63	0.1057

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time (min)	1	1	1173.9288	71.5969	<.0001
Temp C	1	1	1159.3687	70.7089	<.0001
MC %	1	1	51.9283	3.1671	0.0772
Time (min)*Temp C	1	1	1124.1390	68.5602	<.0001
Time (min)*MC %	1	1	1.2286	0.0749	0.7847
Temp C*MC %	1	1	43.4471	2.6498	0.1057

APPENDIX C, con'd

#4 – Part 1 – k values
(For Table 4.5, column a)

Response k value

Summary of Fit

RSquare	0.659495
RSquare Adj	0.633957
Root Mean Square Error	11.1814
Mean of Response	16.18663
Observations (or Sum Wgts)	87

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	19371.800	3228.63	25.8242
Error	80	10001.897	125.02	Prob > F
C. Total	86	29373.697		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	33	3161.181	95.793	0.6582
Pure Error	47	6840.716	145.547	Prob > F
Total Error	80	10001.897		0.8954
				Max RSq
				0.7671

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-143.586	58.91756	-2.44	0.0170
Temp C	3.3322601	0.280872	11.86	<.0001
MC %	-0.507665	0.746551	-0.68	0.4985
Fat %	-0.330035	0.529535	-0.62	0.5349
(Temp C-60.8621)*(MC %-70.3188)	0.0631256	0.182653	0.35	0.7305
(Temp C-60.8621)*(Fat %-5.6161)	-0.027434	0.114869	-0.24	0.8119
(MC %-70.3188)*(Fat %-5.6161)	0.2709161	0.129427	2.09	0.0395

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Temp C	1	1	17597.531	140.7535	<.0001
MC %	1	1	57.813	0.4624	0.4985
Fat %	1	1	48.565	0.3884	0.5349
Temp C*MC %	1	1	14.933	0.1194	0.7305
Temp C*Fat %	1	1	7.131	0.0570	0.8119
MC %*Fat %	1	1	547.786	4.3815	0.0395

APPENDIX C, con'd

#5 – Part 1 – k values
(For Table 4.5, column b)

Response k value

Summary of Fit

RSquare	0.663959
RSquare Adj	0.643797
Root Mean Square Error	12.22015
Mean of Response	17.77034
Observations (or Sum Wgts)	54

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	14752.730	4917.58	32.9305
Error	50	7466.603	149.33	Prob > F
C. Total	53	22219.334		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	27	2215.3711	82.051	0.3594
Pure Error	23	5251.2324	228.314	Prob > F
Total Error	50	7466.6035		0.9941
				Max RSq
				0.7637

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-67.83111	79.2747	-0.86	0.3963
Temp C	3.5752004	0.374369	9.55	<.0001
MC %	-1.797851	1.040144	-1.73	0.0901
(Temp C-60.6481)*(MC %-72.9859)	-0.118713	0.25058	-0.47	0.6377

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Temp C	1	1	13619.305	91.2015	<.0001
MC %	1	1	446.143	2.9876	0.0901
Temp C*MC %	1	1	33.516	0.2244	0.6377

APPENDIX C, con'd

#6 – Part 1 – k values
(For Table 4.5, column c)

Response k value

Summary of Fit

RSquare	0.658678
RSquare Adj	0.623369
Root Mean Square Error	8.944401
Mean of Response	13.5951
Observations (or Sum Wgts)	33

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	4477.2288	1492.41	18.6546
Error	29	2320.0672	80.00	Prob > F
C. Total	32	6797.2960		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	5	730.5840	146.117	2.2063
Pure Error	24	1589.4832	66.228	Prob > F
Total Error	29	2320.0672		0.0870
				Max RSq
				0.7662

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-259.5101	72.65463	-3.57	0.0013
Temp C	2.8552049	0.39008	7.32	<.0001
MC %	1.4992956	0.964787	1.55	0.1310
(Temp C-61.2121)*(MC %-65.9545)	0.3789683	0.241721	1.57	0.1278

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Temp C	1	1	4286.1626	53.5755	<.0001
MC %	1	1	193.2031	2.4150	0.1310
Temp C*MC %	1	1	196.6433	2.4580	0.1278

APPENDIX C, con'd

#7 – Part 2 – Raw data
(For Table 4.6, column a)

Response ln N/No

Summary of Fit

RSquare	0.950327
RSquare Adj	0.947666
Root Mean Square Error	0.769737
Mean of Response	-3.97773
Observations (or Sum Wgts)	60

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	634.78981	211.597	357.1279
Error	56	33.17974	0.592	Prob > F
C. Total	59	667.96955		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	11	14.632414	1.33022	3.2274
Pure Error	45	18.547323	0.41216	Prob > F
Total Error	56	33.179738		0.0026
				Max RSq
				0.9722

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5.8261487	0.413525	14.09	<.0001
Time (min.)	-2.506345	0.093689	-26.75	<.0001
MC	-0.110565	0.006878	-16.08	<.0001
(Time (min.)-1.5)*(MC-54.6679)	-0.063971	0.006485	-9.87	<.0001

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time (min.)	1	1	424.01911	715.6497	<.0001
MC	1	1	153.11004	258.4156	<.0001
Time (min.)*MC	1	1	57.66066	97.3183	<.0001

APPENDIX C, con'd

#8 – Part 2 – Raw data
(For Table 4.6, column b)

Response ln N/No

Summary of Fit

RSquare	0.95163
RSquare Adj	0.949039
Root Mean Square Error	0.759576
Mean of Response	-3.97773
Observations (or Sum Wgts)	60

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	635.66002	211.887	367.2493
Error	56	32.30953	0.577	Prob > F
C. Total	59	667.96955		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	11	13.762207	1.25111	3.0355
Pure Error	45	18.547323	0.41216	Prob > F
Total Error	56	32.309530		0.0041
				Max RSq
				0.9722

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	81.274211	4.997822	16.26	<.0001
Time (min.)	-2.506345	0.092453	-27.11	<.0001
Aw	-83.52486	5.11951	-16.32	<.0001
(Time (min.)-1.5)*(Aw-0.97567)	-48.42232	4.82672	-10.03	<.0001

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time (min.)	1	1	424.01911	734.9246	<.0001
Aw	1	1	153.57389	266.1796	<.0001
Time (min.)*Aw	1	1	58.06702	100.6438	<.0001

APPENDIX C, con'd

#9 – Part 2 – k values
(For Table 4.7, column a)

Response k value

Summary of Fit

RSquare	0.923759
RSquare Adj	0.904699
Root Mean Square Error	0.325197
Mean of Response	2.506333
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	5.1253581	5.12536	48.4654
Error	4	0.4230121	0.10575	Prob > F
C. Total	5	5.5483701		0.0022

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	1	0.07819285	0.078193	0.6803
Pure Error	3	0.34481921	0.114940	Prob > F
Total Error	4	0.42301206		0.4700
				Max RSq
				0.9379

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.990789	0.519584	-1.91	0.1292
mc	0.0639703	0.009189	6.96	0.0022

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
mc	1	1	5.1253581	48.4654	0.0022

APPENDIX C, con'd

#10 – Part 2 – k values
(For Table 4.7, column b)

Response k value

Summary of Fit

RSquare	0.930272
RSquare Adj	0.91284
Root Mean Square Error	0.310997
Mean of Response	2.506333
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	5.1614932	5.16149	53.3657
Error	4	0.3868769	0.09672	Prob > F
C. Total	5	5.5483701		0.0019

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	1	0.04205768	0.042058	0.3659
Pure Error	3	0.34481921	0.114940	Prob > F
Total Error	4	0.38687689		0.5879
				Max RSq
				0.9379

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-44.73762	6.468426	-6.92	0.0023
water act	48.422229	6.628473	7.31	0.0019

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
water act	1	1	5.1614932	53.3657	0.0019

APPENDIX C, con'd

#11 – Part 3 – Raw data
(For Table 4.10, column a)

Response ln N/No

Summary of Fit

RSquare	0.856687
RSquare Adj	0.834294
Root Mean Square Error	1.767196
Mean of Response	-6.13153
Observations (or Sum Wgts)	75

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	10	1194.7705	119.477	38.2574
Error	64	199.8708	3.123	Prob > F
C. Total	74	1394.6413		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	63	199.86015	3.17238	299.2562
Pure Error	1	0.01060	0.01060	Prob > F
Total Error	64	199.87076		0.0459
				Max RSq
				1.0000

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-44.74747	13.38503	-3.34	0.0014
Time (min)	-3.465307	0.253064	-13.69	<.0001
RH %	0.3889134	0.071496	5.44	<.0001
final mc %	0.0818721	0.177325	0.46	0.6459
Fat %	0.1833283	0.132424	1.38	0.1710
(Time (min)-1.53)*(RH %-92.8)	0.1524578	0.09085	1.68	0.0982
(Time (min)-1.53)*(final mc %-67.2343)	-0.127683	0.128414	-0.99	0.3238
(Time (min)-1.53)*(Fat %-7.13527)	-0.099054	0.097182	-1.02	0.3119
(RH %-92.8)*(final mc %-67.2343)	0.0744757	0.063111	1.18	0.2423
(RH %-92.8)*(Fat %-7.13527)	0.0594761	0.048376	1.23	0.2234
(Fat %-7.13527)*(final mc %-67.2343)	-0.031204	0.033059	-0.94	0.3488

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time (min)	1	1	585.58976	187.5099	<.0001
RH %	1	1	92.40874	29.5899	<.0001
final mc %	1	1	0.66574	0.2132	0.6459
Fat %	1	1	5.98543	1.9166	0.1710
Time (min)*RH %	1	1	8.79463	2.8161	0.0982
Time (min)*final mc %	1	1	3.08754	0.9887	0.3238
Time (min)*Fat %	1	1	3.24446	1.0389	0.3119
RH %*final mc %	1	1	4.34896	1.3926	0.2423
RH %*Fat %	1	1	4.72066	1.5116	0.2234
Fat %*final mc %	1	1	2.78229	0.8909	0.3488

APPENDIX C, con'd

#12 – Part 3 – Raw data
(For Table 4.10, column b)

Response ln N/No

Summary of Fit

RSquare	0.835362
RSquare Adj	0.802435
Root Mean Square Error	2.062154
Mean of Response	-6.84133
Observations (or Sum Wgts)	37

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	647.30535	107.884	25.3697
Error	30	127.57440	4.252	Prob > F
C. Total	36	774.87975		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-50.53718	23.71511	-2.13	0.0414
Time (min)	-3.480053	0.394869	-8.81	<.0001
RH %	0.3153437	0.126101	2.50	0.0181
final mc %	0.2589522	0.357111	0.73	0.4740
(Time (min)-1.52027)*(RH %-92.7568)	0.3841554	0.184746	2.08	0.0462
(Time (min)-1.52027)*(final mc %-71.6382)	-0.830827	0.360285	-2.31	0.0282
(RH %-92.7568)*(final mc %-71.6382)	0.406191	0.176535	2.30	0.0285

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time (min)	1	1	330.29953	77.6722	<.0001
RH %	1	1	26.59345	6.2536	0.0181
final mc %	1	1	2.23601	0.5258	0.4740
Time (min)*RH %	1	1	18.38672	4.3238	0.0462
Time (min)*final mc %	1	1	22.61361	5.3177	0.0282
RH %*final mc %	1	1	22.51338	5.2942	0.0285

APPENDIX C, con'd

#13 – Part 3 – Raw data
(For Table 4.10, column c)

Response ln N/No

Summary of Fit

RSquare	0.920269
RSquare Adj	0.904838
Root Mean Square Error	1.224489
Mean of Response	-5.44041
Observations (or Sum Wgts)	38

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	536.48950	89.4149	59.6349
Error	31	46.48055	1.4994	Prob > F
C. Total	37	582.97005		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	30	46.469949	1.54900	146.1196
Pure Error	1	0.010601	0.01060	Prob > F
Total Error	31	46.480550		0.0654
				Max RSq
				1.0000

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-24.31497	14.10394	-1.72	0.0947
Time (min)	-3.633116	0.316625	-11.47	<.0001
RH %	0.4177081	0.066414	6.29	<.0001
final mc %	-0.227224	0.195133	-1.16	0.2531
(Time (min)-1.53947)*(RH %-92.8421)	0.2277787	0.097777	2.33	0.0265
(Time (min)-1.53947)*(final mc %-62.9463)	0.0230355	0.105119	0.22	0.8280
(RH %-92.8421)*(final mc %-62.9463)	0.0653434	0.055137	1.19	0.2450

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time (min)	1	1	197.41326	131.6639	<.0001
RH %	1	1	59.31084	39.5571	<.0001
final mc %	1	1	2.03309	1.3560	0.2531
Time (min)*RH %	1	1	8.13693	5.4269	0.0265
Time (min)*final mc %	1	1	0.07200	0.0480	0.8280
RH %*final mc %	1	1	2.10582	1.4045	0.2450

APPENDIX C, con'd

#14 – Part 3 – K values
(For Table 4.11, column a)

Response K value

Summary of Fit

RSquare	0.567857
RSquare Adj	0.24375
Root Mean Square Error	0.375577
Mean of Response	3.439588
Observations (or Sum Wgts)	8

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.7414294	0.247143	1.7521
Error	4	0.5642320	0.141058	Prob > F
C. Total	7	1.3056614		0.2947

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	10.449669	4.121517	2.54	0.0643
RH %	-0.073804	0.044262	-1.67	0.1708
Fat Content	-0.020734	0.022264	-0.93	0.4044
(RH %-93)*(Fat Content-7.05575)	-0.009412	0.007421	-1.27	0.2735

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
RH %	1	1	0.39218796	2.7803	0.1708
Fat Content	1	1	0.12233931	0.8673	0.4044
RH %*Fat Content	1	1	0.22690216	1.6086	0.2735

APPENDIX C, con'd

#15 – Part 3 – K values
(For Table 4.11, column b)

Response K value

Summary of Fit

RSquare	0.020163
RSquare Adj	-0.46976
Root Mean Square Error	0.522504
Mean of Response	3.56325
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.01123600	0.011236	0.0412
Error	2	0.54602165	0.273011	Prob > F
C. Total	3	0.55725765		0.8580

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5.20625	8.103031	0.64	0.5864
RH %	-0.017667	0.087084	-0.20	0.8580

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
RH %	1	1	0.01123600	0.0412	0.8580

APPENDIX C, con'd

#16 – Part 3 – K values
(For Table 4.11, column c)

Response K value

Summary of Fit

RSquare	0.970913
RSquare Adj	0.95637
Root Mean Square Error	0.095421
Mean of Response	3.315925
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.60785412	0.607854	66.7594
Error	2	0.01821031	0.009105	Prob > F
C. Total	3	0.62606443		0.0147

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	15.4005	1.479794	10.41	0.0091
RH %	-0.129942	0.015903	-8.17	0.0147

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
RH %	1	1	0.60785412	66.7594	0.0147

APPENDIX C, con'd

#17 – Part 1 and 2 – Raw values
(For Table 4.12)

Response ln N/No

Summary of Fit

RSquare	0.915669
RSquare Adj	0.910399
Root Mean Square Error	1.229352
Mean of Response	-6.17514
Observations (or Sum Wgts)	35

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	525.11568	262.558	173.7290
Error	32	48.36181	1.511	Prob > F
C. Total	34	573.47748		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	7	34.037903	4.86256	8.4868
Pure Error	25	14.323906	0.57296	Prob > F
Total Error	32	48.361809		<.0001
				Max RSq
				0.9750

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.785543	0.361165	-2.18	0.0371
Time (min)	-3.631953	0.195914	-18.54	<.0001
raw vs. cooked[cooked]	0.4083415	0.209952	1.94	0.0606

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time (min)	1	1	519.39878	343.6753	<.0001
raw vs. cooked	1	1	5.71690	3.7828	0.0606

APPENDIX D: Output from secondary modeling (Part 2)

Part 2-raw model-nonlinear

#1 - Water term = 1/aw

Nonlinear fit
Control panel
Report
Coverged in the gradient

Criterion	Current	Stop limit
Iteration	189	200
Shortening	0	15
Obj change	1.13E-10	1E-07
pmr change	3.82E-05	1E-07
Gradient	3.652	0.000001

Parameter	Current value lock		
a	2.0176	SSE	32.327
Ea	332479	N	60
b1	-21.949		

Confidence Limits
Convergence Criterion 0.05

Solution

SSE	DFE	MSE	RMSE
32.327	58	0.557	0.747

Parameter	Estimate	ApproxStdErr
a	2.02E+62	2.89E+62
Ea	332479	0
b1	-21.949	1.413

APPENDIX D, con'd

#2 - Water term = aw

Nonlinear fit
Control panel
Report
Coverged in the gradient

Criterion	Current	Stop limit
Iteration	177	200
Shortening	0	15
Obj change	2.90E-08	1E-07
pmr change	0.000396	1E-07
Gradient	9.44E-07	0.000001

Parameter	Current value	lock		
a	5.88E+42		SSE	32.518
Ea	332479		N	60
b1	23.04			

Confidence Limits
Convergence Criterion 0.05

Solution

SSE	DFE	MSE	RMSE
32.518	58	0.561	0.749

Parameter	Estimate	ApproxStdErr
a	5.88E+42	8.59E+42
Ea	332479	0
b1	23.04	1.48

APPENDIX D, con'd

#3 - Water term = aw+aw2

Nonlinear fit
Control panel
Report
Coverged in the gradient

Criterion	Current	Stop limit
Iteration	47	200
Shortening	0	15
Obj change	6.58E+10	1E-07
pmr change	0.000136	1E-07
Gradient	2.19E-08	0.000001

Parameter	Current value	lock		
a	4.49E+47		SSE	32.64
Ea	332479		N	60
b1	11.799			

Confidence Limits
Convergence Criterion 0.05

Solution

SSE	DFE	MSE	RMSE
32.64	58	0.563	0.75

Parameter	Estimate	ApproxStdErr
a	4.49E+47	3.32E+47
Ea	332479	0
b1	11.799	0.757

APPENDIX E: Initial microbial counts (Part 2)

	CFU/g		
72.5% MC	**Plate 1**	**Plate 2**	**AVG**
Sample A	20	0	10
Sample B	0	0	0
Sample A	550	360	455
Sample B	610	510	560
AVG			256.25
SD			293.297

	CFU/g		
54.4% MC	**Plate 1**	**Plate 2**	**AVG**
Sample A	20	0	10
Sample B	0	0	0
Sample A	4200	4600	4400
Sample B	620	1220	920
AVG			1332.5
SD			2090

	CFU/g		
37.1% MC	**Plate 1**	**Plate 2**	**AVG**
Sample A	20	80	50
Sample B	150	50	100
Sample A	50	40	45
Sample B	40	10	25
AVG			55
SD			31.8852

OVERALL AVG 547.917
OVERALL SD 1248.27

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